

**ARBUSCULAR MYCORRHIZAL (AM)
FUNGAL DIVERSITY OF DEGRADED IRON
ORE MINE WASTELANDS OF GOA.**

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By

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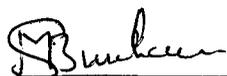
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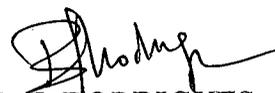
DECLARATION

I hereby declare that the Ph. D. thesis entitled “**ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY OF DEGRADED IRON ORE MINE WASTELANDS 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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I certify that the thesis entitled “**ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY OF DEGRADED IRON ORE MINE WASTELANDS OF GOA**” submitted by Ms. MEHTAB JAHAN BUKHARI is a record of research work done by her during the period from 1999-2002 when she worked under my supervision. The thesis has not formed the basis of any Degree, Diploma, Associate-ship or Fellowship to Ms. MEHTAB JAHAN BUKHARI.

I affirm that the thesis submitted by Ms. MEHTAB JAHAN BUKHARI incorporates the independent research work carried out by her under my supervision.



A handwritten signature in black ink, appearing to read "B. F. Rodrigues", written in a cursive style.

B. F. RODRIGUES

(Signature of the Guide)



*DEDICATED TO
MY PARENTS*

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INTRODUCTION

GENERAL INTRODUCTION

Mining is one of the most common activities of ancient and highly sophisticated capital intensive industry of the modern world. Its reference is even found in the Vedas. Mining is regarded as the second largest industry after agriculture and has played a vital role for the development of civilization from ancient days. Much of the world's wealth such as metals, chemicals, fuel for energy, rocks and stones for building comes from mining (Trivedy, 1990). From the mid 19th century onwards, the industry expanded rapidly so that by the 1950's there were extensive mining enterprises throughout the world. There is not a single industry, which can do without minerals or their products. Minerals thus form a part and parcel of our daily life. Minerals are the backbone of economic growth of any nation. The area of land currently disturbed by mining is approximately 8,24,000 hectares per year. Therefore, in this quarter of the Century alone, the mine degraded land would amount to nearly 24 million hectares or 0.2% of the earth's total surface (Soni and Vasistha, 1986).

MINING INDUSTRY IN GOA

Mining is the dominant industry in Goa. The State of Goa is rich in minerals such as iron ore, manganese ore, bauxite, silica sand, high magnesia, limestone and clay (Swaminathan, 1982). Geographically, the State of Goa is located along the mid-west coast of India. It covers an area of about 3702 km from North to South, the coastline stretches to a length of about 105 km and from East to West, it is 65 km wide. It is bound between the coordinates 15° 48' 00" N and 14° 53' 54" N Latitude and 74° 20' 13" E and 73° 40' 33" E Longitude.

The first reference to the mineral contents in Goan soil dates back to the 16th century. A Dutch traveler by name John H.V. Linschoten had written that in Goa there are many stones containing iron (Gune, 1979). In the year 1905, a few French and German companies had carried out prospects for iron and manganese ore in Goa. Goa has been a prime exporter of iron ore since 1950. The areas under mining in Goa are Bardez, Bicholim, Canacona, Sanguem, Tiswadi, Mormugao, Ponda, Salcete, Pernem and Satari. Mining contributes to about 10% of the total economy (Sardesai, 1985), with iron ore being the most predominant in terms of both production and exports. During 1971-80, Goa accounted for 32% of the country's total iron ore production and 55% of its exports (Swaminathan, 1982). The estimated reserves of iron ore as of today is 1400 million tons and is expected to last for another 25-30 years (i.e., by the year 2020 to 2030) and hence the reject material will be over 5 billion tons excluding the present (already existing 1 billion tons) at the present rate of mining (Nayak, 2002).

MINE DUMPS AND TAILINGS

The mining operation are such that, two classes of wastes are produced *viz.*, (i) piles of surface overburden waste rock and lean ore, which constitutes the reject dumps, and (ii) a fine grained waste resulting from the ore beneficiation process and deposited in large man-made basin called tailing ponds. The latter kinds of waste materials are termed as tailings (Rodrigues, 1997).

MINING HAZARDS - IMPACT OF MINING ON ENVIRONMENT AND HUMAN HEALTH

Mining appears to be one of the most degrading actions of man on earth as it physically tears up the earth surface, producing gaping holes and barren heaps, changes the geomorphic pattern and contaminates the environment.

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

Mining accounts for a substantial proportion of the loss of land of primary production. In India, 7, 85,000 hectares of land is reported to be under mining operations (Baliga, 1985). Due to large-scale deforestation, the

aggregate forest area in the country dwindled by 16.1% from 5.62 lakh⁵sq. km. in 1972-75 to 4.63 lakhs sq. km. in 1980-82 according to estimates made by the National Remote Sensing Agency (NRSA).

In Goa, deforestation was inevitable as 70% of the mining activity is carried out in forest area (D'Souza and Nayak, 1994). Indiscriminate mining since 1961 has destroyed 50,000 hectares of forest in Goa and it is estimated that during all these years as much as 900 to 1000 million tones of waste rock, low-grade ores and tailings have been accumulated near mining areas. The waste material consists mainly of laterites, phyllites, quartzites, manganiferous and other types of clays, slimes *etc.*

Over 30 million tones of iron ore rejects are scattered over an area of 10,000 hectare of paddy and coconut groves thus killing the fertility of the soil. This in many cases drastically changes the microclimatic conditions, which lead to degradation of flora and fauna. Sliding and slumping of land due to large-scale excavations in surface mining is the basic cause of land degradation. Large-scale surface mining produces enormous quantities of overburden, which are spread over large areas making them unusable.

Iron ore belts in mining region are aquifers, which store water (D'Souza and Nayak, 1994). In mines where workings have gone below the water table, pumping out the water from the mining pits, besides polluting the water stream has resulted in the depression of water table thus depriving the neighbouring villages of well water supply, especially during the summer

months. This eventually affects the biodiversity in the forest and rivers due to shortage of water. Silting of waterways over the years have caused flooding of adjacent fields (Ghosh, 1990). Deforestation of all quarry sites is prerequisite for any mining operation. It is found that besides the quarry area, vast expanses of other adjacent areas have been deforested.

About 1000-5000 kg of explosives is used for blasting per mine per month (D' Souza, 1991). With the increase of mining operation heavy blasting has to be resorted to, thus leading to ground vibrations which may lead to damage of structures in the vicinity of mining centers and may also cause irritation to the people inhabiting nearby (Paliwal, 1989). Heavy noise causes disturbances to sense organs, cardiovascular systems, while physical pains results at a level of 140 perceived noise decibels.

When mining operations are in full swing, the air is prominently visible. Inside the mine, the mining activities raise dust into the atmosphere causing air pollution. Thus, the particulate matter remains suspended in the air due to the continuous day and night operations causing diseases such as silicosis, tuberculosis and allergic diseases like asthma in mine workers and inhabitants of the area. The dust poses a serious nuisance as it contains numerous metallic compounds which has its effects on nearby communities, industrial machinery and demanding effects on vegetation by blocking plant pores as well as reducing high penetration and photosynthesis (Rodrigues, 1997).

In mining industry, water pollution mainly occurs in the form of mine drainage. Mining affects the hydrological regime by direct discharge of the mine water to the streams and, due to erosion and wash off from mined out area and waste dumps (Chaudhari, 1994).

Studies have shown that there was significant effect of mining on the fauna (Ganihar, 1990). Mining is found to have an adverse effect on the activity of microorganisms, nitrogen fixers, ammonifiers, cellulolytic bacteria, phosphorus solubilizing bacteria which are important organisms generating the essential nutrients to plants via the food chain which are reduced due to the toxic effects of mine materials (D'Souza and Nayak, 1994).

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

Damage to environment by the mining activity has been caused largely by reject dumps, pumping out of muddy waters from the working pits including those where the mine working have gone below the water level, and slimes from the beneficiation plant. The damage is more conspicuous during monsoon, when the rainwater carries the washed out materials from the mine

waste dumps to the adjoining agricultural fields and water streams. The slimes and silts, which enter the agricultural fields are of such character, they get hardened on drying, thus making aeration and root penetration difficult.

ECOSYSTEM, PLANT SUCCESSION AND MINING

The vegetation together with the soil in which it has its roots, the associated fauna, and the environment that surrounds them form a closely interrelated and interdependent system. They interact and support each other to constitute an ecosystem. Although ecosystems are sensitive to the outside influences, they are self-sustaining. Once properly established, they need no further support. This is because of natural cycling of accumulated materials, which maintains the vegetation and the other organisms within it. The ecosystems have a capacity to develop. In nature, after a major disturbance, vegetation slowly and gradually develops over a period of time. This process is termed as Plant Succession.

- If the above two properties (self-sustaining and capacity to develop) of the ecosystem are considered, then one may presume that after mining disturbance there is no need of any revegetational efforts *i.e.*, a self-sustaining vegetation cover will develop naturally. This, of course, is true but the process of natural succession will take many years. As mining leaves behind several problems, such as bare rock faces of materials contaminated with heavy metals, the process of natural succession will be even much slower. It is quite possible that further degradation could take place, especially by erosion, which could have serious effects on surrounding land also. The use of expensive

input is inappropriate for developing countries where there is general reduction to increase mining costs because of limited financial resources. 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 like arbuscular mycorrhizal fungi.

MYCORRHIZAE

Mycorrhizae are symbiotic associations that form between the roots of most plant species and fungi. Bi-directional movement of nutrients characterizes these symbioses where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil.

Mycorrhizae have been categorized into two major groups based on their infection anatomy (Frank, 1885) viz.,

- 1) **Ectomycorrhizae**- are characterized by (a) a mantle of fungal tissue around the host rootlets and (b) penetration of the fungus between cells of the rootlet cortex ('Hartig net') but not into the cells themselves.
- 2) **Endomycorrhizae**- are characterized by the penetration of the root cortex cells by the fungus. In this case there is no Hartig net and no mantle.

In Endomycorrhiza the fungus grows inter- and intra-cellularly and forms within cortical cells specific fungal structures. In Ectomycorrhiza often a thick hyphal mantle is formed around feeder roots and these roots are

morphologically altered. Description of different types of mycorrhizae as classified by Harley, (1989) has been outlined in **Table-1**.

Of all the several kinds of mycorrhizae, arbuscular mycorrhizal fungi are the most prevalent ones. They are ubiquitous in distribution and are found in most ecosystems including dense Rain Forest, Open Wood lands, Savanna, Grasslands, Heaths, Sand dunes, Semi-arid deserts and Mine wastelands.

ARBUSCULAR MYCORRHIZAL FUNGI

The diagnostic feature of arbuscular mycorrhizae (AM) is the development of a highly branched arbuscules within root cortical cells. The fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within the cell. The general term for all mycorrhizal types where the fungus grows within cortical cells is endomycorrhiza. They are widespread in occurrence and occur in all types of soils from mine spoils to agricultural soils. The majority of higher green plants and large numbers of fungi are involved in mycorrhiza formation. About 80% species of Angiosperms, 100% of the Gymnosperms and 70% of the Pteridophytes were found to be mycorrhizal (Harley and Harley, 1987).

There are several consistent structure/function relationships associated with all arbuscular mycorrhizal symbiosis, which are critical to understanding their role in ecosystem dynamics. The arbuscule is an important point of contact for their exchange of resources between plant and fungus. Both organisms retain a membrane to separate their adjacent cells (Cox and Tinker,

**Table- 1. Characteristics of important kinds of mycorrhizas in their mature state
(Harley, 1989).**

| Character | Kinds of mycorrhiza | | | | | | |
|------------------------------|----------------------|-----------------|--------------------|---------------------|------------------------|--------------------|-------------------|
| | Vesicular-arbuscular | Ecto-mycorrhiza | Ectendo-mycorrhiza | Arbutoid mycorrhiza | Monotropoid mycorrhiza | Ericoid mycorrhiza | Orchid mycorrhiza |
| Fungus | | | | | | | |
| Septate | | + | + | + | + | + | + |
| Aseptate | + | (+) | - | - | - | - | + |
| Hyphae enter cells | + | - | + | + | + | + | - |
| Fungal sheath present | - | + | + or - | + | + | - | + |
| Hartig net formed | - | + | + | + | + | - | - |
| Hyphal coils in cells | + | - | + | + | | + | - |
| Haustoria | | | | | | | |

Cont.

| | | | | | | | |
|------------------------------|-----------------------------------|-------------------------------------|------------------|----------|----------------------|------------------------------|-------------|
| Dichotomous | + | - | - | - | | - | - |
| Non dichotomous | - | - | - | - | + | - | + or - |
| Vesicles in cells or tissues | + or (-) | - | - | - | - | - | - |
| Achlorophyll | - or (+) | - | - | - or (+) | + | - | + |
| Fungal taxon | Phyco | Basidio Asco Phyco Deutero | Basidio Asco? | Basidio | Basidio | Asco (Basidio) Deutero | Basidio |
| Host taxon | Bryo Pterido Gymno Angio | Gymno Angio (Pterido) | Gymno Angio | Ericales | Monotropic- aceae | Ericales | Orchidaceae |

1976). Enzymes such as phosphatases are concentrated along the interface, presumably facilitating nutrient movement from fungus to plant. Intraradical structures, because they exist, create an altered physiology of the host. Several research laboratories are presently studying the mechanisms whereby signals are exchanged between host and fungus, which are regulating their development (Giovannetti *et al.*, 1994).

Associated with the hyphae that penetrate a root is an external matrix that radiates out into the soil. There are important architectural features associated with these extrametrical hyphae. These include runner, or arterial hyphae that are thick walled and often-traverse long distances to invade uncolonized roots (Mosse, 1959 ; Friese and Allen, 1991). These hyphae may be a major carbon sink in storing and transporting a large amount of energy necessary for colonizing new roots. These hyphae may also serve as bridges between plants, transporting resources between individuals (Read, 1992).

When a hypha penetrates the root, the runner hypha produces finer branched hypha that radiates out into the soil matrix. Friese and Allen (1991) suggested that this pattern was a distinct architectural feature of the symbiosis that is critical to arbuscular mycorrhizal functioning.

Vesicles develop to accumulate storage products in many arbuscular mycorrhizal associations as they are initiated soon after the first arbuscules, but continue to develop when the arbuscules senesce. Vesicles are hyphal swellings in the root cortex that contain lipids and cytoplasm. These may be

inter- or intra-cellular. Vesicles can develop thick walls in older roots and may function as propagules (Biermann and Linderman 1983). Some fungi produce vesicles which are similar in structure to the spores they produced in soil, but in other cases they are different.

TYPES OF ARBUSCULAR MYCORRHIZAL FUNGI

There are two types of arbuscular mycorrhizal (AM) fungi (Barker *et al.*, 1998).

Arum type and 2) Paris type

Many herbaceous plants exhibit the Arum colonization type which involves extensive intercellular growth of the fungus as it penetrates the root cortex, followed later in the colonization by formation of arbuscules. In Paris type of colonization growth into the root is slow, being primarily intra-cellular, and the fungus forms coils inside each cell with rare or minimally structured arbuscules (Gallaud, 1905).

STAGES OF DEVELOPMENT OF ARBUSCULAR MYCORRHIZAL FUNGI

The development of arbuscular mycorrhizal colonization in roots can be divided into the following four stages (Tommerup and Briggs, 1981).

- Spores germination and hyphal growth from infective propagules of arbuscular mycorrhizal fungi.
- Growth of hyphae through soil to host roots. The mycelial system surrounding the roots is dimorphic (Mosse, 1959 ; Nicolson, 1967).

- Penetration and successful initiation of colonization of roots. Hyphae penetrate mechanically and enzymatically into cortical cells (Kinden and Brown, 1975). At the point, penetrating hyphae may or may not form appresoria (Abbott, 1982).
- Spread of colonization and development of internal hyphal system, arbuscules, which bifurcates inside a cell and bring about nutritional transfer between the two symbionts and vesicles, which develop as terminal or intercalary swellings in inter- or intra-cellular hyphae. They are also responsible for storage and vegetative reproduction.

The term vesicular-arbuscular mycorrhiza (VAM) was originally applied to symbiotic associations formed by all fungi in the Glomales, but because a major suborder lacks the ability to form vesicles in roots, arbuscular mycorrhiza (AM) is now the preferred acronym. The order Glomales is further divided into families and genera according to the method of spore formation. The spores of arbuscular mycorrhizal fungi are very distinctive (Morton and Benny, 1990). Schubler *et al.*, (2001), based on comprehensive SSU rRNA analysis, and on the basis of natural relationship of arbuscular mycorrhizal fungi and the related fungi, recognized a new fungal phylum *Glomeromycota*.

ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI

Arbuscular mycorrhizal (AM) fungi play a very important role in the improvement of plant growth. Most vascular plants require mycorrhizae for

their survival. They are vital for the uptake and accumulation of ions from the soil and their translocation to the hosts because of their high metabolic rate and strategically diffuse distribution in the upper soil layers. The fungus serves as a highly efficient extension of the host root system (Bolan, 1991). Minerals like N, P, K, Ca, S, Zn, Cu, and Sr are absorbed from the soil by arbuscular mycorrhizal fungi and are translocated to the host plant (Mosse, 1957). Improved nutrient uptake, especially phosphorus (P) is the primary cause for improved plant productivity. Earlier studies (Baylis, 1967; Sanders and Tinker, 1971; Marschener and Dell, 1994; Fidelibus, *et al*, 2001) have revealed that mycorrhizal roots absorbed phosphate from low P status at a greater rate per unit length of root than non-mycorrhizal roots. The high efficiency in nutrient uptake by mycorrhizal roots is mainly due to the activity of the hyphal network developing from the roots into the surrounding soil thus, increasing the absorptive root surface area for nutrients and their translocation to the host plant (Allen *et al.*, 1981; Berta *et al.*, 1995). Arbuscular mycorrhizal fungi have great potential to enhance plant growth by increasing nutrient uptake (Bagyaraj, 1992; Vasanthakrishna and Bagyaraj, 1993). They also act as potential factors in determining diversity in ecosystem (Giovannetti and Gianinazzi-Pearson, 1994).

Land disturbances such as surface mining can disrupt mycorrhizal populations and often results in growth media with minimal levels of endophyte inoculum. This results in poor plant growth and survival (Khan, 1978; Miller, 1979; Reeves *et al.*, 1979; Allen and Allen, 1980). The occurrence of arbuscular mycorrhizal fungi in the mine spoils has been

documented in studies by Ponder, (1979) and Kiernan *et al.*, (1983). Number of studies by earlier workers (Daft and Nicolson, 1974; Daft *et al.*, 1975; Khan, 1978; Reeves *et al.*, 1979; Lambert and Cole, 1980; Khan, 1981) has reported the positive effect of arbuscular mycorrhizal fungi in rehabilitation of disturbed lands.

Mycorrhizae have also been reported in plants growing on heavy metal contaminated sites (Shetty *et al.*, 1995; Weissenhorn and Leyval, 1995; Pawlowska *et al.*, 1996; Chaudhry *et al.*, 1998 & 1999) indicating that these fungi have evolved a heavy metal tolerance and that they may play a role in the phytoremediation of the site. Noyd *et al.*, (1996) reported that arbuscular mycorrhizal fungal infectivity of native prairie grasses increased over three seasons on a coarse taconite iron ore tailing plots which helped to establish a sustainable native grass community that will meet reclamation goals. Khan *et al.*, (2000) have reported the role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Similarly, Pawlowska *et al.*, (2000) reported the effects of metal phytoextraction practices on the indigenous community of arbuscular-mycorrhizal fungi at a metal-contaminated landfill.

In fact, understanding the role of mycorrhizal associations in relation to establishment and development of plant communities could offer solutions to many of the problems that are encountered in the mine wastelands. Arbuscular mycorrhizal fungi enables the plant to grow and survive better under stress conditions through an increased uptake of nutrients especially phosphorus, zinc, copper and water. There appears to be a considerable scope

for decreasing the need for fertilizers, particularly P, by manipulating the arbuscular mycorrhizal fungal symbiosis in phosphate deficient soils such as mine wastelands.

The present investigation was carried out to determine the status of arbuscular mycorrhizal fungi in iron ore mine wastelands of Goa. The main objectives of the present study are as follows.

1. Survey of vegetation of various mine sites to identify the dominant plant species.
2. Assessment of arbuscular mycorrhizal colonization in the plants growing on the mine sites and the assessment of spore density in the rhizosphere soil.
3. To study the effect of severe land disturbances on arbuscular mycorrhizal fungal populations due to mining.
4. Survey for the occurrence of native arbuscular mycorrhizal fungal spores and assessment of root colonization in the mine reject dumps of varying years.
5. To study the seasonal variation in arbuscular mycorrhizal fungi with respect to root colonization, spore density and edaphic factors.
6. Isolation and taxonomic identification of the arbuscular mycorrhizal fungal spores, and
7. To study the responses of selected arbuscular mycorrhizal fungal species on selected tree species.

REVIEW OF LITERATURE

Mycorrhiza refers to an association between certain soil fungi and plant roots during periods of active plant growth. The association is characterized by the movement of plant-produced carbon to the fungus and fungal-acquired nutrients to the plant. Klironomos and Kendrick, (1993) estimated that between 1915 and 1990, there were only 8900 papers published, on mycorrhizas but almost 3500 from 1991-1995 alone. In 1950s, there were about 10 papers per year published on arbuscular mycorrhizal (AM) research while, in 1990s, this figure exceeded 450 papers per year which includes nearly 170 on the ecology of arbuscular mycorrhizal (AM) fungi in field, as opposed to pot studies.

The term mycorrhiza, which literally means *fungus-root*, was first applied to fungus-tree associations described in 1885 by the German forest pathologist A.B. Frank. Since then, it is learnt that a vast majority of land plants form symbiotic associations with fungi. An estimated 80% of all plant species belong to genera that characteristically form mycorrhizae. The mycorrhizal condition among plants is a rule rather than an exception.

The arbuscular mycorrhiza has undergone several name changes from endomycorrhiza to vesicular arbuscular mycorrhiza (VAM) to arbuscular mycorrhiza (AM). The shift from endomycorrhiza to VAM followed the recognition that evolutionary and functionally VAM differed from other types of mycorrhizas that penetrated the root cells. The fungi forming arbuscular mycorrhizae were all Zygomycetes in the order Glomales (Morton and Benny,

1990). They form easily distinguishable structures including arbuscules, vesicles, coils and distinctive hyphae. More recently, 'V' in VAM was dropped because members belonging to Gigasporaceae do not form vesicles within host roots (Morton and Benny, 1990).

Until recently, it was believed that land plants became established during the late Silurian. However, recent evidence from several disciplines suggests that they may have emerged earlier, during the Ordovician period (Taylor *et al.*, 1995). The arbuscular mycorrhizal fungi are one of the few plant-fungus relationships that have a fossil record (Simon *et al.*, 1993) and it is generally accepted that early vascular plants were associated with arbuscular mycorrhiza-like fungus, and that their origin and ability to colonize land was highly dependant upon the association (Lewis, 1987; Allen, 1991; Selosse and Le Tacon, 1998). Ribosomal DNA sequencing by Simon *et al.*, (1993) place the origin of arbuscular mycorrhiza-like fungi between 462 and 363 million years ago, within the Ordovician, Silurian and Devonian periods. These dates would easily place them at the time of land plant emergence.

Weiss (1904) first discovered fossil mycorrhizas in Lower Carboniferous strata. The earliest and best examples of endomycorrhizas are from the Rhynie Chert fossils, from the Devonian period, discovered by Kidstone and Lang (1921). These showed fungal structures resembling vesicles and spores from the fungus *Palaeomyces* associated with the rhizoid of plants such as *Rhynia* and

Asteroxylon. Recent reappraisal of the Rhynie Chert plants suggest those primitive plants may have been associated with fungi very similar to modern arbuscular mycorrhizal fungi by the early Devonian period (410 to 360 million years ago) (Pirozynski and Dalpé, 1989). The most important recent discoveries of mycorrhizal fossils are the arbuscules found by Stubblefield *et al.*, (1987) in Triassic strata in Antarctica. These fossils show arbuscules organized like those of modern day arbuscular mycorrhizal fungi, indicating that the structural characteristics of the arbuscular mycorrhiza were well developed by the Triassic, even if the functional properties may not have been.

From the first comprehensive description of an arbuscular mycorrhizal fungi (Gallaud, 1905) until workers in the 1950s demonstrated convincingly that arbuscular mycorrhizal fungi could enhance plant growth (Nicolson, 1967), research was confined to the range of plants forming these associations and the taxonomic position of the symbiotic fungi. However, this was a crucial period because these observations established that mycorrhizas were widespread (Janse, 1896; Lohman, 1927) and ancient (Kidstone and Lang, 1921). Janse (1896) undertook the first broad scale survey in Java, showing that the great majority of tropical plants formed mycorrhizas. Stahl, (1900) categorized plants into obligatory mycotrophic, facultatively mycotrophic and non-mycotrophic families. The first description of fungi in the Endogonaceae (*Glomus*) appeared in the mid-1800s (Tulasne and Tulasne, 1844). Butler (1939) linked members of the Endogonaceae with the arbuscular mycorrhizal (AM) type.

ECOLOGY AND DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL FUNGI

The arbuscular mycorrhizal association is a widespread phenomenon, which occurs in 80% of the plant species including Angiosperms, Gymnosperms and Pteridophytes showing a little host specificity (Baylis, 1975; Azcon, 1994). These organisms are ubiquitous in distribution and occur in all kinds of ecosystems ranging from arid drylands, Grasslands, Tropics, *etc.* Arbuscular mycorrhizas can also be found in unexpected plant groups and locations *viz.*, arboreal habitats (Janos, 1993); aquatic trees (Khan, 1993); parasitic plants (Stenlund and Charvat, 1994); proteaceae (Bellgard *et al.*, 1994) and in extreme habitats like deserts (Jacobson *et al.*, 1993) and the Anaconda (Dhillion *et al.*, 1995). Importantly, arbuscular mycorrhiza appear to be missing in islands formed in arctic soils (Christie and Nicolson, 1983) while, DeMars and Boerner (1995) found that the plants without mycelium in the Antarctic readily formed arbuscular mycorrhiza in pot cultures where inoculum was present.

Arbuscular mycorrhizal (AM) fungi are readily dispersed by rodents (Maser *et al.*, 1978; McGee and Baczocha, 1994) but poorly by wind in humid conditions (Allen, 1988) which predominate in the arctic or on islands at high altitudes. In arid lands, where soils are dry, reinvasion is relatively rapid (Allen, 1988). In a cold desert steppe, arbuscular mycorrhiza rapidly invaded a disturbed site, primarily by wind (Allen, 1988). On Mount St. Helens, reinvasion followed animal migration routes (Allen *et al.*, 1992). Interestingly, arbuscular mycorrhiza

appears to colonize tropical islands readily. In Hawaii; Gemma and Koske, (1990) were able to show that arbuscular mycorrhizal plants could wash out to sea, and wash back in with viable inoculum remaining in the roots.

FACTORS INFLUENCING ARBUSCULAR MYCORRHIAL FUNGI

SEASON

Seasonal fluctuation in number of mycorrhizal roots and spore numbers have been examined in deciduous forests (Brundrett and Kendrick, 1988; Mayer and Godoy, 1989), grasslands (Rabatin, 1979; Gay *et al.*, 1982; Sanders and Fitter, 1992), salt marshes (Van Duin *et al.*, 1989), sand dunes (Giovannetti, 1985; Sylvia, 1986; Gemma *et al.*, 1989; Bhaskaran and Selvaraj, 1996; Beena *et al.*, 1997), tropical forests (Louis and Lim, 1987; Mohankumar and Mahadevan, 1988; Brundrett and Abbott, 1994), nutrient deficient tropical soils (Muthukumar *et al.*, 1998) and arid communities (Allen, 1983). Gemma and Koske (1988) found that seasonal differences in abundance of spores was poorly related to seasonal differences in infectivity because there were marked seasonal variations in spore germination.

TEMPERATURE AND LIGHT

Both temperature and light have a significant influence on arbuscular mycorrhizal fungal colonization and sporulation. High soil temperature favours colonization and sporulation (Furlan and Fortin, 1973) while, lower temperature favours arbuscule formation (Schenck and Schroder, 1974). Some species of *Glomus* are shown to be more adapted to high temperature than others (Schenck and

Schroder, 1974; Schenck *et al.*, 1975). Thus, soil temperature alters the physiology of mycorrhizal symbiosis by influencing the root morphology, nutrition and growth. Koske (1981) found optimum temperature for germination of *Gigaspora gigantea* from Rhode Island to be 30°C, while Daniels and Trappe, (1980) observed that *Glomus epigeum* from Oregon germinated best at 22°C. Under poor light and temperature conditions, the host endophyte balance tilts from mutualistic symbiosis to slight parasitism (Hayman, 1974). Studies have indicated that increased light intensity generally increases percent root colonization (Hayman, 1974; Furlan and Fortin, 1977). Longer days also increase root colonization (Boullard, 1957 and 1959; Johnson *et al.*, 1982). A photoperiod of 12 hours or more may be more important than light intensity in providing high levels of root colonization (Hayman, 1974).

MOISTURE

Soil moisture influences arbuscular mycorrhizal colonization and growth (Redhead, 1975). Both excessive and low soil water potential decreased mycorrhizal colonization and sporulation (Nelsen and Safir, 1982a). Studies by Anderson *et al.*, (1983); Allen and Allen, (1984); Douds and Schenck, (1991); Muthukumar *et al.*, (1994 & 1998) also suggested the significant effect of soil moisture on root colonization, arbuscular mycorrhizal fungal spore germination and spore abundance.

RAINFALL AND HUMIDITY

Redhead, (1975) found that colonization by arbuscular mycorrhizal fungi was lower under naturally low rainfall than under adequate rainfall. Michelini *et al.*, (1993); Braunberger *et al.*, (1994) related rainfall with root colonization. Sward *et al.*, (1978) and Walker *et al.*, (1982) observed positive correlation between spore production and humidity.

pH AND ELECTRICAL CONDUCTIVITY (EC)

The distribution of arbuscular mycorrhizal fungi has been shown to be correlated with soil pH (Abbott and Robson, 1977; Wang *et al.*, 1985). Varying soil pH affects the development and functioning of arbuscular mycorrhizae (Mosse, 1972; Abbott and Robson, 1985; Hayman and Tavares, 1985) and also the germination and hyphal growth of some arbuscular mycorrhizal fungi (Green *et al.*, 1976; Hepper, 1984). Hayman (1978), Johnson *et al.*, (1991) and Khalil and Loynachan, (1994) did not find any relationship between soil pH and either root colonization or spore number. Seikh *et al.*, (1975) and Ho, (1987) found that the spore abundance in soil was related to pH. There have been few studies on the effect of salinity on the formation of arbuscular mycorrhizal fungi. Excessive sodium chloride levels in soil inhibit mycorrhizal formation and restrict the activity of most mycorrhizal fungi, but some are known to adapt and tolerate these conditions (Juniper and Abbott, 1993).

SOIL FERTILITY

A key factor, which affects the potential benefit to plants by mycorrhizas in particular soils, is the availability of phosphate and nitrogen (Abbott and Robson, 1991). Phosphorus (P) is generally considered to be the most important factor which limits plant growth in most tropical soils, which could be supplied by mycorrhizal association because many abiotic and biotic factors can restrict its mobility and availability in soils (Harley and Smith, 1983; Hayman, 1983; Marschner, 1986 and Bolan, 1991). Reductions in mycorrhizal benefit occur with increasing soil P levels (Schweiger *et al.*, 1995). Several studies have suggested that root colonization by arbuscular mycorrhizal fungi is inhibited at high P level because of decreased root exudation (Ratnayake *et al.*, 1978; Graham *et al.*, 1981; Muthukumar *et al.*, 1994 and Udaiyan *et al.*, 1996).

ORGANIC MATTER

Soil organic matter has been reported to influence arbuscular mycorrhizal fungi. Nicolson, (1960) reported decreased mycorrhizal colonization levels with increasing organic matter content. However, Gemma *et al.*, (1989), found no correlation between sporulation and organic matter content. Harinikumar, *et al.*, (1990) observed increased mycorrhizal activity in plots amended with farmyard manure. Mixed cropping of soybean and maize along with organic amendments stimulated the proliferation of arbuscular mycorrhizal fungal spores compared to monocropping with either maize or soybean (Harinikumar and Bagyaraj, 1989).

HOST PLANT

Presence or absence of a suitable host plant plays a major role in colonization and sporulation. Studies have indicated that presence of non-mycorrhizal plants reduces colonization in mycorrhizal hosts (Hayman *et al.*, 1975; Morley and Mosse, 1976), possibly because of toxic non host-root exudates (Hayman *et al.*, 1975; Iqbal and Qureshi, 1976). Schenck and Kinloch (1980) observed that the incidence of arbuscular mycorrhizal fungal species depends upon the kind of plant species colonized. They also observed that colonization in low mycotrophic hosts could be increased when grown in the presence of strongly mycotrophic nurse plants.

Hayman (1975) and Iqbal *et al.*, (1975) recorded difference in spore numbers between plant species. Although, arbuscular mycorrhizal fungi have extremely wide host range and the existence of host preference has often been suggested (Fox and Spasoff, 1971; Mosse, 1975). Gerdemann, (1965) and, Manjunath and Bagyaraj, (1982), have shown that the colonization pattern of arbuscular mycorrhizal fungal species can be distinctly different in various plant species or between cultivars of a plant species.

INTERACTION WITH OTHER SOIL ORGANISMS

Mycorrhizal fungi interact with a wide range of organisms in the rhizosphere. Invertebrates like earthworms, millipedes, wasps and ants play an important role in dissemination of arbuscular mycorrhizal fungi through ingestion of arbuscular

mycorrhizal fungal propagules with feed or by bringing arbuscular mycorrhizal fungal propagules to surface soil, favouring further dissemination by wind and water (Bagyaraj, 1991). Singh (1998) found that micro-arthropods, negatively influenced the distribution and density of arbuscular mycorrhizal external hyphae through grazing, thus, disrupting the nutrient uptake by mycorrhizal fungi.

VEGETATION

In some natural ecosystems there is a close positive correlation between plant cover and spore numbers (Anderson *et al.*, 1984; Miller, 1987). In some environments, cultivation has led to a reduction in the diversity of arbuscular mycorrhizal fungi (Schenck and Kinloch, 1980; Hetrick and Bloom, 1983), whereas in others, agricultural practices may lead to greater diversity (Abbott and Robson, 1977).

SOIL DISTURBANCE

Propagules of arbuscular mycorrhizal fungi may be absent in severely disturbed soils where the topsoil has been lost, or where host plants are sparse due to adverse soil or site factors such as salinity, aridity, waterlogging, or climatic extremes (Brundrett, 1991). Most studies on mycorrhizal associations in highly disturbed habitats such as mine sites have found reduced levels of mycorrhizal propagules (Danielson 1985; Jasper *et al.*, 1992; Pflieger *et al.*, 1994 and Brundrett *et al.*, 1996b). Less severe forms of soil disturbance, including agricultural tillage, soil animal activities, fire and erosion can also reduce levels of mycorrhizal

fungus propagules (Habte *et al.*, 1988, O'Halloran, *et al.*, 1986; Read and Birch, 1988; Vilarino and Arines, 1991).

TAXONOMY

Peyronel (1923 & 1924) was the first to recognize that the arbuscular mycorrhizal fungi were the members of Endogonales, rather than *Chytrids*, *Pythium* spp., or other fungi as suggested by earlier workers. Peyronel's discovery followed the revision of the Endogonaceae by Thaxter (1922), who had not realized the mycorrhizal involvement of the family. Gerdemann and Nicolson (1963) then developed procedures for collecting arbuscular mycorrhizal fungal spores from soil and described new species. The family was monographed in 1974 with segregation of the genus *Endogone* into seven genera. Since then rapid and sustained reports of undescribed arbuscular mycorrhizal fungal species have occurred during the past 30 years. Gerdemann and Trappe (1974) reported 30 species of fungi while, Trappe (1982) in his synoptic key to the Endogonaceae listed 77 species, excluding *Endogone*. Hall (1984) in his dichotomous keys to the Endogonaceae listed 67 species, excluding *Endogone*. Berch (1988) listed 126 species, excluding *Endogone*. Approximately 150 species have been described based on morphological characters of the spores by Schenck and Perez (1990).

EARLIER CLASSIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI

When the first fungi in the genus *Glomus* were described, they were known only from the clusters of spores (so-called sporocarps) found in the upper layers of soil

(Tulasne and Tulasne, 1844; Thaxter, 1922). The history of their study was summarized by Butler (1939), by which time the vesicles and arbuscules, clearly illustrated in the 19th century (Janse, 1896), and were already recognized as being produced by a root colonizing fungal symbiont. In the early 1950s, Barbara Mosse, at East Mallinga (UK), first showed experimentally that a fungus, later described as *Glomus mosseae*, was responsible for the mycorrhizal colonization of strawberry roots (Mosse, 1953).

Morphologically, the nearest similar group of fungi with known sexuality belongs to the genus *Endogone*, and by analogy the arbuscular mycorrhizal fungi were placed with them in a single family, the *Endogonaceae* (*Zygomycota*).

A comprehensive review of the group carried out (Gerdeemann and Trappe, 1974), during which two new genera (*Acaulospora* and *Gigaspora*) were erected within the *Endogonaceae*. The fungi within this rather unnatural grouping were eventually formally accommodated in their own order, the *Endogonales*, though without further taxonomic clarification above genus level (Benjamin, 1979). A cladistic analysis, mainly of morphological features, produced a 'species tree' with a new order, *Glomerales* containing two suborders and three families (Morton and Benny, 1990). However, some of the conclusions of this work were questioned.

CLASSIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI WITHIN THE FUNGI

The kingdom fungi has been circumscribed by the use of morphological, biochemical and molecular studies. Schubler *et al.*, (2001), based on the comprehensive SSU rRNA analysis, separated the arbuscular mycorrhizal fungi in a monophyletic clade, which is not related to any zygomycetous group. But probably shares common ancestry with the Ascomycota-Basidiomycota clade. They recognized a new, fungal phylum based on natural relationships for the arbuscular mycorrhizal fungi, the Glomeromycota with a single class Glomeromycetes (Cavalier-Smith, 1998). The class Glomeromycetes was circumscribed for the phylum, containing more than 150 described species, some of which are synonyms (Walker and Vestberg, 1998; Walker and Trappe, 1993).

FUNGAL MYCELIUM AND ITS PARTS

Morphological, architectural and histochemical properties of intraradical hyphae were shown to have taxonomic value at the genus levels or above (Abbott and Robson, 1979) in that *Glomus* has long infecting units with 'H' connections between parallel strands; pale staining with points in *Acaulospora* and *Entrophospora* and coiled irregularly swollen hyphae with lateral projections or knots in *Gigaspora* and *Scutellospora* (Morton and Bentivenga, 1994). The mycelia of arbuscular mycorrhizal fungi also have certain meristic (repeating) structures that are conserved enough to define higher taxonomic groups (Morton and Benny, 1990). Morton, (1990) used arbuscules as the only character to unite Glominae and Gigasporinae in Glomales. However, a more detailed study of

arbuscule morphology by Brundrett and Kendrick (1990) revealed distinct differences between isolates of *Gigaspora* and *Glomus* species. Intraradical vesicles are formed inclusively in Glomaceae and Acaulosporaceae, but distinct differences are evident in each family (Abbott, 1982), with differences in vesicle morphology among sporocarpic species in *Glomus* has been reported by McGee, (1986).

SPORES

Arbuscular mycorrhizal fungi are obligate biotrophs (Lewis, 1973) that can only be maintained pure in pot cultures with host plants. Many attempts to grow these organisms *in vitro* in association with genetically transformed root were implemented in the last decades (Becard and Piche, 1992; St. Arnaud *et al.*, 1996; Declerck *et al.*, 1998). However most of them form relatively large asexual spores in soil and their identification has therefore, traditionally been based almost exclusively on morphological descriptions of the different spore types, giving rise progressively to more accurate classification of the different taxa (Gerdemann and Trappe, 1974; Morton and Benny, 1990). Many new species of arbuscular mycorrhizal fungi have been reported since historical revision of the Endogonaceae by Thaxter (1922). Walker, (1992) discussed the problems involved in taxonomy of arbuscular mycorrhizal fungi. Morton and Benny, (1990) gave keys to differentiate genera and Schenck and Perez, (1990) provided the manual for identification of arbuscular mycorrhizal fungal species.

Recently, the newly recognized fungal phylum, *Glomeromycota* (Schubler *et al.*, 2001), formerly circumscribed only as an order, *Glomerales* is divided into four statically highly supported mainclades. The order *Glomerales* still representing many of the 'classical glomeralean' species (Morton ad Benny, 1990), the Diversisporales, and the two 'ancestral' linkages, Paraglomerales, and Archaeosporales.

As to the family structure within those orders, the largest 'Genus' within the arbuscular mycorrhizal fungi, *Glomus*, clearly is nonmonophyletic and represents at least three families. One of them is represented by the newly proposed family *Diversisporaceae* fam. Ined. (Schwarzott, *et al.*, 2001) which is monophyletic with the *Gigasporaceae* and *Acaulosporaceae*. The Glomeraceae will represent either *Glomus*-Group A or B depending upon the phylogeny of the species.

WALL STRUCTURE AND CYTOCHEMISTRY

Scanning Electron Microscope (SEM) studies have confirmed light microscope observations that different ornamentations can be observed on spore walls (Koske and Walker, 1985; Maia and Kimbrough, 1993). Comparative Transmission Electron Microscopy (TEM) investigations have revealed variations in the fine architecture of wall components of both spores and hyphae.

ONTOGENY OF SPORES

Taxonomic characters of spore and sporocarp ontogeny in descriptions of new species or studies of the established ones have been reported earlier (Morton and

Benny, 1990; Walker, 1992). Ontogenetic diversity has been considered diagnostic in the differentiation of two genera, *Acaulospora* and *Entrophospora* (Ames and Schneider, 1979). Almeida and Schenck, (1990) transferred most of the species of *Sclerocystis* (except *Sclerocystis coremioides*) to *Glomus* based on spore ontogeny. However, Wu (1993a) grouped all the species with highly organized sporocarp into *Sclerocystis*.

SPORE GERMINATION

In *Gigaspora* germination takes place directly through the spore wall whilst in *Scutellospora*, it occurs from a germination shield formed upon or within the inner wall layer (Walker and Sanders, 1986). Although arbuscular mycorrhizal fungi are obligate biotrophs, isolated spores germinate on nutrient or water agar, (Mosse, 1962; Hepper, 1981). The germinating ability, the pattern of germination and the quantity of mycelium produced are characters that can all show a high degree of variations (Hepper and Smith, 1976; Pons and Gianinazzi-Pearson, 1984; Giovannetti *et al.*, 1991). Spores of *Gigaspora* species germinate rapidly in water agar and produce extensive hyphal growth, while *Acaulospora* species are generally slow, and *Glomus* species vary in their rates and levels of germination (Tommerup, 1983 b; Pons and Gianinazzi-Pearson, 1984).

MOLECULAR CHARACTERS

Isoenzymes and serological approaches can, to a limited extent be used to differentiate arbuscular mycorrhizal fungi within plant roots (Cordier *et al.*, 1996; Goebel *et al.*, 1998; Hepper *et al.*, 1986; Rosendahl *et al.*, 1989). Molecular

techniques using PCR (Mullis *et al.*, 1986; Saiki *et al.*, 1988), PCR associated with systematic sequencing of RFLP (Di Bonito *et al.*, 1995; Redecker *et al.*, 1997; Sanders *et al.*, 1995) or RADP (Lanfranco *et al.*, 1995), have been applied to detect Glomalean fungi singly colonizing roots or to monitor diversity of their spores. Recently, Redecker *et al.*, (2000b) transferred *Sclerocystis coremioides* the only species retained by Almeida and Schenck (1990) in the genus *Sclerocystis* to *Glomus* based on molecular studies.

Recently Morton and Redecker (2001), based on concordant molecular and morphological characters discovered two new families of Glomales, Archaesporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*.

SPORE-IN- SPORE SYNDROME

Arbuscular mycorrhizal fungi are associated with a wide range of soil fungi (Lee and Koske, 1994). Spores of arbuscular mycorrhizal fungi inside the spores of other arbuscular mycorrhizal fungi have been reported by Koske, (1981; 1984) from sand dunes of Atlantic Coast. Wu and Chen (1993), reported *Glomus*-like spores within spores of *Glomus sinuosum* from Taiwan. Muthukumar *et al.*, (1993) and Muthukumar and Udaiyan (1999), have reported the occurrence and seasonality of arbuscular mycorrhizal fungal spores inside the spore of other arbuscular mycorrhizal fungi in a semi-arid tropical grassland in Western Ghats from Southern India. Recently, Jaiswal (2002) reported the occurrence of

Acaulospora spinosa inside the spores of *Scutellospora* in sand dune vegetation of Goa.

MYCORRHIZAL BENEFITS TO PLANTS

NUTRIENT BENEFITS

PHOSPHORUS

Mycorrhizal association can enhance the uptake of P by plant roots particularly in low fertility soils (Tinker, 1978; Bolan, 1991). Arbuscular mycorrhizal fungal hyphae are able to absorb P directly from the soluble P pool in the soil and translocate it to the host roots (Cooper and Tinker, 1978 & 1981; Rhodes and Gerdemann, 1980). Mycorrhizal colonization also improves plant growth and P uptake from highly phosphate fixing soils and from latosols which contain insoluble iron and aluminium phosphate (Powell, 1980). Mycorrhizal plant can actively hydrolyze Pyrate, the major source of organic phosphorus than the non-mycorrhizal plants both in soil (Gianinazzi *et al.*, 1981) and in axenic culture (Allen *et al.*, 1981).

NITROGEN

Mycorrhizal plants can derive N from both organic as well as inorganic sources that are not available to non-mycorrhizal plants (Ames *et al.*, 1984; Barea *et al.*, 1989). Uptake of N¹⁵ from labelled ammonium salts have been reported by Johansen *et al.*, (1992, 1994).

The interaction between rhizobia and arbuscular mycorrhizal fungi has received considerable attention because of the relatively high P demand of N₂ fixation. The two symbionts typically act synergistically, resulting in greater N and P content in combination than when each is inoculated individually. Legumes are typically coarse-rooted and therefore inefficient in absorbing P from the soil. The arbuscular mycorrhizal fungi associated with legumes are an essential link for adequate phosphorus nutrition, leading to enhanced nitrogenase activity which in turn promotes root and mycorrhizal growth. Plants colonized with arbuscular mycorrhizal fungi have a lower tissue nitrogen concentration than those without mycorrhizae (Daft *et al.*, 1975; Ames *et al.*, 1983).

OTHER NUTRIENTS

Uptake of nutrients such as Ca, Mg, Fe, Cu, Mn and Zn can be influenced by both soil P levels and mycorrhizal colonization. Mycorrhizal colonization relieved zinc deficiency in peach and apple (Gilmore, 1971; Benson and Covey, 1976).

NON NUTRIENT BENEFITS

Arbuscular mycorrhizal fungi can bind and aggregate soil particles through the extensively growing mycelium (Sutton and Sheppard, 1976). Further, the mycelium of arbuscular mycorrhizal fungi not only loosely aggregates soil particles, but the hyphae bind to them through amorphous polysaccharides. The mycelium also contributes to the formation of water-stable aggregates necessary for good soil tilth (Jeffries and Barea, 2000).

Arbuscular mycorrhizal fungi are known to improve drought tolerance in plants (Aldon, 1975; Lindsey *et al.*, 1977; Nelsen and Safir, 1982a). Mycorrhizal plants are known to be less susceptible to wilting and transplant shock than nonmycorrhizal plants (Barrows and Roncadori, 1977; Menge *et al.*, 1978; Levy and Krikun, 1980; Janos, 1980; Hardie and Leyton, 1981; Sieverding, 1981; Biermann and Linderman, 1983; Cooper, 1984; Michelson and Rosendahl, 1990). Allen *et al.*, (1980) have reported increased cytokinin activity in leaves and roots. Mycorrhizal association is known to increase the resistance to nematode infestation by depressing root penetration and larval development (Sikora, 1978). The stronger vascular system of mycorrhizal plants (Schoenbeck, 1979) increases the flow of nutrients, imparts greater mechanical strength, and diminishes the effect of vascular pathogens. In addition, mycorrhizal plants are shown to have greater tolerance to heavy metal toxicity, high soil temperature, and adverse soil pH than non-mycorrhizal plants (Shetty *et al.*, 1995; Weissenhorn and Leyval, 1995; Pawlowska *et al.*, 1996; Chaudhry *et al.*, 1998; Chaudhry *et al.*, 1999), indicating that these fungi have evolved a HM-tolerance and that they may play a role in the phytoremediation of the site. Arbuscular mycorrhizal fungi also help to control pests (*eg.* Nematodes) and fungal pathogens (Azcon-Aguilar and Barea, 1996).

ARBUSCULAR MYCORRHIZAE IN MINE WASTE LANDS

Schramm (1966) found that only ectotrophic or nitrogen fixing plant species were able to colonize the black wastes from anthracite mining in Pennsylvania. Later,

a majority of herbaceous plants colonizing coal wastes in Scotland, and Pennsylvania were observed to contain arbuscular mycorrhizae and/or root nodulating bacteria (Daft and Nicolson, 1974; Daft *et al.*, 1975; Daft and Hacskaylo, 1976). Gould and Hendrix (1998) conducted studies related to changes in mycorrhizal fungal community with time following reclamation.

Daft and Hacskaylo (1977) obtained five fold growth responses to mycorrhiza for red maple in anthracite waste fertilized with bonemeal. Aldon, (1978) reported improved growth and survival of mycorrhizal shrubs in acid spoils. Lambert and Cole, (1980) studied the effect of mycorrhizae on establishment and performance of forage species in mine spoil. Khan (1981) and, Zak and Parkinson (1982) studied the effect of amendment of mine spoil on arbuscular mycorrhizal fungi in slender wheat grass. Jasper *et al.*, (1991) studied the effect of soil disturbance on arbuscular mycorrhizal fungi in soils from different vegetation type. Pawlowska *et al.*, (1996) studied the mycorrhizal status of plants colonizing a calamine spoil mound in Southern Poland. Gould *et al.*, (1996) reported the population dynamics of mycorrhizal spore and propagule population density of five reclaimed areas on the Muhlenberg mine site. In Tropical Australia soils Brundrett *et al.*, (1996 a & b) studied the distribution and abundance of mycorrhizal fungal propagules in soil from natural habitats and mine sites.

Jamaluddin and Chandra (1995), studied the development of arbuscular mycorrhiza in tree species planted in coal mine dumps in Maharashtra while, Bisen *et al.*, (1996) studied arbuscular mycorrhizal colonization in tree species planted in Cu, Al, and Coal mines of Madhya Pradesh. Lakshman (1997) studied the occurrence of arbuscular mycorrhizal fungi in some tropical herbs and shrubs in stockpiled mine spoil. Sastry and Johri (1999) studied arbuscular mycorrhizal fungal diversity in some plant species from iron stressed sites of Balidala iron ore mine sites in Madhya Pradesh in India. Uniyal, (2001) reported the incidence of arbuscular mycorrhizal fungi from ecologically revegetated mined area of Doon valley and reported 19 arbuscular mycorrhizal fungal species with *Glomus* being the most dominant genera. Preliminary investigation of arbuscular mycorrhizal colonization from some herbs and tree species from iron ore mine wastelands of Goa have been reported earlier (Rodrigues and Bukhari, 1996 ; Rodrigues and Bukhari, 1997). Rodrigues (1999) reported arbuscular mycorrhizal colonization in some seedlings from iron ore mine wastelands of Goa.

CHAPTER-I

**SURVEY OF VEGETATION OF IRON ORE MINES
OF VARYING AGES.**

INTRODUCTION

Of all the activities of man that affect land, plants and animals, mining must be the most destructive. In search of minerals of all sorts, from sand to gravel to base metals, not only the vegetation, animals and soil are eliminated but also the whole landform gets disturbed. Thus, mining causes disturbance to land, soil and the plants, which clothe it. Mining implies selection, which in turn implies the rejection of waste, and the very process of selection will cause an impact on environment. Due to mining activity the original ecosystem is disturbed and in its place appears empty pits, sterile waste heaps or both (Nayak, 2002).

The State of Goa has a total surface area of 3,70,000 ha. of which 66,300 ha. area (almost 18% of the total area) has been leased out for open cast iron ore mining in the northern and central parts of Goa. However, only 18,300 ha. (5% of the total area) has been under actual mining operations. A total of 70 mines covering an area of roughly 6,082 ha. are active and yield more than 80% of the total iron ore production from Goa (Samant, 1989).

Mining in Goa started from 1949-1950. For the first 10 years, it was manual and the production was mainly manganese and ferromanganese. From 1958-59, the production of iron ore picked up and within a period of about 3 years, the iron ore was no longer the pick and shovel exercise as it was a few years ago. Today, mining is a highly sophisticated, capital-intensive industry where larger quantities of ore have to be extracted to avail of the economies of scale (Salgaonkar, 1991).

Goa has been a prime exporter of iron ore since 1950, as much as 350 million tonnes of iron has been exported during all these years. In Goa, deforestation was inevitable as 70% of the mining activity is carried out in forest areas (D'Souza and Nayak, 1994). Deforestation, to a lesser extent is also caused by infrastructural facilities and spreading of overburden. This, in many cases drastically changes the micro-climatic conditions, which leads to degradation of flora and fauna (Paliwal, 1989). Nevertheless, the damage does cause environmental degradation (Subramanayam, 1986).

Indiscriminate mining since 1961 has destroyed 50,000 ha. of forest in Goa. Dumping of mine reject dump has killed the fertility of the forestland. The disturbance of land surface due to mining alters the potential for vegetative growth. The mine spoils are nutritionally and microbiologically impoverished (Vissar *et al.*, 1979) and natural recovery of unattended spoil sites is a slow process. Positive effect of vegetation on soil formation and organic matter accumulation has been documented in studies by Marshall, (1977). During ecosystem development, accumulation of nutrients takes place. The results of these are observable in terms of species composition. Schafer and Nielsen, (1979) indicated that improvement of soil conditions promoted plant succession.

In Goa, as per forest survey of India 1991, the total forest cover is about 33.8% with the major forests located in seven talukas viz., Pernem, Bicholim, Ponda, Satari, Sanguem, Quepem and Canacona. The vegetation of Goa can be broadly classified into 4 categories as follows.

1. Estuarine Vegetation along the banks of rivers.
2. Strand Vegetation along the Coastal belts.
3. Plateau Vegetation- deciduous, confined to the lower elevations of the Ghats.
4. Semi-evergreen and evergreen forests limited to patches along the upper elevation of the Ghats.

The present study was undertaken to survey the mines of varying ages and to find out the dominant and common plant species growing on iron ore mine wasteland of Goa which can tolerate the adverse conditions viz., high temperature, deficiency of mineral nutrients and water prevailing on the mine wastelands.

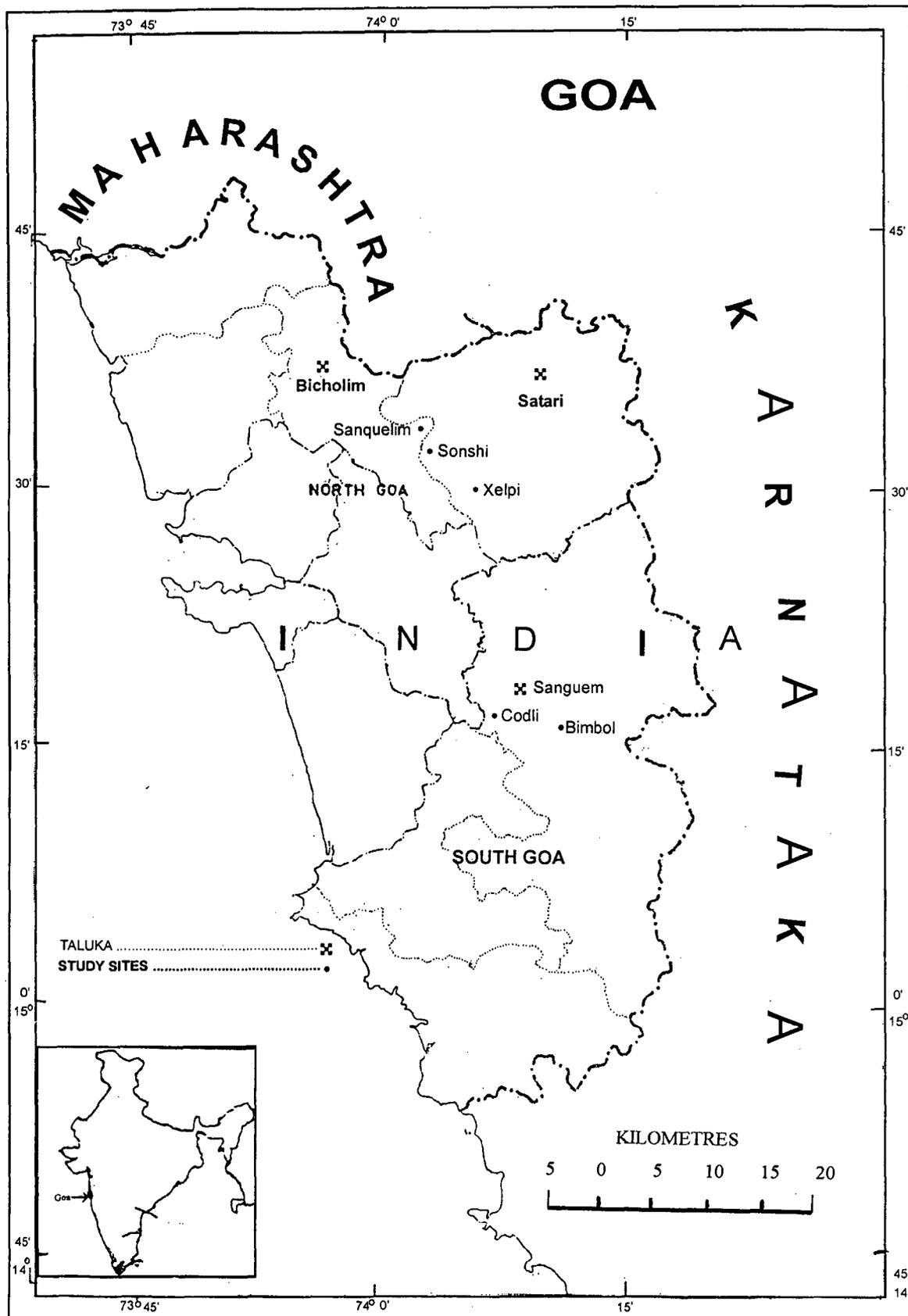
MATERIALS AND METHODS

STUDY SITE

The present study was conducted at five iron ore mine sites viz., Sanquelim, Huldgo Dongor "Bimbol", Sonshi, Codli and Dadiovaril Sodo "Xelpi" which were of varying ages (**Fig. -1**).

SANQUELIM

Sanquelim, a 50-year-old mine is situated at Sanquelim in Bicholim, North Goa, and lies at 15^o 34' 2" N Latitude and 74^o 1' 12" E Longitude. This mine is spread over an area of 203.5 ha. with an actual area of 102 ha. under mining activity until 1988 when it was abandoned after exhausting the mineral ore reserves.



Based upon Survey of India map with the permission of the Surveyor General of India.

Fig. - 1. Map of Goa showing various study sites.

HULDOO DONGOR “BIMBOL”

Huldoo Dongor “Bimbol” mine situated in Sanguem, South Goa and lies at $15^{\circ} 20' 30''$ N Latitude and $74^{\circ} 13' 25''$ E Longitude and is spread over an area of 97.50 ha. Mining at this site started in 1956 and is still continued.

SONSHI

Sonshi mine is 35 year old and is situated at Satari, North Goa, $15^{\circ} 32' 15''$ N Latitude and $74^{\circ} 3' 46''$ E Longitude. This mine is spread over an area of 62 ha., and the entire area is presently under active mining activity.

CODLI

Codli, a 30-year-old mine, situated in Sanguem, South Goa and lies at $15^{\circ} 20' 53''$ N Latitude and $74^{\circ} 8' 33''$ E Longitude and is spread over an area of 300 ha. with an actual area of 290 ha. presently under mining.

DADIOVARIL SODO “XELPI”

Dadiovaril Sodo Xelpi mine is 10 year old and is situated in Satari, North Goa. It lies at $15^{\circ} 29' 45''$ N Latitude and $74^{\circ} 7' 49''$ E Longitude. This mine is spread over an area of 100 ha. with an area of 45 ha. under active mining.

SURVEY OF VEGETATION

Vegetation survey was conducted in early monsoon (July–August) at five iron ore mines viz., Sanquelim (50), Bimbol (45), Sonshi (35), Codli (30) and Xelpi (10) with the age of the mines in years given in parenthesis. Plant representatives of herbs, shrubs, and trees were collected from all the above

sites and were identified using floras (Rao, 1985 & 1986; Matthew, 1991; Mohanan and Henry, 1994; Naithani *et al.*, 1997).

RESULTS

The results of vegetation survey are depicted in **Table-2**, which gives an exhaustive list of plant species reported from various iron ore mine sites undertaken in the present study. Distribution of plant species diversity at iron ores of varying ages are depicted in **Fig.- 2**.

The recently degraded mine *viz.*, Xelpi (10 year old) showed least number of plant species (both naturally occurring and cultivated) *i.e.*, 29 comprising of herbs (20), shrubs (3) and trees (6) with the number of species given in parenthesis (**Fig.- 3 a**).

Codli (30 year old mine) showed a total of 102 plant species (both naturally occurring and cultivated) consisting of herbs (55), twiners (3), shrubs (17) and tree (27) with the number of species given in parenthesis (**Fig.- 3 b**).

Sonshi (35-year-old mine) showed a total of 73 plant species comprising of herbs (41), twiners (3), shrubs (13) and trees (16) with the number of species given in parenthesis (**Fig.- 3 c**).

Bimbol (45-year-old mine) had a total of 115 plant species (both naturally occurring and cultivated) comprising of herbs (59), twiners (3),

| | | |
|--|------|-----------|
| <i>Tridax procumbens</i> L. | Herb | 1,2,3,4,5 |
| <i>Acanthospermum hispidum</i> DC. | Herb | 5 |
| <i>Parthenium hysterophorus</i> L. | Herb | 3,5 |
| <i>Tricholepis glaberrima</i> DC. | Herb | 2,4,5 |
| <i>Senecio bombayensis</i> Balakr. | Herb | 5 |
| <i>Ageratum conyzoides</i> L. | Herb | 1,2,3,4,5 |
| <i>Emilia sonchifolia</i> (L.) DC. | Herb | 1,2,3,4,5 |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Herb | 1,2,3,4,5 |
| <i>Vernonia cinerea</i> (L.) Less. | Herb | 1,2,3,4,5 |
| <i>Blumea mollis</i> (D. Don) Merr . | Herb | 5 |

BALSAMINACEAE

| | | |
|---------------------------------------|------|-----|
| <i>Impatiens kleinii</i> wight & Arn. | Herb | 2,5 |
| <i>Impatiens balsamina</i> L. | Herb | 5 |

BAMBACACEAE

| | | |
|------------------------|------|-----|
| <i>Bombax ceiba</i> L. | Tree | 4,5 |
|------------------------|------|-----|

BIGNONIACEAE

| | | |
|-------------------------------------|------|---|
| <i>Jacaranda mimosifolia</i> D. Don | Tree | 5 |
| <i>Tecoma stans</i> (L.) Kunth. | Herb | 5 |

CAESALPINIACEAE

| | | |
|--|-------|-----------|
| <i>Bauhinia purpurea</i> L. | Tree | 2,3,4,5 |
| <i>Caesalpinia pulcherrima</i> (L.) Sw. | Shrub | 2,5 |
| <i>Cassia absus</i> L. | Herb | 5 |
| <i>Cassia fistula</i> L | Tree | 2,5 |
| <i>Cassia mimosoides</i> L. | Herb | 2,5 |
| <i>Cassia siamea</i> Lam. | Tree | 4,5 |
| <i>Cassia tora</i> L. | Herb | 1,2,3,4,5 |
| <i>Delonix regia</i> (Bojer ex Hook.) Raf. | Tree | 2,3,4,5 |

Cont.

TABLE- 2. List of plant species reported from iron ore mines of varying ages.

| PLANT SPECIES (FAMILY AND SCIENTIFIC NAME) | HABIT | LOCATION |
|--|--------------|-----------------|
| ACANTHACEAE | | |
| <i>Adathoda zeylanica</i> (Medic.) kus | Shrub | 5 |
| <i>Andrographis paniculata</i> (Burm. f.) Wallich ex Nees | Herb | 2,5 |
| <i>Justicia procumbens</i> L. | Herb | 1,2,3,4,5 |
| <i>Lepidogathis lutea</i> Dalz. | Herb | 5 |
| <i>Rungia linifolia</i> Nees | Herb | 2,4,5 |
| AMARANTHACEAE | | |
| <i>Achyranthes aspera</i> L. | Herb | 2,4,5 |
| <i>Alternanthera sessilis</i> (L.) R. Br. | Herb | 4,5 |
| <i>Amaranthus viridis</i> L. | Herb | 5 |
| <i>Celosia argenticornis</i> L. | Herb | 2,3,4,5 |
| AMARYLLIDACEAE | | |
| <i>Cryinum viviparum</i> var <i>viviparum</i> Ansari & Nair | Shrub | 2,4,5 |
| ANACARDIACEAE | | |
| <i>Anacardium occidentale</i> L. | Tree | 1,2,3,4,5 |
| <i>Lannea coromandelica</i> (Houtt.) Merr. | Herb | 5 |
| <i>Mangifera indica</i> L. | Tree | 2,4,5 |
| ANNONACEAE | | |
| <i>Annona squamosa</i> L. | Tree | 2,5 |

Cont.

| | | |
|---|------|-------|
| <i>Michaelia champaca</i> L. | Tree | 5 |
| <i>Polyalthia longifolia</i> (Sonn.) Thwaites | Tree | 2,4,5 |

APIACEAE

| | | |
|------------------------------------|------|---------|
| <i>Hydrocotyle asiatica</i> L. | Herb | 2,5 |
| <i>Mullugo pentaphylla</i> L. | Herb | 2,3,4,5 |
| <i>Pimpinella adscendens</i> Dalz. | Herb | 5 |

APOCANACEAE

| | | |
|--|-------|---------|
| <i>Ervatamia heyneana</i> (Wall.) Cooke | Tree | 4,5 |
| <i>Rauwolfia serpentina</i> (L.) Benth. ex Kurz. | Shrub | 4,5 |
| <i>Carissa carandas</i> L. | Shrub | 2,4,5 |
| <i>Catharanthus roseus</i> (L.) G. Don. | Shrub | 5 |
| <i>Vinca rosea</i> L. | Shrub | 2,5 |
| <i>Plumeria rubra</i> L. | Tree | 4,5 |
| <i>Alstonia scholaris</i> (L.) R. Br. | Tree | 2,3,4,5 |
| <i>Ichnocarpus frutescens</i> (L.) R. Br. | Shrub | 5 |

ARECACEAE

| | | |
|---|------|-------|
| <i>Amorphophallus commutatus</i> Engler | Herb | 2,4,5 |
| <i>Caryota urens</i> L. | Tree | 4,5 |
| <i>Cocos nucifera</i> L. | Tree | 2,5 |
| <i>Colocasia esculenta</i> (L.) Schott | Herb | 5 |

ASCLEPIDIACEAE

| | | |
|--|--------|-----------|
| <i>Calotropis gigantea</i> (L.) R. Br. | Shrub | 1,2,3,4,5 |
| <i>Gymnema sylvestre</i> (Retz.) R. Br. ex R. & S. | Tree | 5 |
| <i>Hemidesmus indicus</i> (L.) R. Br. | Twiner | 2,3,4,5 |
| <i>Cryptolepis buchanani</i> Roemer & Schultes | Shrub | 5 |

ASTERACEAE

| | | |
|-----------------------------|------|-------|
| <i>Eclipta prostrata</i> L. | Herb | 3,4,5 |
|-----------------------------|------|-------|

Cont.

| | | |
|--|------|-----------|
| <i>Tridax procumbens</i> L. | Herb | 1,2,3,4,5 |
| <i>Acanthospermum hispidum</i> DC. | Herb | 5 |
| <i>Parthenium hysterophorus</i> L. | Herb | 3,5 |
| <i>Tricholepis glaberrima</i> DC. | Herb | 2,4,5 |
| <i>Senecio bombayensis</i> Balakr. | Herb | 5 |
| <i>Ageratum conyzoides</i> L. | Herb | 1,2,3,4,5 |
| <i>Emilia sonchifolia</i> (L.) DC. | Herb | 1,2,3,4,5 |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Herb | 1,2,3,4,5 |
| <i>Vernonia cinerea</i> (L.) Less. | Herb | 1,2,3,4,5 |
| <i>Blumea mollis</i> (D. Don) Merr . | Herb | 5 |

BALSAMINACEAE

| | | |
|---------------------------------------|------|-----|
| <i>Impatiens kleinii</i> wight & Arn. | Herb | 2,5 |
| <i>Impatiens balsamina</i> L. | Herb | 5 |

BAMBACACEAE

| | | |
|------------------------|------|-----|
| <i>Bombax ceiba</i> L. | Tree | 4,5 |
|------------------------|------|-----|

BIGNONIACEAE

| | | |
|-------------------------------------|------|---|
| <i>Jacaranda mimosifolia</i> D. Don | Tree | 5 |
| <i>Tecoma stans</i> (L.) Kunth. | Herb | 5 |

CAESALPINIACEAE

| | | |
|---|-------|-----------|
| <i>Bauhinia purpurea</i> L. | Tree | 2,3,4,5 |
| <i>Caesalpinia pulcherrima</i> (L.) Sw. | Shrub | 2,5 |
| <i>Cassia absus</i> L. | Herb | 5 |
| <i>Cassia fistula</i> L | Tree | 2,5 |
| <i>Cassia mimosoides</i> L. | Herb | 2,5 |
| <i>Cassia siamea</i> Lam. | Tree | 4,5 |
| <i>Cassia tora</i> L. | Herb | 1,2,3,4,5 |
| <i>Delonix regia</i> (Bojer ex Hook.) Raf | Tree | 2,3,4,5 |

Cont.

| | | |
|--|-------|---------|
| <i>Tamarindus indica</i> L. | Tree | 2,3,4,5 |
| <i>Cassia alata</i> L. | Shrub | 4,5 |
| <i>Peltophorum pterocarpum</i> (DC.) Baker ex Heyne | Tree | 2,3,4,5 |

CARECACEAE

| | | |
|-------------------------|------|---|
| <i>Carica papaya</i> L. | Tree | 5 |
|-------------------------|------|---|

CASUARINACEAE

| | | |
|---|------|---------|
| <i>Casuarina equisetifolia</i> Forster & Forster f. | Tree | 2,3,4,5 |
|---|------|---------|

CLUSIACEAE

| | | |
|--------------------------------|------|-----|
| <i>Garcinia indica</i> Choiss. | Tree | 4,5 |
|--------------------------------|------|-----|

COMBRETACEAE

| | | |
|--|-------|-----------|
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | Shrub | 1,2,3,4,5 |
| <i>Terminalia bellerica</i> (Gaertner) Roxb. | Tree | 2,4,5 |
| <i>Terminalia catappa</i> L. | Tree | 2,3,4,5 |
| <i>Terminalia crenulata</i> Roth. | Tree | 1,2,3,4,5 |
| <i>Terminalia paniculata</i> Roth. | Tree | 1,2,3,4,5 |
| <i>Murdannia semiteres</i> (Dalz.) Sant. | Herb | 5 |
| <i>Randia rugulosa</i> (Thw.) Hk. f. | Tree | 4,5 |

COMMELINACEAE

| | | |
|--------------------------------------|-------|-----|
| <i>Commeline forskalaei</i> Vahl | Herb | 4,5 |
| <i>Cynaotis cristata</i> (L.) D. Don | Herbs | 5 |

CONVOLVULACEAE

| | | |
|---------------------------------------|--------|-----|
| <i>Canscora perfoliata</i> Lam. | Herb | 5 |
| <i>Evolvulus alsinoides</i> (L.) L. | Herb | 4,5 |
| <i>Merremia tridentata</i> Hallier f. | Twiner | 5 |

Cont.

Ipomoea obscura (L.) Ker-Gawler Twiner 5

CUCURBITACEAE

Mukia maderaspatana (L.) M. Roemer Twiner 5

Solena amplexicaulis (Lam.) Gandhi Climber 5

CYPERACEAE

Pycnus pumilus (L.) Nees ex C. B. Clarke Herb 5

Cyperus odoratus L. Herb 4,5

Cyperus rotundus L. Herb 5

Cyperus iria L. Herb 1,2,3,4,5

Fimbristylis dichotoma (L.) Vahl Herb 4,5

Fimbristylis ferruginea (L.) Vahl Herb 5

Rhynchospora wightiana Steud. Herb 5

Cyperus pulcherrimus Willd. Herb 5

Cyperus compressus L. Herb 5

DIOSCOREACEAE

Dioscorea pentaphylla L. Twiner 5

Dioscorea bulbifera L. Twiner 3,4,5

Drosera indica L. Herb 5

ERIOCAULACEAE

Eriocaulon cinereum R. Br. Herb 5

Eriocaulon xeranthemum Mart. Herb 5

EUPHORBIACEAE

Bridelia retusa (L.) Sprengel Tree 5

Bridelia scandes (Roxb.) Wild. Shrub 5

Euphorbia hirta L. Herb 2,3,4,5

Euphorbia notoptera Boiss. Herb 5

Euphorbia thymifolia L. Herb 2,4,5

Cont.

| | | |
|---|-------|---------|
| <i>Jatropha curcas</i> L. | Shrub | 5 |
| <i>Macaranga peltata</i> (Roxb.) Muell. Arg | Tree | 5 |
| <i>Phyllanthus emblica</i> L. | Tree | 2,5 |
| <i>Phyllanthus fraternus</i> Webster | Herb | 5 |
| <i>Phyllanthus madraspatensis</i> L. | Herb | 5 |
| <i>Phyllanthus simplex</i> Retz. | Herb | 2,5 |
| <i>Ricinus communis</i> L. | Shrub | 2,3,4,5 |
| <i>Sapium insigne</i> (Royle) Benth. ex Trim. | Tree | 4,5 |

FABACEAE

| | | |
|--|--------|------------|
| <i>Alysicarpus bupleurifolius</i> (L.) DC. | Herb | 5 |
| <i>Alysicarpus vaginalis</i> (L.) | Herb | 2,3,4,5 |
| <i>Atylosia scrabaeoides</i> Benth. | Twiner | 5 |
| <i>Crotalaria filipes</i> Benth. | Herb | 5 |
| <i>Crotalaria pallida</i> Dryand. | Herb | 5 |
| <i>Crotalaria prostrata</i> Rottler ex Willd. | Herb | 1,2 ,3,4,5 |
| <i>Dalbergia sissoo</i> Roxb. | Tree | 5 |
| <i>Dalbergia sympathetica</i> Nimmo ex Grah | Tree | 5 |
| <i>Derris scandens</i> (Roxb.) | Twiner | 5 |
| <i>Desmodium heterocarpon</i> (L.) DC. | Shrub | 5 |
| <i>Desmodium triflorum</i> (L.) DC. | Herb | 1,2,3,4,5 |
| <i>Erythrina variegata</i> L. | Tree | 4,5 |
| <i>Geissaspis cristata</i> Wight & Arn. | Herb | 1,2,3,4,5 |
| <i>Geissaspis tenella</i> Benth. | Herb | 4,5 |
| <i>Gliricidia sepium</i> (Jacq.) Kunth ex Walp . | Tree | 5 |
| <i>Indigofera prostrata</i> Willd. | Herb | 1,2,3,4,5 |
| <i>Indigofera tinctoria</i> L. | *Shrub | 3,4,5 |
| <i>Pongamia pinnata</i> (L.) Pierre | Tree | 5 |
| <i>Pueraria tuberosa</i> (Roxb. ex Willd.) DC. | Twiner | 2,5 |
| <i>Smithia conferta</i> Sm. | Herb | 5 |
| <i>Smithia salsuginea</i> Hance | Herb | 1,2,3,4,5 |

Cont.

| | | |
|--|--------|---------|
| <i>Smithia sensitiva</i> Ait. | Herb | 2,3,4,5 |
| <i>Tephrosia coccinea</i> Wall. | Herb | 5 |
| <i>Tephrosia purpurea</i> (L.) Pers. | Herb | 5 |
| <i>Teramnus labialis</i> (L.f.) Sprengel | Twiner | 5 |
| <i>Zornia gibbosa</i> Spanoghe | Herb | 5 |

GENTIANACEAE

| | | |
|--------------------------------------|------|---------|
| <i>Canscora diffusa</i> (Vahl) R.Br. | Herb | 2,3,4,5 |
| <i>Exacum lawaai</i> C.B. Clarke | Herb | 5 |

LAMIACEAE

| | | |
|--|-------|---------|
| <i>Colebrookea oppositifolia</i> Smith | Shrub | 5 |
| <i>Hyptis suaveolens</i> (L.) Poit. | Herb | 3,5 |
| <i>Ocimum tenuiflorum</i> L. | Herb | 2,3,5 |
| <i>Leucas aspera</i> Spreng. | Herb | 2,3,4,5 |

LECYTHIDACEAE

| | | |
|-----------------------------|------|-------|
| <i>Careya arborea</i> Roxb. | Tree | 2,4,5 |
|-----------------------------|------|-------|

LEEACEAE

| | | |
|------------------------------------|-------|---|
| <i>Leea asiatica</i> (L.) Ridsdale | Shrub | 5 |
| <i>Leea indica</i> (Burm.f.) Merr. | Shrub | 5 |

LILIACEAE

| | | |
|----------------------------|---------|---|
| <i>Gloriosa superba</i> L. | Climber | 5 |
| <i>Smilax zeylanica</i> L. | Climber | 5 |

LYTHRACEAE

| | | |
|----------------------------------|-------|-----|
| <i>Woodfordia tomentosa</i> Bedd | Tree | 5 |
| <i>Lawsonia inermis</i> L. | Shrub | 2,5 |

Cont.

MALVACEAE

| | | |
|--|-------|-----------|
| <i>Sida rhombifolia</i> L. | Herb | 1,2,3,4,5 |
| <i>Gossypium arboreum</i> L. | Shrub | 5 |
| <i>Hibiscus rosa-sinensis</i> L. | Shrub | 5 |
| <i>Sida cordifolia</i> L. | Herb | 2,3,4,5 |
| <i>Thespesia populnea</i> (L.) Sol. ex Corr. Serr. | Tree | 5 - |
| <i>Sida acuta</i> Burm. f. | Herb | 1,2,3,4,5 |
| <i>Urena lobate</i> L. | Shrub | 5 |
| <i>Measa indica</i> (Roxb.) DC. | Tree | 5 |

MELASTOMACEAE

| | | |
|--|-------|-----|
| <i>Melastoma malabathricum</i> L. | Shrub | 5 |
| <i>Memecylon umbellatum</i> Burm.f. | Shrub | 3,5 |
| <i>Osbeckia truncata</i> Don ex Wt. & Arn. | Herb | 5 |

MELIACEAE

| | | |
|-------------------------------------|------|-------|
| <i>Naregamia alata</i> Wight & Arn. | Herb | 2,4,5 |
|-------------------------------------|------|-------|

MENISPERMACEAE

| | | |
|--|--------|---------|
| <i>Anamirta cocculus</i> (L.) Wight & Arn. | Twiner | 5 |
| <i>Cocculus hirsutus</i> (L.) | Herb | 5 |
| <i>Cyclea peltata</i> (Lamk.) Hk.f. & Th. | Twiner | 5 |
| <i>Tinospora cordifolia</i> (Wild.) Miers | Twiner | 2,3,4,5 |
| <i>Tinospora malabarica</i> Miers | Twiner | 5 |

MIMOSACEAE

| | | |
|---|-------|-----------|
| <i>Acacia pennata</i> (L.) Willd. | Shrub | 5 |
| <i>Acacia torta</i> (Roxb.) Craib | Shrub | 5 |
| <i>Leucaena leucocephala</i> (Lam.) de Wit. | Tree | 2,3,4,5 |
| <i>Mimosa pudica</i> L. | Herb | 1,2,3,4,5 |
| <i>Samanea saman</i> Merr. | Tree | 2,3,4,5 |
| <i>Acacia auriculiformis</i> A. Cunn. ex Benth. | Tree | 1,2,3,4,5 |

Cont.

| | | |
|--|-------|-----------|
| <i>Acacia mangium</i> Willd. | Tree | 1,2,3,4,5 |
| <i>Acacia nilotica</i> (L.) Del. | Tree | 4,5 |
| <i>Parkia biglandulosa</i> Wight & Arn. | Tree | 5 |
| <i>Pithecellobium dulce</i> (Roxb.) Benth. | Tree | 2,4,5 |
| <i>Wagatea spicata</i> Dalz. | Shrub | 5 |

MORACEAE

| | | |
|---------------------------------------|------|-------|
| <i>Artocarpus heterophyllus</i> Lamk. | Tree | 2,4,5 |
| <i>Ficus heterophylla</i> L. | Tree | 5 |
| <i>Ficus hispida</i> L.f. | Tree | 5 |
| <i>Ficus benghalesis</i> L. | Herb | 5 |
| <i>Ficus racemosa</i> L. | Tree | 5 |
| <i>Ficus religiosa</i> L. | Tree | 2,5 |

MUSACEAE

| | | |
|----------------------------|------|-----|
| <i>Musa paradisiaca</i> L. | Tree | 4,5 |
|----------------------------|------|-----|

MYRTACEAE

| | | |
|--------------------------------------|-------|-------|
| <i>Psidium guajava</i> L. | Tree | 2,5 |
| <i>Eucalyptus tereticornis</i> Smith | Tree | 3,4,5 |
| <i>Eugenia corymbosa</i> Lam. | Shrub | 4,5 |
| <i>Syzygium cumini</i> (L.) Skeels | Tree | 4,5 |

NYCTAGINACEAE

| | | |
|---|-------|---|
| <i>Bougainvillea spectabilis</i> Willd. | Shrub | 5 |
|---|-------|---|

OCHNACEAE

| | | |
|---------------------------|-------|---|
| <i>Ochna obtusata</i> DC. | Shrub | 5 |
|---------------------------|-------|---|

ONAGRACEAE

| | | |
|----------------------------------|------|-----------|
| <i>Ludwigia parviflora</i> Roxb. | Herb | 1,2,3,4,5 |
|----------------------------------|------|-----------|

Cont.

ORCHIDACEAE

Habenaria marginata Coleb Herb 5

OXALIDACEAE

Biophytum sensitivum (L.) DC. Herb 2,4,5

PASSIFLORACEAE

Passiflora foetida L. Twiner 5

PEDALIACEAE

Sesamum mulayanum Nair Herb 5

PIPERACEAE

Peperomia pellucida (L.) H. B. & K. Herb 2,4,5

POACEAE

Arundinella spicata Dalz. Herb 4,5

Cynodon dactylon L. Pers. Herb 1,2,3,4,5

Dactyloctenium aegyptium (L.) P. Beauv. Herb 1,2,3,4,5

Digitaria ciliaris (Retz.) Korler Herb 4,5

Dimeria woodrowii strapf Herb 5

Echinochloa colona (L.) Link Herb 5

Eleusine indica Gaertn. Herb 5

Eragrostis unioloides (Retz.) Nees ex Steudel Herb 2,3,4,5

Heteropogon contortus (L.) P.
Beauv. Ex Roem. & Schult. Herb 2,3,4,5

Isachne elegans Dalz. Herb 5

Ischaemum semisagittatum Roxb. Herb 1,2,3,4,5

Panicum notatum Retz. Herb 5

Paspalum scrobiculatum L. Herb 2,4,5

Vetiveria zizanioides (L.) Nash Herb 5

Oplismenus burmannii (Retz.) P. Beauv. Herb 3,5

Cont.

| | | |
|---|-------|---------|
| <i>Aristida hystrix</i> L. f. | Herb | 5 |
| POLYGALACEAE | | |
| <i>Polygala elongata</i> Klein ex Willd. | Herb | 2,4,5 |
| PTERIDACEAE | | |
| <i>Adiantum philippense</i> L. | Herb | 2,3,4,5 |
| <i>Pteris pellucida</i> Presl | Herb | 2,4,5 |
| <i>Cheilanthes tenuifolia</i> (Burm.) Swartz | Herb | 5 |
| RHAMNACEAE | | |
| <i>Zizyphus rugosa</i> Lamk. | Shrub | 2,3,4,5 |
| <i>Zizyphus mauritiana</i> Lamk. | Tree | 5 |
| <i>Zizyphus oenoplia</i> (L.) Mill. | Shrub | 2,3,4,5 |
| RUBIACEAE | | |
| <i>Hedyotis corymbosa</i> (L.) Lam. | Herb | 5 |
| <i>Ixora coccinea</i> L. | Shrub | 2,3,4,5 |
| <i>Spermacoce hispida</i> L. | Herb | 2,3,4,5 |
| <i>Spermacoce ocymoides</i> Burm. f. | Herb | 5 |
| <i>Spermacoce stricta</i> L.f. | Herb | 5 |
| <i>Mussaenda laxa</i> Hutchin. | Shrub | 5 |
| <i>Neanotis foetida</i> (Hook.f.) W.H. Lewis | Herb | 2,3,4,5 |
| <i>Wendlandia thyrsoides</i> (Roemer & Schultes) Steudel | Tree | 5 |
| RUTACEAE | | |
| <i>Zanthoxylum rhetsa</i> (Roxb.) DC. | Tree | 5 |
| SCHIZAEACEAE | | |
| <i>Lygodium flexuosum</i> (L.) Swartz | Herb | 2,3,4,5 |

Cont.

SCROPHULARIACEAE

| | | |
|---|------|---------|
| <i>Lindernia viscosa</i> (Hornem.) Boldingh | Herb | 5 |
| <i>Centranthera hispida</i> R. Br. | Herb | 4,5 |
| <i>Lindernia crustacea</i> (L.) F. Muell. | Herb | 2,3,4,5 |
| <i>Lindernia oppositifolia</i> Mukr. | Herb | 5 |
| <i>Ramphicarpa longiflora</i> Benth. | Herb | 3,4,5 |
| <i>Striga asiatica</i> (L.) O. Kuntze | Herb | 5 |
| <i>Scoparia dulcis</i> L. | Herb | 5 |

SELAGINELLACEAE

| | | |
|--|------|-----|
| <i>Selaginella tenera</i> (Hook. & Grev.) Spring | Herb | 2,5 |
|--|------|-----|

SOLANACEAE

| | | |
|---------------------------|------|-----|
| <i>Physalis minima</i> L. | Herb | 2,5 |
|---------------------------|------|-----|

STERCULIACEAE

| | | |
|----------------------------------|-------|-------|
| <i>Helicteres isora</i> L. | Shrub | 2,4,5 |
| <i>Melochia corchorifolia</i> L. | Herb | 2,4,5 |
| <i>Sterculia urens</i> Roxb. | Tree | 4,5 |
| <i>Waltheria indica</i> L. | Herb | 5 |

TILIACEAE

| | | |
|------------------------------------|-------|---------|
| <i>Microcos paniculata</i> L. | Shrub | 2,3,4,5 |
| <i>Corchorus tridens</i> L. | Herb | 5 |
| <i>Triumfetta rhomboidea</i> Jacq. | Herb | 4,5 |
| <i>Corchorus capsularis</i> L. | Herb | 5 |

ULMACEAE

| | | |
|------------------------------------|------|-----------|
| <i>Trema orientalis</i> (L.) Blume | Tree | 1,2,3,4,5 |
|------------------------------------|------|-----------|

VERBENACEAE

| | | |
|--|-------|-----------|
| <i>Clerodendron serratum</i> (L.) Moon Spreng. | Shrub | 1,2,3,4,5 |
|--|-------|-----------|

Cont.

| | | |
|--|-------|---------|
| <i>Clerodendron viscosum</i> Vent. | Shrub | 2,4,5 |
| <i>Clerodendron inerme</i> (L.) Gaertner | Shrub | 2,3,4,5 |
| <i>Lantana camara</i> L. | Shrub | 2,3,4,5 |
| <i>Vitex negundo</i> L. | Shrub | 2,3,5 |
| ZINGIBERACEAE | | |
| <i>Curcuma decipiens</i> Dalz. | Herb | 5 |

Numbers indicate various mine sites

- 1- Xelpi (10 year old iron ore mine)
- 2- Codli (30 year old iron ore mine)
- 3- Sonshi (35 year old iron ore mine)
- 4- Bimbol (45 year old iron ore mine)
- 5- Sanquelim (50 year old iron ore mine)

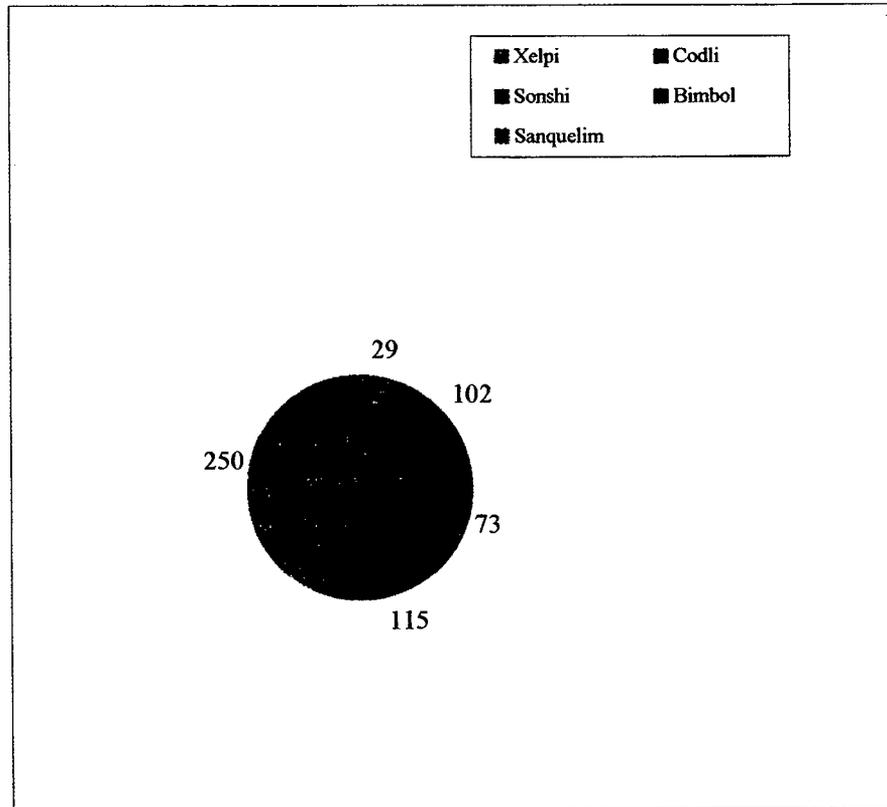
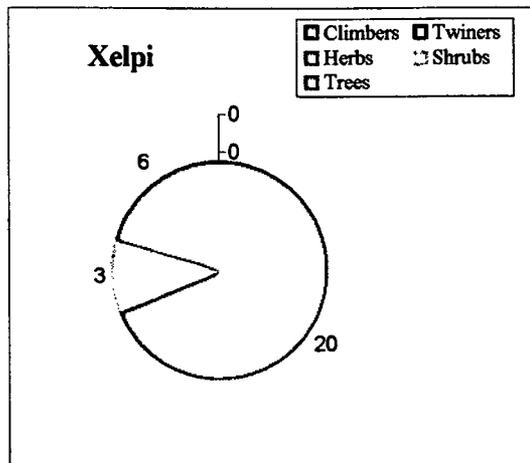
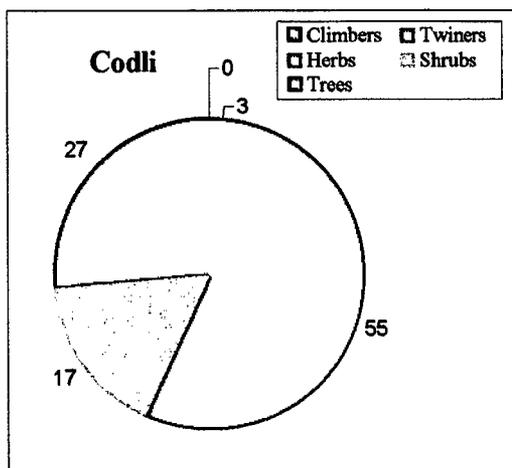


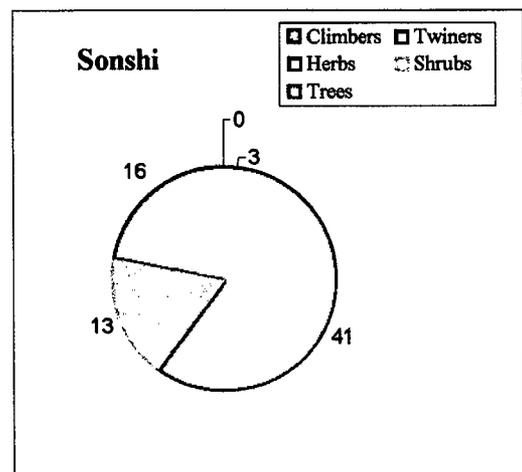
Fig.- 2. Distribution of plant species diversity at Xelpi, Codli, Sonshi, Bimbol and Sanquelim iron ore mines.



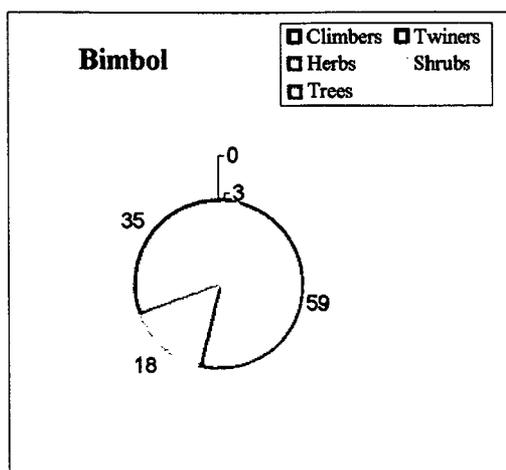
(a)



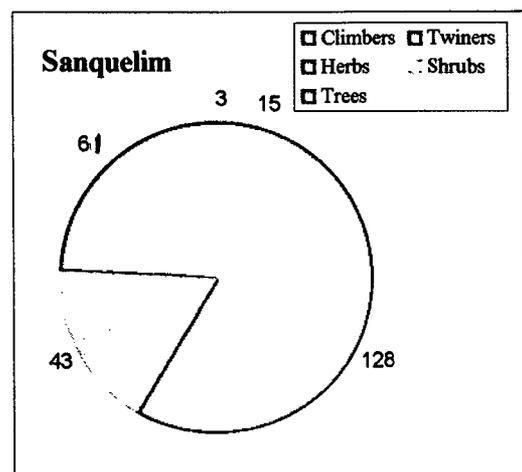
(b)



(c)



(d)



(e)

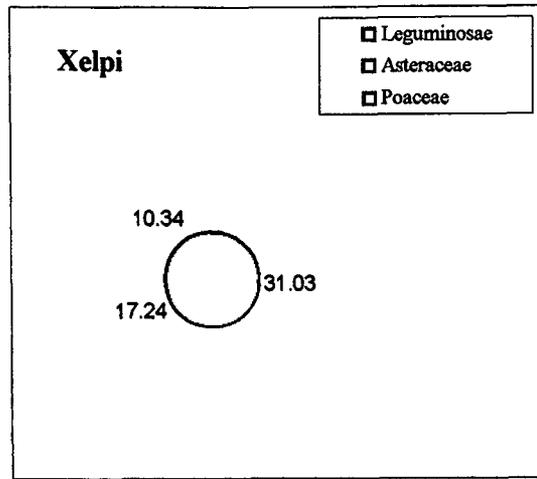
Fig. -3. Distribution of major plant groups viz., climbers, twiners, herbs, shrubs and trees at (a) Xelpi, (b) Codli, (c) Sonshi, (d) Bimbol and (e) Sanquelim iron ore mines.

shrubs (18) and trees (35) with the number of species given in parenthesis (Fig.- 3 d).

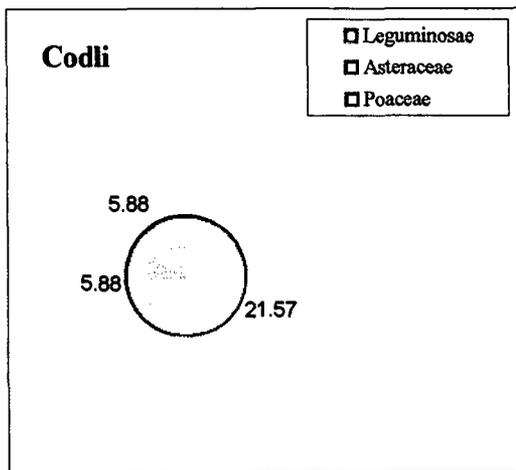
The oldest mine viz., Sanquelim exhibited a rich plant diversity with a total of 250 plant species (both naturally occurring and cultivated) which included 128 herbs, 15 twiners, 3 climbers, 43 shrubs, and 61 tree species (Fig.- 3 e).

The legume species were most dominant followed by species of Asteraceae and Poaceae. In the recently degraded mine viz., Xelpi, the percentage of leguminous species was 31.03 followed by Asteraceae (17.24%) and Poaceae (10.34%). At Codli (30 year old mine), the percentage of leguminous species was 21.57 followed Asteraceae (5.88%) and Poaceae (5.88%). At Sonshi (35 year old mine), the percentage of leguminous species was 24.65 followed by Asteraceae (9.59%) and Poaceae (8.22%). At Bimbol (45 year old mine), the legume percentage was 20.87 followed by Asteraceae (6.09%) and Poaceae (6.6%). At the oldest mine i.e., Sanquelim (50 year old mine), percentage of leguminous species was 19.2, followed by Asteraceae (4.4%) and Poaceae (6.4%) (Fig.- 4).

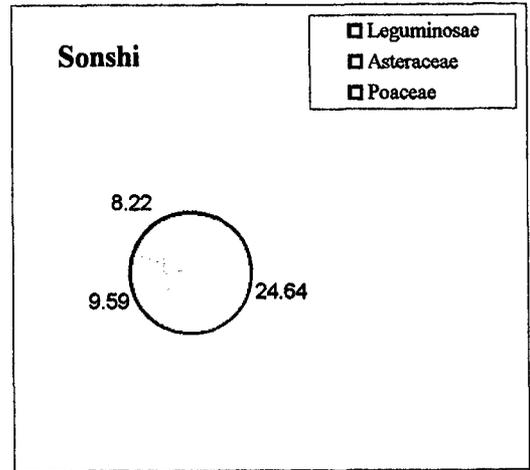
A total of 29 species viz., *Justicia procumbens* L., *Anacardium occidentale* L., *Calotropis gigantea* (L.) R. Br., *Tridax procumbens* L., *Ageratum conyzoides* L., *Emilia sonchifolia* (L.) DC., *Chromolaena odoratum* (L.) King & Robinson, *Vernonia cinerea* (L.) Less., *Cassia tora* L., *Calycopteris floribunda* (Roxb.) Lam., *Terminalia crenulata* Roth.,



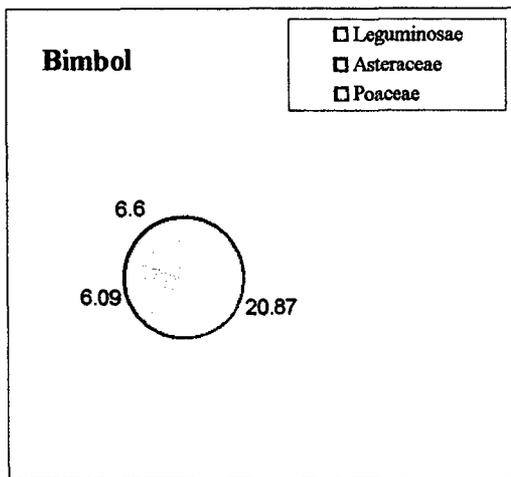
(a)



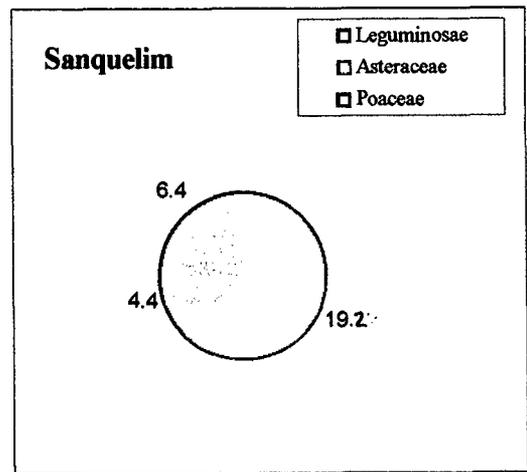
(b)



(c)



(d)



(e)

Fig.- 4. Percent distribution of Leguminosae, Asteraceae, and Poaceae at (a) Xelpi, (b) Codli, (c) Sonshi, (d) Bimbol and (e) Sanquelim iron ore mines.

Terminalia paniculata Roth., *Cyperus iria* L., *Crotalaria prostrata* Rottler ex Willd., *Desmodium triflorum* (L.) DC., *Geissaspis cristata* Wight & Arn., *Indigofera prostrata* Willd., *Smithia salsuginea* Hance, *Sida rhombifolia* L., *Sida acuta* Burm. f., *Mimosa pudica* L., *Acacia auriculiformis* A. Cunn. ex Benth., *Acacia mangium* Willd., *Ludwigia parviflora* Roxb., *Cynodon dactylon* L. f., *Dactyloctenium aegyptium* (L.) P. Beauv., *Ischaemum semisagittatum* Roxb., *Trema orientalis* (L.) Blume, *Clerodendron serratum* (L.) Spreng. were found common to all the sites undertaken for the study.

DISCUSSION

Survey of vegetation revealed that the diversity of plant species increased with increase in age of mine dumps. These observations are in accordance with Rodrigues *et al.*, (1997) who have conducted vegetation survey of eight fairly established mine dumps viz., Lisboa, Elba, Dhat, Torino, Napoli, Sardinha and Plot No. 1 in Sanquelim and Orasso Dongor mine in Assonora, surrounding vegetation in Sanquelim and the vegetation of five plateaus, and reported a total of 414 plant species. The present study revealed that Sonshi (35-year-old mine) exhibited less species diversity as compared to Codli (30-year-old mine). This could be possibly attributed to the fact that Codli had an area of 290 ha. under mining activity as against only 62 ha. under mining at Sonshi. Another probable reason is that the surrounding vegetation at Codli exhibited higher plant diversity than at Sonshi.

The present study indicated that among the plant species the legumes were most dominant. Leguminous species are important colonizers on

abandoned mine spoils (Jefferies *et al.*, 1981). A good number of legume species is an indication of gradual improvement of the fertility status of the spoil as majority of them are potential nitrogen fixers. These results are in accordance with Benerjee *et al.*, (1999). Johnson and Bradshaw (1979) listed out 19 perennial legumes useful in reclamation. Fox *et al.*, (1982) reported the arrival of 17 woody legumes in rehabilitated bauxite mines in Western Australia with time. However, earlier workers (Prasad and Pandey, 1985; Russel, 1985; Jha and Singh, 1990) have reported predominance of Poaceae in mine lands. A good number of leguminous species with dual symbiotic association are successful as pioneer colonizers, due to their ability to compensate for the infertility of the habitat.

Among the shrubs, *Calycopteris floribunda*, *Cassia alata*, *Calotropis gigantea* and *Clerodendron serratum* were common to all the sites. This indicates that these species are better adapted and can tolerate the stress conditions prevailing on the mine sites. Among the herbs *Mimosa pudica*, *Justicia procumbens*, *Tridax procumbens*, *Ageratum conyzoides*, *Emilia sonchifolia*, *Chromolaena odoratum*, *Cassia tora*, *Vernonia cinerea*, *Ischaemum semisagittatum*, *Dactyloctenium aegyptium*, *Cynodon dactylon*, *Cyperus iria*, *Ludwigia parviflora*, *Sida acuta*, *Sida rhombifolia*, *Indigofera prostrata*, *Geissaspis cristata*, *Smithia salsuginea* *Crotalaria prostrata* and *Desmodium triflorum* were common to all the sites indicating that these species have a wide adaptability.

Presence of trees in a community is an indication of its maturity. In older disturbed sites leguminous trees like *Bauhinia purpurea*, *Cassia siamea*, *Delonix regia*, *Tamarindus indica*, *Peltophorum pterocarpum*, *Leucaena leucocephala*, *Samanea saman*, *Acacia auriculiformis*, *Acacia mangium*, *Acacia nilotica*, and *Pithecellobium dulce* were well established as late colonizing species. The presence of various tree species at different mine sites is an indication of gradual venture of invading the dumps and succession. The list also includes noxious weeds like *Chromolaena odoratum* and *Parthenium hysterophorus*. The use of these species should be avoided as these species tend to dominate other native species (Rodrigues, 1997).

Thus, the kinds of plants change continuously with succession. Those species that are important in the pioneer stages are not likely to be important in the climáx. Some species have wider tolerance or niche preferences than others do and therefore, persist over a longer period of time. The diversity of species tends to increase with succession.

The species reported in the present survey study can tolerate the stress conditions encountered on the mine reject dumps and hence, may be screened for their potentiality to grow on mine rejects. It is certain that screening of these plant species would help to increase the biodiversity of the mining area. It is also important that the introduction of exotic species should be made with great care and after consultation, as these species may be very successful and escapes out into the neighbouring areas, and may turn out to be a nuisance. However, these may be used as nurse plants. Initially, a thick plantation of

these species would protect the land against erosion and help in soil stabilization and building up of soil organic matter. However, it is essential to replace these species by native species in the later stages. This is necessary to avoid monocultures and to bring about plant biodiversity.

CHAPTER-II

**STATUS OF ARBUSCULAR MYCORRHIZAL (AM)
FUNGI IN IRON ORE MINE WASTELANDS
AT CODLI-GOA.**

INTRODUCTION

Mining is one of the most degrading actions of man on the earth as it physically tears up the earth's surface, producing gaping holes and barren heaps, changes the geomorphic pattern and contaminates the environment. Surface mining disrupts mycorrhizal population thus leaving minimal levels of endophyte inoculum (Khan, 1978; Miller, 1979; Reeves *et al.*, 1979; Allen and Allen, 1980). Natural re-establishment of vegetation on dry, nutrient poor abandoned mined land is a slow process even when plant propagules are available. To facilitate natural succession and to reclaim the drastically disturbed sites that lack topsoil, low-cost techniques of establishing vegetation must be developed. A more economical and long lasting alternative is to reintroduce mycorrhizal fungi adapted to local natural vegetation on the sites identified for revegetation (Parkinson, 1978).

Several studies have dealt with the role of arbuscular mycorrhizal fungi in reclaimed and disturbed soils (Daft *et al.*, 1975; Aldon, 1978). The occurrence of arbuscular mycorrhizal (AM) fungi in mine spoils has been reported earlier (Ponder, 1979; Zak and Parkinson, 1982; Kiernan *et al.*, 1983; Waaland and Allen, 1987). The occurrence of arbuscular mycorrhizal fungal association in herbaceous plants growing in mine spoils has been documented by Daft and Nicolson, (1974). Similarly, Allen and Allen (1980) reported mycorrhizal colonization in plants growing on reclaimed strip mine in Wyoming. These investigations stress the importance of arbuscular mycorrhizal association in allowing successful recolonization, establishment, and growth of herbaceous plant species on disturbed sites.

Arbuscular mycorrhizal fungi by virtue of their symbiotic associations with roots of most vascular plants are among the most significant microbes in terrestrial ecosystems. They offer good scope for their use in plant growth improvement because of their nutrient mobilization capacity and moisture retention capacity. Mycorrhizae are not only more efficient in utilizing available nutrients from the soil (Smith, 1980; Tinker, 1975; Bowen and Smith, 1981), but also are involved in transfer of nutrients from components of soil minerals and organic residues to solution and in nutrient cycling in an ecosystem (Jeffries and Barea, 1994).

Arbuscular mycorrhizal fungi are sometime reported to be an important associate of many pioneer plants, which may require arbuscular mycorrhizal colonization in order to survive on disturbed lands (Jehne and Thompson, 1981). They are particularly useful in detoxifying heavy minerals by chelation (Lamont, 1978; Khan *et al.*, 2000).

Studies related to the diversity of arbuscular mycorrhizal fungi from iron ore mines are very scarce (Rodrigues, 1999; Sastry and Johri, 1999). Hence, the present investigation was carried out with an aim to study the colonization and diversity of native arbuscular mycorrhizal fungal species in the rhizosphere soils from iron ore mine wastelands at Codli Goa.

MATERIALS AND METHODS

STUDY SITE

Codli a 30-year-old mine, situated in Sanguem, South Goa (15° 20' 53" N Latitude and 74° 8' 33" E Longitude) is spread over an area of 300 ha. with an actual area of 290 ha. presently under mining (**Plate-I**).

SOIL ANALYSIS

For soil analysis, mine reject samples were collected from a depth of 0-25 cm from five different locations of Codli mine and were brought to the laboratory in polyethylene bags. Samples were passed through 2mm sieve to remove the larger soil particles and were mixed thoroughly to obtain a composite sample. Later, the composite sample was processed three times to get the mean value.

Soil pH was measured after dilution with distilled water (1:1 w/v soil: water) soon after the samples were brought to the laboratory. Electrical Conductivity (EC) was determined in 1:1 water: waste extracts (Bower and Wilcox, 1965). Total nitrogen was determined by micro-Kjeldahl method (Jackson, 1971). Total phosphorus was determined by molybdenum blue method (Jackson, 1971). Total potassium was determined by flame photometric method (Jackson, 1971). Total calcium and magnesium were determined by titrimetric method. Organic carbon and organic matter content were analyzed by Walkley and Black's rapid titration method (Jackson, 1971).

SAMPLING

Fifty-five plant species, which includes Pteridophytes, herbs, shrubs and trees belonging to 29 families, were taken up in the present study. For shrubs and trees, the roots were dug and traced back to plant, which ensured that the roots belonged to the intended plant species. Root samples of herbs were collected by uprooting the entire plant. In case of bulbs, tubers and corms, the plants were uprooted along with the bulbous portion and the finer roots. Rhizosphere soils samples were collected from a depth of 15-20 cm in polyethylene bags and were brought to the laboratory and were stored at 4°C till further processing.

ASSESSMENT OF ARBUSCULAR MYCORRHIZAL (AM) COLONIZATION AND SPORE DENSITY

The roots were freed from the adhering soil, gently washed and cut into 1cm segments. Later the root bits were cleared with 10 % KOH, acidified with 1N HCl stained with (0.05%) trypan blue in lactophenol (Phillips and Hayman, 1970) and were left overnight for staining. Percentage of root colonization was carried out by using slide method (Giovannetti and Mosse, 1980). In case of bulbs, corms and tubers, besides staining roots, freshly collected bulbs, corms, and tubers were also examined for arbuscular mycorrhizal colonization after staining. In case of pteridophytes the root bits were cleared with 2.5% KOH (Koske and Gemma, 1989), acidified with 5N HCl and stained with 0.05% trypan blue.

Hundred grams of rhizosphere soil sample was taken from each plant and assayed for spore count using wet sieving and decanting technique

(Gerdemann and Nicolson, 1963). Soil sample was dispersed in 1 liter of water and the suspension was left undisturbed for 1-2 minutes to allow the heavier soil particles to settle down. Later, the suspension was passed through a series of sieves of various mesh sizes stacked in a descending order (500 μ m – 37 μ m). This washing and decanting process was repeated twice in order to increase the likelihood that majority of the spores are recovered. After ensuring that all the colloidal particles have passed through the sieve, the remains on each of the sieves were washed and the washings were collected separately in beakers. Estimation of spore density was carried out as per the procedure given by Gaur and Adholeya, (1994) which is outlined below.

1. A Filter paper (Whatman No. 1, size 11 cm diameter is taken and given fold.
2. This is followed by a second fold.
3. This filter paper is reopened, two lines are drawn to divide the filter paper in four equal quadrants.
4. Vertical lines are drawn on one half of the filter paper so as to divide it into approximately fifteen columns with each column about 0.5 mm apart. Each column is numbered and the direction of counting marked.
5. The filter paper is then folded in such a way 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 the rest of the filter paper is retained without spores.

6. This filter paper with the sample spore is spread on a bigger petri-plate and was observed under stereomicroscope and by moving the petri-plate the spore were counted in every space between the lines numbered.

Intact spores were picked up using a wet needle and were mounted in polyvinyl alcohol lacto-glycerol (PVLG) (Koske and Tessier, 1983) on a glass slide for identification.

IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL (AM) FUNGAL SPECIES

Intact and crushed spores in polyvinyl alcohol lacto-glycerol (PVLG) with or without Melzer's reagent were examined under Leica compound microscope. Taxonomic identification of spores to species level was based on spore morphology, ornamentation, wall characteristics using various bibliographies (Schenck and Perez, 1990; Almeida and Schenck, 1990; Walker and Vestberg, 1998; Redecker, *et al.*, 2000b; Morton and Redecker, 2001; Schubler *et al.*, 2001) by matching original descriptions and those provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (<http://invam.caf.wvu.edu>). Spore colour was examined under Leica stereomicroscope using intact spores immersed in water. Voucher specimens of arbuscular mycorrhizal fungi have been retained in the Botany Department, Goa University, Goa.

Spores in the rhizosphere soil were multiplied using *Eleusine coracana* (L.) Gaertn, *Lycopersicum esculentum* Mill., *Allium cepa* L. and *Coleus sp.* as host plants. The spores isolated from the trap cultures were later used for confirming the identified spores recovered during the study period (Plate- XXVI).

PLANT IDENTIFICATION

Plants collected in the present study were identified using floras (Rao, 1985 & 1986; Matthew, (1991); Mohanan and Henry, (1994) and Naithani *et al.*, 1997).

STATISTICAL ANALYSIS

The data on mycorrhizal colonization (AM) was arcsine square root transformed and spore numbers were log-transformed prior to statistical analysis. Pearson's correlation was used to understand the relationship between root colonization and spore density. Frequency of occurrence was calculated by using the formula given below.

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

RESULTS

Soil analysis results revealed that mine rejects were slightly acidic with pH of 6.06 (0.154), and Electrical Conductivity (EC) of 0.11 (0.01) mmho cm⁻¹. Results also indicate acute deficiencies of both macro- and micro-nutrients, organic carbon and organic matter content in the mine rejects (Table- 3).

Table- 3. Soil characteristics at Codli iron ore mine site.

| Parameters | |
|--------------------------------------|--------------|
| pH | 6.06 ± 0.054 |
| Electrical Conductivity (EC) | 0.11 ± 0.01 |
| Nitrogen (mg 100 g ⁻¹) | 52.6 ± 3.22 |
| Phosphorus (mg 100 g ⁻¹) | 124 ± 5.47 |
| Potassium (mg 100 g ⁻¹) | 48.8 ± 1.30 |
| Calcium (mg 100 g ⁻¹) | 6.17 ± 0.54 |
| Magnesium (mg 100 g ⁻¹) | 1.596 ± 0.57 |
| Organic carbon (%) | 0.22 ± 0.01 |
| Organic matter (%) | 0.374 ± 0.01 |

Values are mean of five readings.

± - indicates Standard deviation.

Arbuscular mycorrhizal colonization was recorded in all the plant species examined in the study. However, the extent of colonization exhibited variations (Table- 4). The mycorrhizal colonization was characterized by intraradical and extramatrical hyphae, intracellular hyphal coils, inter or intracellular vesicles and/ or arbuscules (Plate II, III, & IV). In pteridophytes, root colonization ranged from 15% (*Lygodium flexuosum*) to 30% (*Adiantum philippense*). All the three species of tuber and corms exhibited varied mycorrhizal colonization. The degree of mycorrhizal colonization was maximum in *Crinum vivipara* (40%) followed by *Puereria tuberosa* (35%) while least colonization was recorded in *Amorphophallus commutatus* (20%). Root^t colonization in *Crinum vivipara* was characterized \ t by hyphae, vesicles and arbuscules, whereas the arbuscules were absent in *Amorphophallus commutatus* and *Puereria tuberosa*. Besides roots, arbuscular mycorrhizal colonization was also recorded in the outermost parenchymatous tissues in bulb, corm and tubers examined. Besides pteridophytes, bulb, corm and tuber, the mycorrhizal colonization in other herbs ranged from 8% in *Biophytum sensitivum* to 99% in *Cassia tora*. Among the shrubs, percent root colonization ranged from 20% in *Lantana camera* to 75% in *Calotropis gigantea*. Among tree species root colonization ranged from 15% in *Acacia mangium* to 75% in *Samanea saman*. Average root colonization in herbs, shrubs and trees was 50.25%, 50.25% and 47.43% respectively.

Spore density exhibited great variations among various plant groups (Table-4). Among the pteridophytes, the spore density ranged from 14 spores

Table-4. Status of arbuscular mycorrhizal fungi from iron ore mine wastelands at Codli.

| Family and Scientific name | Habit | *Spore density 100 g ⁻¹ soil | * Root colonization (%) | AM fungal structures | | |
|--|-----------------|---|-------------------------------|----------------------------|---|---|
| | | | | H | V | A |
| Acanthaceae | | | | | | |
| <i>Justicia procumbens</i> L. | Herb | 38 ± 4.58 | 48 ± 11.79 | + | + | + |
| <i>Andrographis paniculata</i> (Burm. f.) Wallich ex Nees | Herb | 55 ± 7.0 | 34 ± 3.61 | + | + | + |
| Amaryllidaceae | | | | | | |
| <i>Crynum vivipara</i> var <i>viviparum</i> Ansari & Nair | Shrub (bulb) | 90 ± 2.0 | 40 ± 7.94 | + | + | + |
| Anacardiaceae | | | | | | |
| <i>Anacardium occidentale</i> L. | Tree | 200 ± 12 | 30 ± 2.66 | + | + | + |
| Araceae | | | | | | |
| <i>Amorphophallus commutatus</i> Engler | Herb (corm) | 34 ± 3.61 | 20 ± 2.00 | + | + | - |
| Asclepiadiaceae | | | | | | |
| <i>Calotropis gigantea</i> (L.) R. Br. | Shrub | 80 ± 6.24 | 75 ± 7.0 | + | + | + |
| <i>Hemidesmus indicus</i> R. Br. | Herb | 29 ± 4.36 | 34 ± 5.57 | + | + | - |
| Asteraceae | | | | | | |
| <i>Ageratum conyzoides</i> L. | Herb | 110 ± 5.57 | 39 ± 3.46 | + | + | + |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Herb | 200 ± 7.0 | 53 ± 8.89 | + | + | + |
| <i>Vernonia cinerea</i> (L.) Less. | Herb | 396 ± 4.25 | 66 ± 8.89 | + | + | + |
| <i>Tricholepis glaberrima</i> DC. | Herb | 350 ± 2.12 | 80 ± 5.57 | + | + | + |
| <i>Emilia sonchifolia</i> (L.) DC. | Herb | 65 ± 2.65 | 94 ± 2.0 | + | + | + |
| Balsaminaceae | | | | | | |
| <i>Impatiens kleinii</i> Wight & Arn. | Herb | 98 ± 7.6 | 9 ± 1 | + | + | - |
| Caesalpiniaaceae | | | | | | |
| <i>Delonix regia</i> (Hook.) Raf. | Tree | 96 ± 5.57 | 60 ± 8.0 | + | + | + |
| <i>Tamarindus indica</i> L. | Tree | 108 ± 5.57 | 50 ± 6.56 | + | + | + |
| <i>Peltophorum pterocarpum</i> (DC.) Backer ex K. | Tree | 96 ± 3.0 | 38 ± 3.61 | + | + | + |
| <i>Cassia tora</i> L. | Herb | 86 ± 2.0 | 99 ± 1.0 | + | + | + |

Cont.

| | | | | | | |
|--|-----------------|------------|-----------|---|---|---|
| Casuarinaceae | | | | | | |
| <i>Casuarina equisetifolia</i> Foster & Foster f. | Tree | 72 ± 9.64 | 18 ± 4.36 | + | + | + |
| Combretaceae | | | | | | |
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | Shrub | 164 ± 7.81 | 57 ± 6.0 | + | + | + |
| <i>Terminalia paniculata</i> Roth. | Tree | 192 ± 9.85 | 67 ± 5.57 | + | + | + |
| <i>Terminalia crenulata</i> Roth. | Tree | 72 ± 2.65 | 65 ± 4.0 | + | + | + |
| Euphorbiaceae | | | | | | |
| <i>Euphorbia hirta</i> L. | herb | 144 ± 5.29 | 90 ± 4.0 | + | + | + |
| <i>Phyllanthus simplex</i> Retz. | Herb | 70 ± 2.65 | 70 ± 3.61 | + | + | + |
| <i>Euphorbia thymifolia</i> L. | Herb | 96 ± 4.36 | 90 ± 5.0 | + | + | + |
| <i>Ricinus communis</i> L. | Shrub | 168 ± 6.24 | 46 ± 7.55 | + | + | + |
| Fabaceae | | | | | | |
| <i>Crotalaria prostrata</i> Rottler ex Willd | Herb | 24 ± 4.58 | 68 ± 2.65 | + | + | + |
| <i>Pueraria tuberosa</i> (Roxb. Ex Willd.) DC. | Herb (tuber) | 30 ± 7.0 | 35 ± 3.47 | + | - | + |
| <i>Smithia salsuginea</i> Hance | Herb | 290 ± 6.24 | 88 ± 3.61 | + | + | + |
| Gentianaceae | | | | | | |
| <i>Canscora diffusa</i> (Vahl) R. Br. | Herb | 9.0 ± 1.7 | 10 ± 4.58 | + | + | - |
| Lecythidaceae | | | | | | |
| <i>Careya arborea</i> Roxb. | Tree | 132 ± 6.24 | 60 ± 4.36 | + | + | - |
| Lamiaceae | | | | | | |
| <i>Leucas aspera</i> Spreng. | Herb | 15 ± 2.64 | 20 ± 3.0 | + | + | - |
| <i>Ocimum tenuiflorum</i> L. | Herb | 184 ± 5.57 | 61 ± 4.36 | + | + | + |
| Malvaceae | | | | | | |
| <i>Sida acuta</i> Burm. F. | Herb | 128 ± 6.56 | 83 ± 5.0 | + | + | + |
| <i>Sida cordifolia</i> L. | Herb | 38 ± 4.58 | 25 ± 5.29 | + | + | + |
| <i>Sida rhombifolia</i> L. | Herb | 280 ± 7.55 | 54 ± 3.61 | + | + | + |
| Mimosaceae | | | | | | |
| <i>Acacia auriculiformis</i> A. Cunn. Ex Benth. | Tree | 148 ± 5.29 | 45 ± 3.61 | + | + | + |
| <i>Acacia mangium</i> Willd. | Tree | 102 ± 2.65 | 15 ± 5.0 | + | + | + |
| <i>Mimosa pudica</i> L. | Herb | 154 ± 8.54 | 76 ± 6.24 | + | + | + |
| <i>Leucaena leucocephala</i> (Lam.) De Wit. | Tree | 20 ± 5.0 | 40 ± 3.0 | + | + | + |
| <i>Samanea saman</i> Merr. | Tree | 116 ± 5.57 | 75 ± 7.81 | + | + | + |

Cont.

| | | | | | | |
|---|-------|------------|-----------|---|---|---|
| Moraceae | | | | | | |
| <i>Artocarpus heterophyllus</i> Lam. | Tree | 85 ± 3.61 | 46 ± 6.56 | + | + | + |
| Onagraceae | | | | | | |
| <i>Ludwigia parviflora</i> L. | Herb | 68 ± 6.08 | 70 ± 6.24 | + | + | + |
| Oxalidaceae | | | | | | |
| <i>Biopytium sensitivum</i> (L.) DC. | Herb | 19 ± 3.0 | 8 ± 2.65 | + | - | - |
| Poaceae | | | | | | |
| <i>Cynodon dactylon</i> L. f. | Herb | 64 ± 5.57 | 48 ± 6.24 | + | + | + |
| <i>Dactyloctenium aegyptium</i> (L.) P. Beauv. | Herb | 140 ± 0.54 | 65 ± 5.29 | + | + | + |
| <i>Ischaemum semisagittatum</i> Roxb. | Herb | 102 ± 5.0 | 86 ± 6.08 | + | + | + |
| Pteridaceae | | | | | | |
| <i>Adiantum philippense</i> L. | Herb | 15 ± 4.36 | 30 ± 5.57 | + | + | - |
| Rubiaceae | | | | | | |
| <i>Neanotis foetida</i> (Hook. F.) W. H. Lewis | Herb | 112 ± 7.0 | 15 ± 2.65 | + | - | - |
| Solanaceae | | | | | | |
| <i>Physalis minima</i> L. | Herb | 24 ± 3.0 | 16 ± 5.29 | + | + | + |
| Tiliaceae | | | | | | |
| <i>Microcos paniculata</i> L. | Shrub | 160 ± 3.0 | 60 ± 6.0 | + | + | + |
| Ulmaceae | | | | | | |
| <i>Trema orientalis</i> (L.) Blume | Tree | 44 ± 4.0 | 55 ± 3.61 | + | + | + |
| Verbinaceae | | | | | | |
| <i>Lantana camara</i> L. | Shrub | 20 ± 1.0 | 20 ± 3.0 | + | + | - |
| <i>Clerodendron viscosum</i> Vent. | Shrub | 140 ± 4.0 | 52 ± 6.56 | + | + | + |
| Schizaceae | | | | | | |
| <i>Lygodium flexuosum</i> (L.) Swartz | Herb | 20 ± 5.29 | 15 ± 1.73 | + | - | + |
| Selaginellaceae | | | | | | |
| <i>Selaginella tenera</i> (Hook. & Grev.) Spring | Herb | 14 ± 4.36 | 20 ± 6.0 | + | - | + |

* - Mean of three samples.

H - Hyphal colonization; A- Arbuscular colonization; V- Vesicular colonization.

± - Standard deviation

PLATE II

Arbuscular mycorrhizal fungal colonization (a-d)

- (a) Hyphal colonization with hypha running parallel forming H connections and Y shaped structures.
- (b) Hyphal coils (arrowed).
- (c) Initial stage in arbuscular colonization.
- (d) Arbuscules forming a broom like structure (arrowed).

(Scale bar: a = 50 μm ; b, c = 22 μm ; d = 10 μm)

PLATE III

Variation in shapes of vesicles (a-f)

- (a) Intercellular vesicles.
- (b) Vesicles.
- (c) Group of vesicles.
- (d) Globular vesicles.
- (e) Lobed vesicles.
- (f) Elliptical vesicles.

(Scale bar: a = 100 μm ; b-d = 22 μm ; e, f = 50 μm).

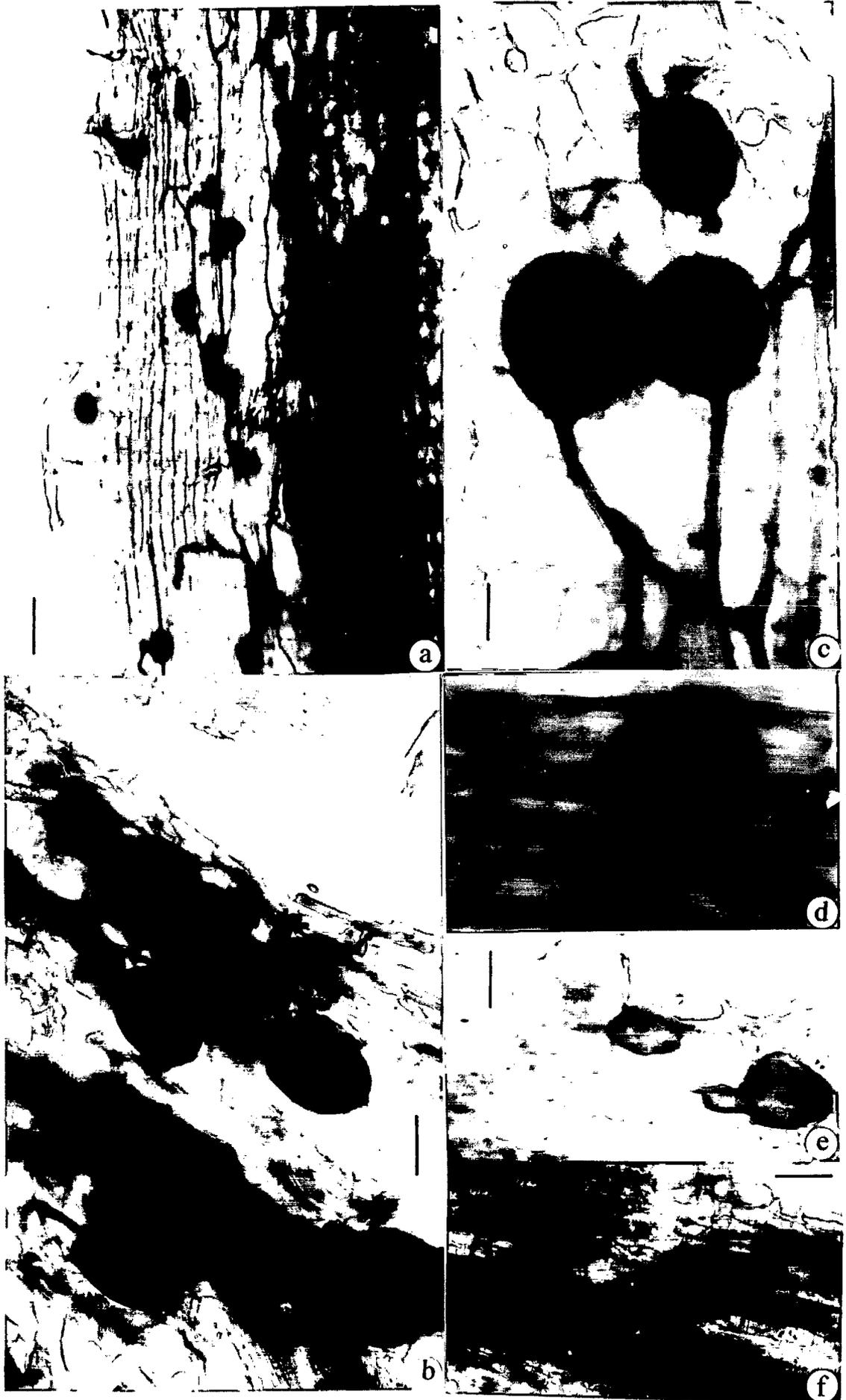


PLATE - III

PLATE IV

Arbuscular mycorrhizal fungal structures (a-c)

(a) Vesicles with oil globules (og).

(b) Vesicles with oil globules (og).

(c) Extramatrical spores of arbuscular mycorrhizal fungi.

(Scale bar: a, b = 50 μm ; c = 100 μm).

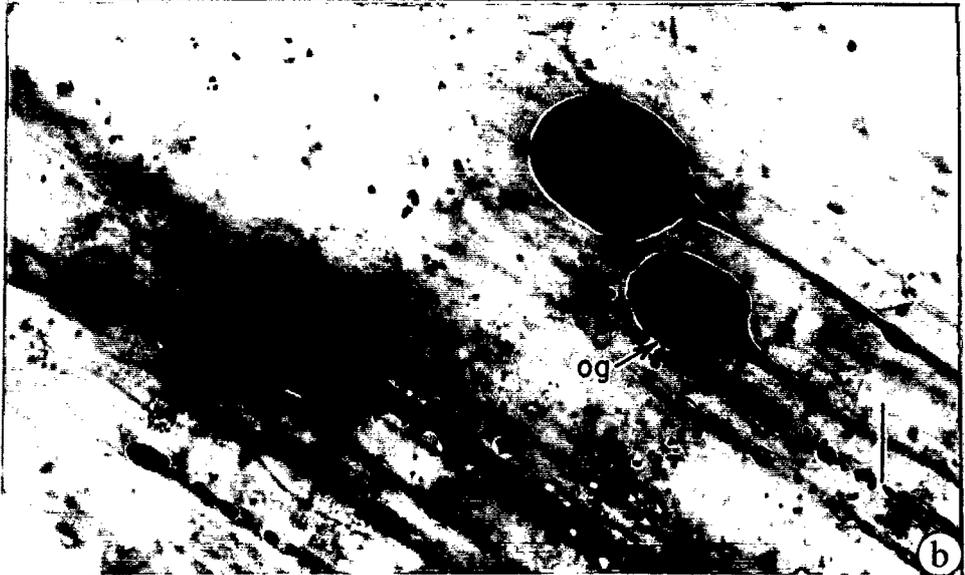
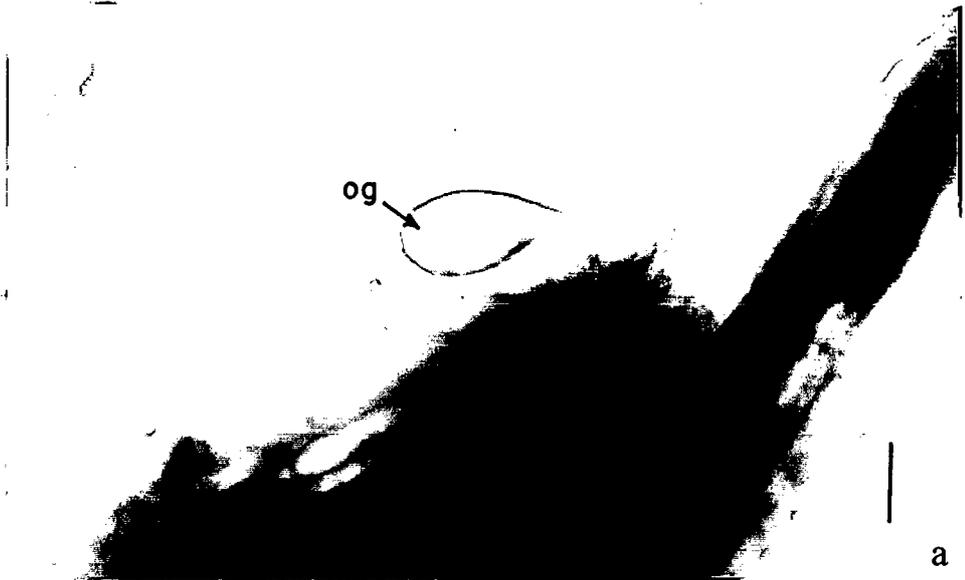


PLATE - IV

to 20 spores 100 g⁻¹ soil. Maximum spores were recovered from *Lygodium flexuosum* (20 spores 100 g⁻¹ soil) while minimum were recovered from *Selaginella tenera* (14 spores 100 g⁻¹ soil). Among bulb, corm and tuber, maximum spores were recovered from *Crimum vivipara* (90 spores 100 g⁻¹ soil) followed by *Amorphophallus commutatus* (34 spores 100 g⁻¹ soil) and least number of arbuscular mycorrhizal fungal spores were recovered from *Puereria tuberosa* (30 spores 100 g⁻¹ soil).

Besides pteridophytes, bulb, corm and tuber, the spore density in other herbs ranged from 9 spores 100 g⁻¹ in *Canscora diffusa* to 396 spores 100 g⁻¹ rhizosphere soil in *Vernonia cinerea*. Among the six shrubs studied, the spore density ranged from 20 spores 100 g⁻¹ rhizosphere soil in *Lantana camera* to 168 spores 100 g⁻¹ rhizosphere soil in *Ricinus communis*. Among the 15 tree species studied, the spore density ranged from 20 spores 100 g⁻¹ soil in *Leucaena leucocephala* to 200 spores 100 g⁻¹ rhizosphere soil in *Anacardium occidentale*. Average spore density recorded was higher in shrubs (122 spores 100 g⁻¹ soil) followed by herbs (102.5 spores 100 g⁻¹ soil) and was least in trees (98.87 spores 100 g⁻¹ soil).

The present study indicated a positive ($r=0.534$, $P<0.01$) correlation between spore number and root colonization. Forty arbuscular mycorrhizal fungal species were recovered from the rhizosphere of 55 plant species from iron ore mine wastelands at Codli (Table- 5). The arbuscular mycorrhizal fungal species recorded belonged to four genera viz., *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*.

Table- 5. Arbuscular mycorrhizal fungal species associated with plants from iron ore mine site at Codli.

| Family and Scientific name | Identified arbuscular mycorrhizal fungal species |
|--|---|
| Acanthaceae | |
| <i>Justicia procumbens</i> L. | <i>Gi. margarita, G. intraradices, G. macrocarpum.</i> |
| <i>Andrographis paniculata</i> (Burm. f.) Wallich ex Nees | <i>G. etunicatum, G. globiferum, G. taiwanensis, S. weresubiae.</i> |
| Amaryllidaceae | |
| <i>Crynum vivipara</i> var <i>viviparum</i> Ansari & Nair | <i>A. spinosa, A. undulata, G. fasciculatum, G. reticulatum, G. macrocarpum, G. sinuosum, S. gregaria.</i> |
| Anacardiaceae | |
| <i>Anacardium occidentale</i> L. | <i>A. spinosa, G. constrictum, S. gregaria, S. pellucida, S. reticulata</i> |
| Araceae | |
| <i>Amorphophallus commutatus</i> Engler | <i>G. geosporum, G. macrocarpum, G. monosporum, G. taiwanensis.</i> |
| Asclepidiaceae | |
| <i>Calotropis gigantea</i> (L.) R. Br. | <i>A. laevis, A. scrobiculata, Gi. rosea, G. constrictum, G. fasciculatum, S. gregaria, S. pellucida, S. reticulata.</i> |
| <i>Hemidesmus indicus</i> R. Br. | <i>Gi. margarita, G. etunicatum, G. fasciculatum, S. pellucida.</i> |
| Asteraceae | |
| <i>Ageratum conyzoides</i> L. | <i>Gi. margarita, A. scrobiculata, G. constrictum, G. rubiforme, G. sinuosum, G. taiwanensis, S. gregaria.</i> |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | <i>A. scrobiculata, Gi. margarita, G. etunicatum, G. geosporum, G. macrocarpum, G. rubiforme, G. sinuosum, G. taiwanensis, S. gregaria.</i> |
| <i>Vernonia cinerea</i> (L.) Less. | <i>A. spinosa, Gi. margarita, Gi. .decipiens, G. constrictum, G. taiwanensis.</i> |
| <i>Tricholepis glaberrima</i> DC. | <i>G. constrictum, G. dimorphicum, G. dominikii, G. fasciculatum, G. geosporum.</i> |

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| <i>Emilia sonchifolia</i> (L.) DC. | <i>G. constrictum</i> , <i>G. macrocarpum</i> , <i>G. sinuosum</i> , <i>S. gregaria</i> , <i>S. pellucida</i> , <i>S. werresubiae</i> . |
| Balsaminaceae | |
| <i>Impatiens kleinii</i> Wight & Arn. | <i>A. spinosa</i> , <i>G. fasciculatum</i> , <i>G. claroideum</i> , <i>G. monosporum</i> |
| Caesalpiniaceae | |
| <i>Delonix regia</i> (Hook.) Raf. | <i>A. bireticulata</i> , <i>A. scrobiculata</i> , <i>A. spinosa</i> , <i>G. geosporum</i> , <i>G. sinuosum</i> , <i>S. weresubiae</i> . |
| <i>Tamarindus indica</i> L. | <i>A. spinosa</i> , <i>G. constrictum</i> , <i>G. etunicatum</i> , <i>G. globiferum</i> , <i>G. claroideum</i> , <i>G. taiwanensis</i> , <i>S. reticulata</i> . |
| <i>Peltophorum pterocarpum</i> (DC.) Backer ex K. | <i>A. spinosa</i> , <i>A. undulata</i> , <i>Gi. albida</i> , <i>G. macrocarpum</i> , <i>G. sinuosum</i> , <i>S. pellucida</i> . |
| <i>Cassia tora</i> L. | <i>A. spinosa</i> , <i>Gi. margarita</i> , <i>G. fasciculatum</i> , <i>G. macrocarpum</i> , <i>G. taiwanensis</i> , <i>S. weresubiae</i> . |
| Casuarinaceae | |
| <i>Casuarina equisetifolia</i> Foster & Foster f. | <i>A. laevis</i> , <i>A. scrobiculata</i> , <i>A. spinosa</i> , <i>G. sinuosum</i> . |
| Combretaceae | |
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | <i>G. fasciculatum</i> , <i>G. claroideum</i> , <i>S. gregaria</i> , <i>S. reticulata</i> , <i>S. pellucida</i> . |
| <i>Terminalia paniculata</i> Roth. | <i>G. geosporum</i> , <i>G. lacteum</i> , <i>G. microaggregatum</i> , <i>G. monosporum</i> , <i>G. multicaule</i> , <i>G. clavisporum</i> , <i>G. coremioides</i> , <i>S. pellucida</i> . |
| <i>Terminalia crenulata</i> Roth. | <i>A. scrobiculata</i> , <i>Gi. margarita</i> , <i>G. fasciculatum</i> , <i>G. macrocarpum</i> , <i>S. gregaria</i> . |
| Euphorbiaceae | |
| <i>Euphorbia hirta</i> L. | <i>A. spinosa</i> , <i>G. constrictum</i> , <i>G. etunicatum</i> , <i>G. globiferum</i> , <i>G. macrocarpum</i> , <i>G. sinuosum</i> , <i>G. taiwanensis</i> . |
| <i>Phyllanthus simplex</i> Retz. | <i>A. scrobiculata</i> , <i>G. geosporum</i> , <i>S. pellucida</i> , <i>S. weresubiae</i> . |
| <i>Euphorbia thymifolia</i> L. | <i>A. scrobiculata</i> , <i>A. spinosa</i> , <i>G. fasciculatum</i> , <i>G. mosseae</i> , <i>G. taiwanensis</i> , <i>S. reticulata</i> . |
| <i>Ricinus communis</i> L. | <i>A. delicata</i> , <i>G. geosporum</i> , <i>G. taiwanensis</i> , <i>S. pellucida</i> . |
| Fabaceae | |
| <i>Crotalaria prostrata</i> Rottler ex Willd | <i>Gi. margarita</i> , <i>G. globiferum</i> , <i>S. pellucida</i> . |
| <i>Pueraria tuberosa</i> (Roxb. Ex Willd.) DC. | <i>Gi. margarita</i> , <i>G. geosporum</i> , <i>G. intraradices</i> . |

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| <i>Smithia salsuginea</i> Hance | <i>Gi. margarita</i> , <i>G. fasciculatum</i> , <i>G. claroideum</i> , <i>G. clavisorum</i> , <i>G. taiwanensis</i> , <i>S. gregaria</i> , <i>G. sp.</i> |
| Gentianaceae | |
| <i>Canscora diffusa</i> (Vahl) R. Br. | <i>A. scrobiculata</i> , <i>Gi. albida</i> , <i>G. etunicatum</i> , <i>S. reticulata</i> . |
| Lecythidaceae | |
| <i>Careya arborea</i> Roxb. | <i>A. laevis</i> , <i>G. constrictum</i> , <i>G. formosanum</i> , <i>G. globiferum</i> , <i>G. macrocarpum</i> , <i>G. rubiforme</i> . |
| Lamiaceae | |
| <i>Leucas aspera</i> Spreng. | <i>G. macrocarpum</i> , <i>S. gregaria</i> , <i>S. weresubiae</i> . |
| <i>Ocimum tenuiflorum</i> L. | <i>G. macrocarpum</i> , <i>G. geosporum</i> , <i>S. reticulata</i> , <i>S. pellucida</i> . |
| Malvaceae | |
| <i>Sida acuta</i> Burm.f. | <i>A. scrobiculata</i> , <i>G. constrictum</i> , <i>S. reticulata</i> , <i>G. taiwanensis</i> . |
| <i>Sida cordifolia</i> L. | <i>G. macrocarpum</i> , <i>G. constrictum</i> , <i>G. globiferum</i> , <i>G. geosporum</i> , <i>S. pellucida</i> . |
| <i>Sida rhombifolia</i> L. | <i>Gi. margarita</i> , <i>G. fasciculatum</i> , <i>G. mosseae</i> , <i>G. taiwanensis</i> . |
| Mimosaceae | |
| <i>Acacia auriculiformis</i> A. Cunn. ex Benth. | <i>A. scrobiculata</i> , <i>A. spinosa</i> , <i>G. macrocarpum</i> , <i>S. weresubiae</i> . |
| <i>Acacia mangium</i> Willd. | <i>A. scrobiculata</i> , <i>A. spinosa</i> , <i>G. geosporum</i> , <i>G. macrocarpum</i> , <i>S. pellucida</i> . |
| <i>Mimosa pudica</i> L. | <i>Gi. margarita</i> , <i>G. globiferum</i> , <i>G. macrocarpum</i> , <i>G. rubiforme</i> , <i>G. taiwanensis</i> , <i>S. gregaria</i> , <i>S. reticulata</i> , <i>S. sp.</i> , <i>G. sp.</i> |
| <i>Leucaena leucocephala</i> (Lam.) De Wit. | <i>A. scrobiculata</i> , <i>Gi. margarita</i> , <i>S. gregaria</i> , <i>S. weresubiae</i> . |
| <i>Samanea saman</i> Merr. | <i>A. scrobiculata</i> , <i>G. fasciculatum</i> , <i>G. globiferum</i> , <i>G. macrocarpum</i> , <i>S. gregaria</i> , <i>S. pellucida</i> . |
| Moraceae | |
| <i>Artocarpus heterophyllus</i> Lam. | <i>Gi. margarita</i> , <i>A. scrobiculata</i> , <i>G. constrictum</i> , <i>G. rubiforme</i> , <i>G. taiwanensis</i> , <i>S. pellucida</i> . |
| Onagraceae | |
| <i>Ludwigia parviflora</i> L. | <i>A. scrobiculata</i> , <i>Gi. margarita</i> , <i>S. pellucida</i> , <i>S. weresubiae</i> . |
| Oxalidaceae | |
| <i>Biopytium sensitivum</i> (L.) DC. | <i>A. scrobiculata</i> , <i>G. fasciculatum</i> , <i>G. globiferum</i> . |
| Poaceae | |
| <i>Cynodon dactylon</i> L. f. | <i>A. spinosa</i> , <i>G. claroideum</i> , <i>S. gregaria</i> , <i>S. reticulata</i> , <i>S. pellucida</i> . |
| <i>Dactyloctenium aegyptium</i> (L.) P. Beauv. | <i>Gi. rosea</i> , <i>S. gregaria</i> , <i>S. reticulata</i> , <i>S. pellucida</i> , <i>S. weresubiae</i> . |
| <i>Ischaemum semisagittatum</i> Roxb. | <i>Gi. decipiens</i> , <i>Gi. margarita</i> , <i>G. fasciculatum</i> , <i>S.</i> <i>nigra</i> . |

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| Pteridaceae | |
| <i>Adiantum philippense</i> L. | <i>A. spinosa</i> , <i>G. mosseae</i> , <i>G. sinuosum</i> , <i>G. taiwanensis</i> . |
| Rubiaceae | |
| <i>Neanotis foetida</i> (Hook. F.) W. H. Lewis | <i>G. macrocarpum</i> , <i>A. undulata</i> |
| Solanaceae | |
| <i>Physalis minima</i> L. | <i>A. scrobiculata</i> , <i>A. spinosa</i> , <i>Gi. albida</i> , <i>S. gregaria</i> |
| Tiliaceae | |
| <i>Microcos paniculata</i> L. | <i>A. spinosa</i> , <i>G. macrocarpum</i> , <i>G. taiwanensis</i> , <i>S. pellucida</i> , <i>S. weresubiae</i> . |
| Ulmaceae | |
| <i>Trema orientalis</i> (L.) Blume | <i>Gi. margarita</i> , <i>G. flavisporum</i> , <i>G. multicaule</i> , <i>S. reticulata</i> . |
| Verbinaceae | |
| <i>Lantana camara</i> L. | <i>A. spinosa</i> , <i>A. scrobiculata</i> , <i>G. geosporum</i> , <i>G. taiwanensis</i> . |
| <i>Clerodendron viscosum</i> Vent. | <i>G. macrocarpum</i> , <i>G. claroideum</i> , <i>G. clavisporum</i> , <i>G. taiwanensis</i> , <i>S. reticulata</i> . |
| Schizaceae | |
| <i>Lygodium flexuosum</i> (L.) Swartz | <i>A. spinosa</i> , <i>G. dimorphicum</i> , <i>G. etunicatum</i> , <i>G. lacteum</i> , <i>G. rubiforme</i> , <i>S. sp.</i> |
| Selaginellaceae | |
| <i>Selaginella tenera</i> (Hook. & Grev.) Spring | <i>Gi. margarita</i> , <i>G. geosporum</i> , <i>G. taiwanensis</i> . |

Fungal genera are abbreviated as:

A.- Acaulospora, Gi.- Gigaspora, G.- Glomus, S.- Scutellospora.

Among the pteridophyte examined, maximum arbuscular mycorrhizal fungal species were found in the rhizosphere of *Lygodium flexuosum* (6) followed by *Adiantum philippense* (4) and *Selaginella tenera* (3) with the number of AM species given in parenthesis. Among bulb, corm and tuber, arbuscular mycorrhizal species richness was maximum in *Crinum vivipara* (7) followed by *Amorphophallus commutatus* (4) and *Puereria tuberosa* (3) with the number of arbuscular mycorrhizal fungal species given in parenthesis.

Besides Pteridophytes, bulb, corm and tuber, the arbuscular mycorrhizal fungal species richness in other herbs ranges from 2–9 per plant. Maximum arbuscular mycorrhizal fungal species were recorded in *Chromolaena odoratum* (9) and *Mimosa pudica* (9) and minimum were observed in *Neanotis foetida* (2) with the number of arbuscular mycorrhizal fungal species given in parenthesis.

- Among the shrubs, maximum arbuscular mycorrhizal fungal species were recorded in *Calotropis gigantea* (8) and minimum were recorded in *Lantana camera* (4) and *Ricinus communis* (4). In tree species, maximum arbuscular mycorrhizal fungal species were recovered from *Terminalia paniculata* (8) and minimum of 4 arbuscular mycorrhizal fungal species were recovered from *Casuarina equisetifolia*, *Leucaena leucocephala* and *Trema orientalis* with the number of arbuscular mycorrhizal fungal species given in parenthesis.

Table- 6. Frequency of occurrence of arbuscular mycorrhizal fungi in selected host plants from iron ore mine wastelands at Codli.

| ARBUSCULAR MYCORRHIZAL FUNGAL SPECIES | FREQUENCY (%) |
|--|---------------|
| <i>Acaulospora bireticulata</i> Rothwell & Trappe | 1.82 |
| <i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss | 1.82 |
| <i>Acaulospora laevis</i> Gerd. & Trappe | 5.45 |
| <i>Acaulospora scrobiculata</i> Trappe | 34.55 |
| <i>Acaulospora spinosa</i> Walker & Trappe | 34.55 |
| <i>Acaulospora undulata</i> Sieverding | 5.45 |
| <i>Gigaspora albida</i> Schenck & Smith | 5.45 |
| <i>Gigaspora decipiens</i> Hall & Abbott | 3.64 |
| <i>Gigaspora margarita</i> Becker & Hall | 32.73 |
| <i>Gigaspora rosea</i> Nicolson & Schenck | 3.64 |
| <i>Glomus claroideum</i> Schenck & Smith emend Walker & Vestberg | 10.09 |
| <i>Glomus clavisorum</i> (Trappe) Almeida & Schenck | 5.46 |
| <i>Glomus constrictum</i> Trappe | 21.82 |
| <i>Glomus coremioides</i> (Berk. & Broome) Redecker et Morton, | 1.82 |
| <i>Glomus dimorphicum</i> Boyetchko & Tewari | 3.64 |
| <i>Glomus domnikii</i> Blaszkowski | 1.82 |
| <i>Glomus etunicatum</i> Becker & Gerdemann | 12.73 |
| <i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker and Koske, | 25.45 |
| <i>Glomus flavisorum</i> (M. Lange & Lund) Trappe & Gerdemann | 1.82 |
| <i>Glomus formosanum</i> Wu & Chen | 1.82 |
| <i>Glomus intraradices</i> Schenck & Smith | 3.64 |
| <i>Glomus lacteum</i> Rose & Trappe | 3.64 |
| <i>Glomus geosporum</i> (Nicol. & Gerd.) Walker | 23.63 |
| <i>Glomus globiferum</i> Koske & Walker | 16.36 |
| <i>Glomus macrocarpum</i> Tul. & Tul. | 36.36 |
| <i>Glomus monosporum</i> Gerdemann & Trappe | 5.46 |
| <i>Glomus mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe | 5.46 |
| <i>Glomus multicaule</i> Gerdemann & Bakshi | 3.64 |
| <i>Glomus microaggregatum</i> Koske, Gemma & Olexia | 1.82 |
| <i>Glomus reticulatum</i> Bhattacharjee & Mukerji | 1.82 |
| <i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck | 10.91 |

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| <i>Glomus sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck | 16.36 |
| <i>Glomus taiwanensis</i> (Wu & Chen) Almeida & Schenck | 36.36 |
| <i>Glomus</i> sp. (Unidentified) | 3.64 |
| <i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders | 29.09 |
| <i>Scutellospora nigra</i> (Redhead) Walker & Sanders | 1.82 |
| <i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders | 32.73 |
| <i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders | 23.64 |
| <i>Scutellospora weresubiae</i> Koske & Walker | 20.00 |
| <i>Scutellospora</i> sp. (Unidentified) | 3.64 |

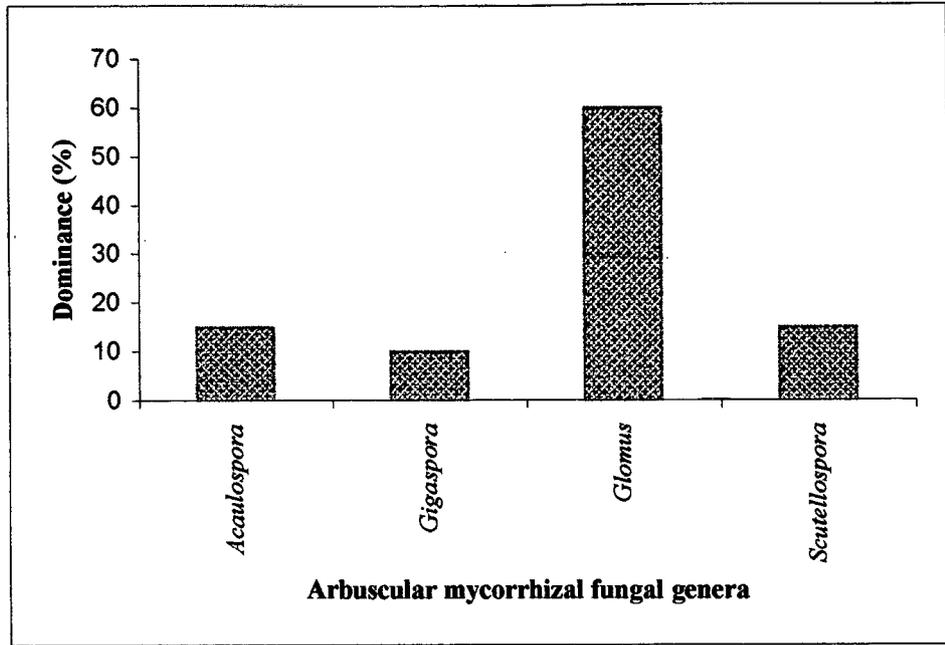


Fig.- 5. Dominance of arbuscular mycorrhizal fungal genera from Codli iron ore mine site.

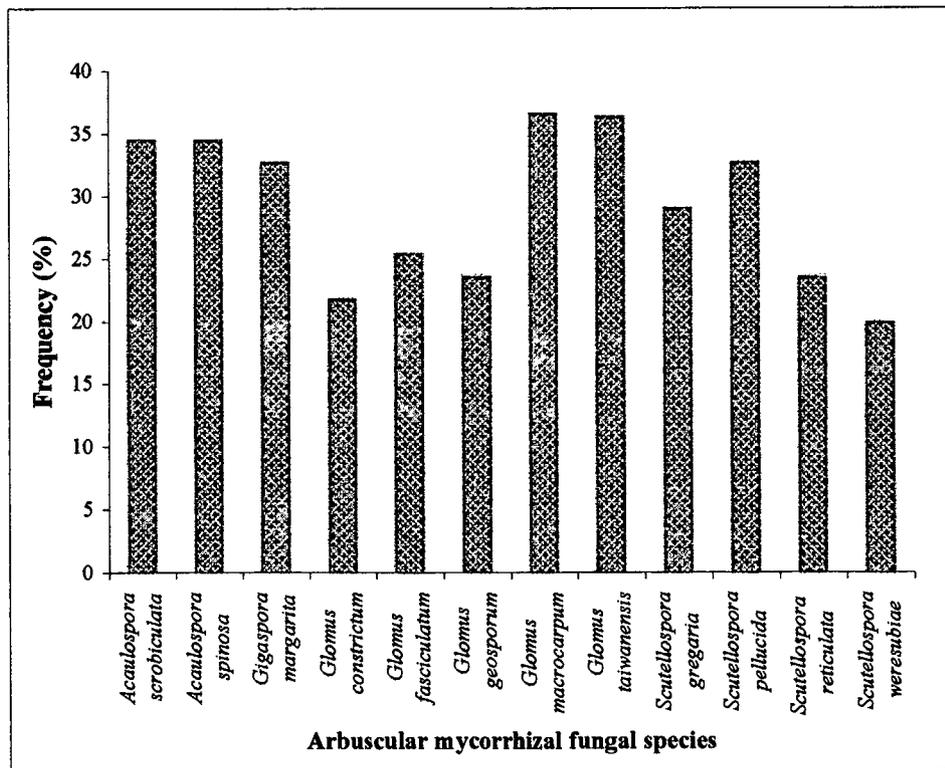


Fig.- 6. Frequency of occurrence of some dominant arbuscular mycorrhizal fungal species from Codli iron ore mine site.

In the present study among the four genera recorded viz., *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* the most dominating genus was *Glomus* followed by *Scutellospora*, *Acaulospora*, and *Gigaspora* (Fig.- 5).

The frequency of occurrence of arbuscular mycorrhizal fungal species recovered from 55 plant species ranged from 1.82% to 36.36% (Table- 6). Arbuscular mycorrhizal fungal species viz., *Acaulospora spinosa* Walker & Trappe (34.55%), *Acaulospora scrobiculata* Trappe (34.55%), *Gigaspora margarita* Becker & Hall (32.73%), *Glomus constrictum* Trappe (21.82%), *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske (25.45%), *Glomus geosporum* (Nicolson & Gerdemann) Walker (23.63%), *Glomus macrocarpum* Tul. & Tul. (36.36%), *Glomus taiwanensis* (Wu & Chen) Almeida & Schenck, *comb. nov.* (36.36%), *Scutellospora gregaria* (Schenck & Nicolson) Walker & Sanders (29.09%), *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders (32.73%), *Scutellospora reticulata* (Koske, Miller & Walker) Walker & Sanders (23.64%), and *Scutellospora weresubiae* Koske & Walker (20%) were the most frequently occurring species at Codli iron ore mine site with the frequency of occurrence given in parenthesis (Fig.- 6).

DISCUSSION

Soil analysis results revealed that the rejects were deficient in Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Organic carbon (%) and Organic matter (%) content.

Electrical Conductivity (EC) was very low indicating that there is no likelihood of salinity problems. The pH of the reject was slightly acidic, thus posing no problems for plant growth. Similar observations have been recorded earlier (Rodrigues *et al.*, 1997).

The results of the present study indicate that there could be significant arbuscular mycorrhizal dependency in plants in order to survive on disturbed lands. Similar observations have been recorded earlier (Reeves, *et al.*, 1979; Miller, 1979) who have stated that the mycorrhizal status of plants on disturbed site is related to the degree of disturbance and harshness of the site. Similarly, Sastry and Johri, (1999) have reported higher dependency of arbuscular mycorrhizal fungi in plants growing on disturbed sites.

Earlier studies by Rodrigues and Bukhari (1996) and (1997) on arbuscular mycorrhizal colonization of plant species found growing in abandoned tailing pond of iron ore mines in Goa and occurrence of arbuscular mycorrhizal fungal colonization in herbaceous plants species growing on iron ore mine wastelands of Goa also showed varied levels of colonization suggesting its importance in the survival of plants in mine wasteland. Similarly Rodrigues, (1999) reported all the plant species to be mycorrhizal in his studies on arbuscular mycorrhizal colonization from seedling growing on mine reject dumps of Goa.

Sastry and Johri, (1999) in their studies on arbuscular mycorrhizal fungal diversity of stressed soils of Bailadila iron ore sites in Bastar region,

Madhya Pradesh reported average to high levels of colonization (25-90 %) and very low spore population ranging from 0-2000 spores kg⁻¹ soil, with a total of 89 arbuscular mycorrhizal fungal species and suggested the arbuscular mycorrhizal fungal dependency of host species in the iron stressed habitats.

In the present study, the extent of root colonization and spore density showed variations between the plant species. Variations in spore number have been reported earlier by Kruckelmann, (1975) who found significant differences in spore number in six different plant species growing in monoculture for sixteen years. The influence of host plant on incidence of arbuscular mycorrhizal fungi has also been observed by Schenck and Kinloch, (1980) on a woodland site newly planted with six agronomic crops and grown in monoculture for seven years. Hayman (1975) and Iqbal *et al.*, (1975) recorded difference in spore numbers between plant species. Mycorrhizal dependency of plant species also contributes to varied levels of colonization

The variations in extent of mycorrhizal colonization among different plant species observed confirm earlier findings of Manjunath and Bagyaraj, (1982), who stated that the extent to which plants respond to arbuscular mycorrhizal colonization varies with plant species. Gerdemann, (1965) has shown that the colonization pattern of arbuscular mycorrhizal fungal species can be distinctly different in various plant species. According to Tommerup, (1992) the fungi vary in their colonization patterns due to differences in rate of intra-radical growth, amount of hyphae per entry point, and growth of external mycelium along roots before entry points are formed. Similarly,

Muthukumar and Udaiyan, (2000 a) in their studies on arbuscular mycorrhizas of plants growing in Western Ghats region of Southern India reported variation in colonization levels in various plant species.

Thus, the presence of arbuscules identifies the arbuscular mycorrhizal colonization in roots and is an indication of mutualism between plants and arbuscular mycorrhizal fungi (Smith and Smith, 1989). Arbuscules are the preferential site for fungus plant metabolite exchange (Cox and Tinker, 1976; Scannerini and Bonfante, 1983). All the endophytic fungi, recognized by Gerdemann and Trappe, (1974) and belonging to Genus *Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocystis* form arbuscules. Nicolson (1959) working on Poaceae observed variation in the morphology of the extramatrical mycelium.

Intracellular coils were common in mycorrhizal roots of most of the plants. Earlier workers (Sward *et al.*, 1978; Abbott and Robson, 1978) have reported coil formation by *Glomus fasciculatum* and *Glomus epigeus* in *Vitis venifera*. According to Abbott (1982) the size of the intracellular hyphae depends upon the type of fungus involved while the host probably influences the amount and the behaviour of the hyphae. Intercellular hyphae produced by the coils or directly by the penetrating hyphal branches are usually found in the intermediate layer of the cortical parenchyma. Thus, they are often intermittent projections and are at times swollen (Abbott and Robson, 1979). Abbott and Robson (1979) has described "H" connections among hyphae running parallel and "Y" shaped junctions are also formed. It has been reported that not all arbuscular mycorrhizal fungi form vesicles within the

roots. *Gigaspora margarita* does not form vesicles while *Acaulospora laevis* formed lobed or irregular vesicles. *Acaulospora trappe* forms smaller unlobed vesicles. *Glomus* gives rise to elliptical, inter- or intra-cellular vesicles (Gerdeemann and Trappe, 1974; Abbott and Robson, 1978; Abbott, 1982).

The occurrence of mycorrhizae in pteridophytes is less documented compared to other groups of plants. Since, 1960, only 180 pteridophytes species have been examined for mycorrhizae as compared to 6000 species of gymnosperms and angiosperms (Newman and Reddell, 1987; Trappe, 1987). About 70% of the pteridophytes were reported to be potentially mycorrhizal in the British flora (Harley and Harley, 1987). Little is known about the occurrence of mycorrhizae in Indian pteridophytes (Mishra *et al.*, 1980; Raghupathy and Mahadevan, 1993). Muthukumar and Udaiyan (2000b) in their study on arbuscular mycorrhizal fungi in pteridophytes of Western Ghats, Southern India found mycorrhiza in 61 of the 71 species they examined with colonization levels ranging from 0% to 78.72%, while, spore density ranged from 2.35 - 39.52 spores 10 g⁻¹ soil.

In pteridophytes, the morphology of the colonization process is very much similar to that found in angiosperms. However, the colonization is not ubiquitous and seems to be dependent on the systematic position of the fern species (Bonfante-Fasolo, 1984; Gemma *et al.*, 1992; Zhi-Wei, 2000). Boullard (1959) suggested that a close correlation exists between arbuscular mycorrhizal fungal colonization and fern evolution.

The occurrence of arbuscular mycorrhizal colonization and arbuscular mycorrhizal fungal spores in non-root underground parts of some plant species

suggest that the arbuscular mycorrhizal fungi besides colonizing the plant roots can also colonize bulbs, corms and tubers. Taber and Trappe (1982) have reported the occurrence of arbuscular mycorrhizal fungi in rhizomes and scale leaves of *Zingiber officinale* and coined the terms 'Mycorrhizome' and 'Mycophyllon' respectively to describe the colonization. Subsequently, Iqbal and Nasim, (1986). also used the term 'Mycorrhizome' to describe the colonization in rhizomes of *Zingiber officinale*. Similarly, the association of arbuscular mycorrhizal fungi with underground corms of *Amorphophallus commutatus* Engler is termed as 'Mycocorm' (Rodrigues, 1995) and with the underground tubers of *Pueraria tuberosa* (Willd.) DC. as 'Mycotuber' (Rodrigues, 1996). Later, Rama Bhat and Kaveriappa (1997) described the association of arbuscular mycorrhizal fungi in the tubers of *Colocasia esculenta* (L.) Schott as 'Mycotuber'. Recently, the arbuscular mycorrhizal association in bulbs of *Allium sativum* L. has been reported by Kunwar *et al.*, (1999).

In the present study, the absence of arbuscules in some plant species suggests that the hyphal coils may serve the function of arbuscules. Similar observations were made by Mago *et al.*, (1992) who has reported absence of arbuscules in Bryophytes. Barker *et al.*, (1998) reported that in Paris type of arbuscular mycorrhizal colonization, growth into the root is slow, being primarily intra-cellular, and the fungus forms coils inside each cell with rare of minimally structured arbuscules.

Among the various families, highest root colonization and spore density was reported from members of Leguminosae followed by Asteraceae

as compared to other families. Legumes are generally known to be highly dependent on arbuscular mycorrhizal association, which are mainly implicated to the higher phosphorus demand for nodulation and nitrogen fixation (Gerdemann, 1968; Crush, 1974; Smith and Daft, 1977; Carling *et al.*, 1978).

Leguminous plants by virtue of their dual symbiotic association that usually results in the fixation of nitrogen and uptake of available phosphorus are successful as pioneer colonizers, due to their ability to compensate for the infertility of the habitat (Harley, 1970). A good number of legume species at Codli mine site is an indication of gradual improvement of the fertility status of the spoil because majority of them are found to be potential nitrogen fixers.

The pre-existing network of soil hyphae is often the main source of arbuscular mycorrhizal inoculum (Powell, 1977; Birch, 1986; McGee, 1989). The existence of a positive correlation between spore number and root colonization suggests that factors that influence root colonization also influences sporulation (Brundrett, 1991).

Present study revealed the occurrence of four arbuscular mycorrhizal fungal genera *viz.*, *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora* species. *Glomus* was the most dominant arbuscular mycorrhizal fungal genera associated with plants growing on nutrient deficient mined soils. Earlier Raman *et al.*, (1993), identified *Glomus* and *Gigaspora spp.* in the mycorrhizospheres of fourteen plant species colonizing a magnesite mine spoil in India. Whereas, Weissenhorn and Leyval (1995) isolated only *Glomus mosseae* and Duek *et al.*, (1986) isolated *Glomus fasciculatum* alone from the

heavy metal polluted soils and, Pawlowska *et al.*, (1996) surveyed a calamine spoil mound rich in Cd., Pb, and Zn in Poland and recovered spores of *Glomus aggregatum*, *Glomus fasciculatum* and *Enterophospora*. Predominance of the genus *Glomus* in the rhizosphere of plants growing on mine wastelands has also been reported by Lakshman, (1997), Sastry and Johri, (1999) and Uniyal, (2001).

Recovery of large arbuscular mycorrhizal fungal diversity in the study site which accounts for nearly 30% of the total known arbuscular mycorrhizal fungi in the study site not only reveals the rich wealth of arbuscular mycorrhizal diversity sheltered in such stressful habitats but also indicates that extreme environments are the centers for evolution and conservation of biodiverse gene pool. These native isolates with the capacity to survive under stress conditions are instrumental in reclamation of disturbed sites. Thus, identification of the dominant native arbuscular mycorrhizal fungal species thriving on mine wastelands, their multiplication and proper utilization would make the re-establishment and regeneration attempts ecologically and economically viable in such constrained ecosystems.

CHAPTER-III

**ARBUSCULAR MYCORRHIZAL FUNGAL
DIVERSITY IN DISTURBED AND UNDISTURBED
AREAS OF IRON ORE MINE SITES.**

INTRODUCTION

Most plants in natural ecosystem have mycorrhizal association which involves three way interactions between fungus, soil and plants (Brundrett, 1991). Consequently, the impact of soil disturbances on these associations will depend on the nature of fungal propagules and changes in soil conditions. Mycorrhizal propagules can be severely influenced by damage to vegetation and soil resulting from natural processes or human interventions. These destructive processes include intense fire (Wicklow-Howard, 1989; Dhillon *et al.*, 1988; Klopatek *et al.*, 1988), exposure of subsurface soil by erosion (Day *et al.*, 1987; Habte, 1989), or by burrowing animals (Koide and Mooney, 1987) and volcanic activity (Allen, 1988). Agricultural practices such as tillage (Evans and Miller, 1988), long fallow periods (Thompson, 1987), soil compaction (Wallace, 1987), biocide application (Medve, 1984; Haas *et al.*, 1987), or flooding for rice culture (Ilag *et al.*, 1987; Nopamornbodi *et al.*, 1987) can also adversely influence mycorrhizae.

Most studies of mycorrhizal association in highly disturbed habitats such as mine sites have found reduced levels of mycorrhizal propagules (Rives *et al.*, 1980; Danielson, 1985; Stahl *et al.*, 1988; Jasper *et al.*, 1992; Pflieger *et al.*, 1994). In undisturbed natural community, a network of hyphae in the soil is considered to be an important source of inoculum (Read and Birch, 1988). Disruption of this network can result in a substantial loss of mycorrhizal colonization (Jasper *et al.*, 1989a, b; Evans and Miller, 1990). Soil structure changes resulting from disturbance could disrupt the spatial association between old and new roots, or reduce the effectiveness of root inocula (Evans

and Miller, 1988; Rives *et al.*, 1980). Changes to soil properties and population of soil organisms, which occur during stockpiling, can reduce the efficacy of surviving arbuscular mycorrhizal endophytes (Waaland and Allen, 1987). Typical changes that occur when soil is disturbed and stockpiled include the loss of organic matter and nutrients, as well as structure and components (Abdul-Kareem and McRae, 1984; Danielson, 1985).

Successional changes in plant population occur during ecosystem recovery from disturbance or establishment on new substrates (Grime, 1979; Barbour *et al.*, 1987). The proportion of mycorrhizal roots has been observed to increase along with plant cover during succession in several natural ecosystems (Khan, 1974; Miller *et al.*, 1983; Lesica and Antibus, 1985).

Arbuscular mycorrhizal fungi play a vital role in primary and secondary succession of plant species especially in low nutrient ecosystem. Measuring biodiversity helps one to understand the ecology of habitat and to develop conservative strategies. The present study reports a comparative account of root colonization, spore density and diversity of arbuscular mycorrhizal fungal species from disturbed and undisturbed areas of a recently degraded iron ore mine and a well established iron ore mine.

MATERIALS AND METHODS

STUDY SITES

Study was conducted at two degraded iron ore mine sites *viz.*, Dadiovaril Sodo “Xelpi” (10) and Bimbol (45) and their surrounding undisturbed areas with age of the mines given in parenthesis.

SURVEY OF VEGETATION

Detailed vegetation survey was conducted in early monsoon (July –August) at both the disturbed sites. Plants were identified using floras (Rao, 1985 & 1986; Matthew, 1991; Mohanan and Henry, 1994; Naithani *et al.*, 1997).

SOIL ANALYSIS

For soil analysis, samples were collected from a depth of 0-25 cm from five different locations from Xelpi and its surrounding undisturbed area and Bimbol and its surrounding undisturbed area. Samples were brought to the laboratory in polyethylene bags, passed through 2mm sieve to remove the larger soil particles and were processed separately.

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 determined by flame photometric method (Jackson, 1971). Organic carbon and organic matter were detected by Walkley and Black’s rapid titration method (Jackson, 1971).

SAMPLING

Ten angiospermic plant species viz., *Chromolaena odoratum* (L.) King & Robinson; *Mimosa pudica* L., *Ischaemum semisagittatum* Roxb. (herbs), *Calycópterus floribunda* (Roxb.) Lam. (shrub); *Acacia auriculiformis* A. Cunn. Ex Benth., *Acacia mangium* Willd., *Anacardium occidentale* L., *Trema orientalis* (L.) Blume, *Terminalia crenulata* Roth. (tree species) were taken up during the study from disturbed and undisturbed areas of Xelpi mine site. While 10 angiospermic plant species viz., *Calotropis gigantea* (L.) R. Br., *Calycópterus floribunda* (Roxb.) Lam., *Careya arborea* Roxb., *Chromolaena odoratum* (L.) King & Robinson, *Delonix regia* (Hook.) Raf., *Microcos paniculata* L., *Mimosa pudica* L., *Randia rugulosa* (Thw.) Hook.f., *Terminalia paniculata* Roth. and *Terminalia crenulata* Roth. were taken up during the study from disturbed and undisturbed areas of Bimbol mine site.

Roots and rhizosphere soils were sampled individually for each plant species from disturbed and undisturbed areas of both the sites during the dry period (March-April). For shrubs and trees, the roots were dug and traced back to plant, which ensured that the roots belonged to the intended plant species. Samples of herbs were usually made by uprooting the entire plant. Plants were uprooted along with the rhizosphere soil and brought to the laboratory in the polyethylene bags. Plants were shaken to remove the adhering soil particles. Feeder roots were cut into 1 cm bits and were processed for further studies.

ASSESSMENT OF ARBUSCULAR MYCORRHIZAL COLONIZATION AND SPORE DENSITY

The root bits were cleared with 10 % KOH, acidified with 1N HCl stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970) and were left overnight for staining. Percentage of root colonization was carried out by using slide method (Giovannetti and Mosse, 1980).

Twenty-five grams of rhizosphere soil of each plant was assayed for spore count using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Estimation of spore density was carried out according to Gaur and Adholeya, (1994). Spores were transferred from filter paper to microscopic slides and mounted in polyvinyl alcohol lacto-glycerol (PVLG) (Koske and Tessier, 1983).

IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGAL SPECIES

Intact and crushed spores in polyvinyl alcohol lacto-glycerol (PVLG) with or without Melzer's reagent were examined under Leica compound microscope. Taxonomic identification of spores to species level was based on spore morphology, ornamentation, wall characteristics using various bibliographies (Schenck and Perez, 1990; Almeida and Schenck, 1990; Walker and Vestberg, 1998; Redecker, *et al.*, 2000b; Morton and Redecker, 2001; Schubler *et al.*, 2001) by matching original descriptions and those provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (<http://invam.caf.wvu.edu>). Spore colour was examined under Leica stereomicroscope using intact spores immersed in water. Voucher specimens

of arbuscular mycorrhizal fungi have been retained in the Botany Department, Goa University, Goa.

Spores in the rhizosphere soil were multiplied using *Eleusine coracana* (L.) Gaertn, *Lycopersicum esculentum* Mill., *Allium cepa* L. and *Coleus sp.* as host plants (**Plate- XXVI**). The spores isolated from the trap cultures were later used for confirming the identified spores recovered during the study period.

STATISTICAL ANALYSIS

The data on mycorrhizal colonization were arcsine square-root transformed and spore numbers were log-transformed prior to statistical analysis. Data on soil factors, mycorrhizal colonization and spore numbers were subjected to Analysis of Variance (ANOVA) to investigate their variations with disturbance and the age of the mine and the means were separated using Duncan's Multiple Range Test (DMRT).

DIVERSITY INDICES

The diversity of arbuscular mycorrhizal fungi in disturbed and undisturbed areas of both the sites were assayed based on diversity indices (Beena *et al.*, 2000).

Species richness is the number of species present in a particular site.

Simpson's index is an index of dominance since the maximum value is obtained when there is only one or few species.

Simpson's index: $D' = \sum (pi)^2$

Shannon Weiner index (H) is an index of diversity in which higher the value, the greater the diversity and the less the site is dominated by one or few species.

Shanon's index: $H' = \Sigma (pi \ln pi)$

Where, pi is the proportion of individuals that species i contribute to the total.

Species evenness indicates the distribution of the individuals within species designations.

The evenness was expressed by:

$$J' = \frac{H'}{H'_{max}}$$

Where H'_{max} is the maximum value of diversity for the number of species present.

Relative abundance and **Frequency of occurrence** of each arbuscular mycorrhizal fungal species was calculated using the formula given below:

$$\text{Frequency (\%)} = \frac{\text{Number of samples containing spores}}{\text{Total number of samples examined}} \times 100$$

$$\text{Relative abundance (\%)} = \frac{\text{Number of spores of each type}}{\text{Number of spores of all types}} \times 100$$

RESULTS

VEGETATION SURVEY

Results depicted in **Table-2** gives the detailed list of plant species reported from recently degraded mine, *i.e.*, Xelpi (10) and a well established mine *i.e.*, Bimbol (45) with age of the mines given in parenthesis.

The oldest mine *viz.*, Bimbol (45 year old) had a total of 115 plant species (both naturally occurring and cultivated) comprising of 59 herbs, 3 twiners, 18 shrubs and 35 trees species while, the recently degraded mine *viz.*, Xelpi (10 year old) showed only 29 plant species (both naturally occurring and cultivated) comprising of 20 herbs, 3 shrubs, and 6 trees species.

Natural undisturbed areas around both the mine sites *viz.*, Xelpi and Bimbol showed dense vegetation cover with a large number of trees, shrubs and herbaceous vegetation cover (**Plate- V**).

SOIL CHARACTERISTICS

Soil characteristics *viz.*, pH, EC, N, P, K, organic carbon, and organic matter of disturbed and undisturbed areas of both the sites are depicted in **Table- 7**, and between only the disturbed areas and, the undisturbed areas are depicted in **Table- 8**. The results indicated that soil nitrogen, organic carbon and organic matter increased with increase in the age of degraded mine site.

Among the various edaphic factors analyzed by employing ANOVA, significant differences ($P < 0.01$) were evident between disturbed and



PLATE - V

undisturbed areas of both the sites. Among the two disturbed sites viz., Xelpi and Bimbol, K did not exhibit significant difference. While, in the undisturbed areas of Xelpi and Bimbol the edaphic factors such as pH, EC and K did not exhibit significant difference (Table- 7 & 8).

XELPI DISTURBED AND UNDISTURBED AREAS

ROOT COLONIZATION, SPORE DENSITY AND SPECIES RICHNESS

Mycorrhizal colonization significantly varied between the disturbed and undisturbed areas of Xelpi mine site (Table- 9). Further, ANOVA on mycorrhizal colonization indicated significant variation in the colonization levels between species, areas, and species and area together (Table- 9).

The significant differences ($P < 0.01$) between species, areas, and species \times area for arbuscular mycorrhizal colonization indicates the difference in arbuscular mycorrhizal colonization with disturbance. Different levels of arbuscular mycorrhizal colonization were recorded at disturbed and undisturbed areas of Xelpi.

Disturbed areas at Xelpi site showed arbuscular mycorrhizal colonization ranging from 10% in *Terminalia crenulata* to 45% in *Anacardium occidentale* while, at the undisturbed area, arbuscular mycorrhizal colonization ranged from 40% in *Acacia auriculiformis* to 65% in *Anacardium occidentale*. Average root colonization was found to be higher in undisturbed area, while it was reported to be low in the disturbed area (Fig.- 7).

Table- 7. Soil characteristics of disturbed and undisturbed areas of Xelpi and Bimbol iron ore mine site.

| Soil factors | Xelpi | | diff. | Bimbol | | diff. |
|-----------------------------|-----------|-------------|-------|-----------|-------------|-------|
| | Disturbed | Undisturbed | | Disturbed | Undisturbed | |
| pH | 6.48 | 5.16 | ** | 6.12 | 5.12 | ** |
| EC | 0.09 | 0.56 | ** | 0.11 | 0.57 | ** |
| N (mg 100 g ⁻¹) | 46.00 | 436.0 | ** | 89.00 | 473.0 | ** |
| P (mg 100 g ⁻¹) | 145.2 | 198.0 | ** | 112.2 | 249.0 | ** |
| K (mg 100 g ⁻¹) | 32.6 | 155.0 | ** | 37.8 | 158.5 | ** |
| OC (%) | 0.02 | 1.93 | ** | 0.27 | 2.09 | ** |
| OM (%) | 0.03 | 3.33 | ** | 0.46 | 3.60 | ** |

Significant at: ** - P< 0.01.

EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter.

diff. – Difference.

Table- 8. Soil characteristics of disturbed areas and undisturbed areas of Xelpi and Bimbol iron ore mine site.

| Soil factors | Disturbed areas | | diff. | Undisturbed areas | | diff. |
|-----------------------------|-----------------|--------|-------|-------------------|--------|-------|
| | Xelpi | Bimbol | | Xelpi | Bimbol | |
| pH | 6.48 | 6.12 | ** | 5.16 | 5.12 | NS |
| EC | 0.09 | 0.11 | * | 0.56 | 0.57 | NS |
| N (mg 100 g ⁻¹) | 46.00 | 89.00 | ** | 436.0 | 473.0 | ** |
| P (mg 100 g ⁻¹) | 145.2 | 112.2 | ** | 198.0 | 249.0 | ** |
| K (mg 100 g ⁻¹) | 32.6 | 37.8 | NS | 155.0 | 158.5 | NS |
| OC (%) | 0.02 | 0.27 | ** | 1.93 | 2.09 | * |
| OM (%) | 0.03 | 0.46 | ** | 3.33 | 3.60 | * |

Significant at: * - P< 0.05, ** - P< 0.01, NS - Not significant.

EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter.

diff. – Difference.

Table- 9. Arbuscular mycorrhizal colonization in selected plant species from disturbed and undisturbed areas of Xelpi mine site.

| Plant species | Family | Root colonization (%) | | diff. |
|---|----------------|-----------------------|-------------|-------|
| | | Disturbed | Undisturbed | |
| <i>Acacia auriculiformis</i> A. Cunn. ex Benth. | Mimosaceae | 28.00 dc | 40.00 e | ** |
| <i>Acacia mangium</i> Willd. | Mimosaceae | 17.00 e | 55.00 b | ** |
| <i>Anacardium occidentale</i> L. | Anacardiaceae | 45.00 a | 65.00 a | ** |
| <i>Calotropis gigantea</i> (L.) R. Br. | Asclepiadaceae | 33.00 bc | 49.00 bcd | ** |
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | Combretaceae | 17.00 e | 53.00 bc | ** |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Asteraceae | 43.00 a | 44.00 cde | NS |
| <i>Ischaemum semisagittatum</i> Roxb. | Poaceae | 40.00 ab | 50.00 bcd | * |
| <i>Mimosa pudica</i> L. | Mimosaceae | 40.00 ab | 52.00 bc | ** |
| <i>Terminalia crenulata</i> Roth. | Combretaceae | 10.00 f | 42.00 de | ** |
| <i>Trema orientalis</i> (L.) Blume | Urticaceae | 23.00 de | 58.00 ab | ** |

| F Statistics | d. f. | F Stats |
|------------------------------------|-------|-----------|
| Plant species (P) | 9 | 19.52** |
| Disturbed and undisturbed area (A) | 1 | 319.96 ** |
| P x A | 9 | 13.07 ** |

Significant at: * - $P < 0.05$, ** - $P < 0.01$, NS - Not significant.

In a column, means followed by a common letter are not significantly different according to DMRT ($P < 0.05$).

diff- Difference; d. f.- Degrees of freedom.

There were significant differences in mean spore densities between species, areas (disturbed and undisturbed), and species x areas. A significant ($P < 0.01$) difference for arbuscular mycorrhizal fungal spore number between plant species, areas (disturbed and undisturbed) and, species x area indicate that spore density vary with species and disturbance (**Table- 10**).

The spore density at disturbed area of Xelpi ranged from 5 spores in *Ischaemum semisagittatum* to 14 spores in *Chromolaena odoratum* 25 g⁻¹ soil. Whereas, at the surrounding undisturbed area, spore density ranged from 33 spores 25 g⁻¹ in *Acacia mangium* to 76 spores 25 g⁻¹ soil in *Calotropis gigantea*. Average spore density recorded was higher in the undisturbed area as compared to the disturbed area (**Fig.- 8**).

Species richness of arbuscular mycorrhizal fungi recovered from individual plant species from disturbed and undisturbed areas of Xelpi site has been depicted in **Fig.- 9**. The number of arbuscular mycorrhizal fungal species recorded from the disturbed area of Xelpi ranged from 2 to 4 arbuscular mycorrhizal species per plant with maximum arbuscular mycorrhizal fungal species retrieved from *Anacardium occidentale* (4) and *Terminalia crenulata* (4) while, minimum arbuscular mycorrhizal fungal species were isolated from *Acacia auriculiformis* (2) whereas, at the undisturbed area, the number of arbuscular mycorrhizal fungal species ranged from 5 arbuscular mycorrhizal fungal species isolated from *Acacia auriculiformis* and *Chromolaena odoratum* to 7 arbuscular mycorrhizal fungal species isolated from *Mimosa pudica* with the number of arbuscular mycorrhizal fungal species given in paenthesis.

Table- 10. Spore density in selected plant species from disturbed and undisturbed areas of Xelpi mine site.

| Plant species | Family | Spore density 25 g ⁻¹ soil | | diff. |
|--|----------------|---------------------------------------|-------------|-------|
| | | Disturbed | Undisturbed | |
| <i>Acacia auriculiformis</i> A. Cunn. Ex Benth | Mimosaceae | 7.00 c | 39.00 ef | ** |
| <i>Acacia mangium</i> Willd. | Mimosaceae | 12.00 ab | 33.00 f | ** |
| <i>Anacardium occidentale</i> L. | Anacardiaceae | 11.00 ab | 63.00 abc | ** |
| <i>Calotropis gigantea</i> (L.) R. Br. | Asclepiadaceae | 9.00 bc | 76.00 a | ** |
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | Combretaceae | 11.00 ab | 68.00 ab | ** |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Asteraceae | 14.00 a | 50.00 cde | ** |
| <i>Ischaemum semisagittatum</i> Roxb. | Poaceae | 5.00 d | 45.00 de | ** |
| <i>Mimosa pudica</i> L. | Mimosaceae | 9.00 bc | 56.00 bcd | ** |
| <i>Terminalia crenulata</i> Roth. | Combretaceae | 12.00 ab | 55.00 bcd | ** |
| <i>Trema orientalis</i> (L.) Blume | Urticaceae | 10.00 bc | 73.00 ab | ** |

| F Statistics | d. f. | F Stats |
|------------------------------------|-------|------------|
| Plant species (P) | 9 | 9.78** |
| Disturbed and undisturbed area (A) | 1 | 1621.40 ** |
| P x A | 9 | 7.30 ** |

Significant at: ** - P < 0.01

In a column, means followed by a common letter are not significantly different according to DMRT (P < 0.05).

diff- Difference; d. f.- Degrees of freedom.

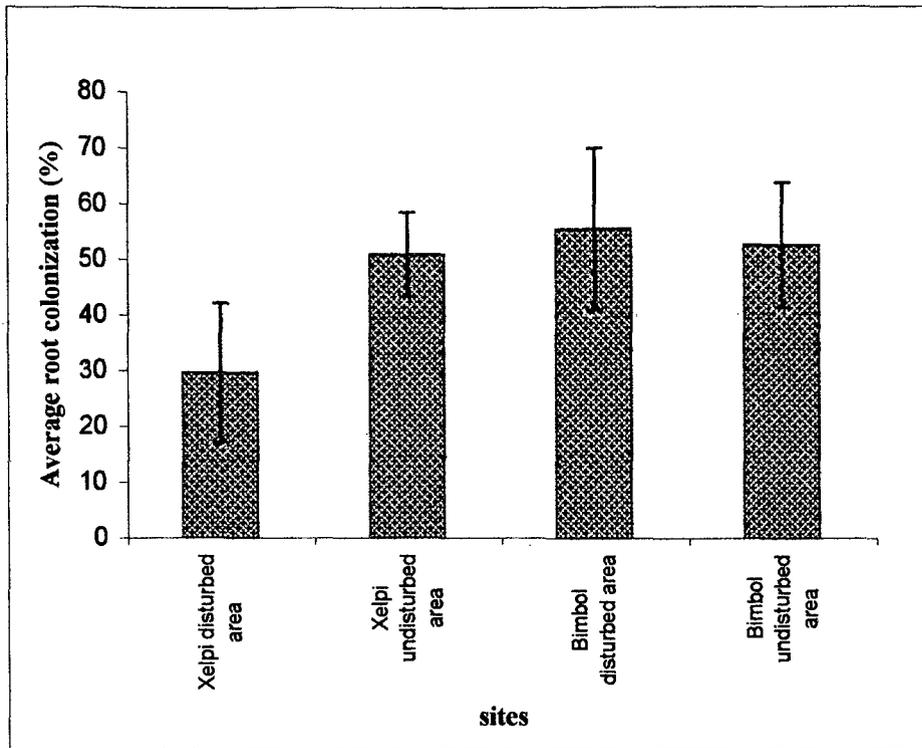


Fig.- 7. Average root colonization of arbuscular mycorrhizal fungi from disturbed and undisturbed areas of Xelpi and Bimbol iron ore mine site. Error bar indicates ± 1 SD

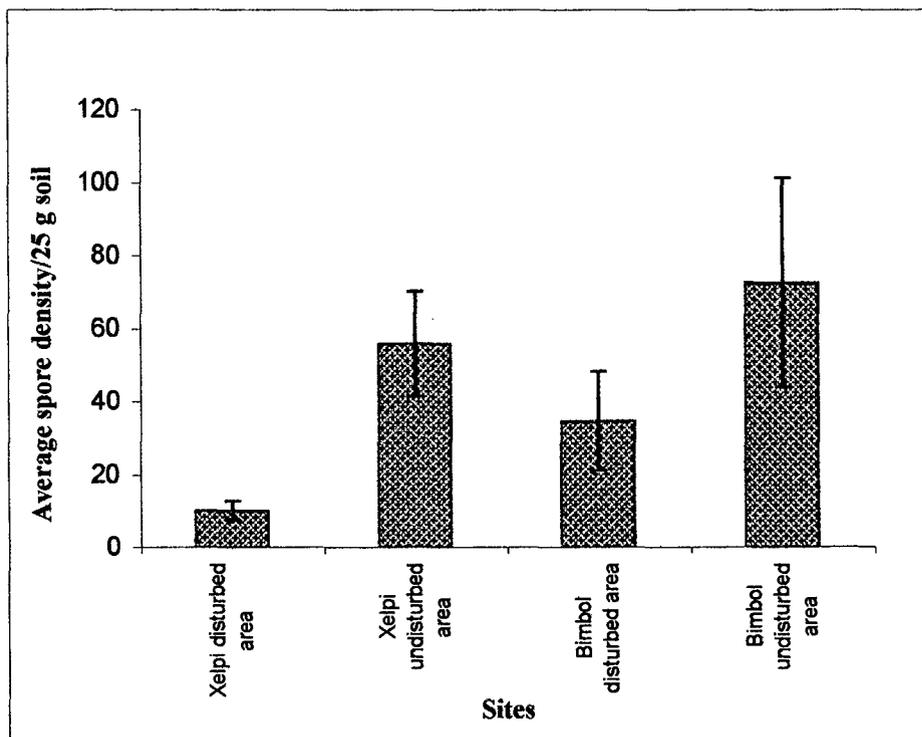


Fig.- 8. Average spore density of arbuscular mycorrhizal fungi from disturbed and undisturbed areas of Xelpi and Bimbol iron ore mine site. Error bar indicates ± 1 SD

OCCURRENCE OF ARBUSCULAR MYCORRHIZAL (AM) FUNGAL SPECIES

Frequency of occurrence and Relative abundance of arbuscular mycorrhizal fungal species in the rhizosphere of disturbed and undisturbed areas of Xelpi mine is depicted in **Table 13 & 14**. The study revealed the occurrence of 23 arbuscular mycorrhizal fungal species from undisturbed area, while only 12 arbuscular mycorrhizal fungal species were reported from disturbed area (**Fig- 11 a**). The arbuscular mycorrhizal fungal genera recorded during the study were *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*.

Pooled data indicated that of the 12 arbuscular mycorrhizal fungal species recovered from disturbed area of Xelpi mine, *Acaulospora spinosa* (70%) was the most dominating species followed by *Acaulospora scrobiculata* (60%), *Scutellospora pellucida* (30%), *Gigaspora margarita* (30%) and *Glomus fasciculatum* (30%). Of the 23 AM species reported from surrounding undisturbed area of Xelpi mine, *Glomus fasciculatum* (80%) was the most dominating species followed by *Acaulospora scrobiculata* (60%), *Acaulospora foveata* (50%), *Gigaspora margarita* (40%), *Glomus multicaule* (40%), *Glomus taiwanensis* (40%), *Glomus macrocarpum* (30%) and *Scutellospora pellucida* (30%). Of the several arbuscular mycorrhizal fungal species reported from the disturbed and undisturbed areas of Xelpi mine site, only 9 arbuscular mycorrhizal fungal species were common to both disturbed and undisturbed areas.

Table- 13. Frequency of occurrence of arbuscular mycorrhizal fungal species from disturbed and undisturbed areas of Xelpi mine site.

| Arbuscular mycorrhizal fungal species | Frequency (%) | |
|--|----------------|------------------|
| | Disturbed area | Undisturbed area |
| <i>Acaulospora foveata</i> Trappe & Janos | 10 | 50 |
| <i>Acaulospora laevis</i> Gerd. & Trappe | - | 10 |
| <i>Acaulospora mellea</i> Spain & Schenck | - | 10 |
| <i>Acaulospora scrobiculata</i> Trappe | 60 | 60 |
| <i>Acaulospora spinosa</i> Walker & Trappe | 70 | 20 |
| <i>Gigaspora margarita</i> Becker & Hall | 30 | 30 |
| <i>Glomus ambisporum</i> Smith & Schenck | - | 10 |
| <i>Glomus claroideum</i> Schenck & Smith emend Walker & Vestberg. | - | 10 |
| <i>Glomus clavisporum</i> (Trappe) Almeida & Schenck | - | 30 |
| <i>Glomus coremioides</i> (Berk. & Broome) Redecker et Morton | - | 10 |
| <i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker and Koske, | 30 | 80 |
| <i>Glomus geosporum</i> (Nicol. & Gerd.) Walker | 10 | 10 |
| <i>Glomus globiferum</i> Koske & Walker | - | 20 |
| <i>Glomus glomerulatum</i> Sieverding | - | 10 |
| <i>Glomus macrocarpum</i> Tul. & Tul. | 20 | 40 |
| <i>Glomus microaggregatum</i> Koske, Gemma & Olexia | 10 | - |
| <i>Glomus mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe | - | 30 |
| <i>Glomus multicaule</i> Gerdemann & Bakshi | - | 40 |
| <i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck | - | 20 |
| <i>Glomus sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck | - | 10 |
| <i>Glomus taiwanensis</i> (Wu & Chen) Almeida & Schenck | 10 | 40 |
| <i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders | 20 | - |
| <i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders | 30 | 30 |
| <i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders | - | 10 |
| <i>Scutellospora verrucosa</i> (Koske & Walker) Walker & Sanders | - | 10 |
| <i>Scutellospora weresubiae</i> Koske & Walker | 10 | - |
| Total No. of samples assessed | 30 | 30 |

Table- 14. Relative abundance of arbuscular mycorrhizal fungal species from disturbed and undisturbed areas of Xelipi mine site.

| Arbuscular mycorrhizal fungal species | Relative abundance (%) | |
|--|------------------------|------------------|
| | Disturbed area | Undisturbed area |
| <i>Acaulospora foveata</i> Trappe & Janos | 2 | 5.9 |
| <i>Acaulospora laevis</i> Gerd. & Trappe | - | 0.36 |
| <i>Acaulospora mellea</i> Spain & Schenck | - | 0.36 |
| <i>Acaulospora scrobiculata</i> Trappe | 34 | 12.72 |
| <i>Acaulospora spinosa</i> Walker & Trappe | 23 | 2.33 |
| <i>Gigaspora margarita</i> Becker & Hall | 4 | 4.45 |
| <i>Glomus ambisporum</i> Smith & Schenck | - | 0.90 |
| <i>Glomus claroideum</i> Schenck & Smith emend Walker & Vestberg. | - | 0.90 |
| <i>Glomus clavisporum</i> (Trappe) Almeida & Schenck | - | 5.91 |
| <i>Glomus coremioides</i> (Berk. & Broome) Redecker et Morton | - | 0.72 |
| <i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker and Koske, | 10 | 25.98 |
| <i>Glomus geosporum</i> (Nicol. & Gerd.) Walker | 2 | 1.25 |
| <i>Glomus globiferum</i> Koske & Walker | - | 1.79 |
| <i>Glomus glomerulatum</i> Sieverding | - | 0.90 |
| <i>Glomus macrocarpum</i> Tul. & Tul. | 9 | 4.12 |
| <i>Glomus microaggregatum</i> Koske, Gemma & Olexia | 1 | - |
| <i>Glomus mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe | - | 3.58 |
| <i>Glomus multicaule</i> Gerdemann & Bakshi | - | 9.13 |
| <i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck | - | 2.69 |
| <i>Glomus sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck | - | 1.61 |
| <i>Glomus taiwanensis</i> (Wu & Chen) Almeida & Schenck | 2 | 9.32 |
| <i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders | 3 | - |
| <i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders | 8 | 4.12 |
| <i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders | - | 0.18 |
| <i>Scutellospora verrucosa</i> (Koske & Walker) Walker & Sanders | - | 0.72 |
| <i>Scutellospora weresubiae</i> Koske & Walker | 2 | - |
| Total number of arbuscular mycorrhizal spores | 300 | 1674 |

DIVERSITY INDICES

Species richness was greater on the surrounding undisturbed area as compared to the disturbed area of the site. Shannon's index of diversity was greater on undisturbed area (4.184) while, it was very low at disturbed area (2.808) of Xelpi site. Simpson's index of dominance was 0.197 in disturbed while it was 0.1167 in the surrounding undisturbed area of Xelpi. Shannon's evenness was 0.7832 in disturbed and 0.9249 in the surrounding undisturbed area. Thus, diversity index was greater on undisturbed area as compared to the disturbed area of the Xelpi iron ore mine site (Fig.- 11 a-d).

BIMBOL DISTURBED AND UNDISTURBED AREAS

COLONIZATION, SPORE DENSITY AND SPECIES RICHNESS

Mycorrhizal colonization significantly varied between the disturbed and undisturbed areas of Bimbol mine site (Table- 11). Further, ANOVA on mycorrhizal colonization indicated significant variation in the colonization levels between species, areas, and species and area together (Table- 11).

The significant differences ($P < 0.01$) between species, areas, and species x area for arbuscular mycorrhizal fungal colonization indicates the difference in arbuscular mycorrhizal colonization with disturbance. Different levels of arbuscular mycorrhizal colonization were recorded at disturbed and undisturbed areas of Bimbol.

Disturbed area at Bimbol mine showed arbuscular mycorrhizal fungal colonization ranging from 18% in *Calotropis gigantea* to 67% in *Terminalia*

paniculata. While, at the undisturbed area of the site, arbuscular mycorrhizal colonization ranged from 36% in *Calotropis gigantea* to 69% in *Calycopteris floribunda*. Average arbuscular mycorrhizal fungal colonization recorded for disturbed area was found to be 55.4 percent while, it was found to be 52.5 percent in the undisturbed area (Fig.- 7).

There were significant differences in mean spore densities between species, areas (disturbed and undisturbed), and species x areas. A significant ($P < 0.01$) difference for arbuscular mycorrhizal fungal spore number between plant species, areas (disturbed and undisturbed) and, species x area indicate that spore density vary with species and disturbance (Table- 12).

The spore density at the disturbed area of Bimbol site ranged from 14 spores 25 g^{-1} soil in *Chromolaena odoratum* to 59 spores 25 g^{-1} soil in *Calotropis gigantea*. Whereas at the surrounding undisturbed area of the site spore density ranged from 40 spores in *Delonix regia* to 130 spores in *Micrøcos paniculata* 25 g^{-1} soil. Average spore density recorded from disturbed area was much lower as compared to the undisturbed area (Fig.- 8).

Species richness of arbuscular mycorrhizal fungi recovered from individual plant species from disturbed and undisturbed areas of Bimbol site has been depicted in Fig.- 10. The number of arbuscular mycorrhizal fungal species recorded from disturbed area of Bimbol site ranged from 4 in *Terminalia crenulata* to 11 in *Terminalia paniculata*. Whereas at the undisturbed area, the number of arbuscular mycorrhizal fungal species ranged

Table- 11. Arbuscular mycorrhizal colonization in selected plant species from disturbed and undisturbed areas of Bimbol mine site.

| Plant species | Family | Root colonization (%) | | diff. |
|--|-----------------|-----------------------|-------------|-------|
| | | Disturbed | Undisturbed | |
| <i>Calotropis gigantea</i> (L.) R. Br. | Asclepiadaceae | 18.00 d | 36.00 f | ** |
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | Combretaceae | 64.00 a | 69.00 a | NS |
| <i>Careya arborea</i> Roxb.. | Lecythidaceae | 60.00 ab | 44.00 def | ** |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Asteraceae | 55.00 b | 40.00 ef | ** |
| <i>Delonix regia</i> (Hook.) Raf. | Caesalpiniaceae | 45.00 c | 52.00 cd | NS |
| <i>Microcos paniculata</i> L. | Tiliaceae | 60.00 ab | 67.00 ab | NS |
| <i>Mimosa pudica</i> L. | Mimosaceae | 60.00 ab | 50.00 d | * |
| <i>Randia rugulosa</i> (Thw.) Hook.f. | Rubiaceae | 60.00 ab | 60.00 bc | NS |
| <i>Terminalia paniculata</i> Roth. | Combretaceae | 67.00 a | 47.00 de | ** |
| <i>Terminalia crenulata</i> Roth. | Combretaceae | 65.00 a | 60.00 bc | NS |

| F Statistics | d. f. | F Stats |
|------------------------------------|-------|----------|
| Plant species (P) | 9 | 34.66 ** |
| Disturbed and undisturbed area (A) | 1 | 4.29 * |
| P x A | 9 | 10.32 ** |

Significant at * - $P < 0.05$, ** - $P < 0.01$, NS - Not significant.

In a column, means followed by a common letter are not significantly different according to DMRT ($P < 0.05$).

diff- Difference; d. f.- Degrees of freedom.

Table- 12. Spore density in selected plant species from disturbed and undisturbed areas of Bimbol mine site.

| Plant species | Family | Spore 25 g ⁻¹ soil | | diff. |
|--|-----------------|-------------------------------|-------------|-------|
| | | Disturbed | Undisturbed | |
| <i>Calotropis gigantea</i> (L.) R. Br. | Asclepiadaceae | 59.00 a | 80.00 bc | ** |
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | Combretaceae | 36.00 c | 94.00 b | ** |
| <i>Careya arborea</i> Roxb. | Lecythidaceae | 39.00 c | 53.00 d | ** |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Asteraceae | 14.00 g | 62.00 d | ** |
| <i>Delonix regia</i> (Hook.) Raf. | Caesalpiniaceae | 25.00 e | 40.00 e | ** |
| <i>Microcos paniculata</i> L. | Tiliaceae | 40.00 c | 130.00 a | ** |
| <i>Mimosa pudica</i> L. | Mimosaceae | 30.00 d | 75.00 c | ** |
| <i>Randia rugulosa</i> (Thw.) Hook.f. | Rubiaceae | 38.00 c | 124.00 a | ** |
| <i>Terminalia paniculata</i> Roth. | Combretaceae | 48.00 b | 91.00 b | ** |
| <i>Terminalia crenulata</i> Roth. | Combretaceae | 18.00f | 94.00 b | ** |

| F Statistics | d. f. | F Stats |
|------------------------------------|-------|------------|
| Plant species (P) | 9 | 66.43 ** |
| Disturbed and undisturbed area (A) | 1 | 1270.44 ** |
| P x A | 9 | 34.37 ** |

Significant at ** - P < 0.01

In a column, means followed by a common letter are not significantly different according to DMRT (P < 0.05).

diff- Difference; d. f.- Degrees of freedom.

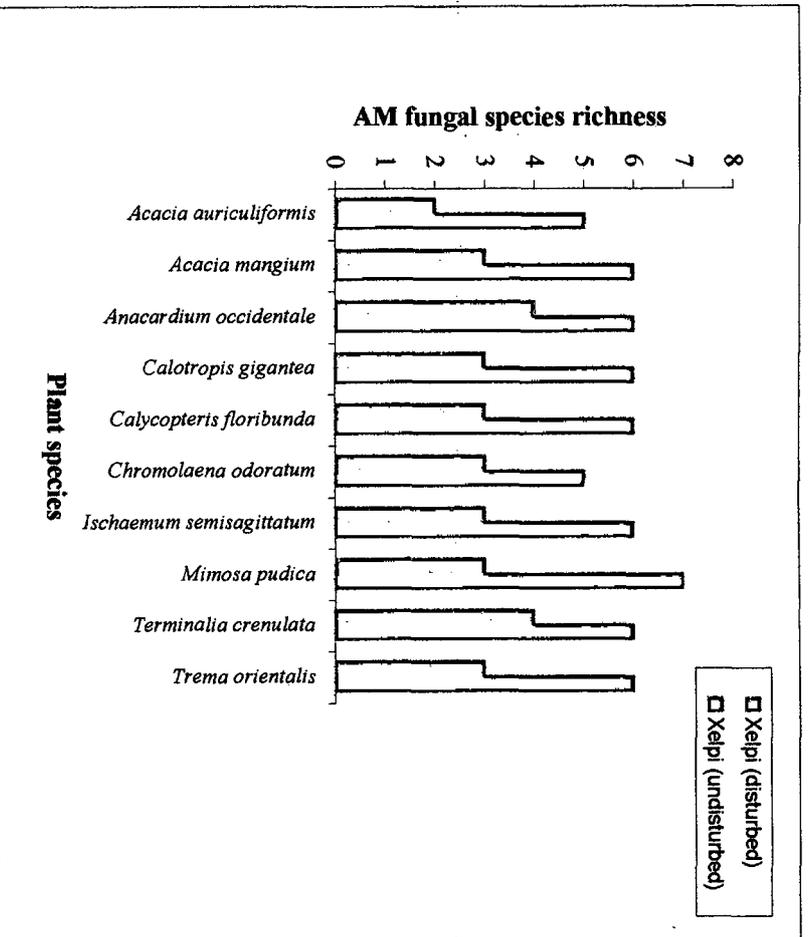


Fig.- 9. Arbuscular mycorrhizal (AM) fungal species richness from individual plant species at disturbed and undisturbed areas of Xelipi iron ore mine site.

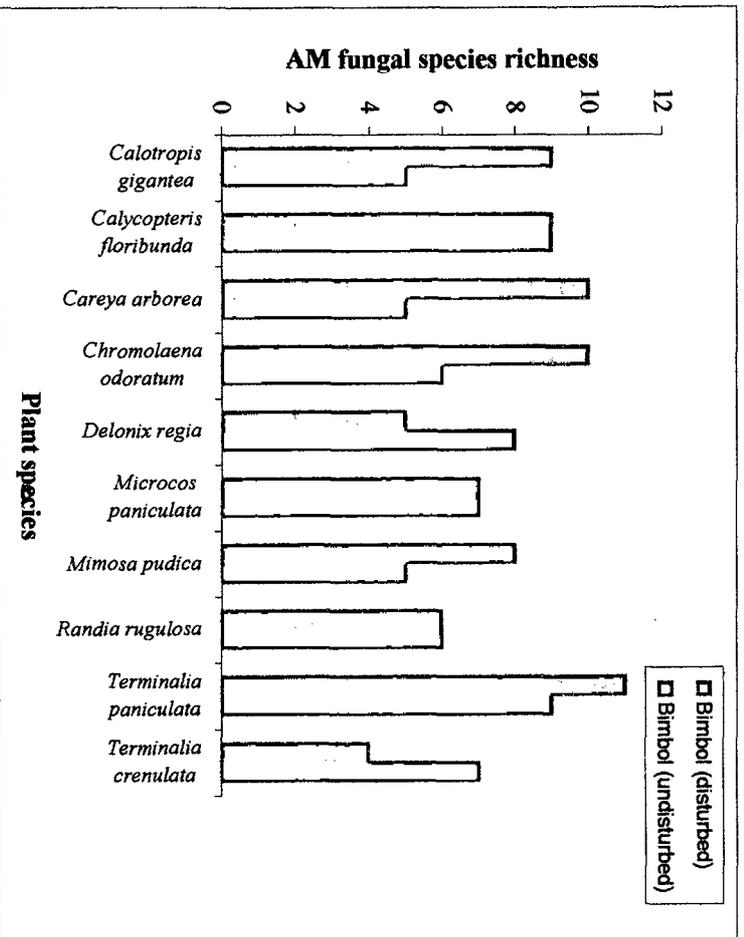


Fig. - 10. Arbuscular mycorrhizal (AM) fungal species richness from individual plant species at disturbed and undisturbed areas of Bimbol iron ore mine site.

from 5 to 9 arbuscular mycorrhizal fungal species per plant with maximum arbuscular mycorrhizal species reported from *Calycopteris floribunda* (9) and *Terminalia paniculata* (9) while, minimum arbuscular mycorrhizal species were recovered from *Calotropis gigantea* (5), *Careya arborea* (5) and *Mimosa pudica* (5) with the number of arbuscular mycorrhizal species given in parenthesis.

OCCURRENCE OF ARBUSCULAR MYCORRHIZAL (AM) FUNGAL SPECIES

Frequency of occurrence and Relative abundance of arbuscular mycorrhizal fungal species in the rhizosphere of disturbed and undisturbed areas of Bimbol mine is depicted in **Table- 15 & 16**. The study revealed the occurrence of 23 arbuscular mycorrhizal fungal species from undisturbed area, while, 31 arbuscular mycorrhizal fungal species were reported from disturbed area (**Fig- 11 a**). The arbuscular mycorrhizal fungal genera recorded during the study were *Acaulospora*, *Archaeospora*, *Gigaspora*, *Glomus* and *Scutellospora*.

Pooled data indicated that of the 31 arbuscular mycorrhizal fungal species reported from disturbed area of Bimbol site *Glomus macrocarpum* (90%) was the most dominant followed by *Acaulospora scrobiculata* (60%), *Glomus geosporum* (50%), *Glomus rubiforme* (50%), *Gigaspora margarita* (40%), *Glomus fasciculatum* (40%), *Acaulospora spinosa* (40%), *Glomus globiferum* (40%) and *Glomus sinuosum* (40%). Of the 23 AM fungal species reported from surrounding undisturbed area of Bimbol site *Glomus fasciculatum* (100%) was the most dominant followed by *Glomus*

Table- 15. Frequency of occurrence of arbuscular mycorrhizal fungal species from disturbed and undisturbed areas of Bimbol mine site.

| Arbuscular mycorrhizal fungal species | Frequency (%) | |
|--|----------------|------------------|
| | Disturbed area | Undisturbed area |
| <i>Acaulospora foveata</i> Trappe & Janos | 10 | 50 |
| <i>Acaulospora laevis</i> Gerd. & Trappe | 30 | 20 |
| <i>Acaulospora mellea</i> Spain & Schenck | 10 | 10 |
| <i>Acaulospora morrowiae</i> Spain & Schenck | 10 | - |
| <i>Acaulospora rhemii</i> Sieverding & Toro | 20 | - |
| <i>Acaulospora scrobiculata</i> Trappe | 60 | 50 |
| <i>Acaulospora spinosa</i> Walker & Trappe | 40 | - |
| <i>Archaeospora leptoticha</i> (Schenck & Smith) Morton & Rdecker | - | 10 |
| <i>Gigaspora margarita</i> Becker & Hall | 40 | 30 |
| <i>Glomus aggregatum</i> Schenck & Smith emend. Koske | 10 | - |
| <i>Glomus ambisporum</i> Smith & Schenck | - | 30 |
| <i>Glomus claroideum</i> Schenck & Smith emend Walker & Vestberg. | 20 | 10 |
| <i>Glomus clavisporum</i> (Trappe) Almeida & Schenck | - | 40 |
| <i>Glomus constrictum</i> Trappe | 40 | 10 |
| <i>Glomus corenioides</i> (Berk. & Broome) Redecker et Morton | 10 | 10 |
| <i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker and Koske, | 40 | 100 |
| <i>Glomus formosanum</i> Wu & Chen | 10 | - |
| <i>Glomus geosporum</i> (Nicol. & Gerd.) Walker | 50 | 20 |
| <i>Glomus globiferum</i> Koske & Walker | 40 | 20 |
| <i>Glomus glomerulatum</i> Sieverding | 10 | 20 |
| <i>Glomus hoi</i> Berch & Trappe | 10 | - |
| <i>Glomus macrocarpum</i> Tul. & Tul. | 90 | 80 |
| <i>Glomus microaggregatum</i> Koske, Gemma & Olexia | 10 | - |
| <i>Glomus monosporum</i> Gerdemann & Trappe | 10 | - |
| <i>Glomus mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe | - | 20 |
| <i>Glomus multicaule</i> Gerdemann & Bakshi | 20 | 60 |
| <i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck | 50 | 10 |
| <i>Glomus sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck | 40 | 10 |
| <i>Glomus taiwanensis</i> (Wu & Chen) Almeida & Schenck | 20 | 40 |
| <i>Scutellospora calospora</i> (Nicol. & Gerd.) Walker & Sanders | 10 | - |

Cont.

| | | |
|--|----|----|
| <i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders | 20 | - |
| <i>Scutellospora nigra</i> (Redhead) Walker & Sanders | 10 | - |
| <i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders | 20 | 10 |
| <i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders | 10 | - |
| <i>Scutellospora verrucosa</i> (Koske & Walker) Walker & Sanders | - | 10 |
| <i>Scutellospora weresubiae</i> Koske & Walker | 20 | - |
| Total No. of samples assessed | 30 | 30 |

Table- 16. Relative abundance of arbuscular mycorrhizal fungal species from disturbed and undisturbed areas of Bimbol mine site.

| Arbuscular mycorrhizal fungal species | Relative abundance (%) | |
|--|------------------------|------------------|
| | Disturbed area | Undisturbed area |
| <i>Acaulospora foveata</i> Trappe & Janos | 0.29 | 5.93 |
| <i>Acaulospora laevis</i> Gerd. & Trappe | 1.15 | 0.71 |
| <i>Acaulospora mellea</i> Spain & Schenck | 0.29 | 0.12 |
| <i>Acaulospora morrowiae</i> Spain & Schenck | 0.29 | - |
| <i>Acaulospora rhemii</i> Sieverding & Toro | 0.58 | - |
| <i>Acaulospora scrobiculata</i> Trappe | 17.29 | 9.02 |
| <i>Acaulospora spinosa</i> Walker & Trappe | 8.65 | - |
| <i>Archaeospora leptoticha</i> (Schenck & Smith) Morton & Rdecker | - | 0.12 |
| <i>Gigaspora margarita</i> Becker & Hall | 4.90 | 1.07 |
| <i>Glomus aggregatum</i> Schenck & Smith emend. Koske | 0.29 | - |
| <i>Glomus ambisporum</i> Smith & Schenck | - | 1.78 |
| <i>Glomus claroideum</i> Schenck & Smith emend Walker & Vestberg. | 1.44 | 0.83 |
| <i>Glomus clavisorum</i> (Trappe) Almeida & Schenck | - | 4.03 |
| <i>Glomus constrictum</i> Trappe | 4.61 | 0.36 |
| <i>Glomus coremioides</i> (Berk. & Broome) Redecker et Morton | 0.58 | 0.24 |
| <i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker and Koske, | 5.76 | 37.96 |
| <i>Glomus formosanum</i> Wu & Chen | 0.58 | - |
| <i>Glomus geosporum</i> (Nicol. & Gerd.) Walker | 3.75 | 0.59 |
| <i>Glomus globiferum</i> Koske & Walker | 3.46 | 0.83 |
| <i>Glomus glomerulatum</i> Sieverding | 0.29 | 1.66 |
| <i>Glomus hoi</i> Berch & Trappe | 0.29 | - |
| <i>Glomus macrocarpum</i> Tul. & Tul. | 27.37 | 5.81 |
| <i>Glomus microaggregatum</i> Koske, Gemma & Olexia | 0.29 | - |
| <i>Glomus monosporum</i> Gerdemann & Trappe | 1.44 | - |
| <i>Glomus mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe | - | 2.37 |
| <i>Glomus multicaule</i> Gerdemann & Bakshi | 0.86 | 11.74 |
| <i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck | 2.31 | 0.95 |
| <i>Glomus sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck | 4.03 | 0.71 |
| <i>Glomus taiwanensis</i> (Wu & Chen) Almeida & Schenck | 1.73 | 12.21 |
| <i>Scutellospora calospora</i> (Nicol. & Gerd.) Walker & Sanders | 0.58 | - |

Cont

| | | |
|--|------|------|
| <i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders | 2.02 | - |
| <i>Scutellospora nigra</i> (Redhead) Walker & Sanders | 0.29 | - |
| <i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders | 2.88 | 0.59 |
| <i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders | 0.29 | - |
| <i>Scutellospora verrucosa</i> (Koske & Walker) Walker & Sanders | - | 0.36 |
| <i>Scutellospora weresubiae</i> Koske & Walker | 1.44 | - |
| Total No. of arbuscular mycorrhizal spores | 1041 | 2178 |

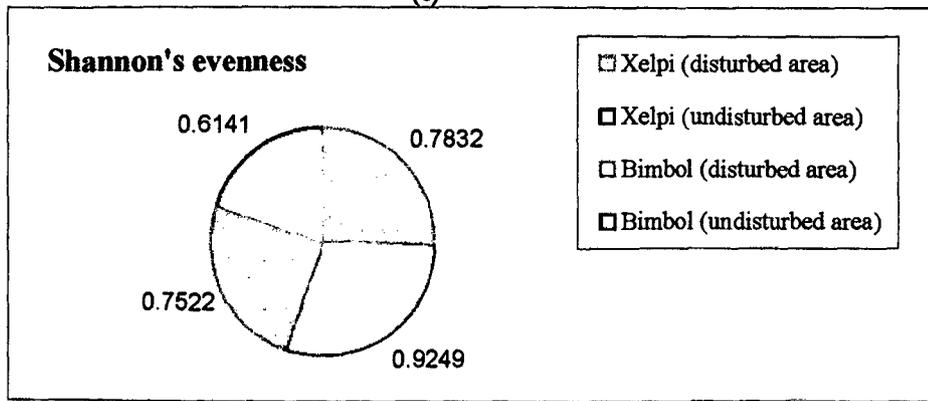
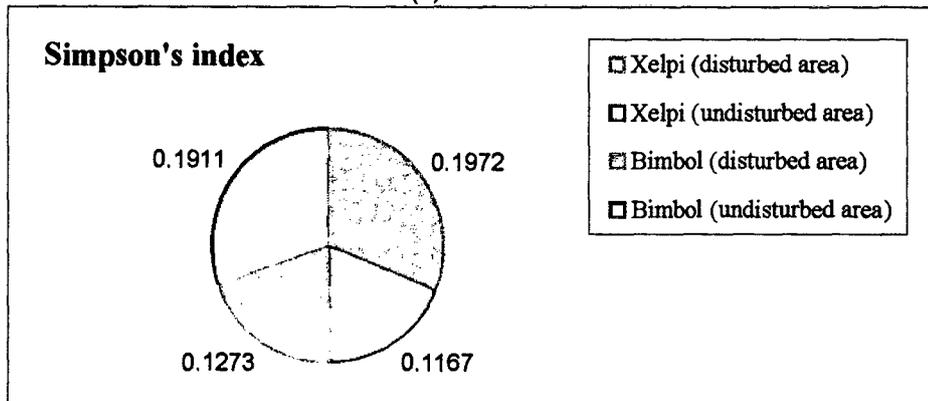
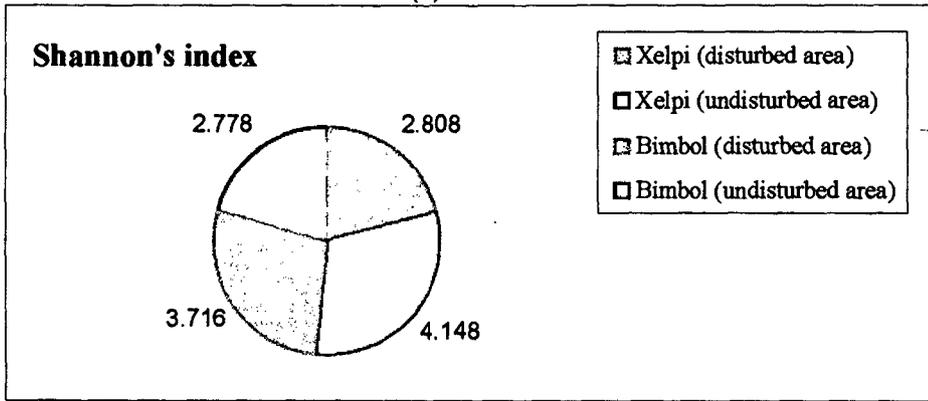
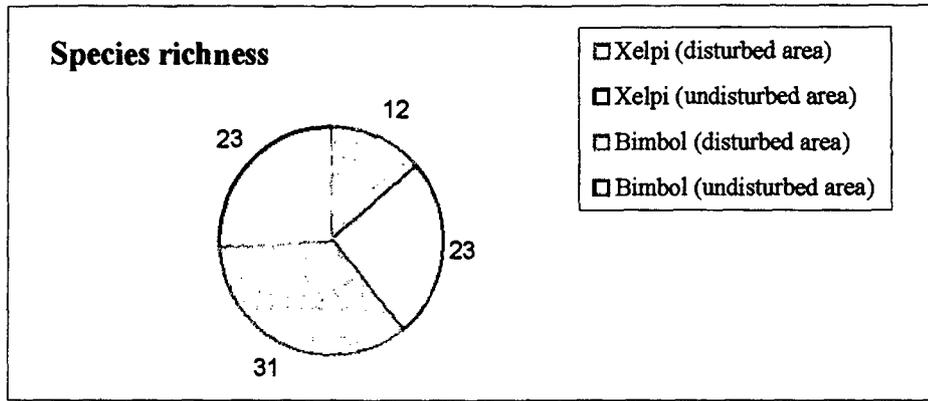


Fig. - 11. Species richness (a) and diversity indices viz., (b) Shannon's (c) Simpson's and (d) Shannon's evenness at disturbed and undisturbed areas of Xelpi and Bimbol iron ore mine sites.

macrocarpum (80%), *Glomus multicaule* (60%), *Acaulospora foveata* (50%), *Acaulospora scrobiculata* (50%), *Glomus clavisporum* (40%) and *Glomus taiwanensis* (40%). Of the several arbuscular mycorrhizal fungal species reported from the disturbed and undisturbed areas of Bimbol site, 19 arbuscular mycorrhizal fungal species were common to both disturbed and undisturbed areas.

DIVERSITY INDICES

Species richness was greater on the disturbed area as compared to the surrounding undisturbed area of the site. Shannon's index of diversity was greater in disturbed area (3.716) and it was less in the surrounding undisturbed area (2.778). Simpson's index of dominance was 0.1273 in disturbed area while it was 0.1911 in the surrounding undisturbed area of the site. Shannon's evenness was 0.7522 in disturbed area and 0.6141 in the undisturbed area. Thus, diversity index was greater on disturbed area as compared to the undisturbed area of Bimbol site (Fig.- 11 a-d).

DISCUSSION

Survey of vegetation revealed increased vegetation cover in terms of species diversity with the elapsed time. These results are in agreement with the earlier studies by Benerjee *et al.*, (1999) who indicated that improvement in soil condition promotes plant succession. Similarly, Schafer and Nielsen, (1979) observed that improvement of soil condition promoted plant succession, microbial activity and sustained stable, productive plant community in coal mine spoil of 1- 50 years old.

There were significant differences in soil properties between disturbed and undisturbed areas of both the sites as well as within the disturbed sites and undisturbed sites. When disturbed sites with sparse vegetation were compared with older disturbed sites with fairly established vegetation cover, increase in soil N, organic carbon and organic matter content was observed in the later. However, this increase was much less than the surrounding undisturbed area even after 45 year of succession. Similar observations have been recorded earlier by Brundrett *et al.*, (1996b) in his studies on bioassay of arbuscular mycorrhizal fungal propagules from disturbed and natural habitats in soils from Tropical Australia. Thus, an increase in the levels of both organic matter and soil nitrogen stimulates plant growth (Francis and Thornes, 1990; Morgan *et al.*, 1990; Vallejo *et al.*, 1999).

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 recently degraded mine as compared to the well established mine 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, inspite 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; Parke *et al.*, 1983; Jasper *et al.*, 1989a, b; 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, some 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). Inoculum of arbuscular mycorrhizal fungi was found to be substantially more abundant in well established mine than in the adjacent climax community. This enhanced mycorrhizal fungal activity may have resulted from greater plant community. According to Collins *et al.*, (1991 & 1992) some species of arbuscular mycorrhizal fungi are more likely to occur in disturbed habitats than others.

When the recently degraded mine site with sparse vegetation was compared with older disturbed site with fairly established vegetation cover, the arbuscular mycorrhizal fungal spore density and diversity was found to be much higher in older disturbed site. Similar observation has been recorded earlier by Gould and Hendrix (1998) who stated that newly reclaimed site contained only few arbuscular mycorrhizal fungal spores whereas, the site that had been reclaimed for several years had much higher population of arbuscular mycorrhizal fungal spores and more species were usually recovered. Sylvia and Will, (1988) in their studies reported that vacant

replenishment sand contained fewer microorganisms than did the sand from root zones of plants in established dunes. Thus, it can be concluded that changes in soil condition will modify the dominance of particular arbuscular mycorrhizal fungal species during mycorrhizal formation. Therefore, studies on the effect of disturbance will play a useful role in assessing likely changes in fungal dominance during colonization of roots. The results in the present study support Connell's hypothesis (1978) which states that the diversity of arbuscular mycorrhizal species is minimal at maximal level of disturbance. Diaz and Honrubia, (1993) in their studies to measure mycorrhizal population in five soils disturbed by mining activity found that the most degraded site showed least spore diversity as compared to the site which had more advanced vegetation. They attributed this difference to soil properties, degree of disturbance and vegetation.

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 while, the established mines possess high arbuscular mycorrhizal fungal diversity as well as plant species diversity and vegetation cover which could possibly be due to improvement in soil conditions. Hence, it can be argued that in a stable ecosystem the increased arbuscular mycorrhizal fungal diversity influences the plant biodiversity.

CHAPTER-IV

**VARIATIONS IN ARBUSCULAR MYCORRHIZAL
(AM) FUNGAL DIVERSITY IN IRON ORE MINES
OF VARYING AGES.**

INTRODUCTION

Successional changes in plant species occurs during ecosystems recovery from disturbance or establishment on new substrates (Grime, 1979; Barbour *et al.*, 1987). Mycorrhizal propagules can be severely influenced by damage to vegetation and soil resulting from natural processes or human interventions. These destructive processes includes intense fire (Dhillon *et al.*, 1988; Klopatek *et al.*, 1988; Wicklow-Howard, 1989) and exposure of subsurface soil by erosion (Day *et al.*, 1987; Habte, 1989).

Mycorrhizae might influence succession by regulating competition, since competition between plants of different successional stages has been cited as a mechanism of succession (Keever, 1950; Connell and Slatyer, 1977; Allen and Allen, 1984), where mycorrhizae influences the competitive interaction between species of different stages of succession, and their abundance may also influence the rate of succession.

The mutualistic relationship of arbuscular mycorrhizal fungi has supported the successional establishment in widely diversified environment *viz.*, agricultural soils (Abbott and Robson, 1978), mine sites (Jasper *et al.*, 1989 a, b), coal wastes (Daft and Hacskeylo, 1976 and 1977; Nicolson and Johnston, 1979), desert soils (Khudairi, 1969; Miller, 1979). Thus, they play a key role in sustainable conservation of tropical gene pool and diversity. Furthermore, through years of succession, evolution, selection, and co-existence, arbuscular mycorrhizal fungi has helped in refining the soil quality, texture, structure, fertility and compatibility to suit the indigenous plant

species. The microbial component especially arbuscular mycorrhizal fungi of the plant rhizosphere endows major task in determining plant diversity (Van der Heijden *et al.*, 1998) and helps in stabilizing highly complex diversity regime of the tropical forests.

Surface mining results in the dumping of huge amount of infertile overburden on unmined land causing inversion of natural soil substratum sequences, disrupting plant communities and their associated mycorrhizal endophytes leaving little viable inoculum capable of inhabiting mycorrhizal colonization in plants colonizing the affected area (Miller, 1979; Reeves *et al.*, 1979; Allen and Allen, 1980). Due to very low organic matter content and lack of vegetation, the mine spoils are nutritionally and microbiologically impoverished (Vissar *et al.*, 1979) and natural recovery of unamended spoils is a very slow process. Marshall (1977) emphasized the positive effect of vegetation on soil formation and organic matter accumulation. During ecosystem development, observable changes in terms of biomass and species richness takes place. Schafer and Neilsen (1979) noted plant succession due to improvement of soil status.

Studies on arbuscular mycorrhizal fungi from mine spoils have been documented in studies by Daft and Nicolson (1974), Daft and Hacskeylo, (1976); Khan, (1978); Miller, (1979); Reeves *et al.*, (1979); Allen and Allen (1980). These investigations have stressed the importance of arbuscular mycorrhizal relationships in allowing successful recolonization and growth of herbaceous plants on mine spoils.

The rate at which plant develops mycorrhizae naturally in habitat disturbed through mining activity will depend in part on the inoculum diversity and species composition of mycorrhizal fungi in the mine spoils following revegetation. The inoculum potential and mycorrhizal species composition of these disturbed habitats is governed by reclamation practices (Allen and Allen, 1980; Rives *et al.*, 1980), plant species composition of the reclaimed site (Schramm, 1966; Miller, 1979; Reeves *et al.*, 1979), chemical and physical characteristics of the spoil (Marx, 1975) and the rate of recolonization by endophytes from undisturbed areas.

If reclamation programme are to allow for the rapid establishment of mycorrhizae in these highly disturbed habitats, than more information is needed on the arbuscular mycorrhizal fungal species diversity that occurs at such sites. Reports on successional establishment with respect to arbuscular mycorrhizal association form mine wastelands are scarce (Daft and HacsKaylo, 1976; Nicolson and Johnston, 1979). Thus, the present investigation is an attempt to study the colonization, species richness and diversity of arbuscular mycorrhizal fungi associated with some dominant and commonly occurring plant species from iron ore mines of varying ages.

MATERIALS AND METHODS

STUDY SITE

Study was conducted at four iron ore mine sites viz., Sanquelim (50), Sonshi (35), Codli (30) and Dadiovaril Sodo "Xelpi" (10) of varying age groups, with age of the mines given in parenthesis.

VEGETATION SURVEY

Survey of vegetation was conducted in early monsoon (July –August) at four iron ore mines viz., Sanquelim (50), Sonshi (35), Codli (30) and Xelpi (10) with age of the mines given in parenthesis. Plants were identified using floras (Rao, 1985 & 1986; Matthew, 1991; Mohanan and Henry, 1994; Naithani *et al.*, 1997).

SOIL ANALYSIS

For soil analysis, samples were collected from a depth of 0-25 cm from five different locations from each mine and were brought to the laboratory in polyethylene bags. Samples from each mine site were passed through 2mm sieve to remove the larger soil particles and for each site, samples collected from five different locations were processed separately.

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 determined by flame photometric method (Jackson, 1971). Organic carbon and organic matter were detected by Walkley and Black's rapid titration method (Jackson, 1971).

SAMPLING

Ten angiospermic plant species belonging to 8 families common to all the four sites were taken up during the study. Roots and rhizosphere soils were

sampled individually for each plant species from all the four mine sites during the dry period (March-April). For shrubs and tree species, the roots were dug and traced back to plant which ensured that the roots belonged to the intended plant species. Samples of herbs were usually made by uprooting the entire plant. Plants were uprooted along with the rhizosphere soil and brought to the laboratory in the polyethylene bags. Plants were shaken to remove the adhering soil particles. Feeder roots were cut into 1cm bits and were processed for further studies.

ASSESSMENT OF ARBUSCULAR MYCORRHIZAL COLONIZATION AND SPORE DENSITY

The root bits were cleared with 10 % KOH, acidified with 1N HCl stained with (0.05%) trypan blue in lactophenol (Phillips and Hayman, 1970) and were left overnight for staining. Percentage of root colonization was carried out by using slide method (Giovannetti and Mosse, 1980).

Twenty-five grams of rhizosphere soil of each plant was assayed for spore count using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Estimation of spore density was carried out according to Gaur and Adholeya, (1994). Spores were transferred from filter paper to microscopic slides and mounted in polyvinyl alcohol lacto-glycerol (PVLG) (Koske and Tessier, 1983).

IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGAL SPECIES

Intact and crushed spores in polyvinyl alcohol-lactic acid-glycerol (PVLG) with or without Melzer's reagent were examined under Leica compound

microscope. Taxonomic identification of spores to species level was based on spore morphology, ornamentation, wall characteristics using various bibliographies (Schenck and Perez, 1990; Almeida and Schenck, 1990; Walker and Vestberg, 1998; Redecker, *et al.*, 2000b; Morton and Redecker, 2001; Schubler *et al.*, 2001) by matching original descriptions and those provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (<http://invam.caf.wvu.edu>). Spore colour was examined under Leica stereomicroscope using intact spores immersed in water. Voucher specimens of arbuscular mycorrhizal fungi have been deposited in the Botany Department, Goa University, Goa.

Spores in the rhizosphere soil were multiplied using *Eleusine coracana* (L.) Gaertn, *Lycopersicum esculentum* Mill, *Allium cepa* L. and *Coleus sp.* as host plants (**Plate XXVI**). The spores isolated from the trap cultures were later used for confirming the identified spores recovered during the study period.

STATISTICAL ANALYSIS

The data on mycorrhizal colonization were arcsine square-root transformed and spore numbers were log-transformed prior to statistical analysis. Data on soil factors, root colonization and spore numbers were subjected to Analysis of Variance (ANOVA) to investigate their variations with age and the means were separated using Duncan's Multiple Range Test (DMRT).

DIVERSITY INDICES

The diversity of arbuscular mycorrhizal fungi from four sites were assayed based on diversity indices (Beena *et al.*, 2000)

Species richness is the number of species present in a particular site.

Simpson's index is an index of dominance since the maximum value is obtained when there is only one or few species.

Simpson's index: $D' = \sum (pi)^2$

Shannon Weiner index (H') is an index of diversity in which higher the value, the greater the diversity and the less the site is dominated by one or few species.

Shannon's index: $H' = \sum (pi \ln pi)$

Where, pi is the proportion of individuals that species i contribute to the total.

Species evenness indicates the distribution of the individuals within species designations.

The evenness was expressed by:

$$J' = \frac{H'}{H'_{max}}$$

Where H'_{max} is the maximum value of diversity for the number of species present.

Relative abundance and Frequency of occurrence of each arbuscular mycorrhizal fungal species was calculated using the formula given below.

$$\text{Frequency (\%)} = \frac{\text{Number of samples containing spores}}{\text{Total number of samples examined}} \times 100$$

$$\text{Relative abundance (\%)} = \frac{\text{Number of spores of each type}}{\text{Number of spores of all types}} \times 100$$

RESULTS

VEGETATION SURVEY

The detailed list of plant species reported from various mine sites is reported in chapter 1 (**Table-2**).

The oldest mine *viz.*, Sanquelim exhibited a rich plant diversity with a total of 250 plant species (both naturally occurring and cultivated) which included 128 herbs, 15 twiners, 3 climbers, 43 shrubs and 61 trees while, at Sonshi (35 year old mine), A total of 73 plant species comprising of 41 herbs, 3 twiners, 13 shrubs and 16 trees were recorded. Codli (30 year old mine) harboured a total of 102 plant species (both naturally occurring and cultivated) which consisted of 55 herbs, 3 twiners, 17 shrubs and 27 tree. The recently degraded mine *viz.*, Xelpi (10 year old) showed the least number of plant species (29) that included both naturally occurring and cultivated with 20 herbs, 3 shrubs and 6 trees.

SOIL CHARACTERISTICS

Soil characteristics of the four mine sites are given in **Table- 17**. Among the various edaphic factors pH, EC, N, P, K, organic carbon and organic matter significantly ($P < 0.01$) varied between sites. Soil nitrogen, organic carbon and organic matter increased with mine age except at Sonshi mine.

ARBUSCULAR MYCORRHIZAL COLONIZATION, SPORE DENSITY AND SPECIES RICHNESS

Mycorrhizal colonization significantly varied between the sites (**Table- 18**). Further, ANOVA on mycorrhizal colonization indicated significant variation in the colonization levels between species, sites, and, species and sites together.

The significant differences ($P < 0.01$) between species, sites, and species x sites for arbuscular mycorrhizal colonization indicates the difference in arbuscular mycorrhizal colonization with mine age. Different levels of mycorrhizal colonization were recorded at different sites.

Arbuscular mycorrhizal colonization at Sanquelim mine ranged from 20% in *Terminalia crenulata* to 72% in *Chromolaena odoratum*. At Sonshi, arbuscular mycorrhizal colonization ranged from 7% in *Acacia auriculiformis* to 73% in *Mimosa pudica*. At Codli, arbuscular mycorrhizal colonization ranged from 9% in *Acacia auriculiformis* to 50% in *Anacardium occidentale*. At the recently degraded mine *i.e.*, Xelpi, arbuscular mycorrhizal colonization ranged from 10% in *Terminalia crenulata* to 45% in *Anacardium occidentale*.

Table- 17. Soil characteristics at iron ore mines of varying ages.

| Sites | pH | EC | Nutrients (mg 100 g ⁻¹) | | | OC (%) | OM (%) |
|---------------------|-------------------|---------------------|-------------------------------------|---------------------|--------------------|---------------------|---------------------|
| | | | N | P | K | | |
| Xelpi (10)* | 6.480 b (0.04) | 0.0874 c (0.002) | 46.00 b (2.45) | 145.200 d (3.96) | 32.600 a (1.82) | 0.017 a (0.003) | 0.0302 a (0.003) |
| Codli (30)* | 6.060 a (0.09) | 0.1122 d (0.004) | 47.200 b (2.05) | 121.200 b (2.77) | 55.600 c (2.51) | 0.2244 c (0.014) | 0.3866 c (0.024) |
| Sonchi (35)* | 6.340 b (0.13) | 0.0704 (0.001) | 36.400 a (4.16) | 138.600 c (3.05) | 56.400 c (2.61) | 0.1104 b (0.012) | 0.1898 b (0.021) |
| Sanquelim (50)* | 6.740 c (0.13) | 0.0566 a (0.002) | 68.200 c (4.32) | 107.400 a (7.80) | 43.200 b (3.42) | 0.2936 d (0.015) | 0.5064 d (0.027) |
| F Statistics | 34.94** | 83.05** | 77.75** | 62.66** | 91.13** | 307.84** | 505.48** |

Significant at: ** - P < 0.01.

In a column, means followed by same letter are not significantly different according to DMRT (P < 0.05).

Values in parenthesis indicates Standard deviation.

* - indicate age of the mine in years.

EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter.

Table- 18. Percent root colonization of arbuscular mycorrhizal (AM) fungi in selected plant species from iron ore mines of varying ages.

| Plant species | Family | Root colonization (%) | | | |
|--|----------------|-----------------------|-------------|--------------|-----------------|
| | | Xelpi (10)* | Codli (30)* | Sonshi (35)* | Sanquelim (50)* |
| <i>Acacia auriculiformis</i> A. Cunn. ex Benth. | Mimosaceae | 28.00 bc | 9.00 d | 7.00 f | 35.00 e |
| <i>Acacia mangium</i> Willd. | Mimosaceae | 17.00 d | 13.00 d | 26.00 d | 60.00 b |
| <i>Anacardium occidentale</i> L. | Anacardiaceae | 45.00 a | 50.00 a | 17.00 c | 70.00 a |
| <i>Calotropis gigantea</i> (L.) R. Br. | Asclepiadaceae | 33.00 b | 23.00 c | 41.00 c | 50.00 cd |
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | Combretaceae | 17.00 d | 33.00 b | 24.00 d | 57.00 bc |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Asteraceae | 43.00 a | 23.00 c | 60.00 b | 72.00 a |
| <i>Ischaemum semisagittatum</i> Roxb. | Poaceae | 40.00 a | 33.00 b | 40.00 c | 51.00 cd |
| <i>Mimosa pudica</i> L. | Mimosaceae | 40.00 a | 38.00 b | 73.00 a | 49.00 d |
| <i>Terminalia crenulata</i> Roth. | Combretaceae | 10.00 a | 25.00 c | 60.00 b | 20.00 f |
| <i>Trema orientalis</i> (L.) Blume | Ulmaceae | 23.00 c | 20.00 c | 70.00 a | 33.00 e |

| F Statistics | d.f. | |
|-------------------|------|----------|
| Plant species (S) | 9 | 83.54** |
| Mine sites (M) | 3 | 224.82** |
| S x M | 27 | 49.33 ** |

Significant at: ** - $P < 0.01$.

In a column, means followed by a common letter are not significantly different according to DMRT ($P < 0.05$).

*- indicate age of the mine in years

d. f. - Degrees of freedom.

Average root colonization at Xelpi, Codli, Sonshi and Sanquelim was found to be 29.6%, 26.7%, 41.8% and 49.7 % respectively (**Fig.- 12**).

There were highly significant differences in mean spore densities between species, mine sites, and species x mine sites. A significant ($P < 0.01$) difference between species, sites, and species x sites for arbuscular mycorrhizal spore density indicates that spore density vary with species and mine age (**Table- 19**).

The spore density at Xelpi mines ranged from 5 spores 25 g^{-1} soil in *Ischaemum semisagittatum* to 14 spores 25 g^{-1} soil in *Chromolaena odoratum*. At Codli mine, spore density ranged from 9 (*Acacia auriculiformis*) to 34 (*Ischaemum semisagittatum*) spores 25 g^{-1} soil. At Sonshi mine, spore density ranged from 12 spores 25 g^{-1} soil in *Acacia auriculiformis* to 55 spores 25 g^{-1} soil in *Chromolaena odoratum*. At Sanquelim mine, spore density ranged from 12 spores 25 g^{-1} soil in *Acacia auriculiformis* to 122 spores 25 g^{-1} soil in *Anacardium occidentale*.

Maximum average spore number recorded at different sites were in the order of Sanquelim > Sonshi > Codli > Xelpi (**Fig.- 13**).

Species richness of arbuscular mycorrhizal fungi recovered from individual plant species from Xelpi, Codli, Sonshi and Sanquelim iron ore mine site has been depicted in **Fig.- 14**. The number of arbuscular mycorrhizal fungal species recorded from Xelpi iron ore mine, ranged from 2

Table- 19. Spore density of arbuscular mycorrhizal (AM) fungi in selected plant species from iron ore mines of varying ages.

| Plant species | Family | Spore 25 g ⁻¹ soil | | | |
|---|----------------|-------------------------------|-------------|--------------|-----------------|
| | | Xelpi (10)* | Codli (30)* | Sonshi (35)* | Sanquelim (50)* |
| <i>Acacia auriculiformis</i> A. Cunn. ex Benth. | Mimosaceae | 7.00 c | 9.00 e | 12.00 f | 12.00 f |
| <i>Acacia mangium</i> Willd. | Mimosaceae | 12.00 ab | 15.00 bcd | 16.00 ef | 20.00 e |
| <i>Anacardium occidentale</i> L. | Anacardiaceae | 11.00 ab | 20.00 b | 26.00 cd | 122.00 a |
| <i>Calotropis gigantea</i> (L.) R. Br. | Asclepiadaceae | 9.00 bc | 11.00 de | 33.00 bc | 47.00 bc |
| <i>Calycopteris floribunda</i> (Roxb.) Lam. | Combretaceae | 11.00 ab | 15.00 bc | 20.00 de | 28.00 d |
| <i>Chromolaena odoratum</i> (L.) King & Robinson | Asteraceae | 14.00 a | 30.00 a | 55.00 a | 101.00 a |
| <i>Ischaemum semisagittatum</i> Roxb. | Poaceae | 5.00 d | 34.00 a | 38.00 b | 44.00 bc |
| <i>Mimosa pudica</i> L. | Mimosaceae | 9.00 bc | 30.00 a | 35.00 bc | 51.00 b |
| <i>Terminalia crenulata</i> Roth. | Combretaceae | 12.00 ab | 13.00 cd | 30.00 bc | 42.00 bc |
| <i>Trema orientalis</i> (L.) Blume | Ulmaceae | 10.00 bc | 16.00 bc | 37.00 b | 36.00 cd |

| F Statistics | d. f. | |
|-------------------|-------|-----------|
| Plant species (S) | 9 | 52.54 ** |
| Mine sites (M) | 3 | 355.28 ** |
| S x M | 27 | 12.95** |

** - Significant at P< 0.01

* - indicate age of the mine in year

In a column , means followed by a common letter are not significantly different according to DMRT (P<0.05)

d. f. - Degrees of freedom.

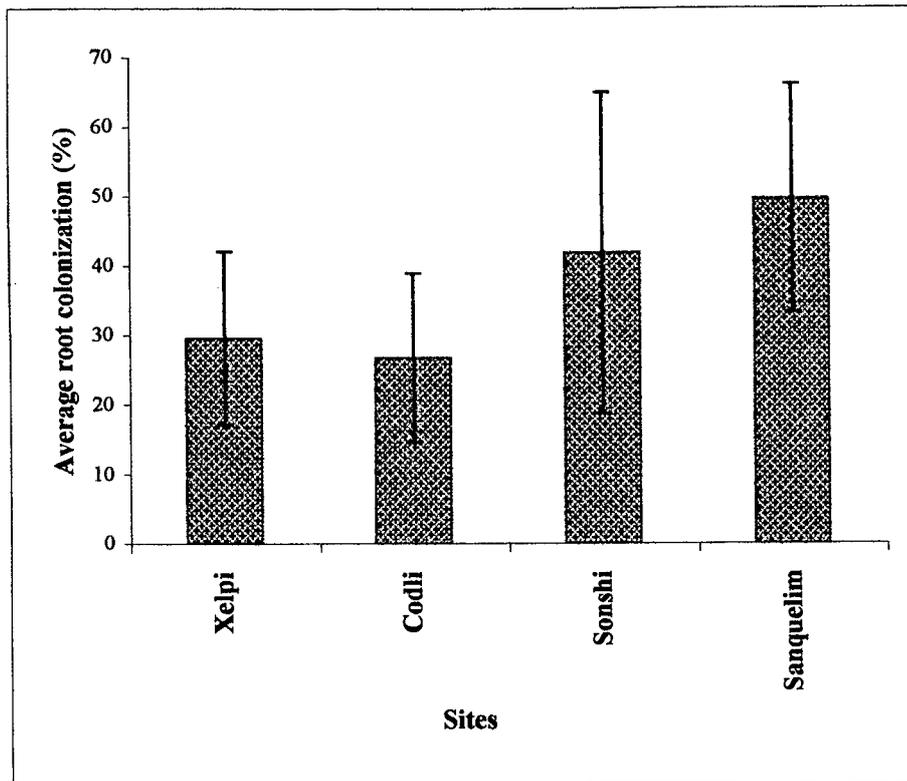


Fig.-12. Average root colonization of arbuscular mycorrhizal fungi at Xelpi, Codli, Sonshi and Sanquelim iron ore mines. Error bar indicates ± 1 SD.

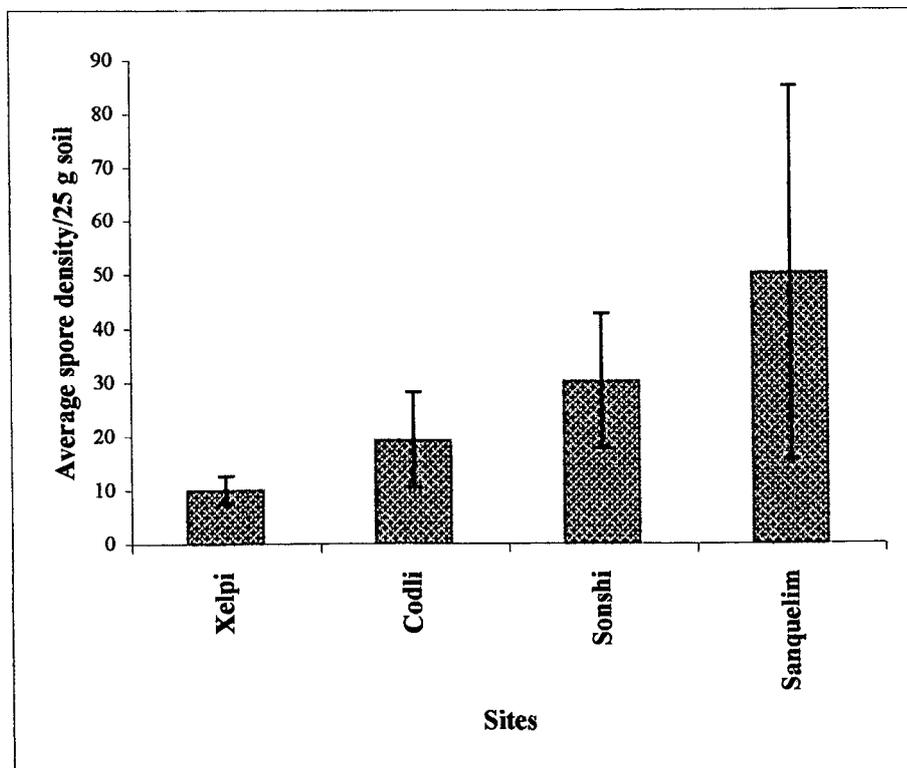


Fig.- 13. Average spore density of arbuscular mycorrhizal fungi at Xelpi, Codli, Sonshi and Sanquelim iron ore mines. Error bar indicates ± 1 SD.

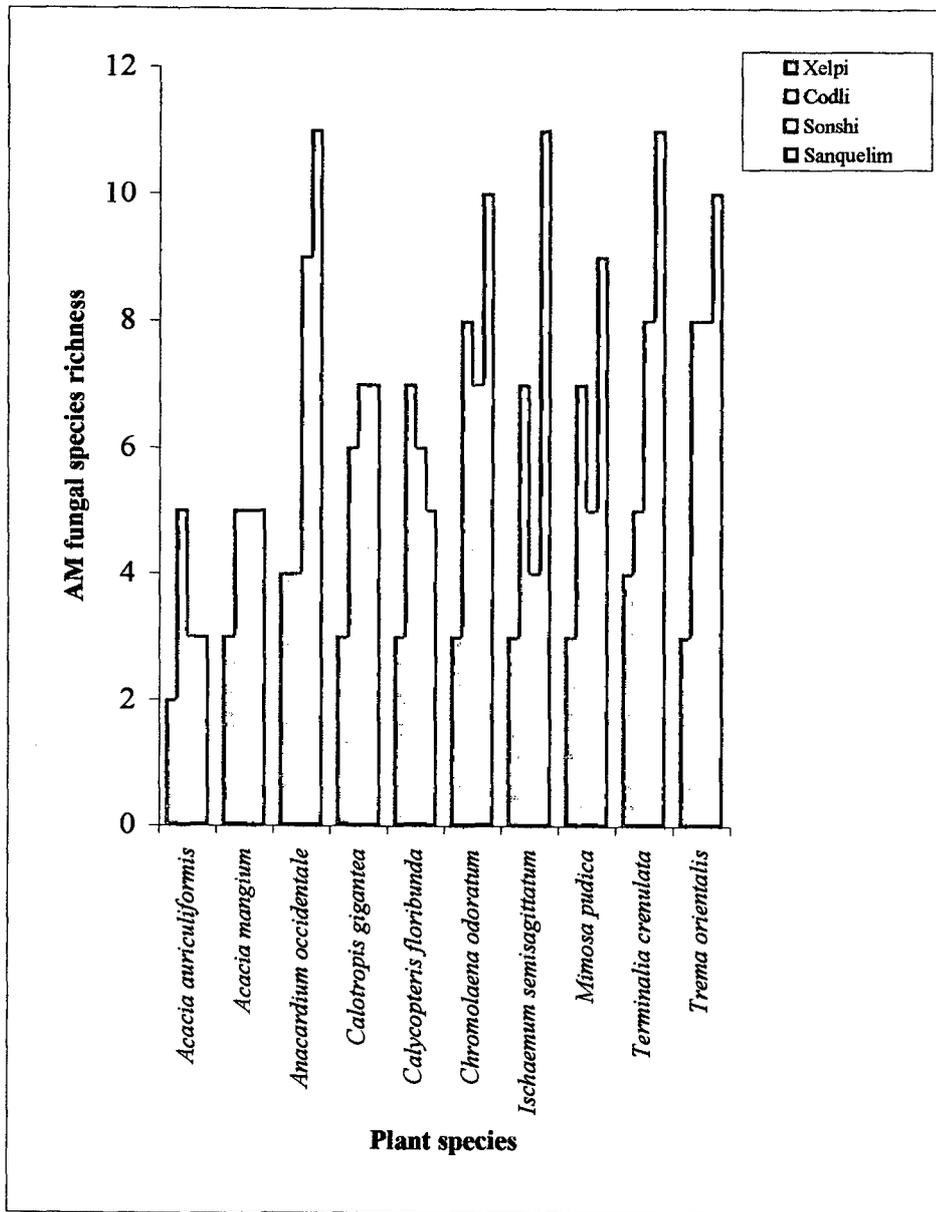


Fig.-14. Arbuscular mycorrhizal fungal species richness from individual plant species at Xelpi, Codli, Sonshi and, Sanquelim iron ore mines.

to 4 arbuscular mycorrhizal fungal species per plant species. At Codli, the number of arbuscular mycorrhizal fungal species ranged from 4 to 8 arbuscular mycorrhizal fungal species per plant. At Sonshi iron ore mine, the number of arbuscular mycorrhizal fungal species ranged from 3 to 9 arbuscular mycorrhizal fungal species per plant species. At Sanquelim mine, number of arbuscular mycorrhizal fungal species ranged from 3 to 11 per plant.

OCCURRENCE OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI

The results of frequency of occurrence and relative abundance of arbuscular mycorrhizal fungi in the rhizosphere of four iron ore mine sites are depicted in **Table- 20 & 21**. The study revealed the occurrence of 33 arbuscular mycorrhizal fungal species from Sanquelim mines, 25 arbuscular mycorrhizal species from Sonshi mine, 22 arbuscular mycorrhizal species from Codli mine while, only 12 arbuscular mycorrhizal fungal species were retrieved from Xelpi mine. The arbuscular mycorrhizal fungal genera recorded during the study belonged to five genera viz., *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*.

Pooled data indicated that *Acaulospora spinosa* was most dominant (30-90%) followed by *Glomus macrocarpum* (20-80%), *Acaulospora scrobiculata* (60%), *Scutellospora gregaria* (20-60%), *Scutellospora pellucida* (30-50%), and *Gigaspora margarita* (10-40%). Of the several arbuscular mycorrhizal fungal species found from the four study sites, *Acaulospora spinosa*, *Acaulospora scrobiculata*, *Glomus fasciculatum*, *Glomus geosporum*,

Table- 20. Frequency of occurrence of arbuscular mycorrhizal (AM) fungal species from iron ore mines of varying ages.

| Arbuscular mycorrhizal fungal species | Frequency (%) | | | |
|--|---------------|-------------|--------------|-----------------|
| | Xelpi (10)* | Codli (30)* | Sonshi (35)* | Sanquelim (50)* |
| <i>Acaulospora foveata</i> Trappe & Janos | 10 | - | - | 20 |
| <i>Acaulospora laevis</i> Gerd. & Trappe | - | 10 | - | 10 |
| <i>Acaulospora mellea</i> Spain & Schenck | - | - | 10 | - |
| <i>Acaulospora morrowiae</i> Spain & Schenck | - | - | - | 10 |
| <i>Acaulospora rhemii</i> Sieverding & Toro | - | - | 10 | - |
| <i>Acaulospora scrobiculata</i> Trappe | 60 | 60 | 60 | 60 |
| <i>Acaulospora spinosa</i> Walker & Trappe | 70 | 90 | 80 | 30 |
| <i>Acaulospora undulata</i> Sieverding | - | - | 10 | 10 |
| <i>Gigaspora albida</i> Schenck & Smith | - | 10 | 10 | 10 |
| <i>Gigaspora decipiens</i> Hall & Abbott | - | - | 10 | - |
| <i>Gigaspora margarita</i> Becker & Hall | 30 | 10 | 40 | 40 |
| <i>Glomus aggregatum</i> Schenck & Smith emend. Koske | - | 10 | - | 10 |
| <i>Glomus claroideum</i> Schenck & Smith emend Walker & Vestberg. | - | 30 | 10 | 20 |
| <i>Glomus clavisorum</i> (Trappe) Almeida & Schenck | - | - | - | 10 |
| <i>Glomus constrictum</i> Trappe | - | 10 | 10 | 10 |
| <i>Glomus coremioides</i> (Berk. & Broome) Redecker et Morton | - | - | 10 | 10 |
| <i>Glomus etunicatum</i> Becker & Gerd. | - | 30 | 10 | 20 |
| <i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker & Koske, | 30 | 40 | 40 | 50 |
| <i>Glomus formosanum</i> Wu & Chen | - | - | - | 10 |
| <i>Glomus geosporum</i> (Nicol. & Gerd.) Walker | 10 | 50 | 40 | 40 |
| <i>Glomus globiferum</i> Koske & Walker | - | - | 10 | 20 |
| <i>Glomus glomerulatum</i> Sieverding | - | 10 | 10 | 10 |
| <i>Glomus hoi</i> Berch & Trappe | - | - | - | 20 |
| <i>Glomus macrocarpum</i> Tul. & Tul. | 20 | 80 | 70 | 60 |
| <i>Glomus microaggregatum</i> Koske, Gemma & Olexia | 10 | - | - | - |
| <i>Glomus microcarpum</i> Tul. & Tul. | - | 10 | - | 10 |
| <i>Glomus monosporum</i> Gerdemann & Trappe | - | 10 | 20 | 10 |
| <i>Glomus mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe | - | - | 10 | - |
| <i>Glomus multicaule</i> Gerdemann & Bakshi | - | - | - | 10 |

| | | | | |
|--|----|----|----|----|
| <i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck | - | 10 | - | 20 |
| <i>Glomus sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck | - | 20 | 10 | 50 |
| <i>Glomus taiwanensis</i> (Wu & Chen) Almeida & Schenck | 10 | 10 | - | 40 |
| <i>Scutellospora calospora</i> (Nicol. & Gerd.) Walker & Sanders | - | - | - | 10 |
| <i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders | 20 | 40 | 20 | 60 |
| <i>Scutellospora nigra</i> (Redhead) Walker & Sanders | - | - | 10 | - |
| <i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders | 30 | 40 | 50 | 50 |
| <i>Scutellospora persica</i> Koske & Walker | - | - | - | 40 |
| <i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders | - | 10 | 20 | 20 |
| <i>Scutellospora weresubiae</i> Koske & Walker | 10 | 30 | 50 | 20 |
| Total number of soil samples assayed | 30 | 30 | 30 | 30 |

* - indicate age of the mine

Table- 21. Relative abundance of arbuscular mycorrhizal (AM) fungal species from iron ore mines of varying ages.

| Arbuscular mycorrhizal fungal species | Relative abundance (%) | | | |
|--|------------------------|-------------|--------------|-----------------|
| | Xelpi (10)* | Codli (30)* | Sonshi (35)* | Sanquelim (50)* |
| <i>Acaulospora foveata</i> Trappe & Janos | 2.0 | - | - | 1.12 |
| <i>Acaulospora laevis</i> Gerd. & Trappe | - | 0.52 | - | 0.40 |
| <i>Acaulospora mellea</i> Spain & Schenck | - | - | 0.33 | - |
| <i>Acaulospora morrowiae</i> Spain & Schenck | - | - | - | 0.40 |
| <i>Acaulospora rhemii</i> Sieverding & Toro | - | - | 0.66 | - |
| <i>Acaulospora scrobiculata</i> Trappe | 34.0 | 17.10 | 15.40 | 13.52 |
| <i>Acaulospora spinosa</i> Walker & Trappe | 23.0 | 15.54 | 20.36 | 9.69 |
| <i>Acaulospora undulata</i> Sieverding | - | - | 0.66 | 0.20 |
| <i>Gigaspora albida</i> Schenck & Smith | - | 1.04 | 0.66 | 0.20 |
| <i>Gigaspora decipiens</i> Hall & Abbott | - | - | 0.33 | - |
| <i>Gigaspora margarita</i> Becker & Hall | 4.0 | 0.78 | 4.97 | 4.98 |
| <i>Glomus aggregatum</i> Schenck & Smith emend. Koske | - | 1.04 | - | 0.40 |
| <i>Glomus claroideum</i> Schenck & Smith emend Walker & Vestberg. | - | 2.07 | 0.33 | 1.74 |
| <i>Glomus clavispurum</i> (Trappe) Almeida & Schenck | - | - | - | 0.99 |
| <i>Glomus constrictum</i> Trappe | - | 1.55 | 0.66 | 0.40 |
| <i>Glomus coremioides</i> (Berk. & Broome) Redecker et Morton | - | - | 0.33 | 0.20 |
| <i>Glomus etunicatum</i> Becker & Gerd. | - | 3.63 | 0.83 | 0.65 |
| <i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker & Koske, | 10.0 | 3.63 | 12.25 | 17.30 |
| <i>Glomus formosanum</i> Wu & Chen | - | - | - | 0.60 |
| <i>Glomus geosporum</i> (Nicol. & Gerd.) Walker | 2.0 | 9.84 | 2.65 | 4.67 |
| <i>Glomus globiferum</i> Koske & Walker | - | - | 0.66 | 1.39 |
| <i>Glomus glomerulatum</i> Sieverding | - | 0.52 | 0.33 | 0.40 |
| <i>Glomus hoi</i> Berch & Trappe | - | - | - | 0.60 |
| <i>Glomus macrocarpum</i> Tul. & Tul. | 9.0 | 21.37 | 15.65 | 9.05 |
| <i>Glomus microaggregatum</i> Koske, Gemma & Olexia | 1.0 | - | - | - |
| <i>Glomus microcarpum</i> Tul. & Tul. | - | 0.52 | - | 0.20 |
| <i>Glomus monosporum</i> Gerdemann & Trappe | - | 1.19 | 1.16 | 0.50 |
| <i>Glomus mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe | - | - | 0.33 | - |
| <i>Glomus multicaule</i> Gerdemann & Bakshi | - | - | - | 0.40 |

| | | | | |
|--|-----|------|------|------|
| <i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck | - | 0.52 | - | 2.58 |
| <i>Glomus sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck | - | 1.04 | 0.66 | 4.17 |
| <i>Glomus taiwanensis</i> (Wu & Chen) Almeida & Schenck | 2.0 | 1.55 | - | 4.57 |
| <i>Scutellospora calospora</i> (Nicol. & Gerd.) Walker & Sanders | - | - | - | 0.40 |
| <i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders | 3.0 | 5.96 | 2.65 | 6.85 |
| <i>Scutellospora nigra</i> (Redhead) Walker & Sanders | - | - | 0.33 | - |
| <i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders | 8.0 | 6.74 | 8.44 | 7.75 |
| <i>Scutellospora persica</i> Koske & Walker | - | - | - | 2.38 |
| <i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders | - | 0.52 | 2.98 | 0.80 |
| <i>Scutellospora weresubiae</i> Koske & Walker | 2.0 | 2.59 | 6.62 | 1.39 |
| Total spore number | 300 | 579 | 906 | 1509 |

* - indicate age of the mine

Glomus macrocarpum, *Scutellospora gregaria*, *Scutellospora pellucida*, *Scutellospora weresubiae* and *Gigaspora margarita* were common to all the sites (Fig.- 15 & 16).

At Xelpi, the most dominating species based on frequency of occurrence was *Acaulospora spinosa* (70%) followed by *Acaulospora scrobiculata* (60%), *Gigaspora margarita* (30%), *Scutellospora pellucida* (30%) and *Glomus fasciculatum* (30%). At Codli, the most dominating species based on frequency of occurrence was *Acaulospora spinosa* (90%) followed by *Glomus macrocarpum* (80%), *Acaulospora scrobiculata* (60%) and *Glomus geosporum* (50%). At Sonshi, the most dominating species was *Acaulospora spinosa* (80%) followed by *Glomus macrocarpum* (70%), *Acaulospora scrobiculata* (60%), *Scutellospora pellucida* (50%) and *Scutellospora weresubiae* (50%). At Sanquelim, the most dominating species was *Acaulospora scrobiculata* (60%), *Glomus macrocarpum* (60%), *Scutellospora gregaria* (60%), *Glomus fasciculatum* (50%), *Glomus sinuosum* (50%), *Scutellospora pellucida* (50%), *Gigaspora margarita* (40%), *Glomus geosporum* (40%), *Glomus taiwanensis* (40%) and *Scutellospora persica* (40%) with percent frequency given in parenthesis. Relative abundance and frequency of occurrence of arbuscular mycorrhizal fungal species common to all the four sites viz., Xelpi, Codli, Sonshi and Sanquelim is depicted in Fig.- 15 & 16.

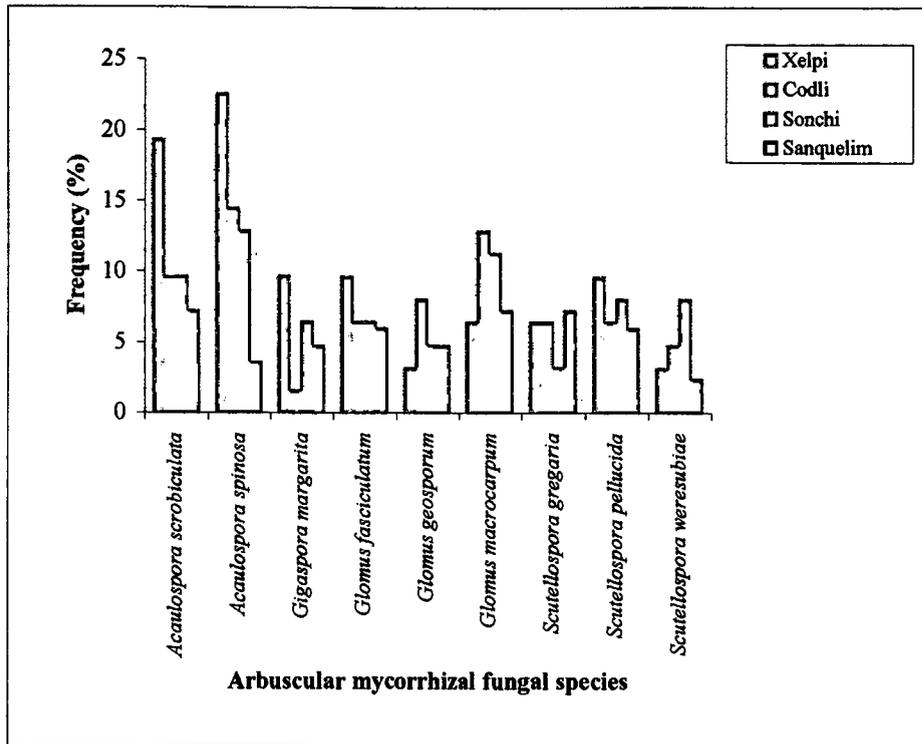


Fig.-15. Frequency of occurrence of arbuscular mycorrhizal fungal species common to Xelpi, Codli, Sonshi and Sanquelim iron ore mines.

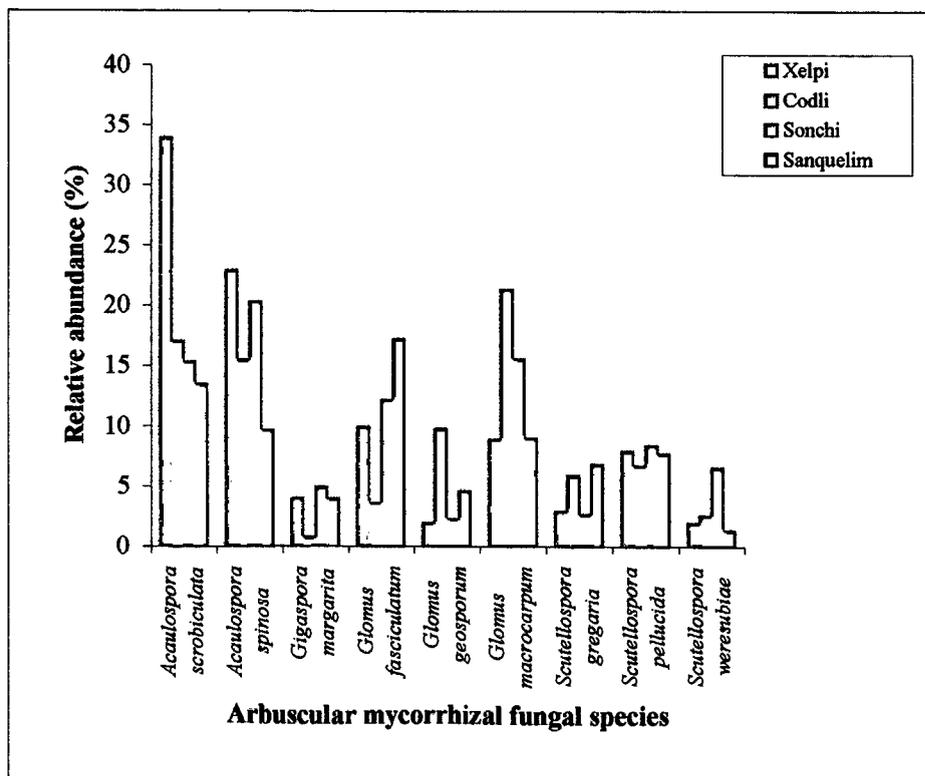
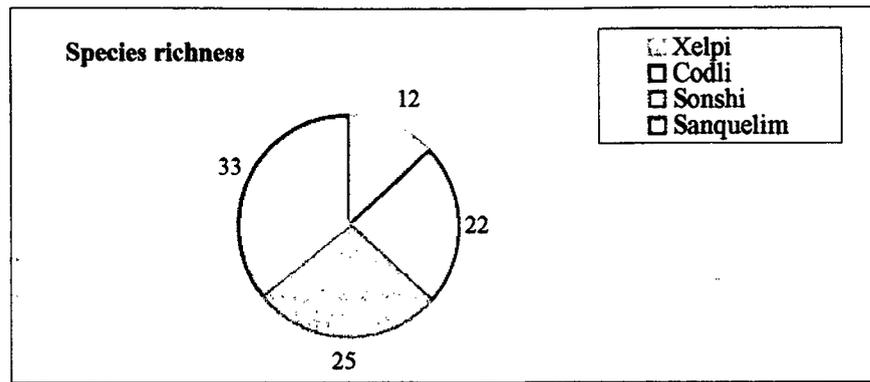
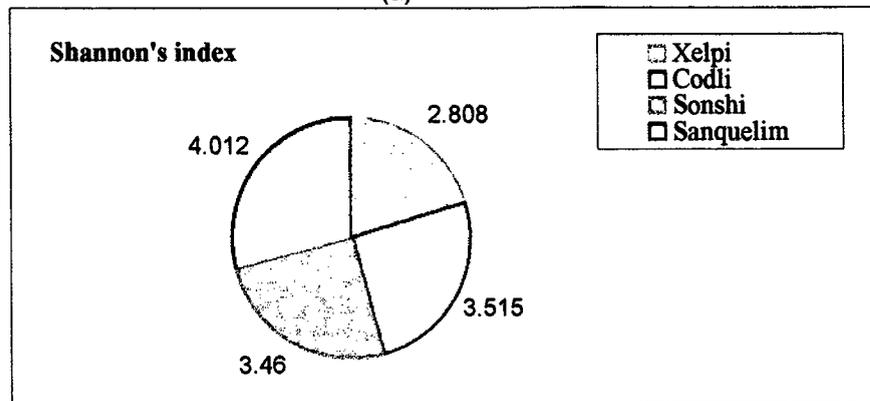


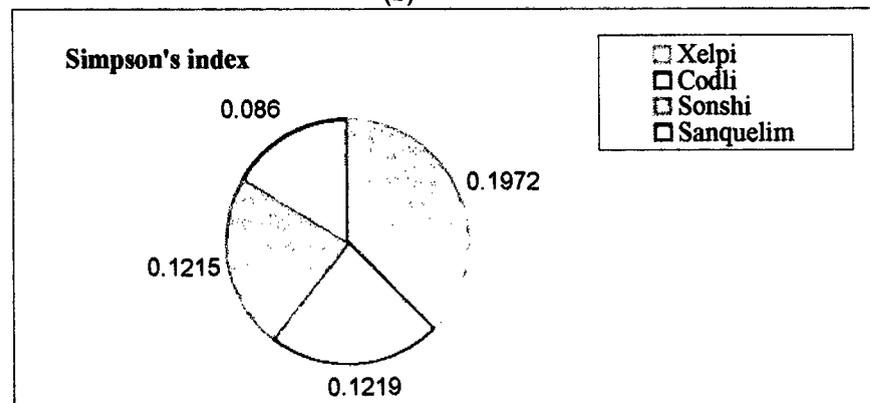
Fig.- 16. Relative abundance of arbuscular mycorrhizal fungal species common to Xelpi, Codli, Sonshi and Sanquelim iron ore mines.



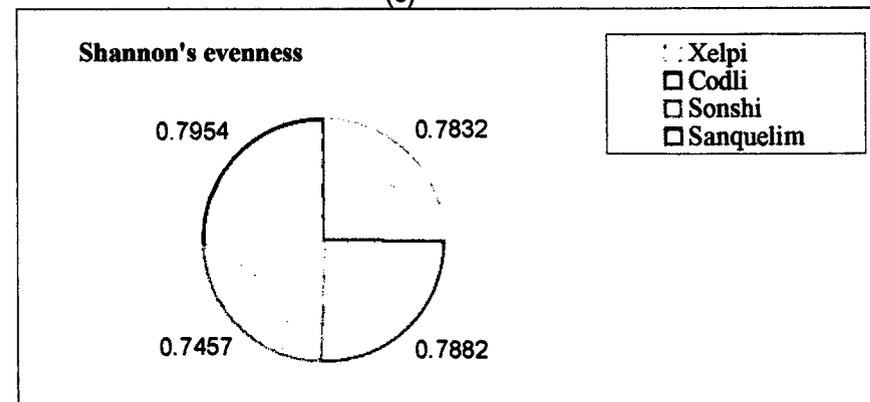
(a)



(b)



(c)



(d)

Fig.- 17. Species richness (a) and diversity indices viz., (b) Shannon's, (c) Simpson's and (d) Shannon's evenness, at Xelpi, Codli, Sonshi and Sanquelim iron ore mines.

DIVERSITY INDICES

Species richness was maximum at Sanquelim mine (33) followed by Sonshi (25), Codli (22) and least arbuscular mycorrhizal fungal species were recovered from Xelpi mines (12) with the number of arbuscular mycorrhizal fungal species given in parenthesis (**Fig.- 17 a**).

Shannon's index of diversity recorded at different sites was in the order of Sanquelim > Codli > Sonshii > Xelpi. Simpson's index of dominance was in the order of Xelpi > Codli > Sonshi > Sanquelim. While, Shannon's Evenness ranged from 0.7457 to 0.7954 at the four iron ore mine sites. Thus, diversity indices were greater on well established mine as compared to the recently degraded mine (**Fig.- 17 b-d**).

DISCUSSION

The present study indicated that spore diversity and mycorrhizal colonization is influenced by age of the mine, host species and soil type.

Studies on vegetation survey revealed increased vegetation cover in terms of species diversity with increase in age of mine sites. Similar observations have been recorded earlier (Benerjee, *et al.*, 1999). Thus, species growing as ground cover (pteridophytes, herbs, shrubs) and to some extent tree species gradually modify the physicochemical properties and nutrient status of the spoil through increased litter production thereby improving the soil conditions. This in turn promotes plant succession.

There were significant differences in soil properties between different mine sites. Soil nitrogen, organic carbon and organic matter increased with mine age and vegetation cover, with younger sites having sparse vegetation compared to older sites with dense vegetation cover except at Sonshi). Similar observations have been made earlier (Brundrett *et al.*, 1996b). Reduced levels of nitrogen, organic carbon, and organic matter at Sonshi could possibly be due to less plant species diversity as compared to Codli mine.

The results on mycorrhizal colonization and spore density revealed significant differences between the sites, which is in accordance with earlier studies (Nicolson and Johnston, 1979; MacMohan and Warner, 1984; Zak and Parkinson, 1982). According to Black and Tinker, (1979) the difference in arbuscular mycorrhizal formation at different sites may be due to difference in physical/chemical properties of the soil and soil pH while, Slankis (1974) attributed these differences in arbuscular mycorrhizal formation to difference in the nutrient concentration of the sites.

In the present study, the extent of mycorrhizal colonization varied with plant species at each site confirming the findings of Manjunath and Bagyaraj, (1982), who found that the extent to which plants respond to colonization varies with the plant species. Gerdemann, (1965) showed that the colonization pattern of arbuscular mycorrhizal fungal species can distinctly differ in various plant species. Similarly, spore density also exhibited variation between plant species at each site. Thus, mycorrhizal formation not only depends on plant

and fungal species but also on the sites conditions (Mason *et al.*, 1983) especially nutrient availability (Hayman, 1982).

Based on the overall level of arbuscular mycorrhizal population distribution in terms of spore density, species diversity and species richness it is evident that arbuscular mycorrhizal fungal population generally increase during plant succession with mine age and vegetation cover reaching exceptionally high levels in some fully vegetated areas (Brundrett *et al.*, 1996 b).

The present study revealed that the recently degraded mine site with lowest plant diversity harboured the least number of arbuscular mycorrhizal fungal species. In contrast, the old disturbed sites with higher plant biodiversity had maximum arbuscular mycorrhizal fungal species. This indicates a close relationship between arbuscular mycorrhizal fungal diversity and plant biodiversity. Koske, (1975) and Koske and Halvorson, (1981) observed an increase in the abundance of arbuscular mycorrhizal fungal species richness and spore with increasing age and stabilization of sand-dunes. Anderson *et al.*, (1984) reported a close positive correlation between plant cover and spore numbers in some natural ecosystems. Thus, mycorrhizal associations are potential factors determining diversity in ecosystems. They can probably modify the structure and functioning of a plant community in a complex and unpredictable way (Grime *et al.*, 1987; Read, 1990). Any shift in the mycorrhizal fungal population could have consequences for the composition of plant communities (survival, competition and floristic diversity), causing changes in biology of natural ecosystems (Miller and Allen,

1992; Molina *et al.*, 1992). Therefore, knowledge of the different factors influencing the population biology of arbuscular mycorrhizal fungi is essential in any attempt to use them in environmental conservation (Allen, 1991), biotechnology (Mulongoy *et al.*, 1992) or in sustainable agriculture (Bethlenfalvay and Linderman, 1992).

In the recently degraded mine, the possible reasons for low propagule density and diversity may be low soil fertility, organic matter, soil texture, soil moisture and severe soil compaction. Miller, (1979) stated that the severity of the disturbance and the harshment of the site controls the occurrence of arbuscular mycorrhizal fungi. While, Allen and Allen, (1980), suggest that inoculum density, edaphic characters, plant cover, host genotype and elapsed time are the critical determinants. Thus, the soil structure changes resulting from disturbance would disrupt the spatial association between old and new roots and reduce the effectiveness of root inoculum (Evans and Miller, 1988; Rives *et al.*, 1980).

The reduction in arbuscular mycorrhizal fungal diversity would result because disturbance itself would eliminate many intolerant fungi, while other with more resistance may not be affected because they have more capacity to adapt to major changes to the environment. The increased diversity at the established mine could be due to increase in organic carbon and organic matter content thus, encouraging mycorrhizal development.

The continuous and constant disturbance on the recently degraded mine and Sonshi mine which is under active mining operations might have severely affected the seedling establishment as well as colonization of arbuscular mycorrhizal fungi. It has been pointed out that below ground diversity of arbuscular mycorrhizal fungi is one of the major factor contributing to the maintenance of plant biodiversity and add to the ecosystem functioning. In the present study it has been observed that well-established mines had high arbuscular mycorrhizal fungal diversity with decreased dominance. Hence, it can be argued that in a stable ecosystem the spores are more in number with equal dominance and greater diversity. In the recently degraded mine, as the conditions are not favourable in terms of vegetation cover and nutrient status only a few species that can tolerate the adverse conditions become more dominant whereas, the rest gets eliminated as a result its probability of the dominant species increases and the diversity decreases. The diversity indices go on decreasing with increase in dominance of species. This hypothesis supports our results, which shows increased diversity and decreased dominance with increase in the age of the mine sites.

In the present study, the impact of disturbance seems to be higher on spore number and species richness than on root colonization. These results support the theory of reduction of arbuscular mycorrhizal fungal propagules under severe disturbance which affect plant community structure, leading to ecosystem instability. The increased arbuscular mycorrhizal fungal activity in established mines may be attributed to the moderate degree of disturbance supporting Connell's (1978) view of intermediate disturbance hypothesis.

According to this hypothesis, the higher diversity is maintained at intermediate scale of disturbance. This hypothesis may be applicable to mine wastelands. It is evident that established mines possess high arbuscular mycorrhizal fungal diversity as well as plant diversity and vegetation cover. The moderate level of disturbance aids in the dispersal of arbuscular mycorrhizal fungal propagules as well as plant propagules to the sites.

Thus, mycorrhizae may influence succession by regulating competition between plants of different successional stages which has often been cited as a mechanism of succession (Connell and Slatyer, 1977; Allen and Allen, 1984). When mycorrhizae influence the competitive interaction between species of different successional stages, their abundance may also influence the rate of succession.

The study clearly indicates that severity of disturbance, harshness of site, low inoculum levels, edaphic characteristics, plant species cover and time certainly influences the rate of arbuscular mycorrhizal fungi. Thus, revegetation of any disturbed site can occur over time with high species richness and diversity of arbuscular mycorrhizal fungi as they are important in the establishment of a healthy plant community and facilitates the plant succession.

CHAPTER-V

**SEASONAL DYNAMICS OF ARBUSCULAR
MYCORRHIZAL (AM) FUNGI IN IRON ORE MINE
WASTELANDS AT CODLI-GOA.**

INTRODUCTION

Widespread in their seasonal occurrence and distribution, arbuscular mycorrhizal fungi are a significant part of every natural and cultivated ecosystem, playing a major role in plant species diversity and survival (Bergelson and Crawley, 1988). However, little is known about phenological variations in mycorrhizal diversity in many habitats (Brundrett, 1991). It is well known that arbuscular mycorrhizal fungal symbiosis facilitate the survival, growth and establishment of plants in extreme habitats (Allen, 1991; Koske and Gemma, 1995).

Environmental conditions greatly influence the density and composition of mycorrhizae. Spore germination, hyphal spread within the soil, colonization levels, fungal survival and growth promotion may all respond differently to any given set of environmental conditions. Thus, it is very difficult to predict the influence of environmental variables on mycorrhizal association in plants.

Seasonal fluctuation in number of mycorrhizal roots and spores have been examined in deciduous forests (Brundrett and Kendrick, 1988; Mayer and Godoy, 1989), grasslands (Rabatin, 1979; Gay *et al.*, 1982; Sanders and Fitter, 1992), salt marshes (Van Duin *et al.*, 1989), sand dunes (Gemma *et al.*, 1989; Giovannetti, 1985; Sylvia, 1986; Bhaskaran and Selvaraj, 1996; Beena *et al.*, 1997), tropical forests (Louis and Lim, 1987; Mohankumar and Mahadevan, 1988), nutrient deficient tropical soils (Muthukumar *et al.*, 1998); and arid communities (Allen, 1983). Influence of edaphic and climatic

factors and host plants in the distribution of arbuscular mycorrhizal fungi has been reported in *Acacia farnesiana* Willd. (Udaiyan *et al.*, 1996) and tropical wild legumes (Muthukumar *et al.*, 1994).

Studies on occurrence of arbuscular mycorrhizal fungi from different mine spoils have been documented earlier (Daft and Nicolson, 1974; Daft and Hacskeylo, 1976; Khan, 1978; Miller, 1979; Reeves *et al.*, 1979; Allen and Allen, 1980; Diaz and Honrubia, 1993). These investigations have stressed the importance of arbuscular mycorrhizal fungal relationships in allowing successful recolonization and plant growth. Studies on occurrence of arbuscular mycorrhizal fungi from iron ore mines are scarce (Sastry and Johri, 1999).

However, no studies have been reported on the seasonal dynamics of arbuscular mycorrhizal fungi in iron ore mine wastelands. Thus, the aim of the present study is to understand the pattern of colonization, spore density and species richness in relation to the seasonal changes in the edaphic factors in some plants species from iron ore mine wastelands at Codli, Goa.

MATERIALS AND METHODS

STUDY SITE

The study was carried out on iron ore mine site at Codli, Goa (15° 20' 53" N Latitude and 74° 8' 33" E Longitude) during pre-monsoon (March), monsoon (July) and post-monsoon (November). Six quadrates each with an area of 10 x 10 sq. mt. were randomly laid at the site. Eight angiospermic plant species

comprising of five herbs viz., *Chromolaena odoratum* (L.) King & Robinson, *Emilia sonchifolia* (L.) DC., *Mimosa pudica* L., *Ludwigia parviflora* L., *Ischaemum semisagittatum* Roxb. and three tree species viz., *Acacia auriculiformis* A. Cunn. ex Benth., *Acacia mangium* Willd., and *Trema orientalis* (L.) Blume common to all the six quadrates were taken up for study. Plants collected in the present study were identified using floras (Rao, 1985 & 1986; Matthew, 1991; 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 January 2000 – December 2000 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

Soil temperature was recorded during pre-monsoon (March), monsoon (July) and post-monsoon (November) with the help of soil thermometer. For analysing soil moisture, pH, EC, N, P, K, Ca, Mg, organic carbon, organic matter and available phosphorus, mine reject samples were collected from a depth of 0-25 cm from five 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 quadrat.

Moisture content and soil pH was measured within 2-3 hours of sample collection. Known amount of soil was dried at 85°C to a constant weight to determine the moisture content and expressed as a percentage of oven dried weight. 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 extract (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 determined by flame photometric method (Jackson, 1971). Exchangeable calcium and magnesium were detected by flame emission spectrophotometry. Organic carbon and organic matter content were detected by Walkley and Black's rapid titration method (Jackson, 1971) while, available phosphorus was detected by Olsen's method (Olsen *et al.*, 1954).

SAMPLING

Roots and rhizosphere soils were sampled individually for each plant species viz., *Chromolaena odoratum*, *Emilia sonchifolia*, *Mimosa pudica*, *Ludwigia parviflora*, *Ischaemum semisagittatum*, *Acacia auriculiformis*, *Acacia mangium* and *Trema orientalis* from all the six quadrates during pre-monsoon (March), monsoon (July) and post-monsoon (November) for a period of one year. For trees, the roots were dug and traced back to plant, which ensured that the roots belonged to the intended plant species. Samples of herbs were

usually made by uprooting the entire plant. Plants were uprooted along with the rhizosphere soil and were brought to the laboratory in polyethylene bags. Plants were shaken to remove the adhering soil particles. Feeder roots were cut into 1cm bits and were processed for further studies.

ASSESSMENT OF ARBUSCULAR MYCORRHIZAL COLONIZATION AND SPORE DENSITY

The 1cm cut root bits were cleared with 10 % KOH, acidified with 1N HCl stained with (0.05%) trypan blue in lactophenol (Phillips and Hayman, 1970) and were left overnight for staining. 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 from : 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, are calculated as

$$\text{Arbuscular colonization (AC)} = 100 (q + s/G).$$

$$\text{Vesicular colonization (VC)} = 100 (r + s/G).$$

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

$$\text{Hyphal colonization (HC)} = 100 \{(G-p)/G\}$$

Hundred grams of rhizosphere soil of each plant from each quadrat was assayed for spore count using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Estimation of spore density was carried out according to Gaur and Adholeya, (1994). Spores were transferred from filter paper to microscopic slides and mounted in polyvinyl alcohol lacto-glycerol (PVLG) (Koske and Tessier, 1983).

IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL (AM) FUNGAL SPECIES

Intact and crushed spores in polyvinyl alcohol lacto-glycerol (PVLG) with or without Melzer's reagent were examined under Leica compound microscope.

Taxonomic identification of spores to species level was based on spore morphology, ornamentation, wall characteristics using various bibliographies (Schenck and Perez, 1990; Almeida and Schenck, 1990; Walker and Vestberg, 1998; Redecker, *et al.*, 2000b; Morton and Redecker, 2001; Schubler *et al.*, 2001) by matching original descriptions and those provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (<http://invarm.caf.wvu.edu>). Spore colour was examined under Leica stereomicroscope using intact spores immersed in water. Voucher specimens of arbuscular mycorrhizal fungi have been retained in the Botany Department, Goa University, Goa.

Spores in the rhizosphere soil were multiplied using *Eleusine coracana* (L.) Gaertn., *Lycopersicon esculentum* Mill., *Allium cepa* L. and *Coleus sp.* as host plants (**Plate-XXVI**). The spores isolated from the trap cultures were later used for confirming the identified spores recovered during the study period.

STATISTICAL ANALYSIS

The data on mycorrhizal colonization and arbuscular mycorrhizal fungal structures were arcsine square-root transformed and spore numbers were log-transformed prior to statistical analysis. Analysis of Variance (ANOVA) on edaphic and mycorrhizal variables was carried out to investigate their variations with space and time. The variations within a year in edaphic variables and mycorrhizal status were examined for each species by ANOVA and the means were separated using Duncan's Multiple Range Test (DMRT). Three way ANOVA was used to study whether the arbuscular mycorrhizal

colonization pattern and spore numbers were seasonal for the whole data set (all the eight plant species). Pearson's correlation was performed using SPSS (1998) to assess the relationship between, edaphic and mycorrhizal variables. The frequency of occurrence was calculated using the formula given below.

$$\text{Frequency (\%)} = \frac{\text{Number of samples containing a species}}{\text{Total number of samples examined}} \times 100$$

RESULTS

Climatic data revealed that the temperature was maximum during pre-monsoon and post-monsoon and was least during monsoon. Maximum rainfall was recorded during monsoon followed by pre-monsoon and post-monsoon. Relative Humidity (RH) was maximum during monsoon followed by post-monsoon and pre-monsoon (**Fig.- 18**). Soil temperature was maximum during pre-monsoon (24- 40⁰C) followed by post-monsoon (22- 35⁰C) and was least during monsoon (20- 28⁰C).

The soil at the study site was deficient in nutrients. A significant difference was observed in the soil characteristics during pre-monsoon, monsoon and post-monsoon (**Table- 22**). Correlation coefficient between the edaphic factors revealed that soil moisture was negatively correlated to EC, N, P, K, calcium, organic carbon and organic matter. Soil nitrogen and soil phosphorus were positively correlated to K, calcium. Soil phosphorus was positively correlated to organic carbon and organic matter. Soil nitrogen was positively correlated to organic carbon. Available phosphorus was positively

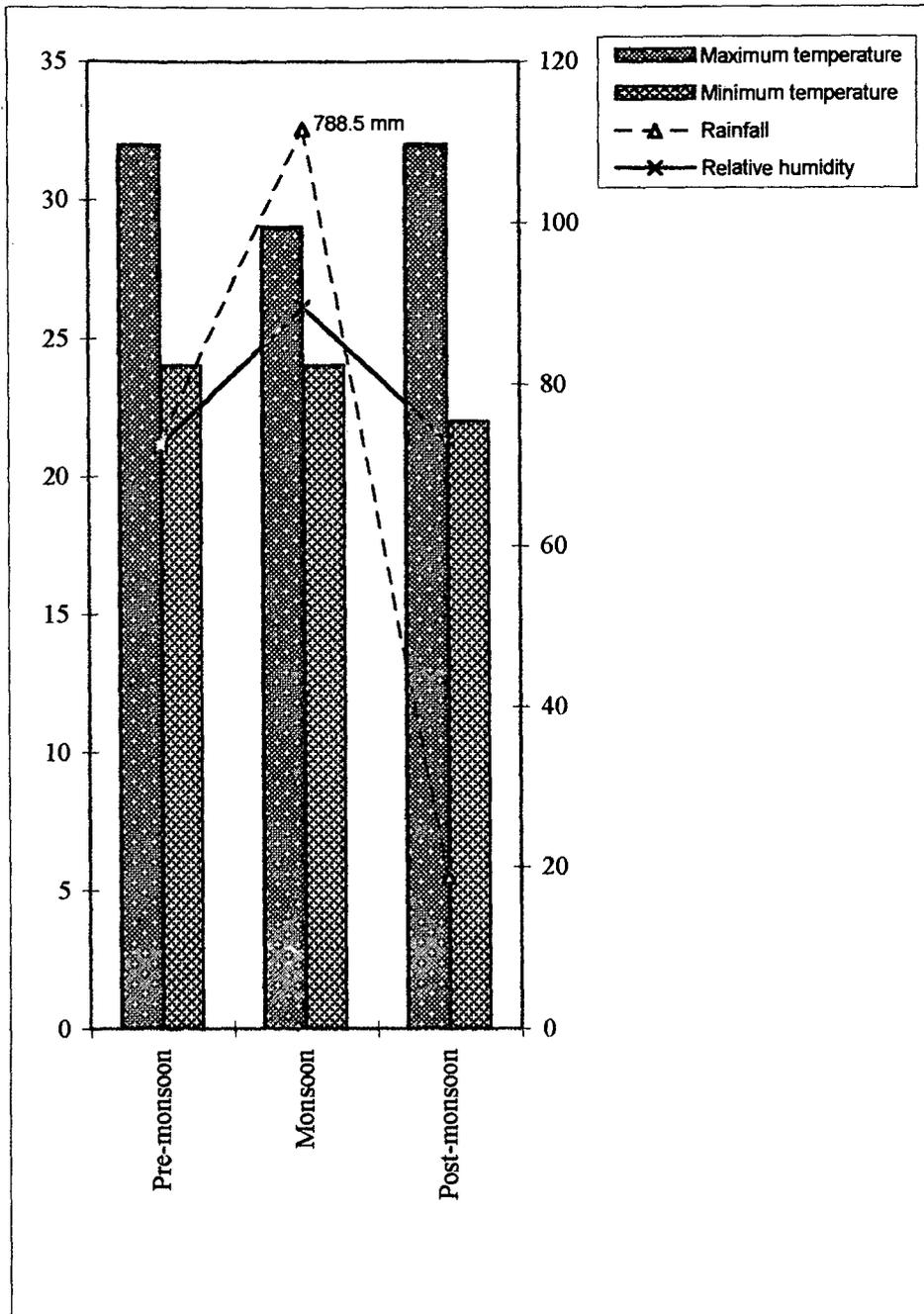


Fig.- 18. Climatic data at Codli iron ore mine site during pre-monsoon, monsoon and post-monsoon

Table- 22. Soil characteristics during pre-monsoon, monsoon and post-monsoon at Codli iron ore mine site.

| Season | Soil moisture (%) | pH | EC | Nutrients (mg 100 g ⁻¹) | | | | | | | OC(%) | OM(%) |
|--------------|-------------------|---------|----------|-------------------------------------|----------|----------|---------|----------|----------|---------|----------|-------|
| | | | | N | P | K | Ca | Mg | AP | | | |
| Pre-monsoon | 3.100 a | 6.400 a | 0.1047 b | 60.33 c | 117.83 c | 40.83 c | 5.94 b | 1.573 ab | 0.1443 a | 0.245 b | 0.4220 b | |
| Monsoon | 11.87 c | 6.300 a | 0.0775 a | 41.50 b | 93.833 a | 27.33 a | 3.867 a | 1.327 a | 0.0860 a | 0.037 a | 0.065 a | |
| Post-monsoon | 7.517 b | 6.633 b | 0.1038 b | 30.83 a | 102.50 b | 31,167 b | 5.338 b | 2.023 b | 0.0983 a | 0.082 a | 0.30 a | |
| F-statistics | ** | ** | NS | ** | ** | ** | ** | NS | NS | ** | NS | |

Significant at: ** - P < 0.01 and NS - Not significant.

Means followed by same letter(s) are not significantly different according to DMRT (P < 0.05)

EC- Electrical conductivity, AP- Available phosphorus, OC- Organic carbon and OM- Organic matter.

correlated to organic matter content. Electrical Conductivity (EC) was positively correlated to potassium, soil phosphorus and calcium. pH was negatively correlated to soil nitrogen (**Table- 23**).

MYCORRHIZAL COLONIZATION AND SPORE NUMBER

Total root length colonization was maximum in *Mimosa pudica* (46.40%) and was least in *Acacia mangium* (23.56%). Similar trend was observed for mean hyphal, arbuscular and vesicular colonization. The root length colonized by hyphae, vesicles and arbuscules exhibited variation between plant species. The mean spore number was higher in *Chromolaena odoratum* (172 spores 100 g⁻¹), *Mimosa pudica* (140 spores 100 g⁻¹), *Trema orientalis* (136 spores 100 g⁻¹) and *Ischaemum semisagittatum* (115 spores 100 g⁻¹) as compared to *Ludwigia parviflora* (73 spores 100 g⁻¹), *Acacia auriculiformis* (63 spores 100 g⁻¹), and *Acacia mangium* (54 spores 100 g⁻¹) for all the three seasons respectively (**Fig.- 19**).

PATTERN OF MYCORRHIZAL COLONIZATION AND ARBUSCULAR MYCORRHIZAL FUNGAL STRUCTURES

Mean colonization levels and the root length colonized by hyphae, arbuscules, vesicles and total root length colonized varied significantly between the seasons (**Fig.- 20-23**). Three way ANOVA on mycorrhizal colonization and arbuscular mycorrhizal fungal structures indicated significant variation in the colonization levels and structures between species, seasons, and species x seasons (**Table- 24**).

Table- 23. Correlation between edaphic variables at Codli iron ore mine site.

| Variable | | | | | | | | | | | |
|------------------------------|------------|---------|----------|----------|------------|---------|---------|--------|--------|----------|----|
| SM (%) | SM | | | | | | | | | | |
| pH | | pH | | | | | | | | | |
| E.C. | -0.705*** | 0.413 | EC | | | | | | | | |
| N (mg 100 g ⁻¹) | -0.605** | -0.525* | 0.189 | N | | | | | | | |
| P (mg 100 g ⁻¹) | -0.864**** | 0.087 | +0.541* | 0.335 | P | | | | | | |
| K (mg 100 g ⁻¹) | -0.872**** | -0.009 | +0.494* | +0.660** | +0.911**** | K | | | | | |
| Ca (mg 100 g ⁻¹) | -0.754**** | 0.364 | +0.629** | 0.403 | +0.478* | 0.429 | Ca | | | | |
| Mg (mg 100 g ⁻¹) | -0.189 | 0.463 | 0.082 | -0.344 | 0.159 | 0.241 | -0.104 | Mg | | | |
| OC (%) | -0.653** | -0.273 | 0.223 | +0.639** | +0.514* | +0.547* | +0.480* | 0.171 | OC (%) | | |
| OM (%) | -0.531* | 0.394 | 0.410 | 0.335 | +0.534* | +0.489* | 0.439 | 0.179 | 0.376 | OM (%) | |
| AP (mg 100 g ⁻¹) | -0.434 | 0.203 | 0.242 | 0.318 | 0.402 | 0.284 | 0.364 | -0.045 | 0.356 | +0.631** | AP |

Significant at : * - P < 0.05, ** - P < 0.01, *** - P < 0.001, **** - P < 0.000 and NS - Not significant respectively.

SM- Soil moisture, EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter and AP- Available phosphorus.

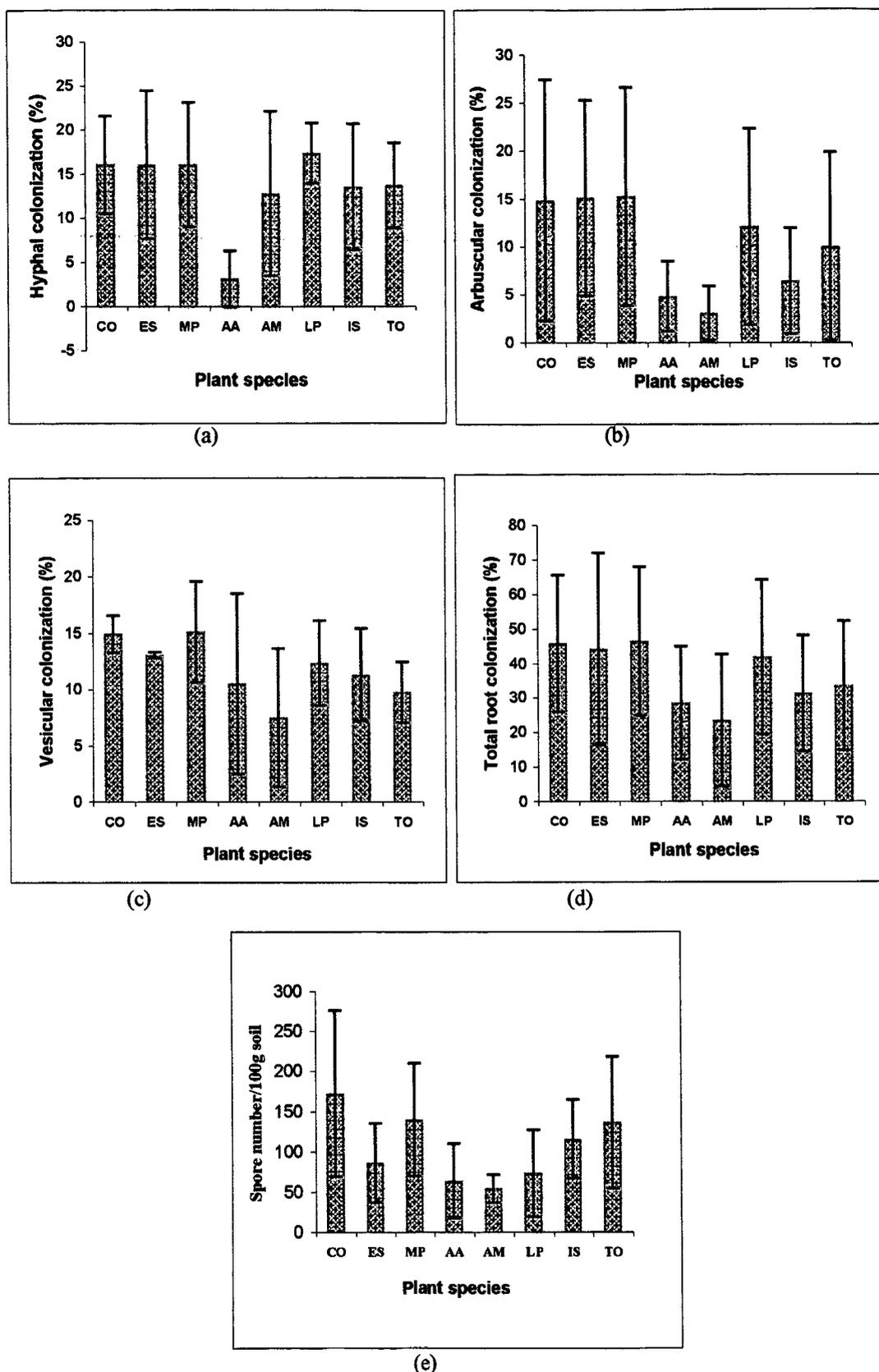


Fig. - 19. Mean value for the morphological characteristics of AM colonization (a-d) and spore number (e) for each species individually. *Chromolaena odoratum* (CO), *Emilia sonchifolia* (ES), *Mimosa pudica* (MP), *Acacia auriculiformis* (AA), *Acacia mangium* (AM), *Ludwigia parviflora* (LP), *Ischaemum semisagittatum* (IS) and *Trema orientalis* (TO). Error bar indicates ± 1 SD.

Table- 24. Three way analysis of variance (ANOVA) of the data on arbuscular mycorrhizal fungal structures and total colonization for eight plant species.

| | | d. f. | F ratio | Significance |
|--------------------------|------------|-------|---------|--------------|
| Plant Species (P) | | 7 | | |
| | Hyphae | | 2.70 | * |
| | Arbuscules | | 23.76 | ** |
| | Vesicles | | 7.14 | ** |
| | Total | | 19.90 | ** |
| Season (S) | | 2 | | |
| | Hyphae | | 47.17 | ** |
| | Arbuscules | | 195.98 | ** |
| | Vesicles | | 8.98 | ** |
| | Total | | 136.13 | ** |
| P x S | | 14 | | |
| | Hyphae | | 1.66 | NS |
| | Arbuscules | | 2.94 | ** |
| | Vesicles | | 4.06 | ** |
| | Total | | 1.78 | * |

Significant at: * - $P < 0.05$, ** - $P < 0.01$, and NS - Not significant
d. f. – Degrees of freedom.

A significant species x seasonal interaction for arbuscular mycorrhizal colonization and structures indicates that different plant species exhibit different patterns of arbuscular mycorrhizal colonization and arbuscular mycorrhizal fungal structures. Different patterns of mycorrhizal colonization were recorded for different seasons. All the plant species studied exhibited high levels of hyphal, and total root length colonization in monsoon followed by post- monsoon while, the colonization levels were least during pre- monsoon. Arbuscular colonization levels in all the plant species except *Acacia auriculiformis* were maximum during monsoon followed by post- monsoon while, the colonization levels were least during pre- monsoon. In *Acacia auriculiformis*, arbuscular colonization was maximum during post- monsoon followed by monsoon and was least during pre- monsoon. The vesicular colonization levels varied for all the plant species and were different for different season (Fig.- 20-23).

RELATIONSHIP BETWEEN ARBUSCULAR MYCORRHIZAL FUNGAL STRUCTURES

Among the various arbuscular mycorrhizal fungal structures, significant ($P < 0.05$, 0.01) positive correlation between hyphal and arbuscular colonization was observed in *Emilia sonchifolia*, *Mimosa pudica*, *Acacia mangium*, *Ischaemum semisagittatum* and *Trema orientalis*. Positive correlation between hyphal and vesicular colonization was recorded in *Acacia mangium* ($P < 0.001$), *Acacia auriculiformis* ($P < 0.01$), *Ischaemum semisagittatum* ($P < 0.01$) and *Trema orientalis* ($P < 0.05$). A significant ($P < 0.01$) positive correlation between hyphal and total root colonization was observed in *Chromolaena odoratum*, *Mimosa pudica* and *Ludwigia parviflora*

Hyphal colonization (%)

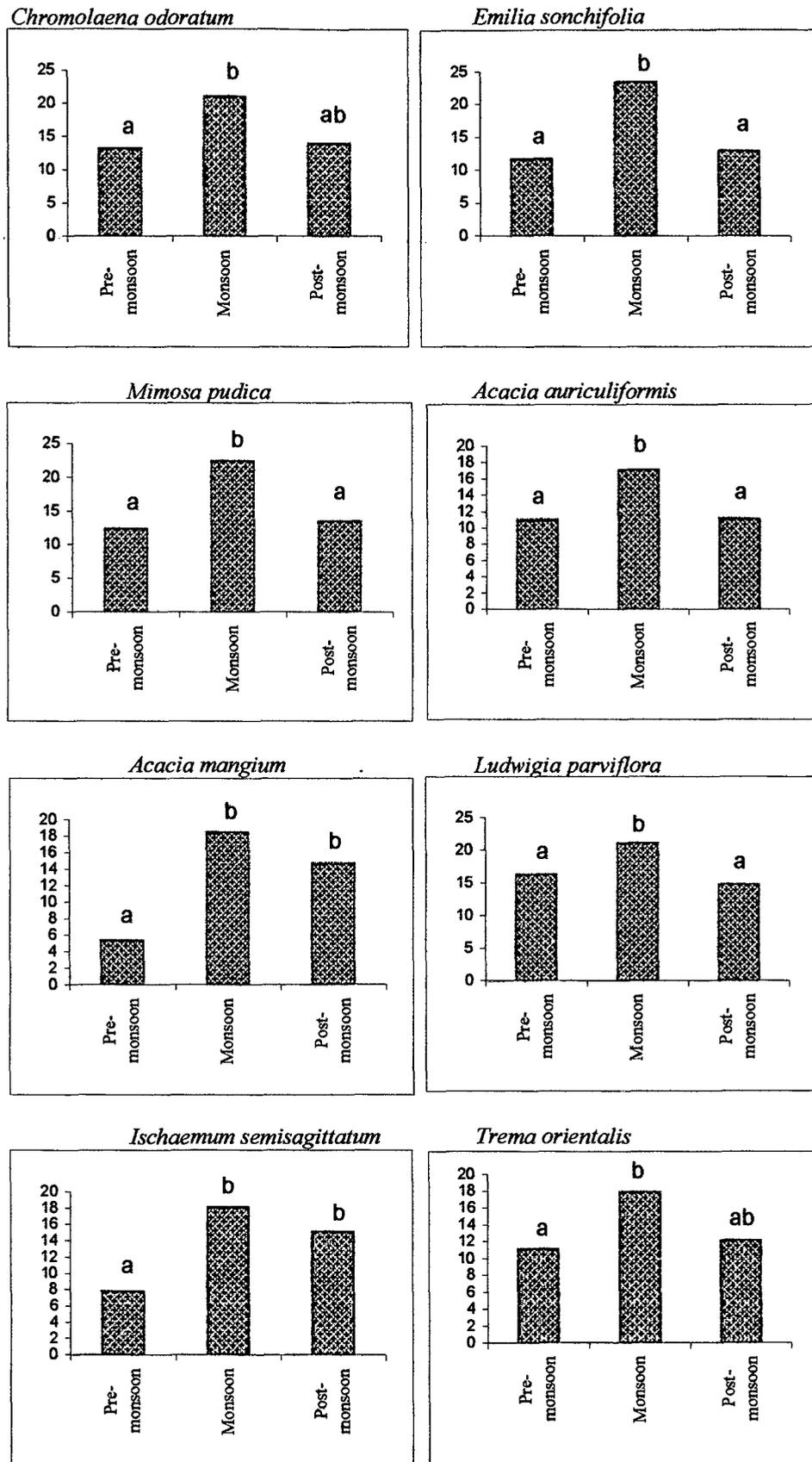


Fig.- 20. Mean hyphal colonization in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter(s) is not significant according to DMRT ($P < 0.05$).

Arbuscular colonization (%)

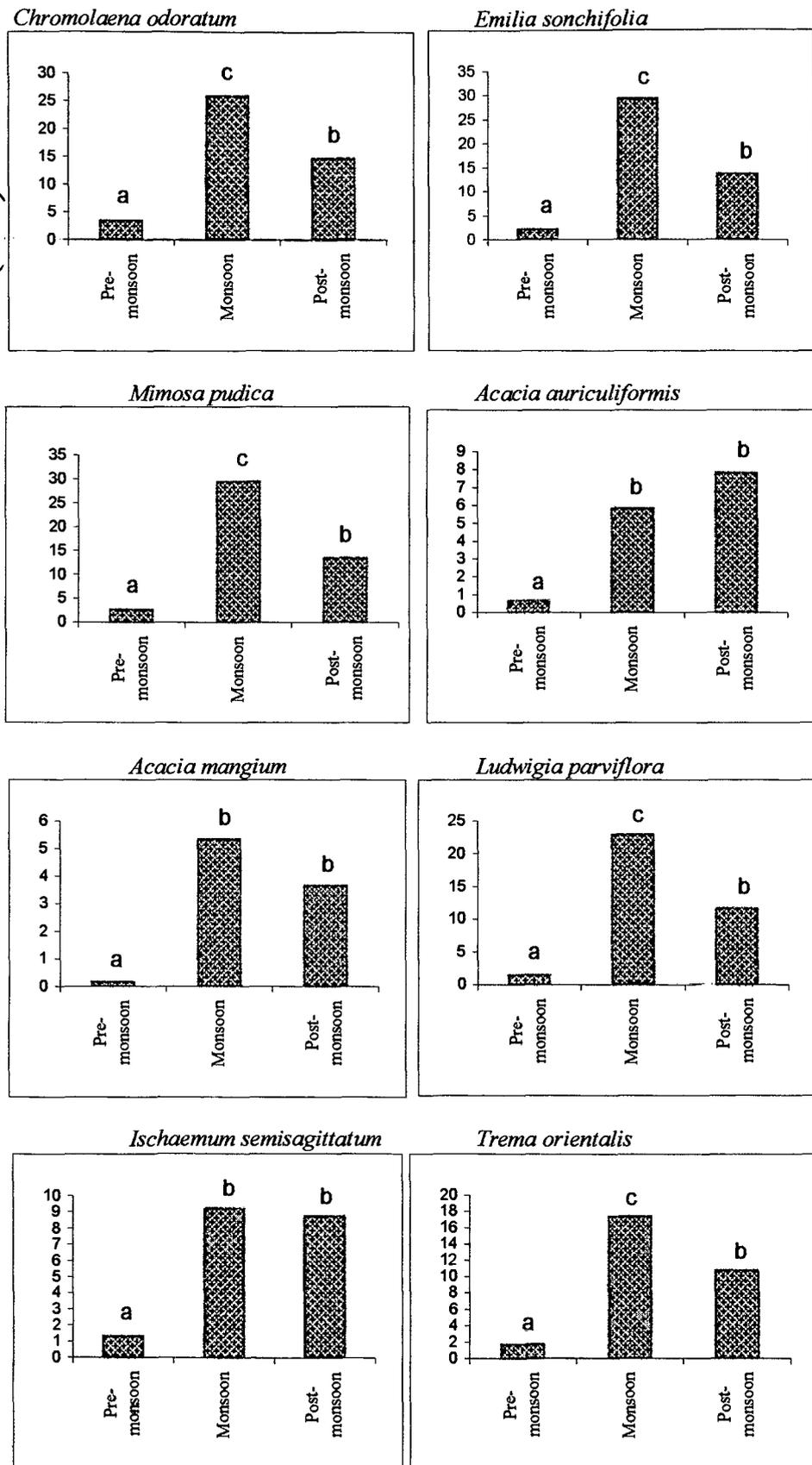


Fig.- 21. Mean arbuscular colonization in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter is not significant according to DMRT (P<0.05).

Vesicular colonization (%)

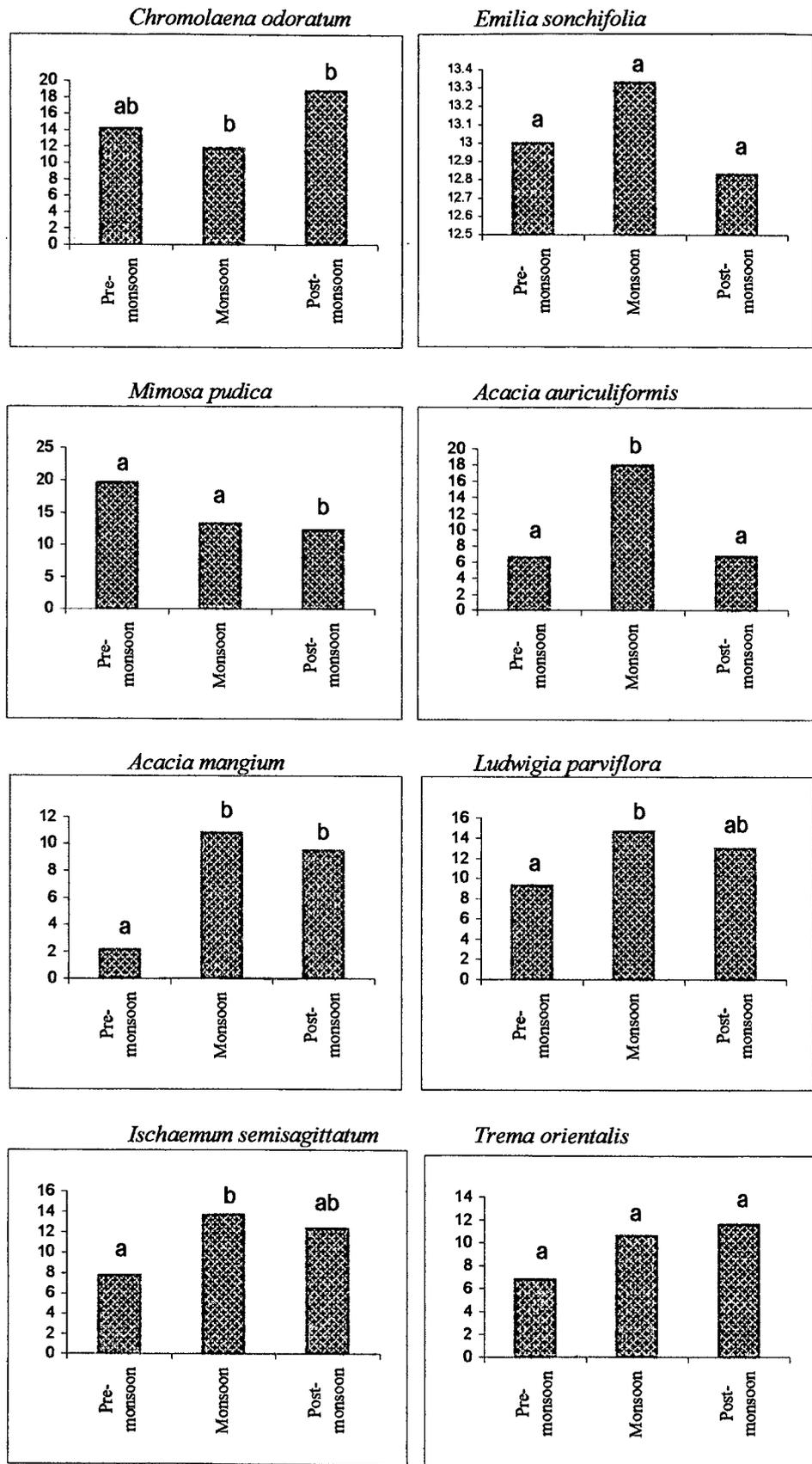


Fig.- 22. Mean vesicular colonization in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter(s) is not significant according to DMRT ($P < 0.05$).

Total root length colonization (%)

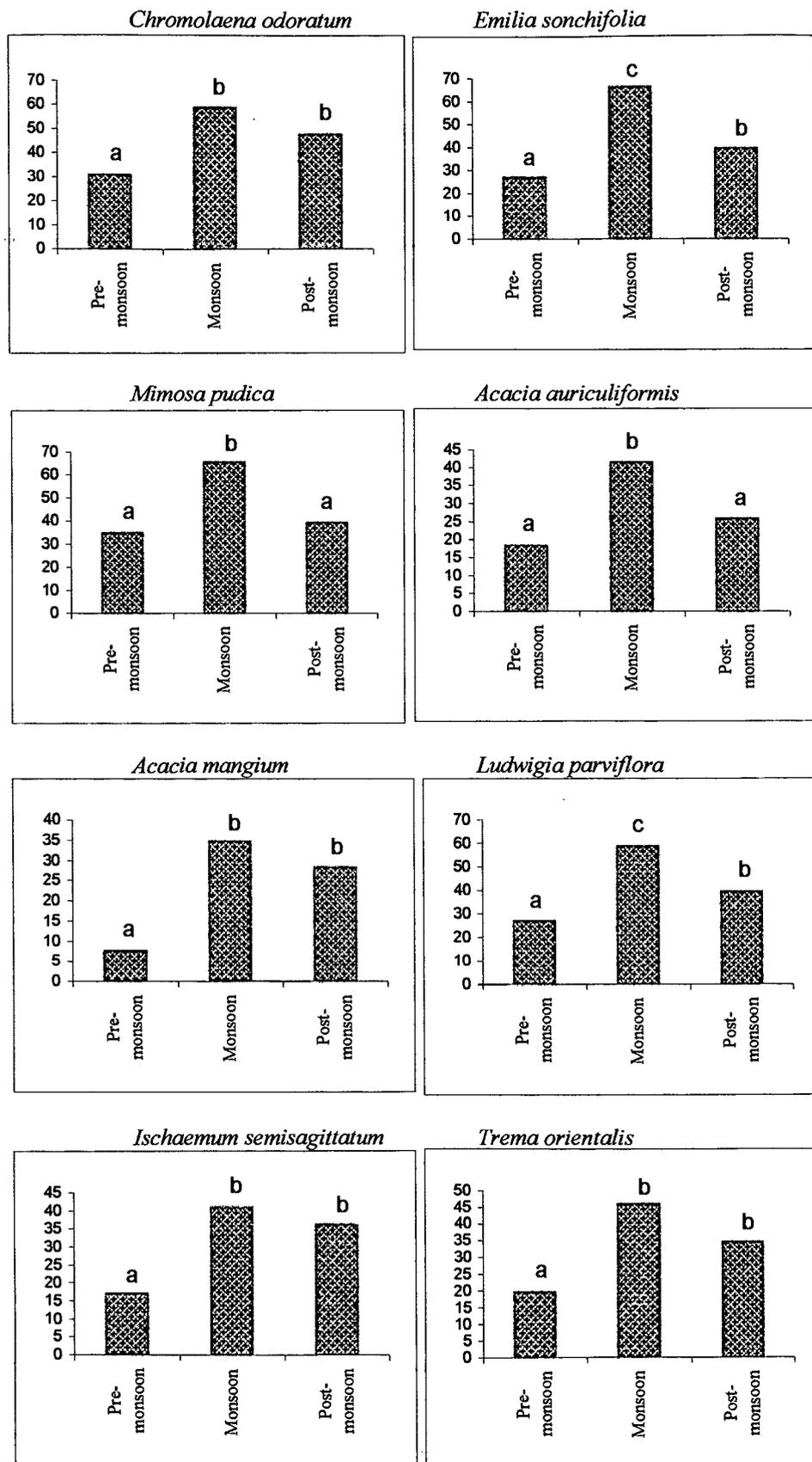


Fig.- 23. Mean total root colonization in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter is not significant according to DMRT ($P < 0.05$).

while, it was highly significant ($P < 0.000$) in *Emilia sonchifolia*, *Acacia auriculiformis*, *Acacia mangium*, *Ischaemum semisagittatum* and *Trema orientalis*. A highly significant ($P < 0.001$, 0.000) positive correlation was noted between arbuscular and total root length colonization in all the eight plant species. A highly significant positive correlation existed between arbuscular and vesicular colonization in *Acacia mangium* ($P < 0.000$), *Ludwigia parviflora* ($P < 0.01$) and *Ischaemum semisagittatum* ($P < 0.05$). A highly significant ($P < 0.01$, 0.000) positive correlation between vesicular and total root colonization was recorded in *Trema orientalis*, *Acacia auriculiformis*, *Acacia mangium*, *Ludwigia parviflora* and *Ischaemum semisagittatum* (Table- 25).

PATTERN OF MYCORRHIZAL SPORE NUMBER

Maximum spore density was recorded during pre-monsoon followed by post-monsoon and least was recorded during the monsoon season. Mean spore density for individual plant species varied significantly between the seasons (Fig.-24). There were highly significant differences in mean spore densities between species, seasons, and species x season together. The significant species x season interaction ($P < 0.01$) suggests the difference in seasonal pattern in spore number (Table- 26).

RELATIONSHIP BETWEEN SPORE NUMBER AND ARBUSCULAR MYCORRHIZAL COLONIZATION

Spore number and hyphal colonization were negatively correlated in *Emilia sonchifolia*, *Acacia mangium*, *Ischaemum semisagittatum* ($P < 0.01$) and *Mimosa pudica* ($P < 0.5$). A highly significant ($P < 0.01$, 0.000) negative

Table- 25. Pearson correlation coefficient for arbuscular mycorrhizal fungal structures.

| Plant species | H x A | H x V | H x T | A x V | A x T | V x T |
|---------------------------------|----------|------------|------------|------------|------------|------------|
| <i>Chromolaena odoratum</i> | 0.393 | -0.263 | +0.596** | -0.057 | +0.913**** | 0.162 |
| <i>Emilia sonchifolia</i> | +0.650** | -0.104 | +0.798**** | -0.010 | +0.892**** | 0.289 |
| <i>Mimosa pudica</i> | +0.488* | -0.353 | +0.688** | -0.317 | +0.849**** | 0.169 |
| <i>Acacia auriculiformis</i> | 0.362 | +0.670** | +0.813**** | 0.395 | +0.711*** | +0.876**** |
| <i>Acacia mangium</i> | +0.638** | +0.715**** | +0.907**** | +0.787**** | +0.850**** | +0.919**** |
| <i>Ludwigia parviflora</i> | 0.407 | 0.384 | +0.635** | +0.617** | +0.917**** | +0.794**** |
| <i>Ischaemum semisagittatum</i> | +0.515* | +0.594** | +0.854**** | +0.541* | +0.836**** | +0.774**** |
| <i>Trema orientalis</i> | +0.495* | +0.517* | +0.832**** | 0.298 | +0.837**** | +0.663** |

Significant at: * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$ and **** - $P < 0.000$.
H- Hypha, A- Arbuscule, V- Vesicle and T- Total root colonization respectively.

Spore number/100g rhizosphere soil

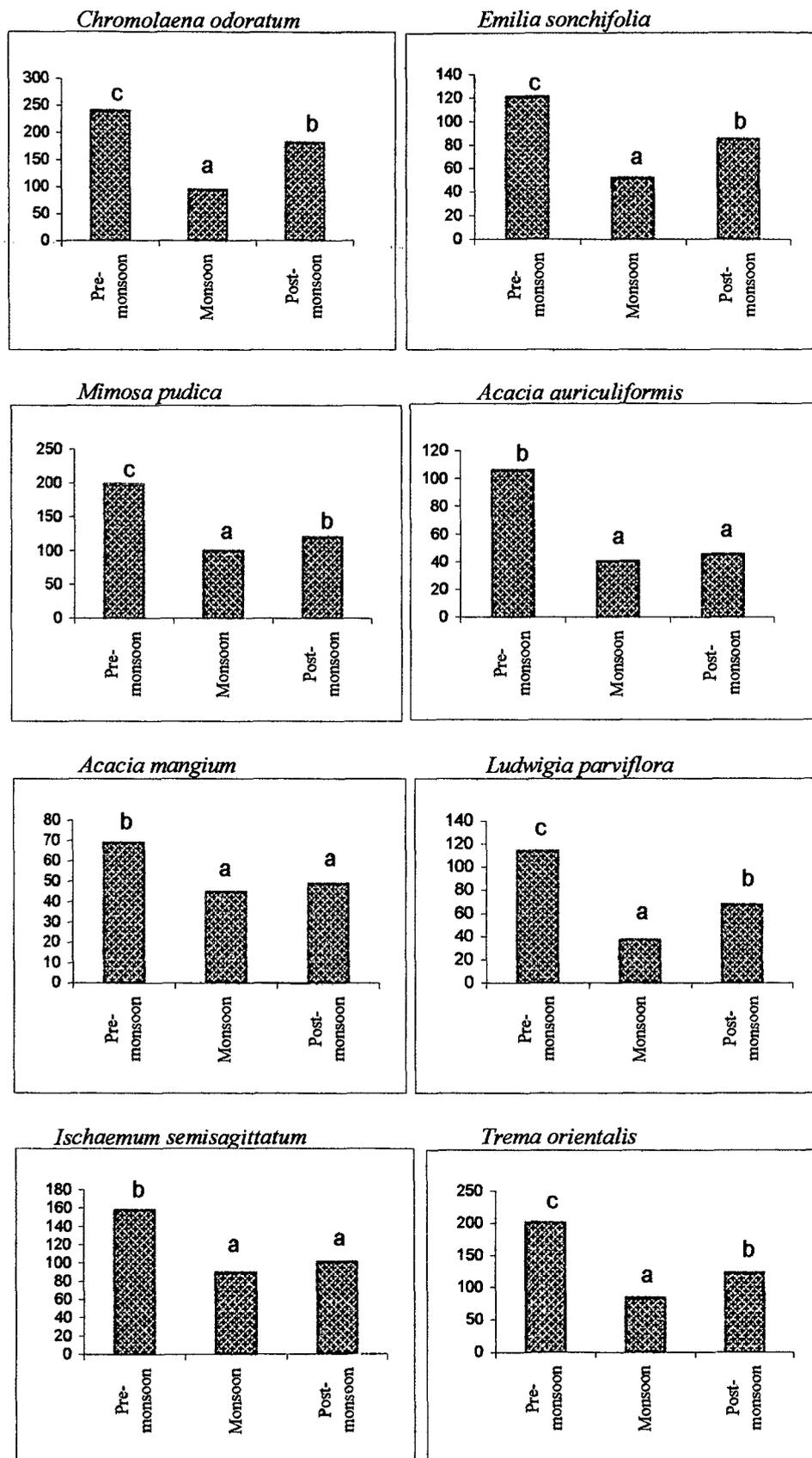


Fig.- 24. Mean spore numbers in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter is not significant according to DMRT (P<0.05).

Table- 26. Three-way analysis of variance (ANOVA) of the data on spore numbers of eight plant species for three season over a year.

| | d. f. | F ratio | Significance |
|--------------------------|--------------|----------------|---------------------|
| Plant species (P) | 7 | 79.18 | ** |
| Season (S) | 2 | 200.37 | ** |
| P x S | 14 | 3.23 | ** |

Significant at: ** - $P < 0.01$.
d. f.- Degrees of freedom.

correlation existed between spore number and arbuscular colonization in all the plant species. Vesicular colonization and spore numbers were negatively correlated to each other in *Acacia auriculiformis*, *Ischaemum semisagittatum*, *Ludwigia parviflora* ($P < 0.05$) and *Acacia mangium* ($P < 0.01$). Total root length colonization and spore number showed significant ($P < 0.01$, 0.001, 0.000) negative correlation in *Chromolaena odoratum*, *Mimosa pudica*, *Acacia auriculiformis*, *Trema orientalis*, *Acacia mangium*, *Emilia sonchifolia*, *Ischaemum semisagittatum* and *Ludwigia parviflora* at different levels of significance (Table- 27).

RELATIONSHIP BETWEEN ARBUSCULAR MYCORRHIZAL FUNGAL COLONIZATION AND EDAPHIC FACTORS

A highly significant ($P < 0.05$, 0.01, 0.001, 0.000) positive correlation existed between soil moisture and root length colonized by hyphae, arbuscules, vesicles, and total root length colonization in all the eight plant species (Table- 28-31).

Electrical Conductivity (EC), calcium, , organic matter. Total soil N, P, K, and organic carbon exhibited significant ($P < 0.05$, 0.01, 0.001, 0.000) negative correlation with hyphal colonization in *Acacia mangium*. A highly significant ($P < 0.05$, 0.01, 0.001) negative correlation existed between root length colonized by hyphae and organic carbon, calcium, soil N, K and P in *Ischaemum semisagittatum*. A significant ($P < 0.05$, 0.001) negative correlation existed between pH, Electrical Conductivity (EC) and root colonized by hyphae in *Ludwigia parviflora*. Available phosphorus exhibited significant

Table- 27. Correlation between arbuscular mycorrhizal (AM) fungal spore number and arbuscular mycorrhizal fungal structures.

| Mycorrhizal structures | Plant species | | | | | | | |
|------------------------|--------------------|-----------------------|------------------|--------------------------|-------------------|----------------------|--------------------------|----------------------|
| | <i>C. odoratum</i> | <i>E. sonchifolia</i> | <i>M. pudica</i> | <i>A. auriculiformis</i> | <i>A. mangium</i> | <i>L. parviflora</i> | <i>I. semisagittatum</i> | <i>T. orientalis</i> |
| Hyphae | -0.377 | -0.678** | -0.490* | -0.361 | -0.592** | -0.414 | -0.675** | -0.375 |
| Arbuscules | -0.755**** | -0.806**** | -0.837**** | -0.783**** | -0.604** | -0.895**** | -0.811**** | -0.845**** |
| Vesicles | 0.281 | -0.250 | 0.423 | -0.494* | -0.658** | -0.487* | -0.589* | -0.253 |
| Total colonization | -0.604** | -0.865**** | -0.657** | -0.680** | -0.703*** | -0.837**** | -0.840**** | -0.677** |

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000

C.- Chromolaena, E.- Emilia, M.- Mimosa, A.- Acacia, L.- Ludwigia, I.- Ischaemum, T.- Trema.

($P < 0.01$) negative correlation with hyphal colonization in *Chromolaena odoratum*. In *Emilia sonchifolia* hyphal colonization exhibited significant ($P < 0.05, 0.01$) negative correlation with Electrical Conductivity (EC), P and K. Organic carbon and K showed negative ($P < 0.05$) correlation with root length colonized by hyphae in *Mimosa pudica*. In *Trema orientalis* hyphal colonization exhibited significant ($P < 0.05, 0.01$) negative correlation with calcium and Electrical Conductivity (EC) (Table- 28).

Root length colonized by arbuscules showed significant ($P < 0.05, 0.01, 0.001, 0.000$) negative correlation with soil N, P, K and organic carbon in all the plant species. A significant ($P < 0.05, 0.01$) negative correlation was observed between arbuscular colonization and organic carbon in all the plant species. Available phosphorus and arbuscular colonization exhibited negative correlation in *Ischaemum semisagittatum* ($P < 0.05$). A significant ($P < 0.05, 0.01$) negative correlation existed between Electrical Conductivity (EC) and arbuscular colonization in all the plant species except *Acacia auriculiformis*. Similarly, arbuscular colonization exhibited significant ($P < 0.05, 0.01$) negative correlation with calcium in *Chromolaena odoratum*, *Mimosa pudica*, *Acacia mangium*, *Emilia sonchifolia*, *Ludwigia parviflora* and *Trema orientalis* (Table- 29).

A highly significant ($P < 0.05, 0.01, 0.001$) negative correlation existed between vesicular colonization and soil phosphorus and, vesicular colonization and soil potassium in *Ischaemum semisagittatum*, *Ludwigia parviflora*, *Trema orientalis* and *Acacia auriculiformis*. A significant ($P < 0.05,$

Table- 28. Correlation between root length colonized by hyphae and edaphic variables.

| Factors | Plant species | | | | | | | |
|------------------------------|--------------------|-----------------------|------------------|--------------------------|-------------------|----------------------|--------------------------|----------------------|
| | <i>C. odoratum</i> | <i>E. sonchifolia</i> | <i>M. pudica</i> | <i>A. auriculiformis</i> | <i>A. mangium</i> | <i>L. parviflora</i> | <i>I. semisagittatum</i> | <i>T. orientalis</i> |
| SM (%) | +0.520* | +0.670** | +0.588** | +0.547* | +0.852**** | +0.507* | +0.739**** | +0.492* |
| pH | -0.390 | -0.381 | -0.147 | -0.387 | 0.021 | -0.493* | 0.031 | -0.194 |
| EC | -0.427 | -0.512* | -0.324 | -0.296 | -0.486* | -0.703*** | -0.444 | -0.674** |
| N (mg 100 g ⁻¹) | -0.057 | -0.204 | -0.181 | -0.010 | -0.756**** | 0.115 | -0.597** | -0.210 |
| P (mg 100 g ⁻¹) | -0.457 | -0.576* | -0.462 | -0.534* | -0.734*** | -0.377 | -0.700*** | -0.375 |
| K (mg 100 g ⁻¹) | -0.304 | -0.683** | -0.557* | -0.550* | -0.802**** | -0.336 | -0.630** | -0.262 |
| Ca (mg 100 g ⁻¹) | -0.383 | -0.445 | -0.388 | -0.311 | -0.654** | -0.411 | -0.656** | -0.558* |
| Mg (mg 100 g ⁻¹) | -0.203 | -0.370 | -0.385 | -0.642** | -0.080 | -0.362 | -0.066 | 0.082 |
| OC (%) | -0.085 | -0.175 | -0.529* | -0.300 | -0.745**** | -0.078 | -0.545* | -0.318 |
| OM (%) | -0.418 | -0.262 | -0.329 | -0.352 | -0.712*** | -0.007 | -0.208 | -0.057 |
| AP (mg 100 g ⁻¹) | -0.637** | -0.170 | -0.039 | -0.067 | -0.443 | -0.124 | -0.177 | -0.124 |

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000

SM- Soil moisture, EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter and AP- Available phosphorus.

C.- *Chromolaena*, *E.*- *Emilia*, *M.*- *Mimosa*, *A.*- *Acacia*, *L.*- *Ludwigia*, *I.*- *Ischaemum*, *T.*- *Trema*.

Table- 29. Correlation between root length colonized by arbuscules and edaphic variables.

| Factors | Plant species | | | | | | | |
|------------------------------|--------------------|-----------------------|------------------|--------------------------|-------------------|----------------------|--------------------------|----------------------|
| | <i>C. odoratum</i> | <i>E. sonchifolia</i> | <i>M. pudica</i> | <i>A. auriculiformis</i> | <i>A. mangium</i> | <i>L. parviflora</i> | <i>I. semisagittatum</i> | <i>T. orientalis</i> |
| SM (%) | +0.834**** | +0.936**** | +0.908**** | +0.640** | +0.765**** | +0.931**** | +0.640** | +0.890**** |
| pH | 0.051 | -0.172 | -0.056 | 0.393 | 0.174 | -0.023 | 0.070 | 0.035 |
| EC | -0.581** | -0.608** | -0.691** | -0.319 | -0.500* | -0.656** | -0.483* | -0.614** |
| N (mg 100 g ⁻¹) | -0.634** | -0.591** | -0.541* | -0.718*** | -0.585** | -0.648** | -0.699*** | -0.657** |
| P (mg 100 g ⁻¹) | -0.734*** | -0.889**** | -0.822**** | -0.691** | -0.627** | -0.881**** | -0.647** | -0.738**** |
| K (mg 100 g ⁻¹) | -0.810**** | -0.916**** | -0.795**** | -0.785**** | -0.655** | -0.926**** | -0.661** | -0.753**** |
| Ca (mg 100 g ⁻¹) | -0.522* | -0.661** | -0.576* | -0.237 | -0.490* | -0.615** | -0.402 | -0.675** |
| Mg (mg 100 g ⁻¹) | -0.176 | -0.223 | -0.179 | -0.012 | -0.071 | -0.126 | -0.092 | -0.006 |
| OC (%) | -0.553* | -0.628** | -0.555* | -0.585** | -0.626** | -0.665** | -0.487* | -0.681** |
| OM (%) | -0.486* | -0.609** | -0.468 | -0.166 | -0.233 | -0.547* | -0.456 | -0.420 |
| AP (mg 100 g ⁻¹) | -0.154 | -0.355 | -0.345 | 0.008 | -0.127 | -0.366 | -0.529* | -0.467 |

Probability levels: * - P < 0.05, ** - P < 0.01, *** - P < 0.001, **** - P < 0.000

SM- Soil moisture, EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter and AP- Available phosphorus.
C.- *Chromolaena*, *E.*- *Emilia*, *M.*- *Mimosa*, *A.*- *Acacia*, *L.*- *Ludwigia*, *I.*- *Ischaemum*, *T.*- *Trema*.

0.01, 0.000) positive correlation existed between vesicular colonization and soil moisture in *Ischaemum semisagittatum*, *Ludwigia parviflora*, *Acacia mangium* and *Acacia auriculiformis*. Vesicular colonization and Electrical Conductivity (EC) exhibited significant ($P < 0.000$) negative correlation in *Acacia auriculiformis*. A significant ($P < 0.05, 0.01$) negative correlation was observed between soil nitrogen and vesicular colonization in *Acacia mangium*, *Ischaemum semisagittatum* and *Trema orientalis* while, it exhibited significant ($P < 0.01$) positive correlation in *Mimosa pudica* (Table- 30).

Soil P, K, and Electrical Conductivity (EC) exhibited significant ($P < 0.05, 0.01, 0.001, 0.000$) negative correlation with total root length colonization in all the plant species except *Ischaemum semisagittatum* where phosphorus exhibited positive ($P < 0.000$) correlation with total colonization. Soil organic matter and total root length colonization exhibited significant ($P < 0.05$) negative correlation in *Chromolaena odoratum* and *Acacia mangium*. Organic carbon and total root length colonization exhibited significant ($P < 0.05, 0.01, 0.000$) negative correlation in *Ischaemum semisagittatum*, *Ludwigia parviflora*, *Acacia auriculiformis*, *Trema orientalis* and *Acacia mangium*. A significant ($P < 0.01, 0.001$) negative correlation existed between calcium and total root length colonization in *Mimosa pudica*, *Acacia auriculiformis*, *Trema orientalis*, *Ischaemum semisagittatum*, *Ludwigia parviflora*, and *Emilia sonchifolia* (Table- 31).

RELATIONSHIP BETWEEN SPORE NUMBER AND EDAPHIC FACTORS

Mean spore numbers exhibited significant ($P < 0.01, 0.001, 0.000$) negative correlation with soil moisture in all the eight plant species. Spore numbers

Table- 30. Correlation between root length colonized by vesicles and edaphic variables.

| Factors | Plant species | | | | | | | |
|------------------------------|--------------------|-----------------------|------------------|--------------------------|-------------------|----------------------|--------------------------|----------------------|
| | <i>C. odoratum</i> | <i>E. sonchifolia</i> | <i>M. pudica</i> | <i>A. auriculiformis</i> | <i>A. mangium</i> | <i>L. parviflora</i> | <i>I. semisagittatum</i> | <i>T. orientalis</i> |
| SM (%) | -0.171 | -0.052 | 0.375 | +0.780**** | +0.646** | +0.533* | +0.535* | 0.353 |
| pH | 0.421 | 0.172 | -0.256 | -0.453 | 0.253 | -0.009 | -0.022 | 0.150 |
| EC | 0.329 | -0.210 | 0.055 | -0.755** | -0.255 | -0.126 | -0.406 | -0.116 |
| N (mg 100 g ⁻¹) | -0.335 | -0.002 | +0.598** | -0.068 | -0.678** | -0.348 | -0.473* | -0.490* |
| P (mg 100 g ⁻¹) | 0.207 | -0.048 | 0.401 | -0.665** | -0.583* | +0.548* | -0.586* | -0.461 |
| K (mg 100 g ⁻¹) | 0.008 | -0.100 | +0.552* | -0.648** | -0.711*** | -0.674** | -0.534* | -0.349 |
| Ca (mg 100 g ⁻¹) | 0.160 | 0.022 | 0.160 | -0.568* | -0.331 | -0.287 | -0.354 | -0.281 |
| Mg (mg 100 g ⁻¹) | 0.334 | 0.293 | 0.086 | -0.451 | -0.241 | -0.251 | -0.000 | 0.221 |
| OC (%) | -0.114 | 0.013 | +0.534* | -0.307 | -0.734*** | -0.433 | -0.267 | -0.447 |
| OM (%) | -0.116 | 0.189 | 0.309 | -0.261 | -0.304 | -0.125 | -0.240 | -0.335 |
| AP (mg 100 g ⁻¹) | -0.154 | 0.291 | 0.135 | -0.127 | -0.077 | -0.112 | -0.373 | -0.347 |

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000

SM- Soil moisture, EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter and AP- Available phosphorus.

C.- *Chromolaena*, *E.*- *Emilia*, *M.*- *Mimosa*, *A.*- *Acacia*, *L.*- *Ludwigia*, *I.*- *Ischaemum*, *T.*- *Trema*.

Table- 31. Correlation between total root length colonization and edaphic variables.

| Factors | Plant species | | | | | | | |
|------------------------------|--------------------|-----------------------|------------------|--------------------------|-------------------|----------------------|--------------------------|----------------------|
| | <i>C. odoratum</i> | <i>E. sonchifolia</i> | <i>M. pudica</i> | <i>A. auriculiformis</i> | <i>A. mangium</i> | <i>L. parviflora</i> | <i>I. semisagittatum</i> | <i>T. orientalis</i> |
| SM (%) | +0.0749**** | +0.835**** | +0.781**** | +0.807**** | +0.852**** | +0.881**** | +0.762**** | +0.788**** |
| pH | -0.015 | -0.275 | -0.300 | -0.210 | 0.150 | -0.233 | 0.001 | -0.048 |
| EC | -0.481* | -0.671** | -0.644** | -0.592** | -0.475* | -0.644** | -0.527* | -0.646** |
| N (mg 100 g ⁻¹) | -0.558* | -0.389 | -0.166 | -0.308 | -0.784**** | -0.413 | -0.692** | -0.569* |
| P (mg 100 g ⁻¹) | -0.630** | -0.792**** | -0.661** | -0.785 | -0.751**** | -0.807**** | +0.748**** | -0.682** |
| K (mg 100 g ⁻¹) | -0.708*** | -0.798**** | -0.605** | -0.813**** | -0.837**** | -0.857**** | -0.716*** | -.605** |
| Ca (mg 100 g ⁻¹) | -0.461 | -0.593** | -0.539* | -0.467 | -0.569 | -0.619** | -0.585** | -0.693** |
| Mg (mg 100 g ⁻¹) | -0.185 | -0.250 | -0.344 | -0.460 | -0.124 | -0.287 | 0.038 | 0.104 |
| OC (%) | -0.460 | -0.462 | -0.398 | -0.480* | -0.771**** | -0.548* | -0.531* | -0.621** |
| OM (%) | -0.562* | -0.445 | -0.394 | -0.333 | -0.509* | -0.411 | -0.360 | 0.373 |
| AP (mg 100 g ⁻¹) | -0.360 | -0.174 | -0.197 | -0.036 | -0.256 | -0.271 | -0.405 | -0.441 |

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000

SM- Soil moisture, EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter and AP- Available phosphorus.

C.- *Chromolaena*, E.- *Emilia*, M.- *Mimosa*, A.- *Acacia*, L.- *Ludwigia*, I.- *Ischaemum*, T.- *Trema*.

showed significant ($P < 0.05$, 0.001, 0.000) positive correlation with percent organic carbon, Electrical Conductivity, total soil phosphorus, potassium and calcium in *Chromolaena odoratum*. While Electrical Conductivity, N, P, K, percent organic carbon showed significant ($P < 0.05$, 0.01, 0.001) positive correlation with spore number in *Mimosa pudica*, *Acacia auriculiformis*, *Trema orientalis*, *Ludwigia parviflora*, *Emilia sonchifolia* and *Acacia mangium*. In *Ischaemum semisagittatum* significant ($P < 0.05$, 0.01, 0.001, 0.000) positive correlation was noted between spore number with available phosphorus, percent organic carbon, total soil N, K and P. Spore numbers exhibited significant ($P < 0.05$) positive correlation with organic matter in *Ludwigia parviflora* (Table- 32).

ARBUSCULAR MYCORRHIZAL FUNGAL SPORES

A total of 42 arbuscular mycorrhizal fungal species belonging to five genera viz., *Acaulospora*, *Gigaspora*, *Glomus*, *Paraglomus* and *Scutellospora* were reported during the study of which 10 arbuscular mycorrhizal fungal species were common to all the three seasons. As regards the species richness, the Genus *Glomus* was dominant in the pre-monsoon season, the Genus *Acaulospora* was dominant in the monsoon season, while the Genus *Scutellospora* was dominant in the post-monsoon season (Table- 33). The arbuscular mycorrhizal fungal species viz., *Acaulospora spinosa* Walker & Trappe, *Acaulospora scrobiculata* Trappe, *Gigaspora margarita* Becker & Hall, *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker and Koske, *Glomus geosporum* (Nicolson & Gerdemann) Walker, *Glomus macrocarpum* Tul. & Tul., *Scutellospora gregaria* (Schenck & Nicolson)

Table- 32. Correlation between arbuscular mycorrhizal (AM) fungal spore number and edaphic variables.

| Factors | Plant species | | | | | | | |
|------------------------------|--------------------|-----------------------|------------------|--------------------------|-------------------|----------------------|--------------------------|----------------------|
| | <i>C. odoratum</i> | <i>E. sonchifolia</i> | <i>M. pudica</i> | <i>A. auriculiformis</i> | <i>A. mangium</i> | <i>L. parviflora</i> | <i>I. semisagittatum</i> | <i>T. orientalis</i> |
| SM (%) | -0.912**** | -0.894**** | -0.868**** | -0.817**** | -0.653** | -0.913**** | -0.735*** | -0.897**** |
| pH | 0.265 | 0.094 | -0.140 | -0.162 | -0.056 | 0.202 | -0.035 | 0.021 |
| EC | +0.695*** | +0.708*** | +0.535* | +0.528* | 0.460 | +0.697*** | 0.465 | +0.497* |
| N (mg 100 g ⁻¹) | 0.395 | +0.525* | +0.686** | +0.785**** | +0.597** | +0.578** | +0.625** | +0.551* |
| P (mg 100 g ⁻¹) | +0.758**** | +0.683** | +0.845**** | +0.735*** | 0.467 | +0.766**** | +0.796**** | +0.859**** |
| K (mg 100 g ⁻¹) | +0.753**** | +0.699*** | +0.864**** | +0.816**** | +0.628** | +0.817**** | +0.727*** | +0.854**** |
| Ca (mg 100 g ⁻¹) | +0.770**** | +0.735*** | +0.556* | +0.616** | +0.575** | +0.809**** | 0.454 | +0.612** |
| Mg (mg 100 g ⁻¹) | 0.222 | 0.149 | 0.113 | -0.072 | -0.065 | 0.085 | -0.022 | 0.242 |
| OC (%) | +0.566* | +0.621** | +0.697*** | +0.646** | +0.491* | +0.576* | +0.490* | +0.682** |
| OM (%) | 0.427 | 0.298 | 0.347 | 0.365 | 0.148 | +0.521* | 0.384 | 0.347 |
| AP (mg 100 g ⁻¹) | 0.205 | 0.206 | .185 | 0.157 | 0.161 | 0.239 | +0.477* | 0.231 |

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000

SM- Soil moisture, EC- Electrical conductivity, OC- Organic carbon, OM- Organic matter and AP- Available phosphorus.

C.- *Chromolaena*, *E.*- *Emilia*, *M.*- *Mimosa*, *A.*- *Acacia*, *L.*- *Ludwigia*, *I.*- *Ischaemum*, *T.*- *Trema*.

Table- 33. Occurrence of arbuscular mycorrhizal (AM) fungal species during pre-monsoon, monsoon and post-monsoon.

| Arbuscular mycorrhizal fungal genera | Number of arbuscular mycorrhizal fungal species | | |
|--------------------------------------|---|-----------|--------------|
| | Pre-monsoon | Monsoon | Post-monsoon |
| <i>Acaulospora</i> | 05 | 11 | 03 |
| <i>Gigaspora</i> | 03 | 01 | 04 |
| <i>Glomus</i> | 16 | 10 | 06 |
| <i>Paraglomus</i> | 01 | - | - |
| <i>Scutellospora</i> | 05 | 04 | 07 |
| Total AM fungal species. | 30 | 26 | 20 |

Table- 34. Seasonal variation in frequency of occurrence of arbuscular mycorrhizal fungal species in selected host plants from iron ore mine wasteland at Codli.

| Arbuscular mycorrhizal fungal species | Frequency (%) | | |
|---|-----------------|---------|------------------|
| | Pre- monsoon | Monsoon | Post- monsoon |
| <i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss | - | 25 | - |
| <i>Acaulospora foveata</i> Trappe & Janos | 25 | - | - |
| <i>Acaulospora laevis</i> Gerd. & Trappe | - | 25 | 12 |
| <i>Acaulospora longula</i> Spain & Schenck | - | 12.5 | - |
| <i>Acaulospora mellea</i> Spain & Schenck | - | 12.5 | - |
| <i>Acaulospora morrowiae</i> Spain & Schenck | - | 12.5 | - |
| <i>Acaulospora rhemii</i> Sieverding & Toro | 25 | 12.5 | - |
| <i>Acaulospora scrobiculata</i> Trappe | 100 | 60 | 37 |
| <i>Acaulospora spinosa</i> Walker & Trappe | 50 | 62.5 | 50 |
| <i>Acaulospora undulata</i> Sieverding | 12 | 12.5 | - |
| <i>Gigaspora albida</i> Schenck & Smith | 12 | - | 12 |
| <i>Gigaspora decipiens</i> Hall & Abbott | - | - | 12 |
| <i>Gigaspora margarita</i> Becker & Hall | 75 | 62.5 | 75 |
| <i>Gigaspora rosea</i> Nicolson & Schenck | 12 | - | 12 |
| <i>Glomus aggregatum</i> Schenck & Smith emend. Koske | 12 | - | - |
| <i>Glomus albida</i> Schenck & Smith | 12 | - | 12 |
| <i>Glomus claroideum</i> Schenck & Smith emend Walker & Vestberg. | 12 | 25 | - |
| <i>Glomus claviforme</i> (Trappe) Almeida & Schenck, <i>comb. nov.</i> | - | 12.5 | - |
| <i>Glomus constrictum</i> Trappe | 37 | - | 25 |
| <i>Glomus coremioides</i> (Berk. & Broome) Redecker et Morton, <i>comb. nov.</i> | 12 | - | - |
| <i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker and Koske, | 62 | 50 | 50 |
| <i>Glomus formosanum</i> Wu & Chen | 12 | - | - |
| <i>Glomus geosporum</i> (Nicol. & Gerd.) Walker | 62 | 37.5 | 37 |
| <i>Glomus globiferum</i> Koske & Walker | 37 | 25 | - |
| <i>Glomus hoi</i> Berch & Trappe | 12 | - | - |
| <i>Glomus macrocarpum</i> Tul. & Tul. | 75 | 75 | 75 |

Cont.

| | | | |
|---|----|------|-----|
| <i>Glomus magnicaule</i> Hall | 12 | - | - |
| <i>Glomus mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe | 12 | 12.5 | - |
| <i>Glomus multicaule</i> Gerdemann & Bakshi | 12 | - | - |
| <i>Glomus rubiforme</i> (Gerd. & Trappe) Almeida & Schenck, <i>comb. nov.</i> | 25 | - | 25 |
| <i>Glomus sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck, <i>comb. nov.</i> | 12 | 12.5 | - |
| <i>Glomus taiwanensis</i> (Wu & Chen) Almeida & Schenck, <i>comb. nov.</i> | 37 | 37.5 | - |
| <i>Paraglomus occultum</i> (Walker) Morton & Redecker | 12 | - | - |
| <i>Scutellospora calospora</i> (Nicol. & Gerd.) Walker & Sanders | - | - | 12 |
| <i>Scutellospora gregaria</i> (Schenck & Nicol.) Walker & Sanders | 75 | 62.5 | 100 |
| <i>Scutellospora nigra</i> (Redhead) Walker & Sanders | - | - | 12 |
| <i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders | 62 | 75 | 37 |
| <i>Scutellospora persica</i> Koske & Walker | 37 | - | 12 |
| <i>Scutellospora reticulata</i> (Koske, Miller & Walker) Walker & Sanders | 25 | 25 | 50 |
| <i>Scutellospora weresubiae</i> Koske & Walker | 25 | 12.5 | 25 |

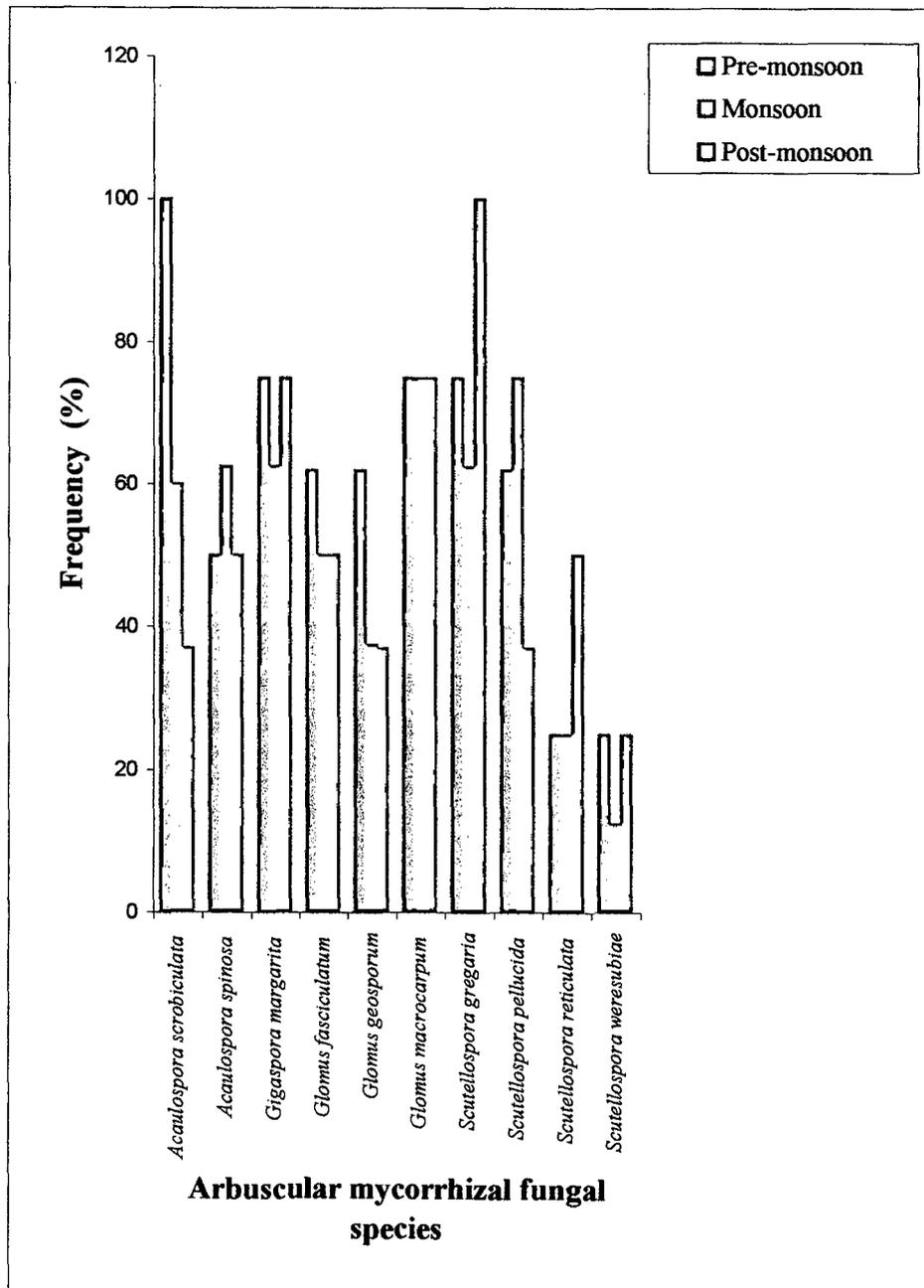


Fig.- 25. Frequency of occurrence of arbuscular mycorrhizal fungal species common to pre-monsoon, monsoon and post-monsoon.

Walker & Sanders, *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders, *Scutellospora reticulata* (Koske, Miller & Walker) Walker & Sanders, and *Scutellospora weresubiae* Koske & Walker commonly occurred in all the three seasons (Fig.- 25 and Table- 34).

DISCUSSION

The result of the present study indicated that spore production and mycorrhizal colonization was strongly influenced by environmental factors together with the host species and soil type. Similar results have been obtained earlier by Udaiyan *et al.*, (1996) and Muthukumar *et al.*, (1998). Within environmental factors, seasonal variations play an important role in influencing mycorrhizal symbiosis.

Present study revealed that among the climatic factors, rainfall and Relative Humidity (RH) played an important role in root colonization and spore formation. Redhead (1975) found that colonization by arbuscular mycorrhizal fungi was lower under naturally low rainfall than under adequate rainfall. Similarly, Michelini *et al.*, (1993) in their studies on *Citrus* related the arbuscular mycorrhizal fungal presence in the roots to high rainfall.

In the present investigation it was observed that arbuscular mycorrhizal colonization reduced during dry season (pre-monsoon). Results reported in the present study are in agreement with Newman *et al.*, (1986) who found reduction in percentage of arbuscular mycorrhizal colonization in the roots of three species of Savanna grasses during dry season in Kenya. In Barbados,

Chinnery *et al.*, (1987) observed a reduction in both percentage and intensity of arbuscular mycorrhizal fungal colonization in the roots of sugarcane early in the dry season. While, Giovannetti (1985) in Italian sand dunes found reduced arbuscular mycorrhizal fungal colonization during summer drought period. Thus, various arbuscular mycorrhizal fungi may respond differently to temperature changes. Soil temperature might alter the physiology of mycorrhizal symbiosis by influencing root morphology, their nutrition and growth. The influence of temperature on arbuscular mycorrhizal fungal colonization and sporulation has been reported by earlier workers (Furlan and Fortin, 1973; Allen, 1983; Udaiyan *et al.*, 1996).

The results of percent root colonization and spore density indicated that extent of mycorrhizal colonization (hyphal, arbuscular, vesicular and total) and spore density varied significantly in pre-monsoon, monsoon and post-monsoon season. Similarly, Muthukumar *et al.*, (1998) reported within-season variations of arbuscular mycorrhizal colonization and spore abundance in the rhizosphere soils in relation to edaphic factors in eight wild legumes. In the present study the colonization levels in most of the plant species varied when the plants were considered individually indicating that different plants species are colonized by different arbuscular mycorrhizal fungal species. This confirms the earlier findings of Gerdemann, (1965) and, Manjunath and Bagyaraj, (1982) who stated that the extent to which plants respond to colonization varies with the plant species. Friese and Allen (1991) and Sanders and Fitter, (1992) suggested that the structure and intensity of arbuscular mycorrhizal colonization varies within plant populations and

depends on factors such as type and spatial availability of inoculum, season, stage of plant development, susceptibility to inoculation and plant nutritional status. The differences in spore number between plant species were also reported by Kruckelmann, (1975), who found significant difference among number of spores in six different plants growing in monoculture for 16 years. Similarly, the influence of host plant on incidence of arbuscular mycorrhizal fungi has also been observed by Schenck and Kinloch (1980) on a woodland site newly planted with 6 agronomic crops and grown in monoculture for 7 years. Hayman, (1975) and Iqbal *et al.*, (1975) also recorded difference in spore numbers between plant species. Variations in spore population in different plant species have been reported earlier (Sparling and Tinker, 1975; Bhaskaran and Selvaraj, 1996; Muthukumar *et al.*, 1998).

In the present study, soil moisture showed positive correlation with mycorrhizal colonization and negative correlation with spore density. Occurrence of maximum arbuscular colonization during monsoon suggests that low temperature may favour its formation as suggested by Schenck and Schroder, (1974). During this period, the fungus is actively involved in nutrient transfer. The production of vesicles increased at the end of the maximum root growth and the formation of new arbuscules ceased and the older ones disintegrated. As a result, the arbuscular colonization was minimum in pre-monsoon during which vesicles became more prominent in the cortex of older roots. Similar observations have been recorded earlier (Saif and Khan, 1975; Mason, 1964).

Thus, proportion of arbuscules to vesicles was influenced by season. High percent of arbuscular mycorrhizal colonization correlates with the active growth of the host plants (Van Duin *et al.*, 1989; Ietswaart *et al.*, 1992, Allen, 1983, Wallace, 1987). Optimum moisture for plant growth may favour mycorrhizal formation due to availability of new roots, which in turn stimulates arbuscular mycorrhizal fungal spore germination as a result maximum colonization has been recorded during monsoon. The changes in colonization pattern with soil moisture are in agreement with Braunberger *et al.*, (1994) who has shown that proportion of root length colonized by hyphae and arbuscules could fluctuate with soil moisture.

Spore density was maximum in all the plants during pre-monsoon and declined in monsoon and post-monsoon. Similar observations have been recorded earlier by Hetrick and Bloom, (1983); Furlan and Fortin (1973) and Hayman, (1970) who suggested that high soil temperature favours sporulation. Maximum spore number in summer in herbs indicates that the senescent roots and the available nutrients in the soil stimulate fungal reproduction as the nutrient requirements by the plants is reduced during these period. The spore population recorded in monsoon suggests that spore germination occurred when the plant roots were growing actively. These results are in accordance with Mason, (1964) who has made similar observations. Similarly, Mason *et al.*, (1992) and Raghupathy and Mahadevan, (1993) have attributed the decrease in spore abundance in rainy season to increased intra-and extamatrixal mycelium which favours spore germination. Spore number in the present study was negatively correlated to hyphal, vesicular, arbuscular and

total mycorrhizal colonization. Khalil *et al.*, (1992) reported a negative correlation between spore number and arbuscular colonization and attributed this to fungal life cycle.

Soil phosphorus was negatively correlated to mycorrhizal colonization. The low soil phosphorus is known to increase root colonization by arbuscular mycorrhizal fungi (Mosse and Hayman, 1971). Muthukumar *et al.*, (1994) and (1998), and Udaiyan *et al.*, (1996) stated that soil phosphorus can reduce arbuscular mycorrhizal (AM) formation and the inhibition may be due to direct effect on the external hyphal growth. While, Sanders (1975) suggested that inhibition of arbuscular mycorrhizal colonization may be indirectly associated with host phosphorus status. Ratnayake *et al.*, (1978) and Graham *et al.*, (1981) have suggested that root colonization by arbuscular mycorrhizal fungi is inhibited at high phosphorus levels because of decreased root exudation. In the present study, spore density was positively correlated to soil phosphorus in all the plant species. Similar results were obtained by Udaiyan *et al.*, (1996).

Soil nitrogen was negatively correlated to mycorrhizal colonization and positively correlated to spore number. These results are in accordance with Redhead, (1975) who found that nitrogen strongly affects mycorrhizal colonization in *Khaya grandifolia*, whereas it enhanced the number of spores in the rhizosphere. Similarly Thomazini, (1974) found that Brazilian soils low in Ca, P, S, N and Zn were associated with good arbuscular mycorrhizal colonization.

In the present study, no marked effect of pH on root colonization and spore number was observed. This results are in agreement with Hayman, (1978); Medina *et al.*, (1988); Johnson *et al.*, (1991); Khalil and Loynachan, (1994) who found no relation between soil pH on either root colonization or spore number. However, a negative correlation between arbuscular mycorrhizal colonization and organic carbon, and organic matter was recorded in the present study. These results are in agreement with Nicolson (1960) who has reported decrease in mycorrhizal colonization with increasing organic matter content.

In the present study, potassium had a negative effect on root colonization. These results are in accordance with Muthukumar *et al.*, (1998). However, the spore number was positively correlated to potassium. Calcium exhibited negative correlation with arbuscular mycorrhizal fungal colonization and positively correlation with arbuscular mycorrhizal fungal spore numbers which suggests that calcium fluctuation in rhizosphere governs the vegetation and reproductive phases of arbuscular mycorrhizal fungi in the mine wastelands. Anderson *et al.*, (1984) reported negative correlation between calcium and plant root length colonization and magnesium and plant root length colonization.

Compared to phosphate and nitrogen, the role of other nutrients on root colonization and spore number of arbuscular mycorrhizal fungi is little known (Marschner and Dell, 1994). Most of the studies on mineral nutrients of plants through arbuscular mycorrhizal fungi come from the experimental work with

agricultural crops and associated arbuscular mycorrhizal fungi. The vegetation and arbuscular mycorrhizal fungi on mine wastelands that are adapted to nutrient deficiencies and stress may have different mechanisms of regulation and exchange of nutrients at the fungus-root interface. Further studies on the function of arbuscular mycorrhizal fungi in nutrient deficient soil may reveal more about the uptake and requirement of nutrients by plants growing on iron ore mine wasteland and arbuscular mycorrhizal fungi.

The total numbers of arbuscular mycorrhizal fungal spore recorded were maximum in pre-monsoon. These results are in agreement with Guadarrama and Alvarez-Sanchez (1999) who observed highest numbers of species and spores of arbuscular mycorrhizal fungi during dry season, with a marked decrease during the rainy season. During the study period, *Glomus* was the most dominating Genera followed by *Acaulospora* and *Scutellospora*. Whereas, during monsoon, *Acaulospora* were more in number followed by *Glomus* and *Scutellospora*. During post-monsoon, *Scutellospora* was most dominant Genera. In the present study, variation in spore population in the soil has been noted which suggest that many species of arbuscular mycorrhizal fungi are worldwide in distribution and possibly the strains and spores may be temperature adapted. In the present study, different species were recovered in different seasons. These results are in accordance with Graham *et al.*, (1982) and Tommerup, (1983) who observed that isolates may differ in their optimum temperature for germination, root colonization and spore production. In the present study a large number of *Acaulospora* species were reported during monsoon while, *Gigaspora* and *Scutellospora* were more in pre-

monsoon and post-monsoon. Thus, the increased soil temperature during pre-monsoon and post-monsoon may favour the formation of *Gigaspora* and *Scutellospora*. These results are in accordance with Daniels and Trappe, (1980) who has made similar observation. Similarly, seasonality in the development of arbuscular mycorrhizal fungi includes the period when the spores are, likely to be released in the soil and presence of some immature spores suggests some production of spores throughout the year (Hayman, 1970).

This study clearly indicates that seasonal variations have a remarkable influence on the occurrence and distribution of arbuscular mycorrhizal fungal spore number and root colonization together with the host species and soil type. Thus, arbuscular mycorrhizal fungi may be of considerable importance to the success of mine wastelands stabilization programmes indicating a good scope for further intensive work which would throw more light on the ecology of arbuscular mycorrhizal fungi on mine wastelands.

CHAPTER-VI

**TAXONOMY OF
ARBUSCULAR MYCORRHIZAL (AM) FUNGI FROM IRON
ORE MINE WASTELANDS AND THE SURROUNDING
UNDISTURBED AREAS.**

INTRODUCTION

Until recently, the order Endogonales (Zygomycotina) has consisted of only one family, the Endogonaceae (Benjamin, 1979; Morton, 1988). Several genera *Acaulospora* Gerdemann and Trappe emend. Berch, *Entrophospora* Ames and Schneider, *Gigaspora* Gerdemann and Trappe, *Glomus* Tulasne and Tulasne, *Sclerocystis* Berkeley 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 Pirozynski and Dalpe containing two Genera, *Glomus* and *Sclerocystis*. Morton and Benny (1990) emended the family Glomaceae and erected a new order Glomales and two new families Acaulosporaceae and Gigasporaceae. Recently, Schubler *et al.*, (2001), based on comprehensive SSU rRNA analysis, and on the basis of natural relationship of arbuscular mycorrhizal fungi and the related fungi, recognized a new fungal phylum *Glomeromycota* with a single class *Glomeromycetes* (Cavalier-Smith, 1998) circumscribed as for the phylum, containing more than 150 described species, some of which are synonyms (Walker and Trappe, 1993; Walker and Vestberg, 1998).

(Table- 35)- Classification of arbuscular mycorrhizal (AM) fungi
(Glomeromycota) (Schubler *et al.*, 2001)

- **Phylum**
 - Class
 - Order
 - Family
 - Genus
- **Glomeromycota**
 - Glomeromycetes
 - Glomerales
 - Glomeraceae
 - *Glomus A.*
 - *Glomus B.*
 - Paraglomerales
 - Paraglomeraceae
 - *Paraglomus*
 - Diversisporales
 - Diversisporaceae *fam. ined.*
 - *Diversispora*
 - Gigasporaceae
 - *Gigaspora*
 - *Scutellospora*

- **Acaulosporaceae**
 - *Acaulospora*
 - *Enterophospora*

 - Archaeosporales
 - **Archaeosporaceae**
 - *Archaeospora*
 - **Geosiphonaceae**
 - *Geosiphon*
-

Phylum: Glomeromycota C. Walker and Schuessler, **phylum nov.** (Schubler *et al.*, 2001).

Fungi with coenocytic to sparsely estate mycelium, living mostly hypogenously, sometimes epigenously. Forming chlamydospores (in some genera) by blastic development of hyphal tip followed by thickening of structural wall components and occlusion by septum, spore- wall thickening, or deposition of an amorphous plug in the lumen of the subtending (sporogenous) hypha and spore. *Complex spores* (in some genera) with a rigid, chitinous structural wall component within a blastic terminal saccule, or by extension of a bulbous base, with or without flexible wall components. *Spores* produced singly, in loose clusters, in tight clusters (without a structured peridium), in sporocarps (with peridial development) or within the roots of the plants.

Class: Glomeromycetes Cavalier-Smith (1998).

Fungi with coenocytic to sparsely septate mycelium. Forming chlamydospores by blastic development. Spores produced singly, in loose clusters, in tight clusters, in sporocarps or within the roots of the plant.

Order: Glomerales (Morton and Benny, 1990)

Fungi mostly hypogeous, sometimes epigeous, forming endomycorrhizas or mycorrhiza-like symbiosis within spore, vesicles and/or arbuscules in plants. *Hyphae* of vegetative mycelium mostly non-septate, though forming septa on older hyphae as cytoplasm is withdrawn or cut off resting spores. *Asexual* reproduction by chlamydospores (termed glomoid spores by Morton and Redecker 2001), mainly terminal, but sometimes intercalary. *Spores* solitary or formed in clusters, or in sporocarps. Differing from other arbuscular mycorrhizal fungi by possession of the rRNA SSU gene sequence signature

Paraglomerales C. Walker and Schuessler **ord. nov.**

Fungi hypogeous, forming endomycorrhizas with arbuscules and intradical mycelium, rarely with vesicles. Producing glomoid spores lacking pigmentation. Differing from other arbuscular mycorrhizal fungi by possession of the rRNA SSU gene sequence signature.

Diversisporales C. Walker and Schuessler **ord. nov.**

Fungi hypogeous, forming endomycorrhizas with arbuscules, often lacking vesicles. With or without hypogeous auxiliary cells. Forming either complex spores produced within a sporiferous saccule (acaulosporoid spore of Morton

and Redecker, 2001), complex spores ('sporangioles'?) developing from a bulbous base on the sporiferous hypha (termed here gigasporoid spores). Differing from the other arbuscular mycorrhizal fungi by possession of the rRNA SSU gene sequence signature.

Archaeosporales C. Walker and Schuessler **ord. nov.**

Fungi hypogenous, forming endocytosymbioses with photoautotrophic prokaryotes, or producing mycorrhizas with arbuscules, with or without vesicles. Spores lacking pigmentation or reaction to Melzer's reagent. Glomoid spores formed singly or in loose clusters on or in the soil, acaulosporoid complex spores formed singly in the soil. Dense spore clusters unknown. Differing from the other arbuscular mycorrhizal fungi by possession of the rRNA SSU gene sequence signature.

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.

Family: Paraglomeraceae Morton et Redecker, **fam. Nov.** (Morton and Redecker, 2001).

Studies of other molecular characters, such as monoclonal antibody specificities (Wright *et al.*, 1987) and fatty acid profiles (Graham *et al.*, 1995) have led to the long-held suspicion that members of this family were

phylogenetically distant from species in *Acaulospora* and *Glomus*, but all of these data did not contain enough phylogenetic information to determine its position relative to other glomalean taxa. Even morphological characters were atypical for the *Acaulospora*-like spores (Morton and Benny, 1990). Small subunit (18S) ribosomal DNA sequences finally provided the character set to position this family as ancestral to both Acaulosporaceae and Glomaceae (Redecker *et al.*, 2000a; Sawaki *et al.*, 1998). The molecular phylogenetic tree suggests that members of this family and Paraglomaceae are more closely related to a nonmycorrhizal fungus, *Geosiphon pyriforme*, a putative Zygomycete fungus, which is the mycobiont in a symbiosis with a *Nostoc* cyanobacterium (the photobiont).

Vegetative structures consist of those inclusive in the suborder Glomineae. There are several characteristics unique to this family, however, in mycorrhizal structures.

Molecular characters which appear to be diagnostic for members of this family:

- (1) Monoclonal antibody cell lines B5 and F8, maintained in the laboratory of Sara Wright (Wright *et al.*, 1987).
- (2) Signature DNA sequence at the 3' end of the 5.8S ribosomal gene: GGCATGTCTGTTTGAGGGCACCA separates members of this family from those of Glomaceae.
- (3) The primer sequence TGCTAAATAGCCAGGCTGY (ARCH1311) also will selectively amplify species of this family.

Genus: *Paraglomus* Morton et Redecker, (Morton and Redecker, 2001).

Development of spores proceeds exactly like that found in *Glomus* (Glomaceae): by blastic expansion of a hyphal tip as shown below. Outer layer of the spore wall often sloughs as the spore ages (in soil or in pot culture storage). Developmentally, this layer is the first component of the spore wall to form in juvenile spores. Usually, organic material will accumulate on the surface; no reaction in Melzer's reagent.

The hypha subtending the spore differentiates at the same rate and synthesizes the same component layers as that found in the spore wall. In some species the subtending hypha of mature spores is so thin that it is hard to see or separates from the spore.

Only two species have been discovered in this genus, both having been classified first as *Glomus* species (based on spore morphology). Faint to invisible mycorrhizal structures provide morphological clues of possible membership in this genus.

Family: Archaeosporaceae Morton et Redecker, fam. nov. (Morton and Redecker, 2001).

Studies of other molecular characters, such as monoclonal antibody specificities (Wright *et al.*, 1987) and fatty acid profiles (Graham *et al.*, 1995) have led to the long-held suspicion that members of this family were phylogenetically distant from species in *Acaulospora* and *Glomus*, but all of

these data did not contain enough phylogenetic information to determine its position relative to other glomalean taxa. Even morphological characters were atypical for the *Acaulospora*-like spores (Morton and Benny, 1990). Small subunit (18S) ribosomal DNA sequences finally provided the character set to position this family as ancestral to both Acaulosporaceae and Glomaceae (Redecker *et al.*, 2000a; Sawaki *et al.*, 1998). The molecular phylogenetic tree suggests that members of this family and Paraglomaceae are more closely related to a nonmycorrhizal fungus, *Geosiphon pyriforme*, a putative Zygomycete fungus which is the mycobiont in a symbiosis with a *Nostoc* cyanobacterium (the photobiont).

Vegetative structures consist of those inclusive in the suborder Glomineae. There are several characteristics unique to this family, however, in mycorrhizal structures.

All species in this family can be distinguished from other glomalean families (including Paraglomaceae) by the signature nucleotide sequence: TCTCKKYTTTCGGYSGAGTCC at position 227 of the 18S rDNA gene (Morton and Redecker, 2001). The degeneracy in this sequence reflects variation among species in the group and has the positive benefit of providing a more lenient diagnostic tool for discovering other species

Genus: Archaeospora Morton et Redecker (Morton and Redecker, 2001).

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All species form *spores* borne laterally from the neck of a pre-differentiated "sporiferous saccule", but mode of formation either is like that of species in *Acaulospora* or from a branch hypha (or "pedicel") to form a *Glomus*-like spore. Some species are dimorphic, forming both acaulosporoid and glomoid spore morphotypes.

Three modes of spore formation occur in this family:

- (1) The spore develops from the side of the subtending hypha of a "sporiferous saccule" similar to that of species in *Acaulospora* (Acaulosporaceae). When the spore detaches, it is sessile (no attached subtending hypha).
- (2) A hypha ("pedicel") first branches from the subtending hypha of a "sporiferous saccule" and the spore develops much like that of species of *Glomus* (Glomaceae) terminally by blastic expansion from this pedicel.
- (3) Species with *Glomus*-like spores forming from a "sporiferous saccule" (2 above) also form separate *Glomus*-like spores directly from external hyphae, some of which also can form saccules (see photo at right).

Spores formed on the fungal thallus are either monomorphic or dimorphic. When monomorphic only acaulosporoid spores are formed. When dimorphic, both acaulosporoid and glomoid spores are formed.

Family: Acaulosporaceae (Stummer and Morton, 1999)

Azygospores formed or within the neck of a sporiferous saccule.

Genus: *Acaulospora* Gerdemann & Trappe (1974) emend. Berch (1985)

Spores arise laterally from the neck of a sporiferous saccule. In this genus, spores are borne laterally from the neck of a pre-differentiated "sporiferous saccule". The *sporiferous saccule* develops blastically from a hyphal tip. After the saccule has become fully expanded, a spore begins to develop from the side of the subtending hypha (termed "saccule neck"). As the spore matures, the saccule loses its contents and eventually sloughs off so that it often is not attached to fully mature spores.

Genus: *Enterophospora* Ames & Schneider (1979)

Spores formed in the neck of the sporiferous saccule.

In this genus, spores are borne from within the neck of a pre-differentiated "sporiferous saccule". Ontogenesis of spore and inner walls mirrors that found in spores of *Acaulospora*.

Family: Glomeraceae

Genus: *Glomus* A

***Glomus* B**

Fungi with coenocytic to sparsely septate mycelium, living mostly hypogeuously, sometimes epigeously. Forming chlamydospores by blastic development of hyphal tip. *Spores* produced singly, in loose clusters, in tight

clusters (without a structured peridium), in sporocarps (with peridial development) or within the roots of plants.

Family: Gigasporaceae Bentivenga and Morton (1995).

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

Genus: *Gigaspora* Gerdemann & Trappe (1974) emend. Walker & Sanders (1986).

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

Spores develop blastically from a hyphal tip, which swells and becomes the "sporogenous cell". After the sporogenous cell reaches its full size (usually about 25-50 μm in most species), the spore begins to develop at the tip. The outer layer and the laminate layer develop simultaneously, and often cannot be distinguished in juvenile spores without the assistance of Melzer's reagent. The laminae then thicken and ultimately develop the warty inner layer, from which multiple germ tubes arise

Genus: *Scutellospora* Walker & Sanders (1986)

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

Early stages in spore development mirror those in the sister genus, *Gigaspora*. Spores develop blastically from a hyphal tip, which swells and becomes the "sporogenous cell". After the sporogenous cell reaches its full size (usually about 25-50 μm in most species), the spore begins to develop at

the tip. The outer layer and the laminate layer develop simultaneously, and often cannot be distinguished in juvenile spores without the assistance of Melzer's reagent. The laminae then thicken and the outer ornamentations develop (if present in the species). Inner walls develop and the last stage is the formation of the germination shield.

The objective of the present work is to describe the morphological features of the arbuscular mycorrhizal fungal spores recovered and identified from the mine wastelands and undisturbed areas of Xelpi and Bimbol iron ore mine.

MATERIALS AND METHODS

Rhizosphere soil samples from various iron ore mines sites *viz.*, Xelpi, Codli, Sonshi, Bimbol and Sanquelim and, the surrounding undisturbed areas of Xelpi and Bimbol were collected from a depth of 15-20 cm in polyethylene bags and were brought to the laboratory and were stored at 4⁰C till further processing.

The spores were isolated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Microscopic observation was done by mounting the spores in polyvinyl alcohol lacto-glycerol (PVLG) (Koske and Tessier, 1983), with or without Melzer's reagent. Spores were transferred from filter paper to microscopic slides and mounted in (PVLG). Intact and crushed spores in polyvinyl alcohol lacto-glycerol (PVLG) with or without Melzer's reagent were examined under Leica compound microscope. Taxonomic identification of spores to species level was based on spore

morphology, ornamentation, wall characteristics using various bibliographies (Schenck and Perez, 1990; Almeida and Schenck, 1990; Walker and Vestberg, 1998; Redecker, *et al.*, 2000b; Morton and Redecker, 2001; Schubler *et al.*, 2001) by matching original descriptions and those provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (<http://invam.caf.wvu.edu>). Spore colour was examined under Leica stereomicroscope using intact spores immersed in water.

Spores in the rhizosphere soil were multiplied using *Eleusine coracana* (L.) Gaertn., *Lycopersicon esculentum* Mill., *Allium cepa* L. and *Coleus sp.* as host plants (Plate- XXVI). The spores isolated from the trap culture were later used for confirming the identified spores recovered during the study period.

RESULTS

In the present study a total of 60 arbuscular mycorrhizal fungal species belonging to six genera *viz.*, *Acaulospora* (13 species), *Archaeospora* (1 species), *Gigaspora* (4 species), *Glomus* (32 species), *Paraglomus* (1 species) and *Scutellospora* (9 species) were recorded. Further, two of the total 60 arbuscular mycorrhizal fungal species recorded in the present study did not fit into the known descriptions and has been described as unidentified species.

In the present investigation, spores of *Glomus microaggregatum* Koske & Gemma inhabited the spores of *Glomus macrocarpum* Tul. & Tul. and, spores and sporocarps of *Glomus sinuosum* with numbers varying

from 1 to 10. The inhabited spores were found to be fresh, while the occupied spores were old and devoid of spore contents (**Plate- XXV**).

DISTINCT MORPHOLOGICAL FEATURES OF THE ARBUSCULAR MYCORRHIZAL (AM) FUNGAL SPECIES.

Acaulospora bireticulata Rothwell & Trappe, *Mycotaxon* **8**: 471-475, 1979.

Spores sessile, borne laterally on the hyphae of the sporiferous saccule; globose to subglobose 120 - 135 μm in diam; subhyaline in youth, becoming light brown at maturity. *Spore wall structure* of three layers, each 0.8 - 1 μm thick, the outer layer dark grayish green to grayish brown, the inner layers hyaline. *Spore surface* ornamented with a polygonal reticulum, the ridges 2 x 1.5 - 2 μm with sinuous, dark grayish-green sides and a paler, depressed central stratum; ridges occasionally branched towards the center of polygons or forming irregular, isolated projections at polygon centers. Polygons 7 - 14 μm long, the enclosed spore surface beset with round-tipped, 4- to 6-sided processes, 1 x 1 μm to give the appearance of an inverted reticulum.

Acaulospora delicata Walker, Pfeiffer & Bloss, *Mycotaxon* **25**: 621-628, 1986
(**Plate- VI a, b**).

Spores formed singly in the soil; laterally on the neck of a sporiferous saccule; hyaline to pale yellowish cream; globose to subglobose 85-140 x 75 -130 μm in diam. *Spore wall structure* of four walls (1-4) in two groups (A, B). Group A of three walls. Wall-1 hyaline, evanescent, 0.5 - 1 μm thick, closely attached to wall two. Wall-2 thick laminated, 2.5 - 3.0 μm thick. Soil particles and debris often adherent to the evanescent outer wall (wall-1).

PLATE VI

Spores of *Acaulospora* (a-d)

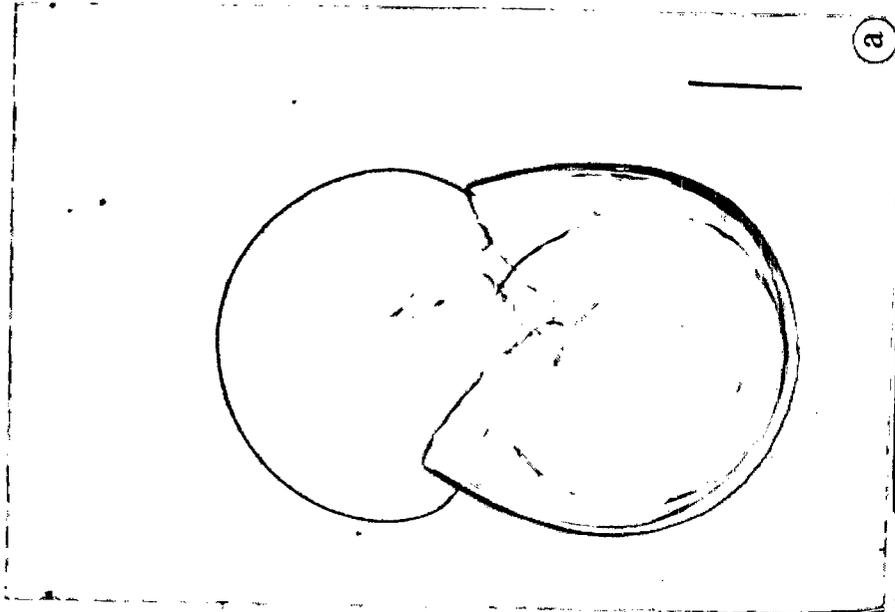
- (a) *Acaulospora delicata*- A fractured spore showing the two wall groups.

- (b) A whole spore of *Acaulospora delicata* mounted in polyvinyl alcohol lacto-glycerol (PVLG) to show the wrinkling of the membranous inner wall group and the debris which typically is adherent to the outer wall (arrowed).

- (c) *Acaulospora foveata* broken spore.

- (d) Spore surface of *Acaulospora foveata* showing details of pitted ornamentation (arrowed).

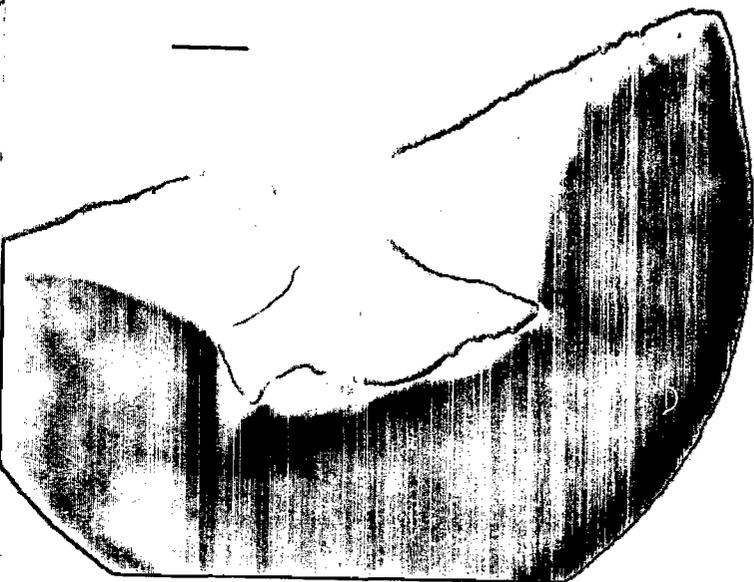
(Scale bar: a, b = 50 μ m; c = 50 μ m; d = 22 μ m).



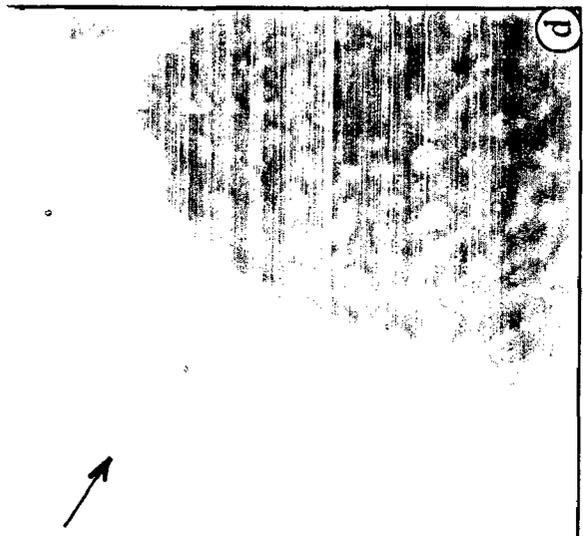
(a)



(b)



(c)



(d)

PLATE - VI

Group B of two thin, hyaline, membranous walls (Walls 3 & 4), both 0.4 - 0.5 μm thick. Wall - 3 covered by minute granular excrescence.

Acaulospora foveata Trappe & Janos, *Mycologia*. 76: 685-699, 1984 (Plate VI- c, d).

Sporocarps unknown. *Spores* formed singly in the soil, laterally on the neck of saporiferous saccule; yellowish-brown to light reddish-brown in youth becoming reddish-brown to brownish-black at maturity; globose to ellipsoid, 250 - 380 x 250- 400 μm . Spore surface uniformly pitted with round to oblong or occasionally irregular depressions 4 - 7 x 8- 15 μm , 1.7 - 2.4 μm deep, with rounded bottoms, separated by ridges 3 - 10 μm broad. Outer surface wall yellowish or reddish brown to brown, 11- 15 μm thick, with an adherent but separable, hyaline inner layer 3 μm thick. Spore contents of small hyaline guttles.

Acaulospora laevis Gerd. & Trappe, *Mycologia Mem.* 5: 33, 1974 (Plate VII a).

Sporocarps unknown. *Spores* formed singly in the soil, laterally on the neck of sporiferous saccule; dull yellow in colour becoming deep yellow to yellow-brown to red-brown or dark olive-brown at maturity, globose to subglobose; 200 - 400 x 150 - 400 μm diam. *Spore wall structure* of three walls (1-3) in two groups (A and B). Group A of a single laminated yellow-to-yellow brown wall (wall-1) 2 - 4 μm thick. Group B of two hyaline membranous inner walls (2 &3) each 0.5 to 1 μm thick. Spore content globose to somewhat polygonal (reticulate in optical section).

Acaulospora longula Spain & Schenck, *Mycologia* 76: 685-699, 1984.

Spores formed singly in the soil, laterally on the neck of sporiferous saccule; hyaline when young to pale yellow-green when mature, globose to subglobose; 95 - 110 x 60 - 80 μm in diam. *Spore wall structure* of five walls (1-5) in two groups (A, B). Composite spore wall 2.5 - 3.5 μm thick. Group A of three walls. Wall-1 mucilaginous, ephemeral, 0.4 - 1.5 μm thick; wall-2 unit wall, 2 - 3 μm thick; wall-3 unit wall 0.5 - 1 μm thick. Group B of a hyaline unit wall 0.5 - 1 μm thick and a membranous wall 0.5 - 1 μm thick. Spore content hyaline to sub hyaline.

Acaulospora mellea Spain & Schenck, *Mycologia*, 76: 685-699, 1984 (Plate-VII b).

Spores formed singly in the soil; borne laterally on the hyphae. *Spores* honey coloured to yellow-brown; globose to subglobose; 85 - 140 x 65 - 85 μm diam. *Spore wall* 5 - 12 μm diam. *Spore wall structure* of five wall layers. Wall-1, yellow-brown 2.5 - 6 μm thick. Wall-2, laminate, inseperable from wall-2 and 0.4 - 0.5 μm thick. Wall-3, membranous, hyaline to light yellow, 0.5 - 0.8 μm . Wall-4 shows beaded appearance, 0.5 - 0.6 μm and wall-5, 0.5 μm thick. Spore content yellow, globular to reticulate in appearance.

Acaulospora morrowiae Spain & Schenck, *Mycologia* 76: 685-699,1984.

Spores formed singly in the soil; laterally on the neck of a sporiferous saccule; young spores with light yellow walls and white content, becoming light yellow with globular transparent contents in reflected light; globose to subglobose; 70 - 100 μm in diam. *Spore wall structure* of five walls (1-5) in three groups

(A, B, C). Group A of a hyaline wall 0.5 - 2 μm thick adherent to wall 2 which is pale yellow, laminated, 2 - 4 μm thick. Group B of a hyaline unit wall (wall-3) 0.8 - 2 μm thick. Group C of two adherent membranous wall-4 and 5, each 0.5 - 1 μm thick.

Acaulospora myriocarpa Spain, Sieverding & Schenck, *Mycotaxon* **25**: 111-117, 1986 (**Plate- VII c**).

Spores formed singly in the soil on the hyphae of a sporiferous saccule; globose to subglobose 35 - 45 μm in diam; hyaline. Composite spore wall hyaline, 1 - 2 μm thick. *Spore wall structure* of three walls (1-3) in one group. Wall-1 rigid, 0.75 - 1.5 μm thick. Wall-2 rigid 0.4 - 0.9 μm . Wall-3 membranous 0.1 - 0.2 μm thick closely appressed to wall-2. Spore contents hyaline and globular.

Acaulospora nicolsonii Walker, Reed & Sanders, *Trans. Br. Mycol. Soc.* **83**: 360-364, 1984 (**Plate- VII d**).

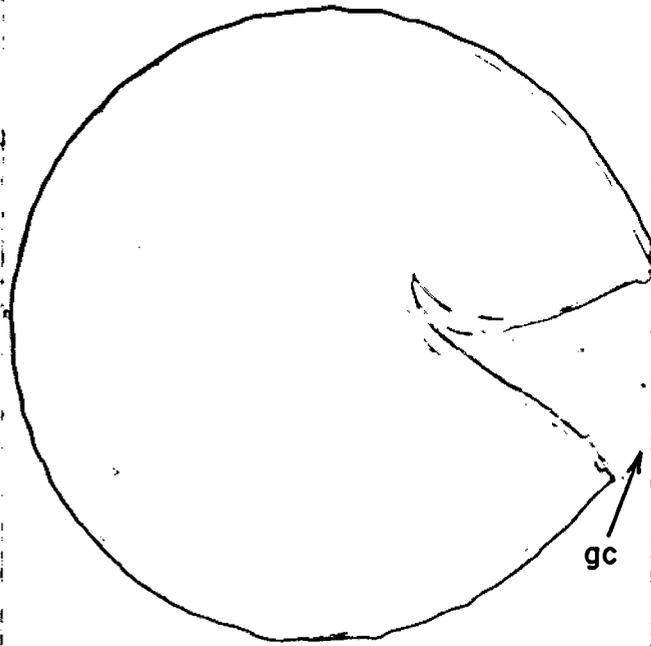
Spores formed singly in the soil; laterally on the neck of a sporiferous saccule; hyaline to pale yellow brown; globose to subglobose to pyriform; 110 - 150 x 125 - 180 μm in diam. *Spore wall structure* of four walls (1-4) in two groups (A, B). Group A of three walls. Wall-1 hyaline, evanescent, 0.5 - 1 μm thick, tightly adherent to wall two. Wall-2 brittle, hyaline to pale yellow-brown, laminated, 3 - 7 μm thick, loosely adherent to wall three. Wall-3, pale yellow, brittle, unit wall, 0.5 - 1 μm thick. Group B of a thin hyaline membranous unit wall 0.5 - 1 μm thick.

PLATE VII

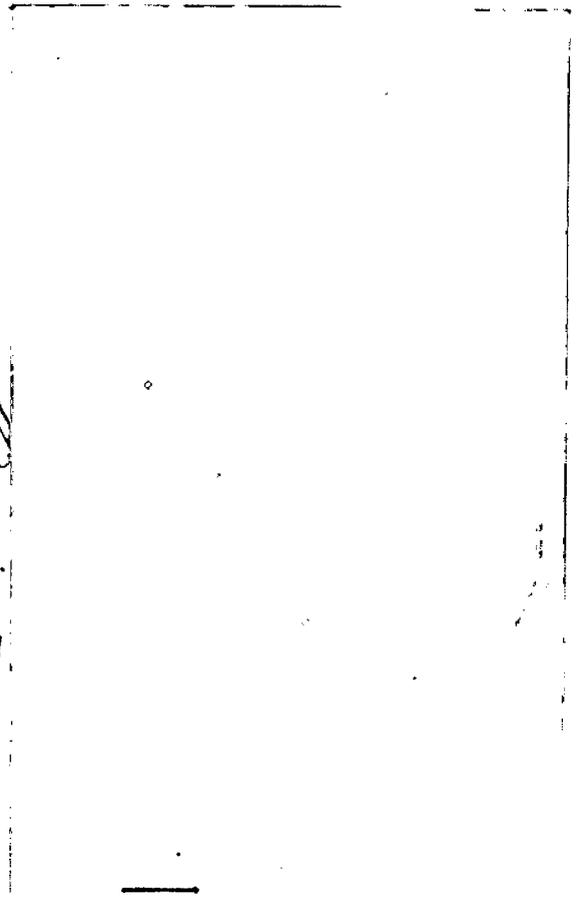
Spores of *Acaulospora* (a-d)

- (a) Young spore of *Acaulospora laevis* showing globular content (gc) and wall layers.
- (b) Intact spore of *Acaulospora mellea* showing details of wall layers (arrowed) and sporiferous saccule (ss).
- (c) *Acaulospora myriocarpa* intact spore.
- (d) *Acaulospora nicolsonii* intact spore.

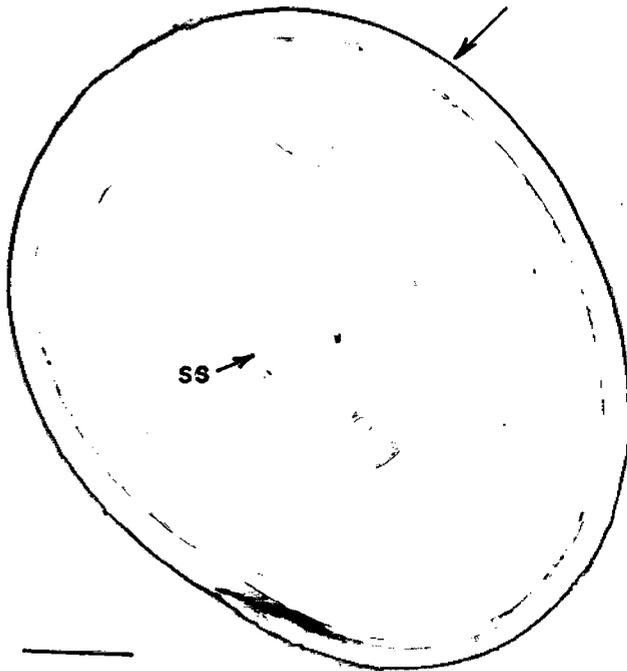
(Scale bar: a = 50 μm ; b = 22 μm ; c = 10 μm ; d = 50 μm).



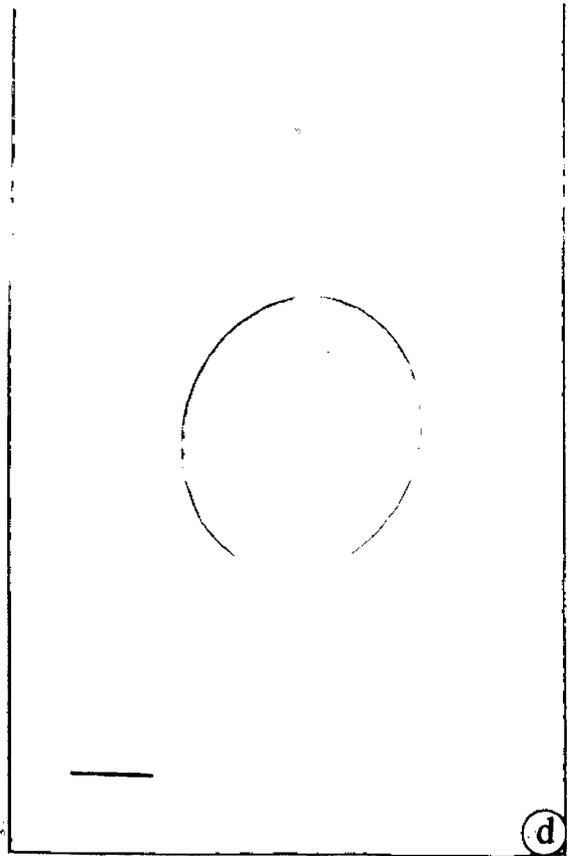
(a)



(c)



b



(d)

PLATE-VII

Acaulospora rhemii Sieverding & Toro *Botanik* **61**: 217-223, 1987.

Spores sessile, borne laterally on the hyphae of the sporiferous saccule; globose to subglobose 100 - 165 μm in diam; light yellow to brown; older spores often appearing dark red brown to black. *Spore wall structure* of four walls (1-4) in three groups (A, B, C). Group A, composed of wall-1, yellow to dark red brown, 3 - 8 μm thick with the ornamentation of labyrinth-form folds 1 - 3 μm wide. Group B is composed of wall-2, hyaline unit wall, 0.5 - 1 μm thick. Group C is composed of wall 3 & 4, hyaline and membranous; wall-3, 0.5 - 1 μm thick, adherent to wall 4, which is 0.4 - 0.5 μm thick.

Acaulospora scrobiculata Trappe. *Mycotaxon* **6**: 359-366, 1977 (Plate- VIII a,b).

Spores sessile, borne laterally on the hyphae of the sporiferous saccule; globose to broadly ellipsoid; hyaline, olive to light brown at maturity 130 - 230 μm diam.; surface evenly pitted with depressions 1 - 1.5 x 1 - 2 μm separated by ridges which are 3 μm thick. The mouths of depressions circular to elliptical or occasionally linear to Y- shaped. *Spore wall structure* of four walls (1-4) in three groups. Group A of an outer pitted wall (wall-1) 3.5 - 6 μm thick. Group B of a single hyaline unit wall (wall-2) 0.5 - 1.5 μm thick. Group C of two membranous walls (wall-3) 0.5 - 0.8 μm thick. Wall-4 minutely roughened, hyaline 0.2 - 0.9 μm thick. Spore contents of small, relatively uniform guttules.

Sporiferous saccule globose; 126 - 165 μm diam. Wall hyaline to off white, smooth, 1.5 - 2 μm thick collapsing at maturity.

PLATE VIII

Spores of *Acaulospora scrobiculata* (a-b)

- (a) *Acaulospora scrobiculata* spore developing on a hyphal stalk (hs) of sporiferous saccule (ss), surface showing details of Y-shaped ornamentation (arrowed).

- (b) Spore surface of *Acaulospora scrobiculata* showing pitted ornamentation with inner wall groups.

(Scale bar: a, b = 22 μm).

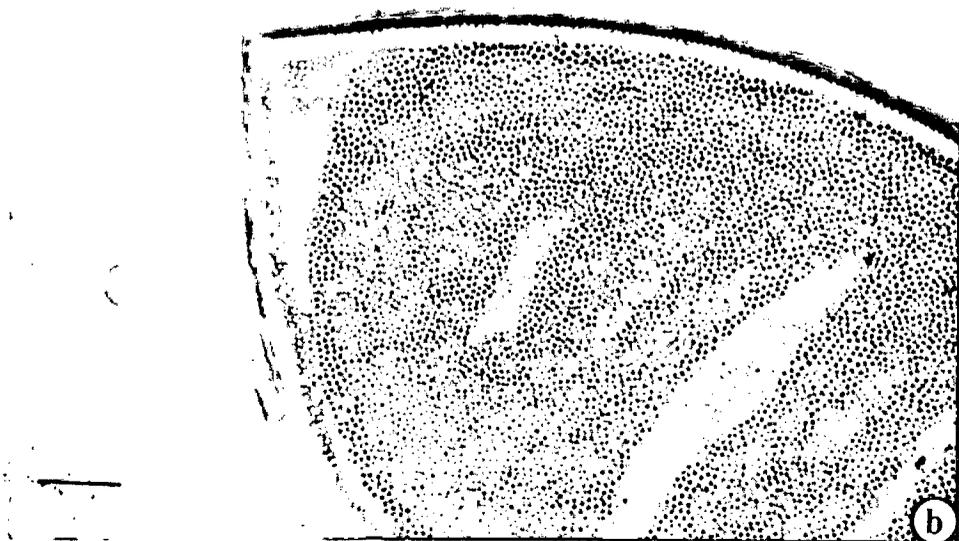
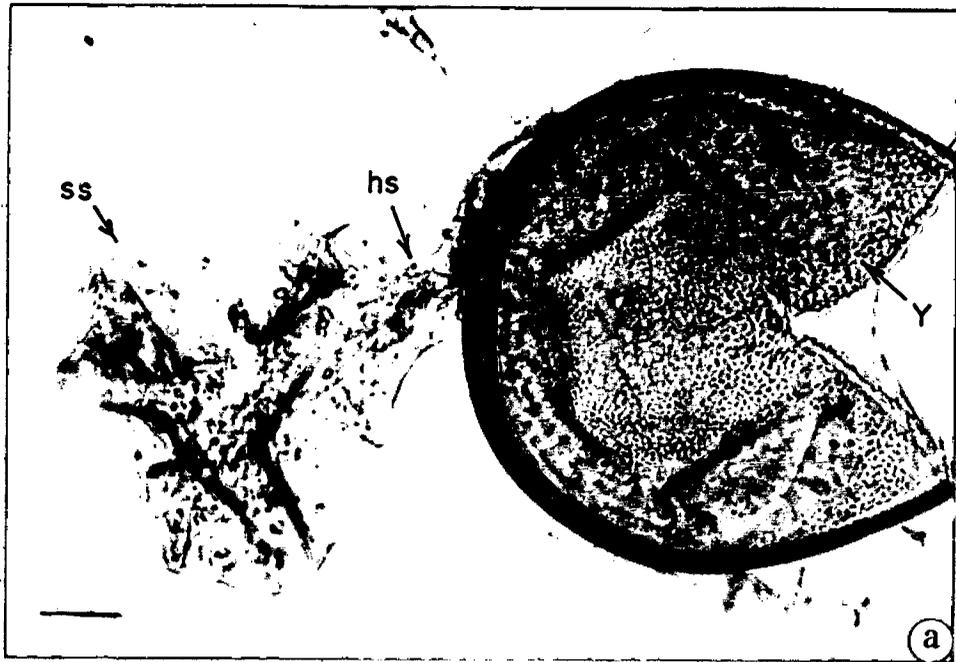


PLATE - VIII

Acaulospora spinosa Walker & Trappe, *Mycotaxon* 12: 515-521, 1981 (Plate- IX a, b, c).

Spores sessile, borne laterally on the hyphae of the sporiferous saccule; globose to subglobose 100 - 250 x 130 - 280 μm in diam.; dull yellowish brown to dark reddish brown. *Spore surface* ornamented with crowded blunt spines; diam. at the polygonal base tapering to 0.5 μm at the tip. *Spore wall structure* of three walls, outer wall light yellowish brown to reddish brown 4 - 7 μm thick including spines and encrustations, enclosing two membranous hyaline walls, each 0.5 and 0.3 μm thick. The inner wall usually slightly thinner.

Sporiferous saccule 120 x 40 μm in diam, attached by a collar 8 - 12 μm broad to the side of a funnel shaped to cylindrical hyphae.

Acaulospora undulata Sieverding, E. *Botanik* 62: 373-380, 1988 (Plate- IX d).

Spores formed singly in the soil, borne laterally on the hyphae of the sporiferous saccule; hyaline to sub hyaline globose to subglobose; 55 - 90 μm in diam. *Spore wall structure* of three walls (1-3) in two groups (A, B). Group A of two walls. Wall-1 hyaline, evanescent, 0.4 - 0.6 μm thick, tightly adherent to wall two. Wall-2 hyaline, 1 - 1.5 μm thick, undulated by regular depressions, 1.5 - 2 μm thick. Each depression circular, ovulate or slightly irregular, 4 - 7 μm wide at periphery of spores; ridges between depressions about 1 μm wide. Group B of a hyaline unit wall 0.5 - 1 μm thick, often minutely roughened.

PLATE IX

Spores of *Acaulospora* (a-d)

- (a) Spore of *Acaulospora spinosa* developing on hyphal stalk of sporiferous saccule (ss).
- (b) Crushed spore of *Acaulospora spinosa* showing details of spine tips and bases.
- (c) Young broken spore of *Acaulospora spinosa* showing spines and pits.
- (d) Spore surface of *Acaulospora undulata* showing pitted ornamentation (arrowed).

(Scale bar: a = 75 μm ; b, c = 22 μm ; d = 10 μm).

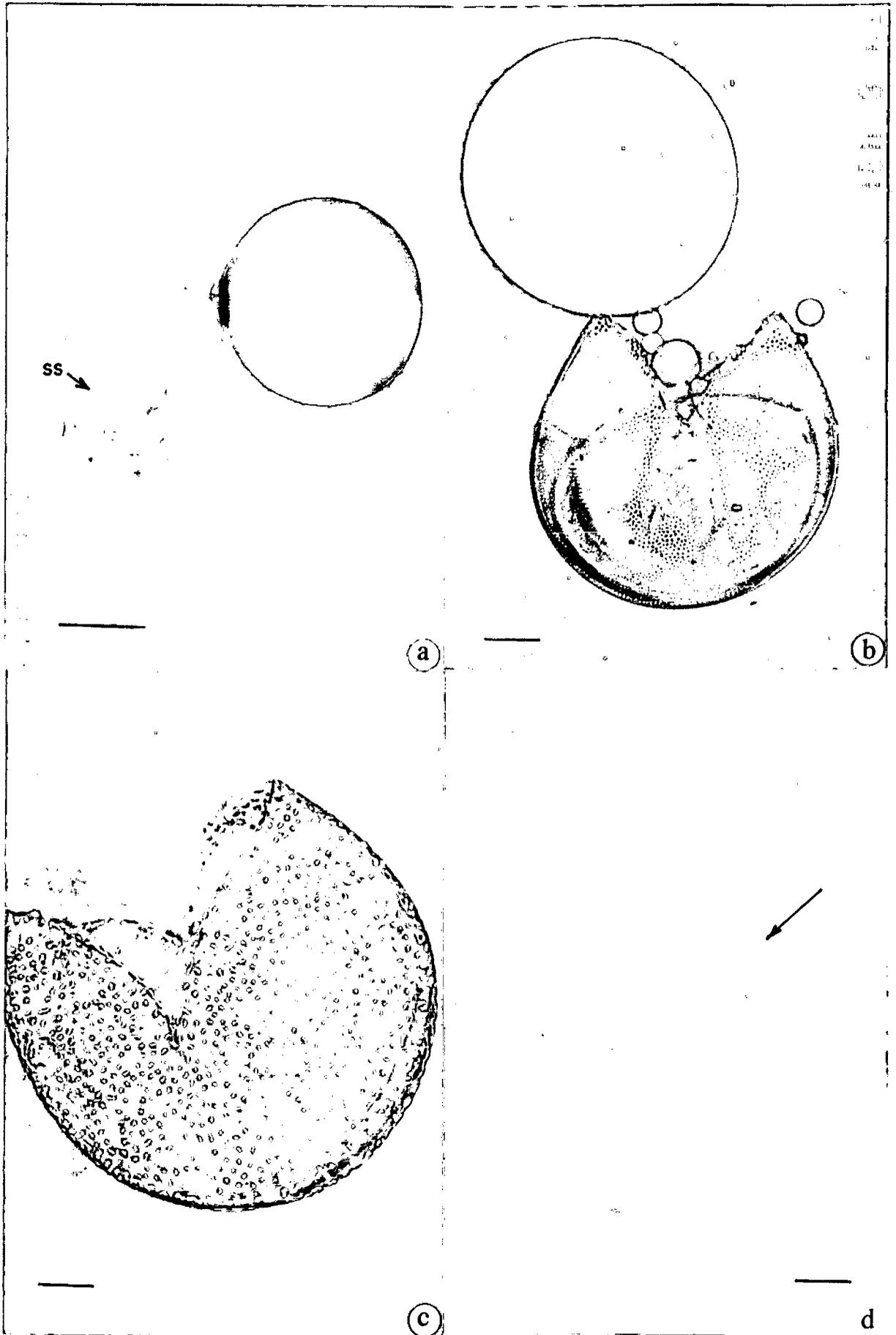


PLATE - IX

Archaeospora leptoticha (Schenck & Smith) Morton & Rdecker *Mycologia*, **93**: 181-195, 2001 (Plate- XIV d).

= *Glomus leptotichum* Schenck & Smith *Mycologia* **74**: 77-92, (1982)

Spores white or pale cream-colored usually appearing dirty because of adherent organic material; 150 - 250 μm diam. *Spore wall structure* consists of two adhaent semiflexible layers. *Spore wall structure* of two wall layers, hyaline to light yellow, 3 - 7 μm thick, with an indistinct alveolate reticulum of shallow ridges. The inner layer is hyaline to pale yellow 3 - 4 μm thick, and forming folds with applied pressure due to flexibility. Spore contents globular white to cream. *Subtending hyphae* 4 - 6 μm wide and 12 - 20 μm at the point of attachment with the spore. Hyphal wall continuous with the spore wall.

Gigaspora albida Schenck & Smith, *Mycologia* **74**: 77-92, 1982.

Spores formed singly in the soil terminally on a sporogenous cell; dull white with a greenish yellow tint; globose 200 - 350 μm in diam. *Spore wall* continuous except for an occluded pore. *Composite spore wall* 3 - 10 μm thick with 1 - 6 walls, outer wall thin smooth 1 μm thick, with 4 - 5 inseparable inner walls.

Sporogenous cell 28 - 50 μm broad; hyaline, smooth. *Subtending hyphae* septate with fine hyphal branches.

Gigaspora decipiens Hall & Abbott, *Trans. Br. Mycol. Soc.* **83**: 203-208, 1984 (Plate- X e, f).

Spores found singly in the soil terminally on a sporogenous cell; white to golden yellow, turning brown to dark brown at maturity; globose to

subglobose; 350 - 450 μm in diam. *Spore wall structure* of three walls (1-3) in one group. Wall-1 hyaline unit wall 2 μm thick. Wall-2 laminated 30 - 34 μm thick. Wall-3, 1.5 - 2 μm thick. Wall of older spores 34 - 36. μm thick with number of laminations. *Sporogenous cell* yellowish brown, bulbous, 63 x 70 μm in diam. *Subtending hyphae* 10 - 12 μm wide, wall of the hyphae 2.5 - 3 μm in thickness hyphae usually coiled often with one or more attached lateral hyphal projections.

Gigaspora margarita Becker & Hall, *Mycotaxon* 4: 155-160, 1976 (Plate- X a-d).

Spores formed singly in the soil terminally on a sporogenous cell; hyaline to golden yellow to reddish brown; globose; 300 - 500 μm in diam. *Spore wall* 10 - 24 μm thick with 8 - 10 fused laminations. Each lamina 0.5 - 5 μm thick. *Sporogenous cell* 48 - 58 μm broad; hyaline to light brown, smooth, walls 1-2.5 - 3.5 μm thick, thickest 8 - 9 μm at the point of attachment to the spore.

Gigaspora rosea Nicolson & Schenck, *Mycologia* 71: 178-198, 1979.

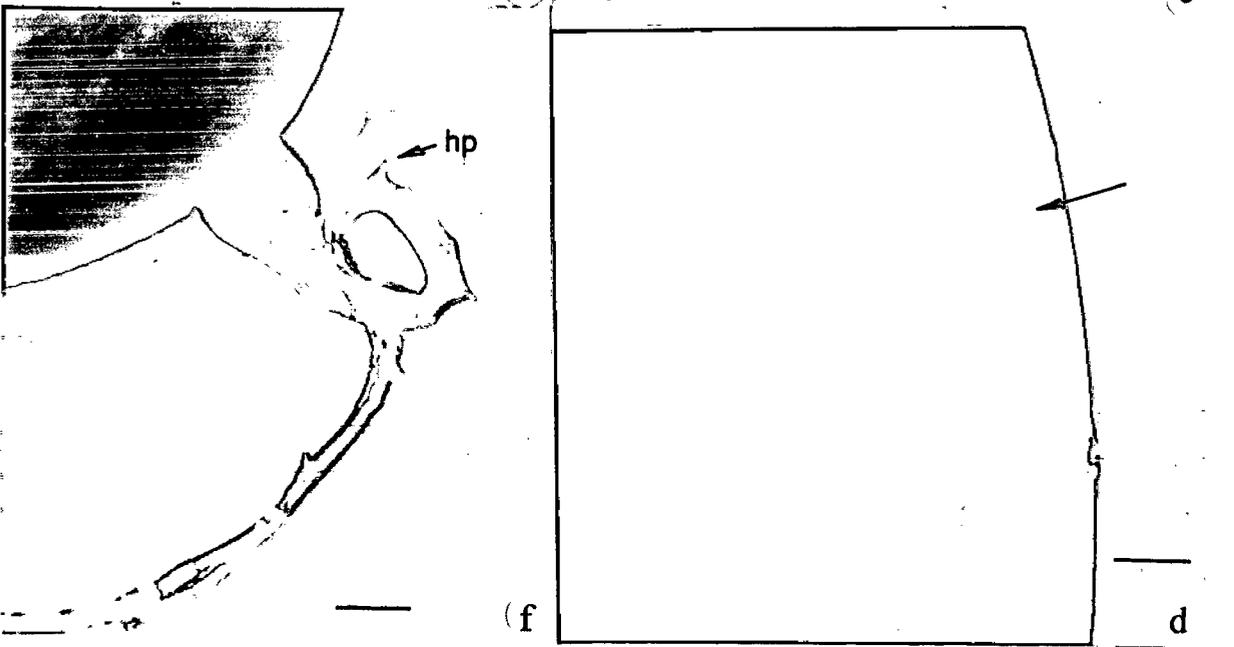
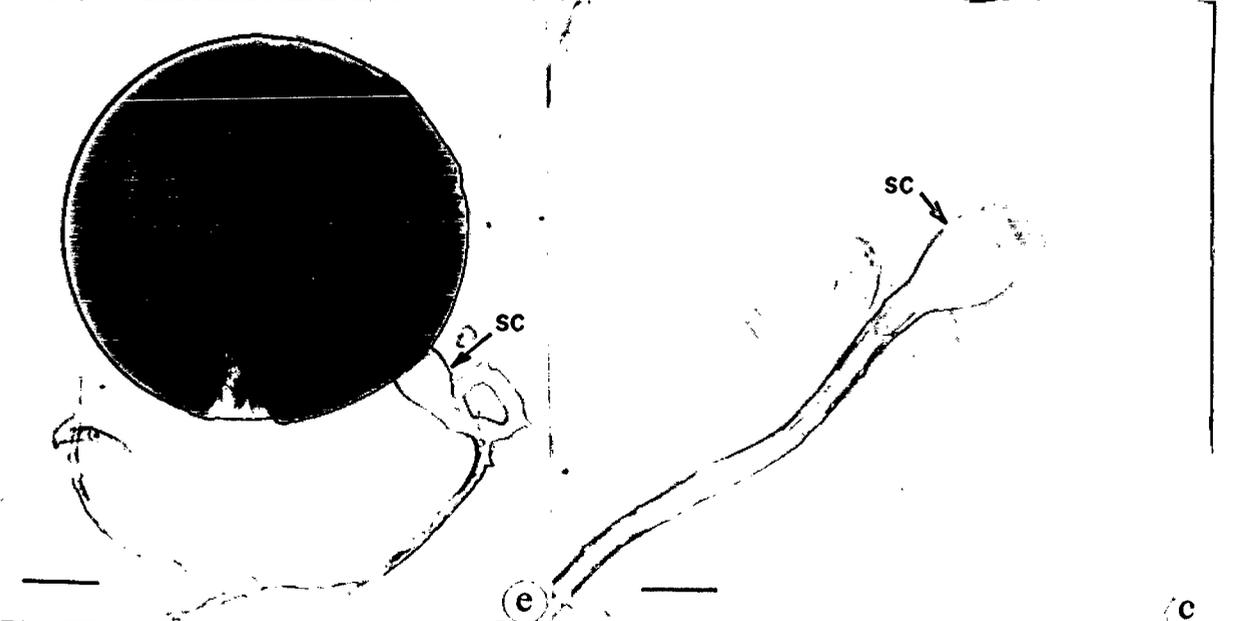
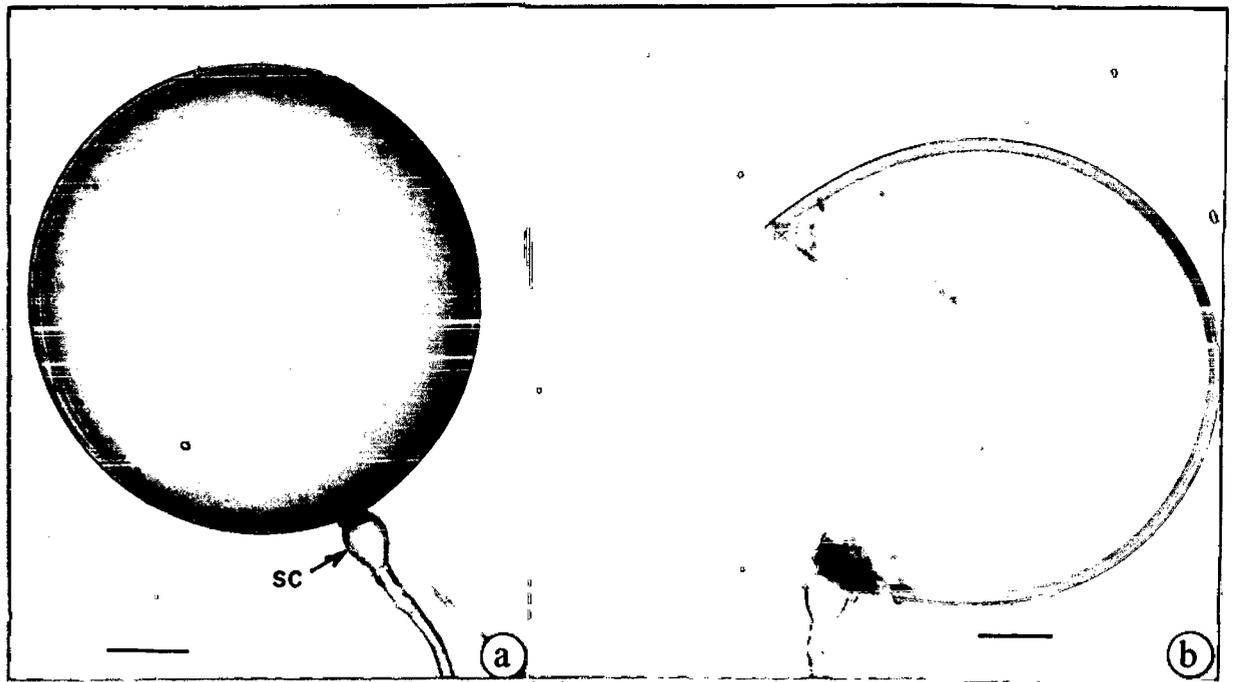
Spores produced singly in the soil terminally on a sporogenous cell; white to cream in colour, with rose pink tint at the hyphal attachment; globose to subglobose; 200 - 280 μm in diam. Composite spore wall 3 - 6.5 μm thick; with 2 - 5 inseparable layers 1 - 2 μm thick, outer wall smooth. *Sporogenous cell* 30 - 39 μm in diam. spherical. *Subtending hypha* 6 - 10 μm wide, hyphal walls 1 - 1.5 μm thick and septate.

PLATE X

Spores of *Gigaspora* (a-f)

- (a) Intact spore of *Gigaspora margarita*.
- (b) Broken spore of *Gigaspora margarita* with spore content.
- (c) A portion of spore of *Gigaspora margarita* showing details of sporogenous cell (sc).
- (d) Wall structure of *Gigaspora margarita* showing laminations of wall.
- (e) A mature spore of *Gigaspora decipiens* showing details of sporogenous cell (sc).
- (f) A portion of spore of *Gigaspora decipiens* showing details of sporogenous cell and coiled subtending hyphae with hyphal protrusions (hp).

(Scale bar: a, b = 100 μm ; c = 50 μm ; d = 20 μm ;
e = 100 μm ; f = 50 μm).



Glomus aggregatum Schenck & Smith emend. Koske, *Mycologia* 77: 619-630, 1985 (Plate- XI a).

Spores in loose clusters in soil and within the roots, globose to subglobose, obovate, cylindrical to irregular, 40 - 85 μm in diam.; hyaline to yellow with a greenish tint. *Spore wall structure* of one or two walls in a single group, yellow to yellow brown, laminated, 1.2 - 2.0 μm thick, consisting of an outer wall slightly thicker and lighter than the inner wall. *Subtending hyphae* 5 - 10 μm wide at the point of attachment with the spore and 5 - 7 μm at the base, pore open. Hyphal attachment straight or recurved sharply at the spore base.

Glomus albidum Walker & Rhodes, *Mycotaxon* 12: 509-514, 1981.

Spore borne singly in the soil, white to off-white, globose to subglobose; 115 - 175 μm in diam.; *Spore wall structure* of two walls (1&2) in one group. Wall-1, hyaline often roughened due to sloughing 0.5 - 2 μm thick. Wall-2, laminated 0.5 - 2 μm thick

Subtending hyphae two walled straight 4 - 10 μm wide, occasionally with a bulging septum 4 - 5 μm distad of the pore, but usually open. Subtending hyphae shrivels and collapses in mature spore.

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

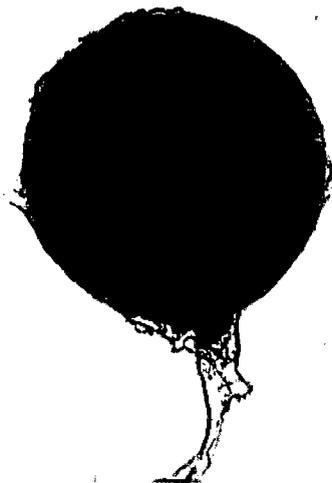
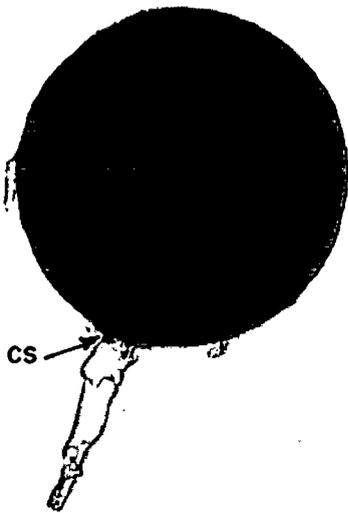
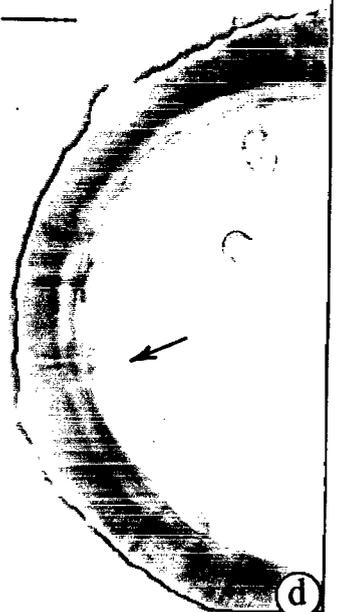
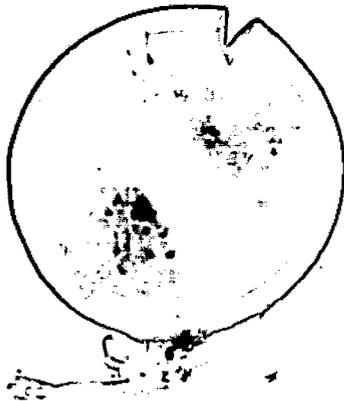
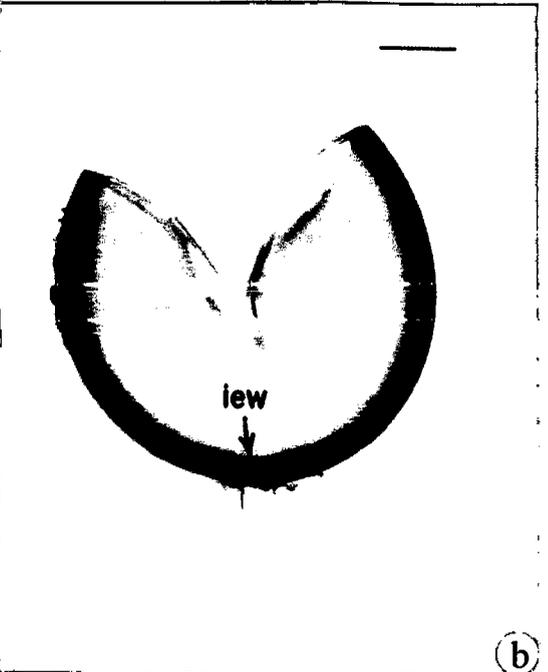
Spores produced in sporocarps, in soil or aggregated around roots, dark brown to black, globose to subglobose 98 - 150 x 95 - 135 μm diam. *Spore wall structure* of three walls (1-3) in one group. Wall-1, reticulate, ephemeral, sub hyaline, 2 - 3.5 μm thick. Wall-2, dark brown to black, 4 - 10 μm thick, laminated. Wall-3, membranous 1 μm thick. *Subtending hyphae* straight 10-

PLATE XI

Spores of *Glomus*

- (a) Cluster of spores of *Glomus aggregatum*.
- (b) Young spore of *Glomus claroideum* forming inner endospore wall (iew) and lacking scalloped shaped ingrowths.
- (c) Spore of *Glomus claroideum* forming multiple branched hyphae.
- (d) Mature spore of *Glomus claroideum* showing scalloped shaped ingrowths (arrowed).
- (e) Intact spore of *Glomus constrictum* showing constriction (cs) of hyphae at the point of attachment to the spore.
- (f) Intact spore of *Glomus constrictum* showing dichotomously branched thin walled hyphae.

(Scale bar: a-c = 50 μm ; d = 22 μm ; e, f = 50 μm)



15 μm wide and 15 - 24 wide at the point of attachment with the spore. Hyphal wall yellow-brown, 4 - 5 μm thick and pore 1.5 - 2 μm wide.

Glomus claroideum Schenck & Smith emend Walker & Vestberg. *Annals of Botany* **82**: 601-624, 1998 (Plate- XI b-d).

= *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 multisubstensum* Mukerji, Bhattacharjee & Tewari. *Trans. Br. Mycol. Soc.* **81**: 3, (1993).

Sporocarps unknown. *Spores* formed singly in the soil one or three subtending hyphae, hyaline to pale straw-coloured to ochraceous; globose to subglobose; 90 - 210 μm in diam.,. *Spore wall structure* of three walls (1-3) in two groups (A, B). Group A of wall-1, hyaline, unit wall 0.3 - 1 μm thick and wall-2 brittle, pale straw-coloured to ochraceous, laminated 4 - 10 μm thick, with 4 - 16 laminae. Group B of a single membranous thin wall-3, 0.5 - 1 μm thick. -Wall-3, in many older spores, bearing domed scalloped ingrowths, 6 - 12 μm diam. *Subtending hyphae* straight to sharply recurved, parallel-sided, or funnel-shaped, sometimes constricted at the spore base 6 - 10 μm long, 5 - 12 μm wide at the point of attachment with the spore. Walls of subtending hyphae 1.4 - 2.5 μm thick.

Glomus clavisorum (Trappe) Almeida & Schenck, *comb. nov. Mycologia*,
82: 703-711, 1990.

(Basionym: *Sclerocystis clavispota* Trappe, *Mycotaxon* 6: 359-361, 1977.

= *Sclerocystis microcarpa* Iqbal & Bushra, *Trans. Br. Mycol. Soc.* 21: 58-59,
1980.

Sporocarps dark brown, globose to subglobose; 190 - 400 μm in diam.
Peridium absent; *Spores* 95 - 110 x 45 - 60 μm , clavate to cylindro-clavate,
brown, arranged in a single tightly packed layer around the central plexus of
hyphae. *Spore wall* laminate, brown, 12 - 20 μm thick at the apex, 3 - 3.5 μm
thick at the sides, generally thickest at the apex, with a small pore opening
into the subtending hyphae.

Glomus constrictum Trappe, *Mycotaxon* 6: 359-366, 1977 (Plate- XI e, f).

Spores formed singly in the soil, subglobose to globose, dark brown to black,
shiny-smooth, 145 - 310 μm diam. *Spore wall structure* of a single wall dark
brown 7 - 10 μm thick. *Subtending hyphae* straight to recurved constricted at
the point of attachment, and just beyond the point of attachment the hyphae is
12 - 19 μm wide, hyphal walls brown, 3 - 5.5 μm thick from which arises
several hyaline to yellow fragile, thin walled hyphae 5 - 7 μm in diam.

Glomus coremioides (Berk. & Broome) Redecker et Morton, *comb. nov.*
Mycologia, 92: 282-285, 2000 (Plate XVII a, b).

= *Sclerocystis coremioides* Berkely & Bromme, *J. Linn. Soc. London* 14: 137,
1875

= *Xenomycetes* Cesati, *Atti, R. Acad. Sci. Fische e Math. Napoli* 8: 26, 1879.

= *Ackermannia* Patouillard, *Bull. Soc. Mycol. France* **18**: 180, 1902.

= *S. coccogena* (Pat.) von Hohn, Sitzungsber. K

= *Sclerocystis dussi* (Patouillard) von Honel, *Mycologia Memoir No. 5*: 76, 1974

Sporocarps brown to yellow brown, subglobose or pulvinate 200 - 250 x 400-480 μm in diam., with or without multihyphal stipe, usually flattened at base.

Peridium composed of thick walled interwoven hyphae 25 - 65 μm thick.

Spores 45 - 60 x 45 - 75 μm , obovoid-ellipsoid to oblong-ellipsoid, to clavate, yellow brown; arranged radially in a hemispherical layer. *Spore wall structure* of single wall, 1.5 - 2.0 μm thick, generally thickest at the base 3.5 - 4 μm thick; *subtending hyphae* long single occasionally two, 4 - 4.5 μm at attachment, then tapered to 2 - 2.5 μm broad. Peridial hyphae, thick walled, 1.3 - 1.5 μm . *Pore* usually open.

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

Spores formed singly in the soil, yellow to reddish brown, 200 - 300 μm in diam., mostly globose. *Spore wall structure* of three walls. Wall-1 hyaline, 1.5 - 1.8 μm thick. Wall-2 laminated yellow wall 4 - 4.5 μm thick and inner wall membranous, 0.8 - 1 μm thick. Hypha at the point of attachment 18 - 21 μm thick and hyphal wall 1.4 - 1.5 μm thick.

Glomus dominikii Blaszkowski, *Karstenia* **27**: 37-42, 1988 (Plate- XII b, c).

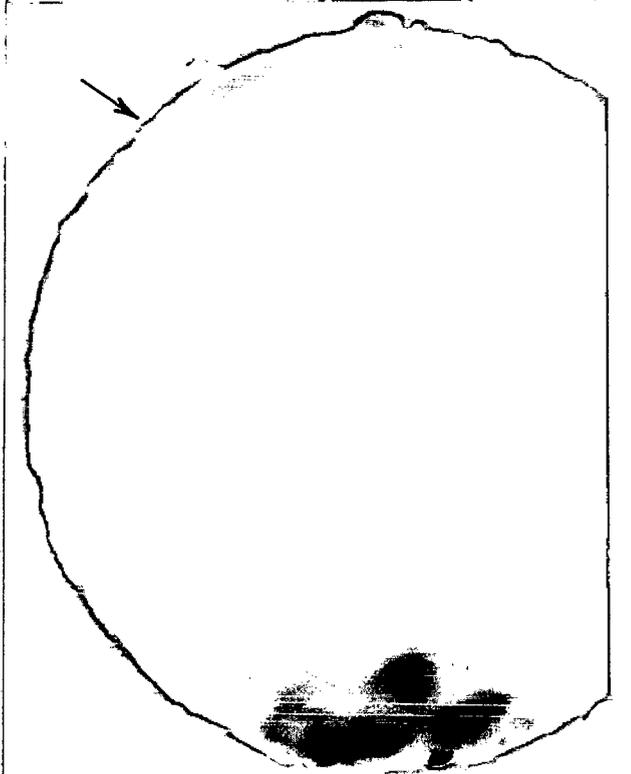
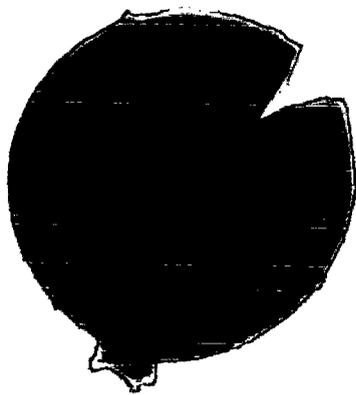
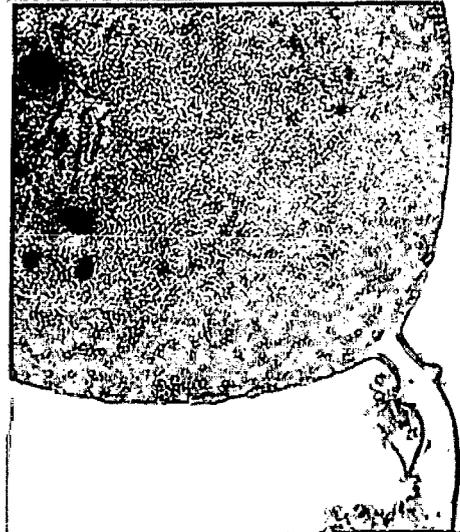
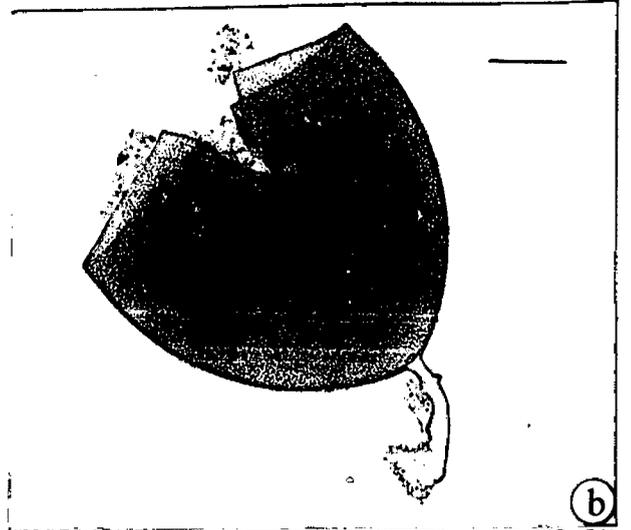
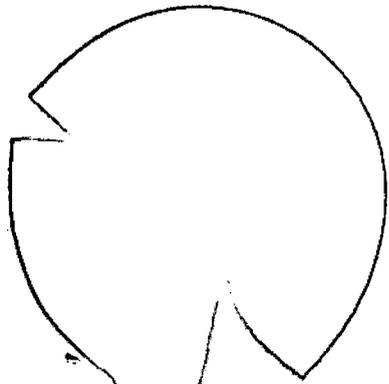
Spores borne singly in the soil, white becoming light yellow to orange-yellow with age, ornamented, globose to subglobose; 110 - 165 μm in diam. *Spore*

PLATE XII

Spores of *Glomus*

- (a) Spore of *Glomus dimorphicum*.
- (b) Broken spore of *Glomus domnikii*.
- (c) A portion of spore of *Glomus domnikii* showing surface ornamentation.
- (d) Intact spore of *Glomus etunicatum* with spore content.
- (e) A portion of spore of *Glomus etunicatum* with outer evanescent wall (arrowed).

(Scale bar: a = 50 μm ; b = 22 μm ; c = 10 μm ; d = 50 μm ;
e = 15 μm).



wall structure of three walls (1-3) in two groups (A, B). Group A of a hyaline, unit wall 1.7 - 3.2 μm thick ornamented with fine warts. Group B, of a hyaline smooth, membranous, wall, 0.4 - 1.1 μm thick, tightly adhering to a hyaline, smooth membranous wall-3, 1.2 - 2.5 μm thick. *Subtending hyphae*, hyaline, straight or slightly recurvate, 120 - 160 μm long, 6 - 10 μm wide, with walls 1.3 - 1.6 μm thick at the spore base without a septum; usually slightly constricted at the point of attachment.

Glomus etunicatum Becker & Gerd., *Mycotaxon* 6: 29-32, 1977 (Plate- XII d, e).

Spores found singly in soil, globose to subglobose; 80 - 150 μm diam., yellow to yellow-brown. *Spore wall structure* of two walls (1&2) in one group. Wall-1 ephemeral hyaline 1.5 - 2 μm thick. Wall-2, 2 - 7 μm thick laminated yellow to brown. *Subtending hyphae* 3 - 8 μm diam., with 5 - 10 μm at the point of attachment with the spore.

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

Spores borne singly in soil, in loose aggregation, in small compact clusters, and in sporocarps; flattened or globose to subglobose, or ellipsoid or irregular, yellow to yellow-brown lacking a peridium. *Spores* 40 - 95 μm diam. *Spore wall structure* of three walls (1-3) in one group. Wall-1 hyaline, smooth, unit wall 0.5 - 2 μm thick. Wall-2 pale yellow to yellow-brown, laminated 2 - 12 μm thick. Wall - 3 hyaline membranous wall 0.4 - 0.5 μm thick, adherent to wall-2. *Subtending hyphae*, straight, flared or slightly constricted at the point

PLATE XIII

Spores of *Glomus*

- (a) Fascicles of spores of *Glomus fasciculatum*.
- (b) Details of a portion of the fascicles of spores of *Glomus fasciculatum*.
- (c) Broken spore of *Glomus formosanum*. Note the branching (arrowed) of the subtending hypha near the point of attachment.
- (d) Intact spore of *Glomus geosporum* with a thickened subtending hypha (sh).
- (e) Spore of *Glomus geosporum* showing subtending hyphae typically breaking off at the point of attachment of spore (arrowed).
- (f) Spore of *Glomus globiferum* with a vesiculate swelling (vs).

(Scale bar: a = 50 μm ; b, c = 22 μm ; d, e = 50 μm ;

f = 100 μm).

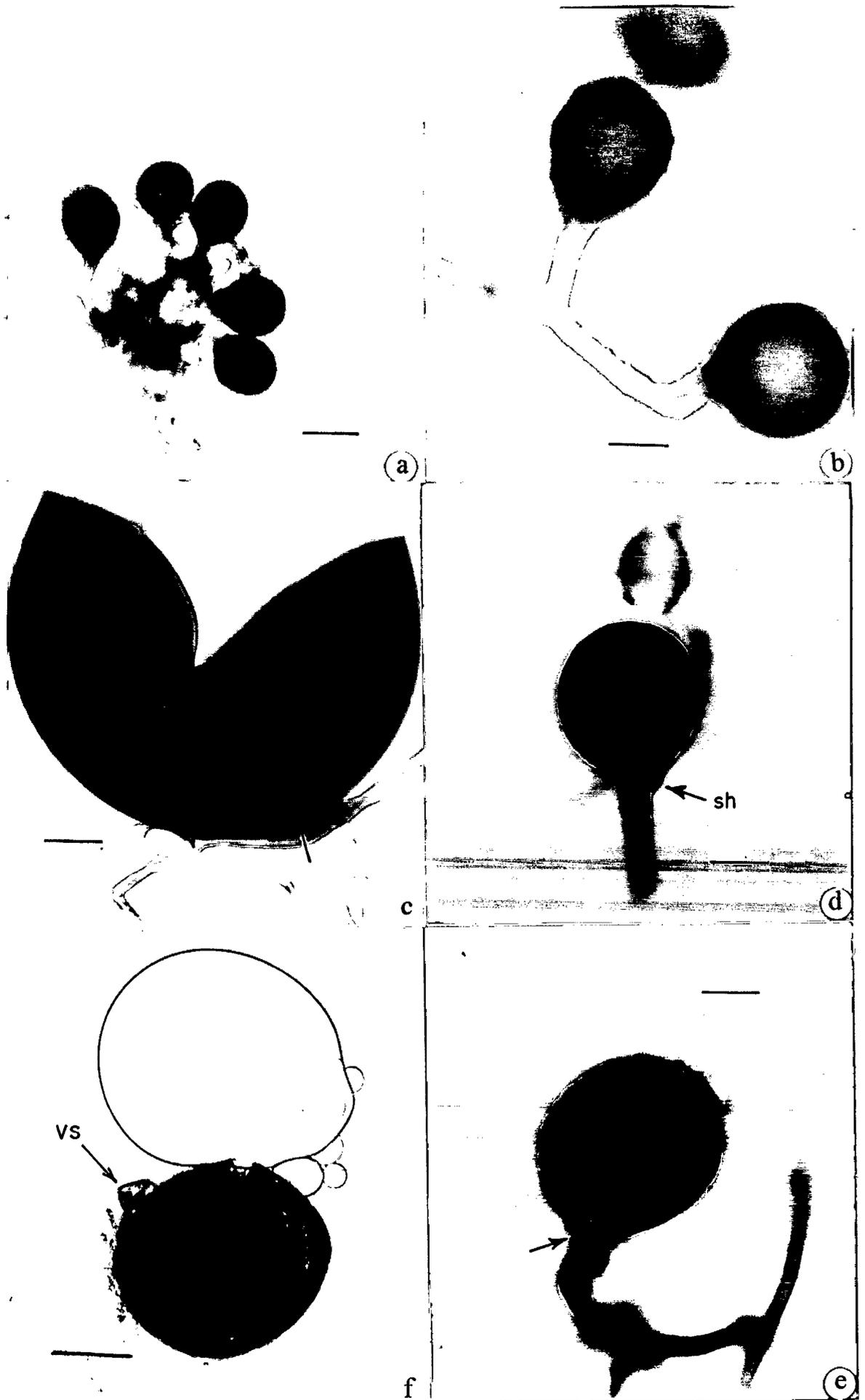


PLATE - XIII

of attachment 3 - 8 μm , 3 - 16 μm wide at the point of attachment with the spore, with a wall thickness of 3.5 - 7.5 μm at the spore base. *Pore* usually open or occluded by thickening of wall-2.

Glomus flavisporum (M. Lange & Lund) Trappe & Gerdemann, *Froesia* **5**: 90-95, 1955.

Spores ovate-oblong, often slightly constricted on the middle, rarely subglobose, 149 - 200 x 90 - 155 μm , wall deep yellowish-brown 6 - 10.5 μm thick, outer thin layer somewhat lamellate, hardly incrustated, ; *stipe* 10-13 mm thick simple.

Glomus formosanum Wu & Chen, *Taiwania* **31**: 65-88, 1986 (Plate- XIII c).

Spores reddish brown, globose to subglobose; 95 - 120 x 95 - 135 μm diam. *Spore wall structure* of single reddish-brown laminated wall 8 - 17 μm thick, but thickest at the point of attachment with the spore up to 18 - 20 μm . *Subtending hyphae* 1 to 3, branched, attached hyphae, frequently, two nearby attached hyphae fused together or closely separated at attachment. *Attached hyphae* 7 - 15.5 μm diam. *Pore* occluded by a wall thickening.

Glomus geosporum (Nicolson & Gerdemann) Walker, *Mycotaxon* **15**: 49-61, 1982 (Plate- XIII d, e).

= *Endogone macrocarpa* (Tul. & Tul.) Tul. & Tul. *Var. geospora* Nicolson & Gerdemann. *Mycologia*, **60**: 318, 1968.

= *Glomus macrocarpum* var, *geosporum* (Nicolson & Gerdemann)

Gerdemann & Trappe, *Mycologia Memoir* No. 5: 55-56, 1974.

Spores found singly in the soil or in loose clusters, globose to subglobose or broadly ellipsoid; 110 - 250 μm diam., yellow-brown to reddish-brown, smooth or often roughened with adherent debris resulting in dull appearance. *Spore wall structure* of three walls (1-3) in one group. Wall-1, 0.4 - 0.5 μm thick, hyaline evanescent. Wall-2, laminated 5 - 14 μm thick. Wall-3, 1 μm thick which forms a septum at the spore base. *Subtending hyphae* straight, recurved or slightly funnel shaped 8 - 11 μm and 13 - 15 μm at the point of attachment with the spore.

***Glomus globiferum* Koske & Walker, *Mycotaxon* 26: 133-142, 1986a(Plate-XIII f).**

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 - 230 x 150-265 μm , excluding peridium. *Peridium* of loosely interwoven hyaline to yellow-brown, coenocytes or sparsely septate, thin walled hyphae, 8 - 18 μm broad, bearing numerous terminal or intercalary globose to ovoid, pale yellow-brown or hyaline vesiculate swellings. *Vesiculate swellings* 15 - 45 x 25 - 75 μm , with wall 1 - 2 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 - 3 μm thick. Wall-2 orange-brown to red-brown, laminate 5 - 20 μm thick with upto 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 - 23 μm wide at the point of attachment with the spore, 18 - 25 μm at the sides, with the walls 2 - 6 μm thick. At the spore base; subtending hyphae usually appears to be inserted into the spore wall.

Glomus glomerulatum Sieverding, *Mycotaxon* 29: 73-79, 1987 (Plate XIV a).

Sporocarps dark brown with a greenish tint, globose, subglobose, rectangular or often flattened or irregular in shape; 300 - 450 μm ; sporocarps formed by interwoven hyaline hyphae; hyphae 3 - 6 μm in diam., non septate. Sporocarps without peridium. *Spores* formed only in sporocarps, and possesses two or more hyphal attachments, yellow to brown, globose to subglobose, 60 - 70 μm in diam. *Spore wall structure* of two walls (1&2) in one group. Wall-1, yellow-brown, 4 - 8 μm thick, laminated. On the surface of this wall a layer of hyphae is often adherent but normally the spore surface is smooth. Wall-2, hyaline, membranous 0.4 - 0.5 μm thick and adherent to wall-1. *Subtending hyphae*, two or more, yellow to brown, straight or recurved; 5 - 7 μm diam. at the point of attachment with the spore. *Pore* of the hyphal attachment is 1 - 2 μm in diam., spore contents hyaline, oily.

Glomus hoi Berch & Trappe, *Mycologia* 77: 654-657, 1985.

Spores borne singly in the soil, light brown, globose to subglobose or irregular; 65 - 120 x 45 - 100 μm in diam. *Spore wall structure* of two walls (1&2) in one group. Wall-1 orange-yellow 3 - 5 μm thick. Wall-2 hyaline or

PLATE XIV

Spores of *Glomus* and *Archaeospora*

- (a) Chlamydo spores of *Glomus glomerulatum* with 2-3 hyphal attachments in loose clusters in a sporocarp.
- (b) Spore of *Glomus intraradices* with wall layers (wls).
- (c) Broken spore of *Glomus lacteum*. Note the hyaline granular spore content (sc).
- (d) Intact spore of *Archaeospora leptoticha*.
- (e) Intact spore of *Glomus mosseae*. Note the funnel shaped hypha (arrowed).

(Scale bar: a, b = 22 μm ; c, d = 50 μm ; e = 35 μm).

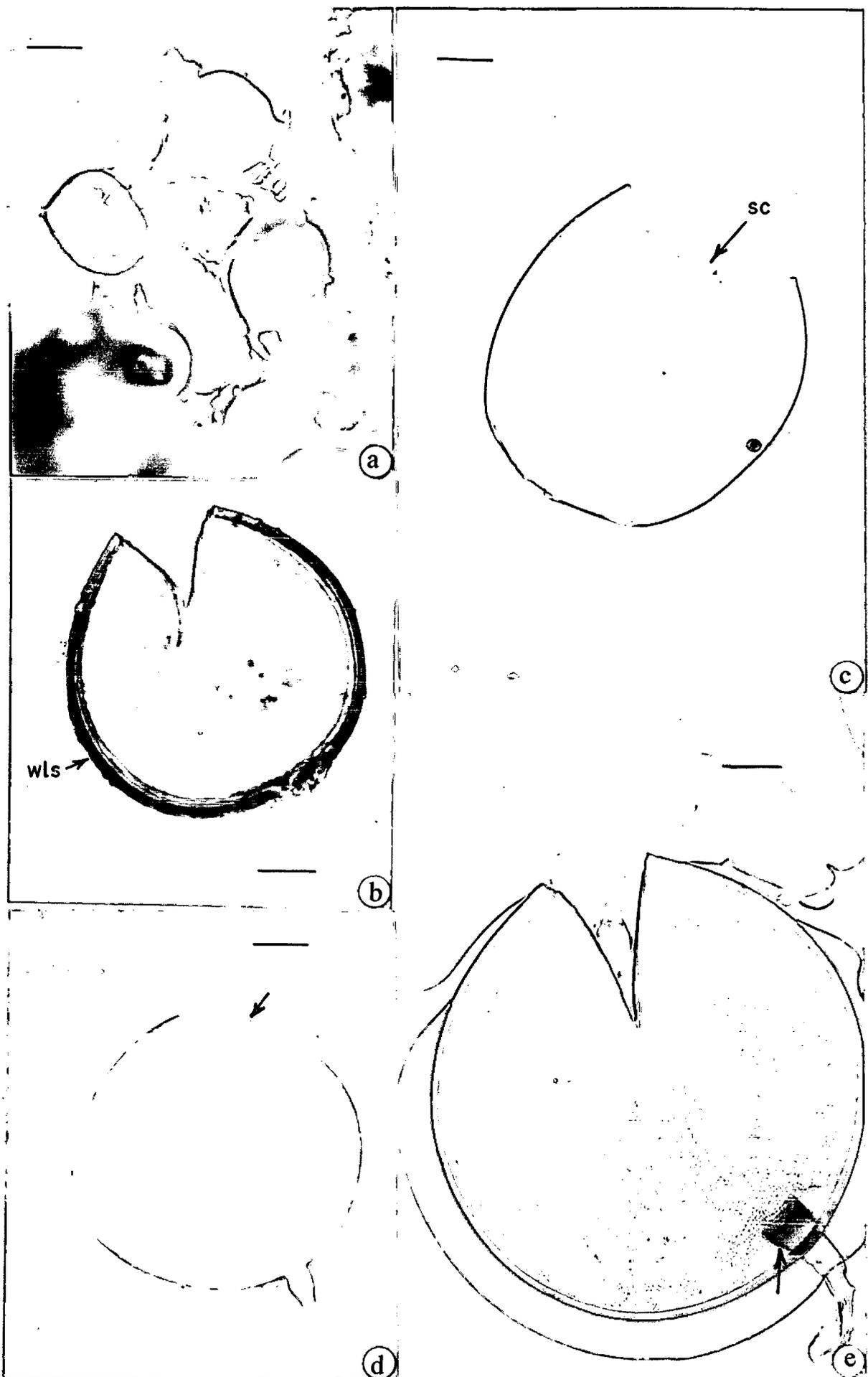


PLATE - XIV

light yellow, membranous, 0.4 - 0.5 μm thick. *Subtending hyphae*, single, cylindrical or slightly flared towards the point of attachment with the spore, where it is 7 - 11 μm wide, with a single wall 2.5 - 3 μm thick, sometimes bearing fine, thin walled, septate, lateral branches.

Glomus intraradices Schenck & Smith, *Mycologia* 74: 78, 1982 (Plate- XIV b).

Spores found frequently within roots and infrequently in loose clusters in the soil. *Spores* yellow brown, globose to subglobose; 95 - 150 μm diam. *Spore wall structure* of two walls (1 & 2) in one group. Wall-1 hyaline, ephemeral 1 - 2 μm thick; wall-2 laminated 2 - 6 μm thick. *Subtending hyphae* 8 - 20 μm wide at the point of attachment with the spore and, with a wall thickness of 1.5 - 4.5 μm .

Glomus lacteum Rose & Trappe, *Mycotaxon* 10: 413-420, 1980 (Plate- XIV c).

Spores borne singly in the soil, opaque, milky white, shiny-smooth, globose to subglobose, 150 - 200 μm diam. *Spore wall structure* of a single wall 3 - 5 μm thick. *Subtending hyphae* 1 - 3, usually one, straight, 7 - 11 μm wide. Spore content hyaline, granular or of globules of varying size.

Glomus macrocarpum Tul. & Tul., *Can. J. Bot.* 61: 2608-2617, 1982 (Plate- XV a, b).

= *Endogone macrocarpa* (Tul. & Tul.) Tul., *Fungi Hypogali* 182, 1851.

= *Endogone guttulata* Fischer, *Schweiz, Pilzkd* 1: 85, 1923.

PLATE XV

Spores of *Glomus*

- (a) A broken portion of sporocarp of *Glomus macrocarpum* showing spores.
- (b) Spores of *Glomus macrocarpum* showing details of spore wall structure. Note the septum (s).
- (c) Intact spore of *Glomus magnicaule*.
- (d) Cluster of spores of *Glomus microaggregatum*.
- (e) Intact spore of *Glomus monosporum*.
- (f) Enlarged view of spore of *Glomus monosporum* showing minute echinulations projecting into the outer wall (arrowed).

(Scale bar: a = 100 μm ; b-e = 50 μm ; f = 35 μm).



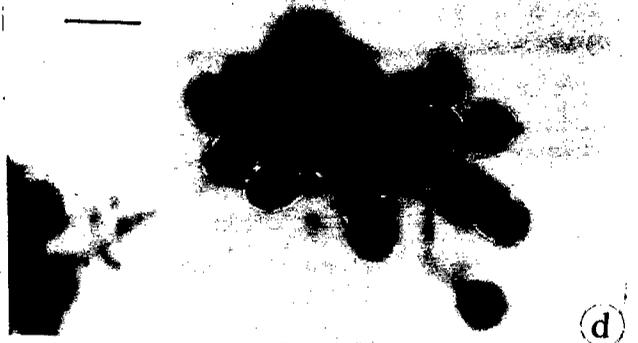
a)



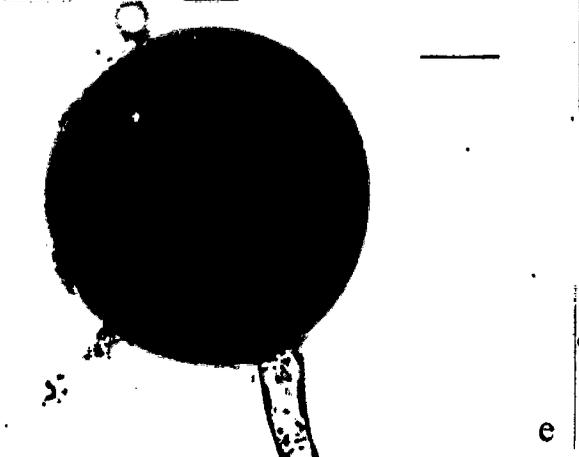
b)



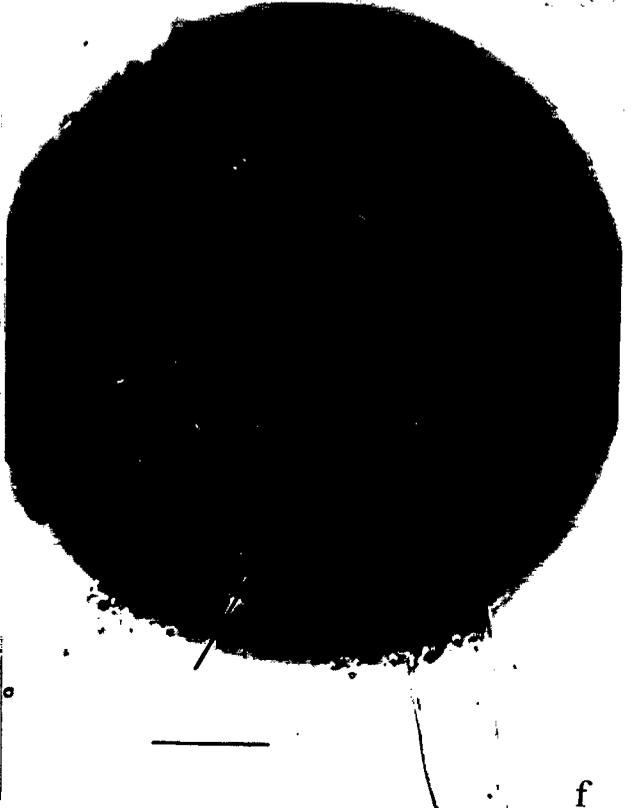
c)



d)



e)



f)

= *Endogone nuda* Petch, *Ceylon J. Sci. Sect. A, Ann. R. Bot. Gard. Pergdeniya*, **9**: 322, 1925.

Fragments 1 to 2 mm in diam. *Spores* yellow to yellow-brown, to dark brown, usually slightly longer than wide, globose to subglobose; 90 - 145 x 70 - 130 µm diam. *Spore wall structure* of three walls in one group. Wall-1 evanescent, hyaline unit wall 1 - 2 µm thick. Wall-2, 6 - 12 µm thick laminated wall. Wall-3, hyaline, unit wall 0.8 - 1 µm thick. *Subtending hyphae* straight 8 - 12 µm with 12 - 18 µm at the point of attachment with the spore. *Pore* closed by a thin septum or occluded by the thickening of wall-2. Some of the spores of *Glomus macrocarpum* were occupied with spores of *Glomus aggregatum* (Plate- XXV a).

Glomus magnicaule Hall, *Trans. Br. Mycol. Soc.* **68**: 341-356, 1977 (Plate- XV c).

Spores formed singly in the soil, brown; globose to subglobose; 150 - 175 µm diam. *Spore wall structure* of two walls (1&2) in one group. Wall-1 brown laminated, 12 - 20 µm thick. Wall-2, light brown 3 µm thick. *Subtending hyphae* 35 - 50 µm wide often slightly pinched at the point of attachment with the spore.

Glomus microaggregatum Koske, Gemma & Olexia, *Mycotaxon* **26**: 125-132, 1986 (Plate- XV d).

Spores found frequently inside dead spores of other fungi and roots, or in loose cluster in soil. Spores hyaline to pale yellow to brownish-yellow, globose to subglobose; 18 - 25 x 20 - 40 µm diam. *Spore wall structure* of

one or two walls (1&2) in one group. Wall-1, 0.8 - 1.8 μm thick. Wall-2, a membranous unit wall, 0.8 - 1 μm thick. *Subtending hyphae* straight 2 - 5 μm diam., and 2 - 8 μm at the point of attachment with the spore.

Glomus microcarpum Tul. & Tul., *Mycologia*, 76: 190-193, 1984.

Spores globose to subglobose; 30 - 45 x 30 - 40 μm diam., yellow-to-yellow brown. *Spore wall structure* of a single wall, 4 - 6 μm thick, yellow-brown, laminated. *Subtending hyphae* straight to slightly funnel shaped 3 - 6 μm diam., and 4 - 7 μm at the point of attachment with the spore. *Pore* usually open or occluded by wall thickening.

Glomus monosporum Gerdemann & Trappe, *Mycologia Memoir* No. 5. 76 pp. 1974 (Plate- XV e, f).

Sporocarp's 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; 200 - 330 μm diam. *Spore wall structure of* two wall (1&2) in one group. Wall-1 hyaline, 0.4 - 0.5 μm thick. Wall-2 dull brown laminate, 5 - 9 μm thick with minute, abundant to scattered echinulations that protrudes into the outer wall; thickening of the inner wall extending into the subtending hyphae. *Subtending hyphae* straight or strongly recurved, 8 - 12 μm wide and appressed to the spore walls.

Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe, *Mycologia Mem.*, **5**: 40, 1974 (Plate XIV e).

Sporocarps rare. *Spores* pale yellow to yellow-brown, globose to subglobose; 150 - 320 μm diam., with one distinct funnel shaped hyphal attachment. *Spore wall structure* of two walls (1&2) in one group. Wall-1, hyaline thin, barely perceptible, 0.4 - 0.5 μm thick. Wall-2, laminated, 3 - 6 μm thick. *Subtending hyphae* 8 - 13 μm diam., and 25 - 35 μm in diam. at the point of attachment with the spore.

Glomus multicaule Gerdemann & Bakshi *Trans. Br. Mycol. Soc.* **66**: 340-343, 1976 (Plate- XVI a, b).

Spores formed singly in soil, dark brown, ellipsoidal, broadly ellipsoidal, subglobose or occasionally triangular, 150 - 240 x 124 - 162 μm diam., with 1-4 hyphal attachments, attachments generally occurring at opposite ends. *Spore wall structure* of a single wall 8 - 28 μm thick, thickest at the point of hyphal attachments, rounded projections 1.2 - 3.5 μm long regularly distributed over wall surface. *Subtending hyphae* usually two at opposite ends, 10 - 12 μm wide, 20 - 24 μm at the point of attachment with the spore with yellow to dark brown wall thickenings, 5 - 7 μm thick, at the spore base, tapering to 2 - 3 μm .

Glomus reticulatum Bhattacharjee & Mukerji, *Sydowia (Annales Mycologici)* **39**: 14-17, 1980.

Spore borne singly in the soil, dark brownish-black, globose, 130 - 160 μm diam. *Spore wall structure* of two walls in one group. Composite spore wall 10 - 13 μm thick, clearly differentiated into an outer and inner wall. Outer

PLATE XVI

Spores of *Glomus* and *Paraglomus*

- (a) Spore of *Glomus multicaule* with two hyphal attachments at opposite ends.
- (b) Surface of broken spore of *Glomus multicaule* showing the regular distribution of protuberances.
- (c) A sub-angular spore of *Paraglomus occultum* with a straight subtending hypha.

(Scale bar: a, b = 22 μm ; c = 22 μm).

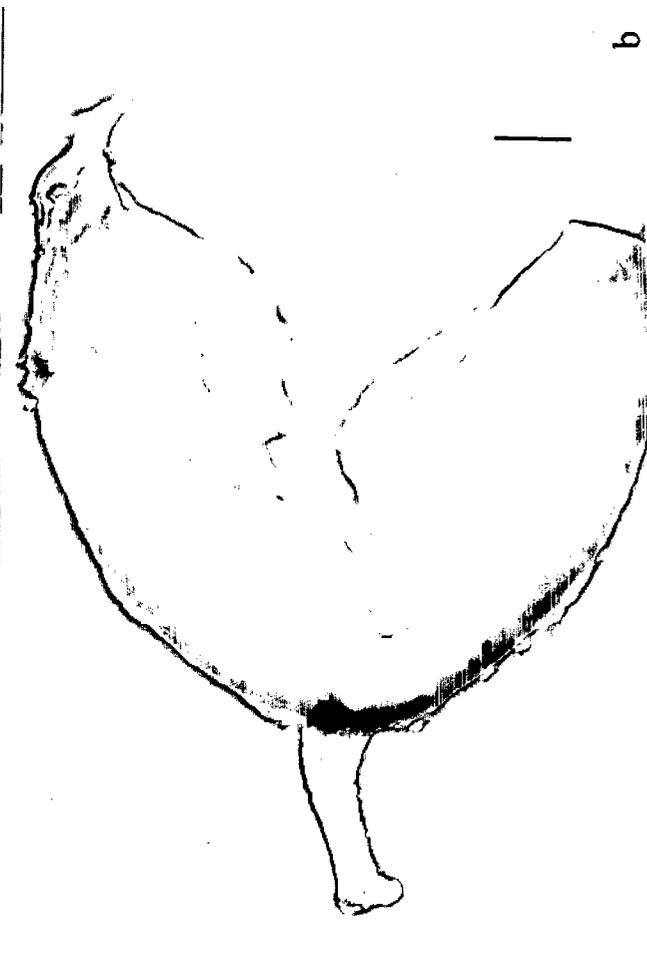
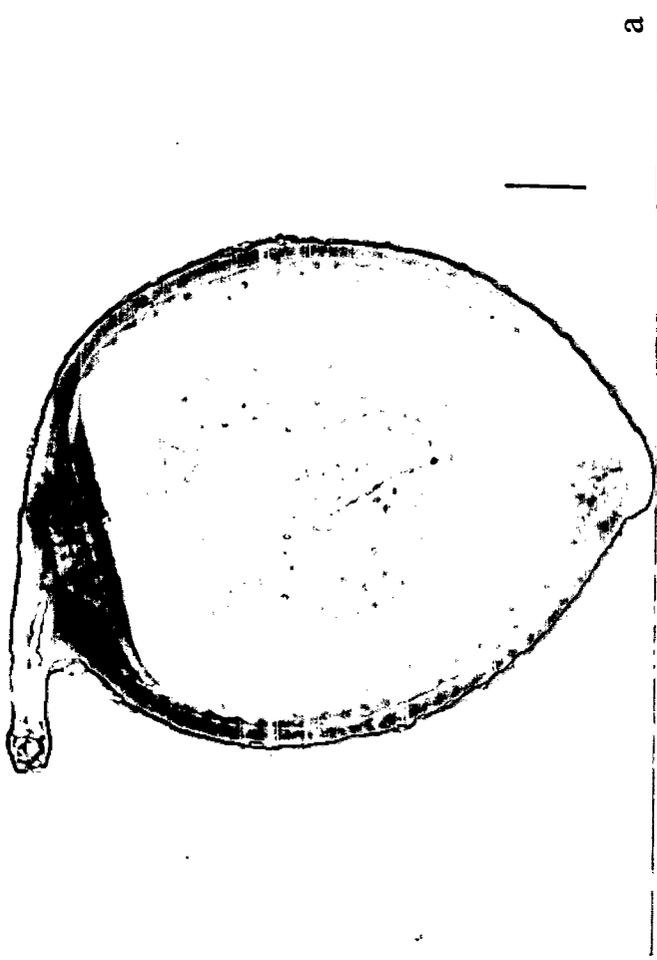


PLATE - XVI

wall, 4 - 7 μm thick, two layered and fissured, outermost layer 1 - 2 μm thick and inner layer 4 - 5 μm thick, inner wall with regular geometric reticulate markings 5.5 - 9.5 μm apart on its outer surface. *Subtending hyphae* single, funnel shaped 4 - 5 μm wide and 8 - 10 μm wide at the point of attachment with the spore.

Glomus rubiforme (Gerdemann. & Trappe) Almeida & Schenck, *comb. nov.*
Mycologia, **82**: 703-711, 1990 (Plate-XVII- c-f).

(Basionym: *Sclerocystis rubiformis* Gerd. & Trappe, *Mycologia Mem.* **5**: 60-63, 1974 .)

= *Sclerocystis indicus*. Bhattachajee, Mukerji & Misra, *Acta Bot. Indica* **8**: 99-100, 1980.

= *Sclerocystis pachycaulis* Wu & Chen, *Taiwania* **31**: 74, 1986.

Sporocarps yellow-brown to dark brown, subglobose to somewhat irregular, *Sporocarps* yellow to yellow-brown 160 - 300 x 180 - 300 μm lacking peridium. In young sporocarps *chlamydospores* are arranged in a hemispherical layer. However, with the subsequent formation of additional chlamydospores, these immature sporocarps are transformed into globular sporocarps with radially arranged chlamydospores. *Chlamydospores* are arranged on a thick walled central plexal cell, which is connected with a broad hyphal stalk. *Chlamydospores* yellow to yellow-brown, obovoid to ellipsoid or subglobose, sometimes irregular, 35 - 51 x 30 - 80 μm . *Spore wall structure* of two walls. Outer wall laminated, 2 - 2.5 μm thick, inner wall, 0.3 - 0.4 μm thick with a small pore opening into the thick walled subtending hyphae. *Subtending hypha* 7 - 12 μm broad at the point of attachment with

PLATE XVII

Spores and sporocarps of *Glomus* (a-f)

- (a) *Glomus coremioides* hemispherical arrangement of chlamydospores around a central plexus of hyphae.
- (b) Chlamydospores of *Glomus coremioides* cut off from the hyphae by a septa at the spore base and a parasitized spore (ps).
- (c) Young sporocarp of *Glomus rubiforme* in fan shaped arrangement connected with hyphal stalk with spores of *Glomus rubiforme* arising from a monohyphal stalk.
- (d) Globular sporocarp of *Glomus rubiforme*.
- (e) Chlamydospores of *Glomus rubiforme* showing details of spore walls and an opening (arrowed) in the pore of subtending hypha.
- (f) Chlamydospores of *Glomus rubiforme* with septa (arrowed) in the subtending hypha.

(Scale bar: a = 100 μm ; b = 20 μm ; c, d = 50 μm ; e, f = 22 μm).

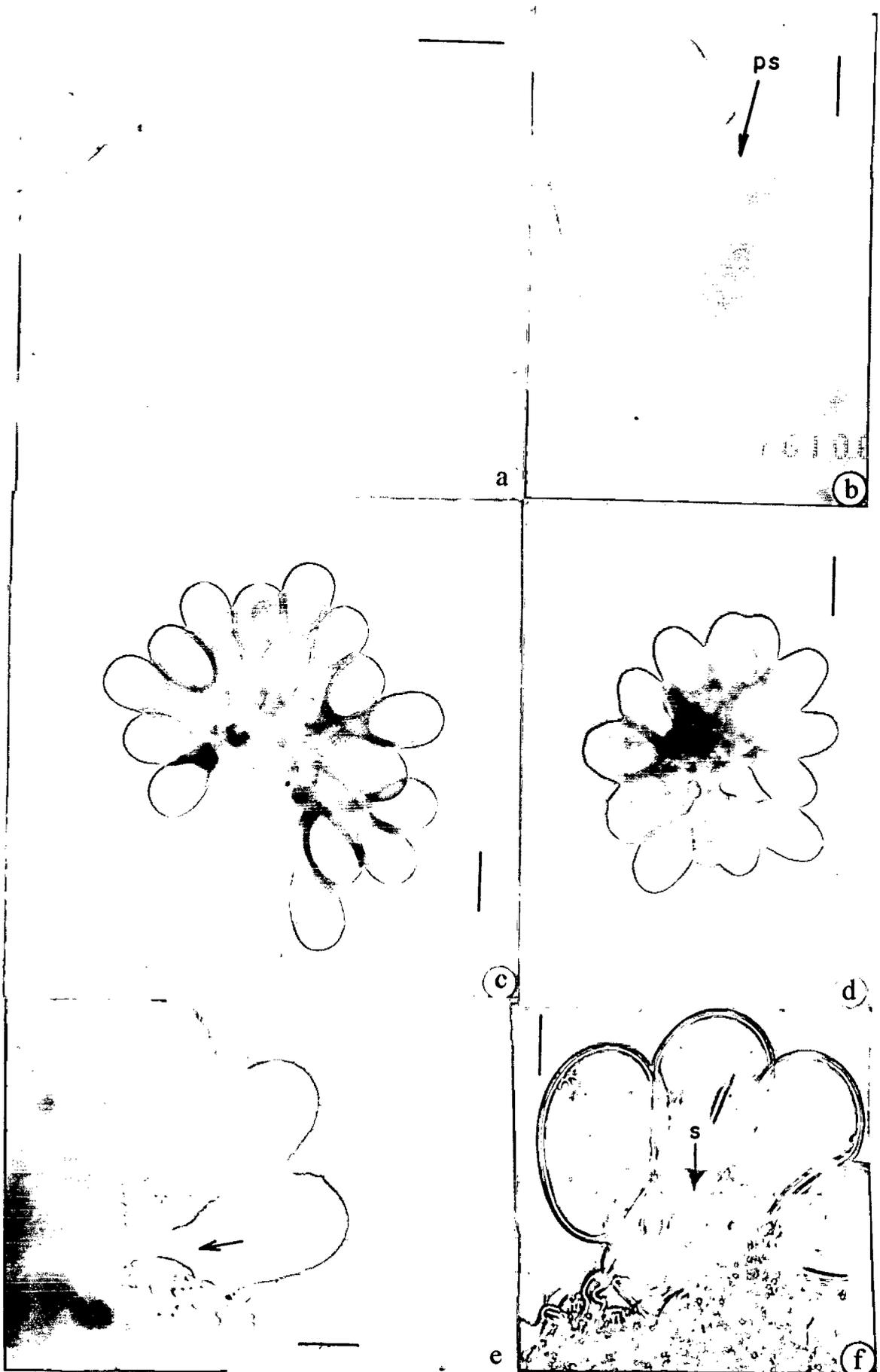


PLATE - XVII

Chlamydo-spore. Thickness of the attached hyphae extending down for some distance.

Glomus sinuosum (Gerd. & Bakshi) Almeida & Schenck, *comb. nov.*
Mycologia, **82**: 703-711, 1990 (Plate-XVIII ; Fig- 26).

(Basionym: *Sclerocystis sinuosa* Gerd. & Bakshi, *Trans. Br. Mycol. Soc.*, **66**:
343, 1976

= *Sclerocystis pakistanica* Iqbal & Bushra, *Trans. Br. Mycol. Soc., Japan* **21**:
59-60, 1980.

Sporocarps reddish-brown to dull brown, found singly or in pairs, globose to subglobose or discoid 300 - 480 x 380 - 600 μm , tuberculate or smooth.

Peridium composed of thick walled septate sinuous hyphae or papillate warts.

Spores 38 - 80 x 50 -138 μm , obovate, elliptical, fusiform-elliptical to clavate, radiating from a central plexus of hyphae. *Spore wall structure* of a single

laminated wall, yellow-brown to brown 1.5 - 5 μm thick at the sides and 2 - 9 μm thick at the apex. *Subtending hyphae* one or two, 2 - 3.4 μm in diam.,

and 3 - 6 μm at the point of attachment with the spore. Spore content delimited by a septum present either at the spore base or in the subtending

hyphae. Sporocarps are often associated with one or three monohyphal stalks and spore development is asynchronous.

The spores and sporocarps of *Glomus sinuosum* exhibited wide range of morphological variations. Similarly spores of *Glomus sinuosum* were occupied with spores of *Glomus aggregatum* (Plate- XXV b).

PLATE XVIII

Spores and sporocarps of *Glomus sinuosum* (a-f)

- (a) Peridial surface of a young sporocarp of *Glomus sinuosum*.
- (b) Young spore of *Glomus sinuosum*. Note the wall thickening at the apex.
- (c) Peridial hyphae of a mature sporocarp of *Glomus sinuosum*.
- (d) Spore of *Glomus sinuosum*. Note the apical spore wall thickening (arrowed).
- (e) & (f)- Spores of *Glomus sinuosum* showing variation in spore shapes.

(Scale bar: a = 100 μm ; b = 44 μm ; c = 50 μm ; d-f = 44 μm)

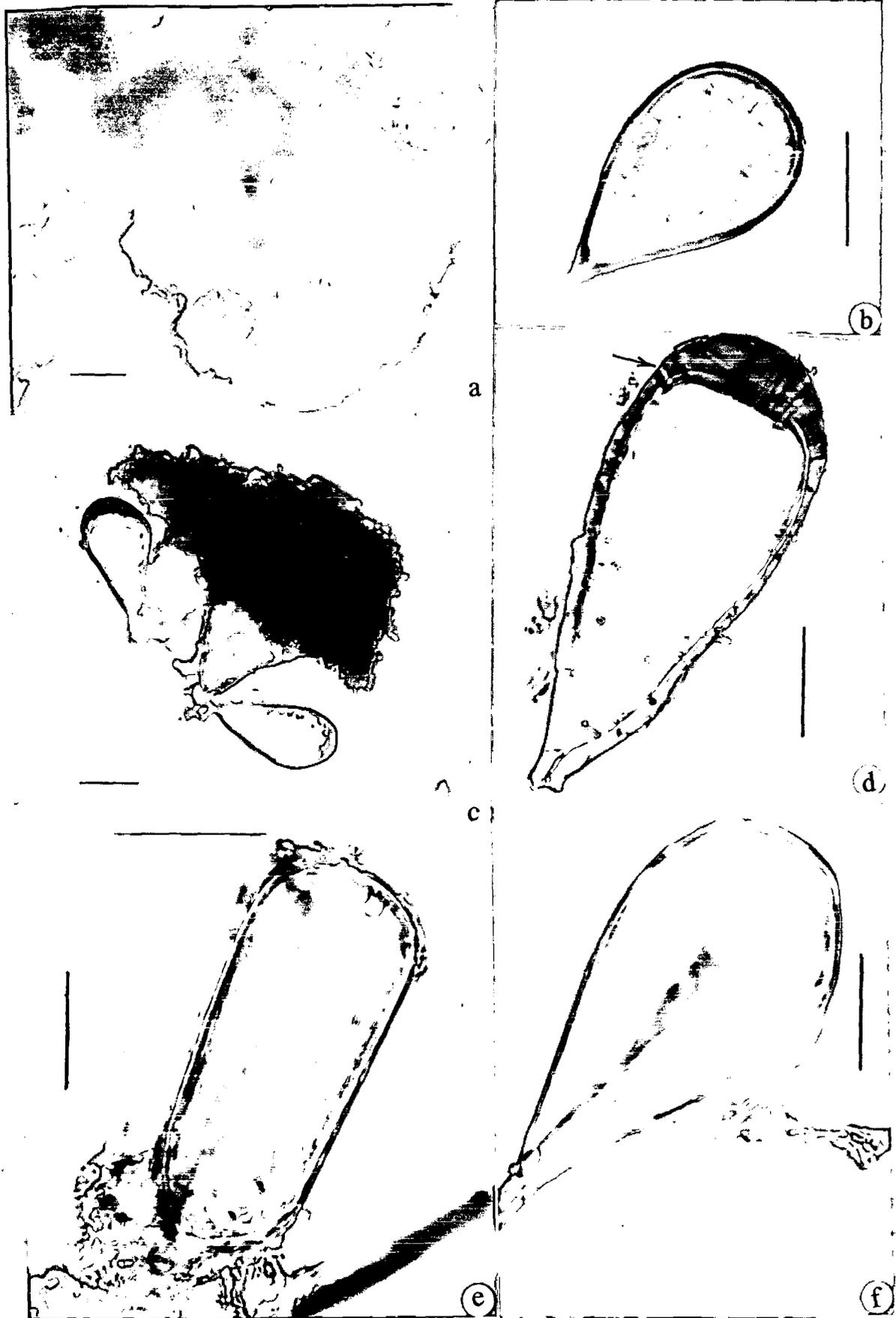


PLATE - XVIII

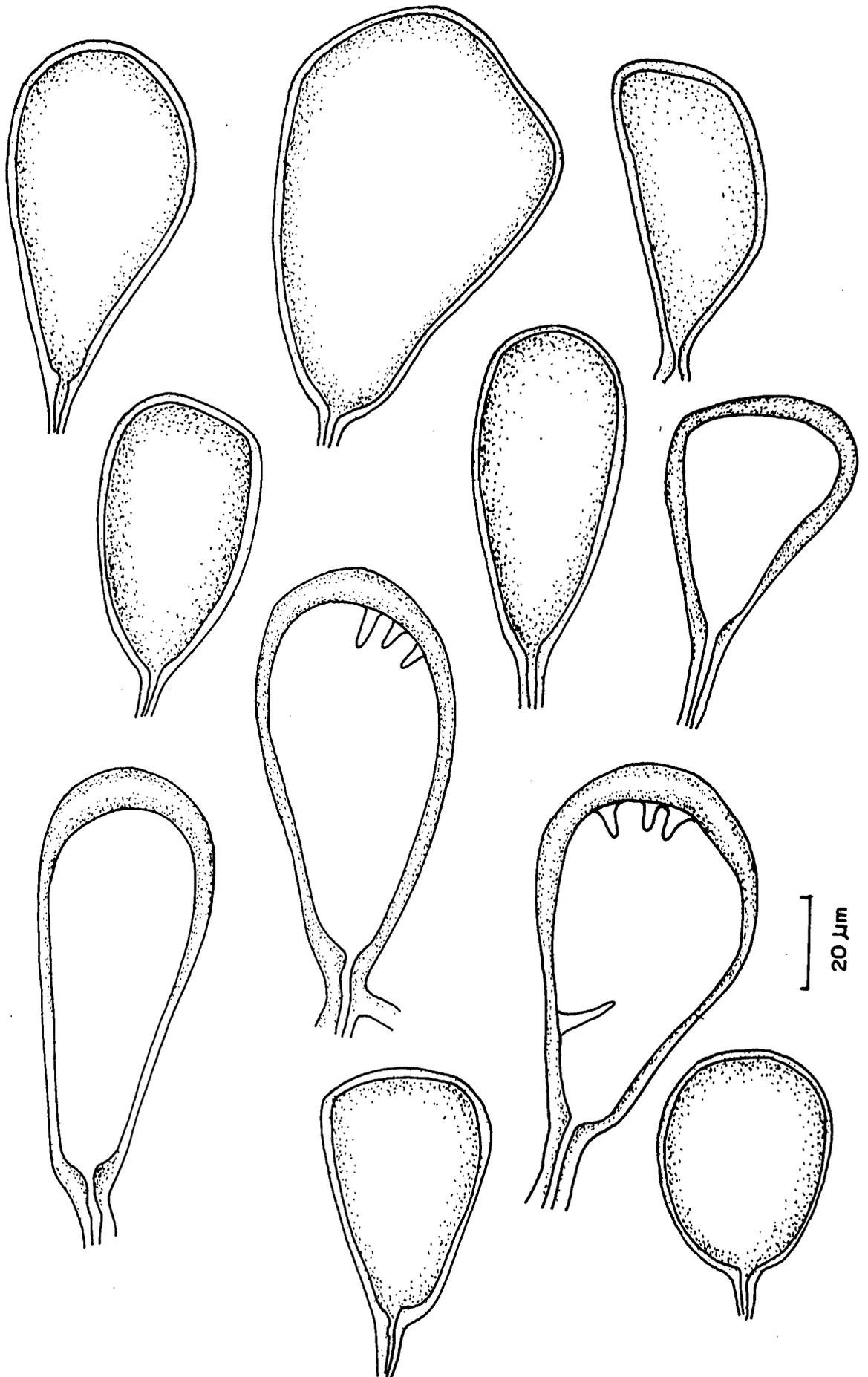


Fig. 26 Heterogenous Chlamydospores of *Gl. sinuatum*

Glomus taiwanensis (Wu & Chen) Almeida & Schenck, *comb. nov.*
Mycologia, **82**: 703-711, 1990 (Plate-XIX; Fig- 27).

(Basionym: *Sclerocystis taiwanensis* Wu & Chen, *Trans. Mycol. Soc. Rep.*
China **2**: 73-83, 1987.)

Sporocarps reddish-brown to dark-brown, globose to subglobose 200 - 300 x 190 - 320 μm with spores arranged in a single tightly packed layer around the central plexus of hyphae. *Peridium* absent. *Spores* yellowish-brown to olive-brown, clavate to cylindro-clavate to obovate; 35 - 75 x 20 - 28 μm . *Spore wall structure* consists of two walls (1&2) in one group. Wall-1 hyaline, 0.5 - 1.5 μm and wall-2 laminated, 6 - 10 μm thick at the apex and 2 - 4 μm at the sides and base. *Subtending hyphae* 1 to 2, 2 - 3.5 μm in diam., and 5 - 8 μm at the point of attachment with the spore. Spore content delimited by a septum present either at the spore base or in the subtending hyphae. Sporocarps are often associated with one or three monohyphal stalks and spore development is asynchronous.

Paraglomus occultum (Walker) Morton et Redecker, *com. Nov. Mycologia*,
93: 181-195, 2001 (Plate- XVI c).

(Basionym: *Glomus occultum* Walker, *Mycotaxon* **15**: 50, 1982).

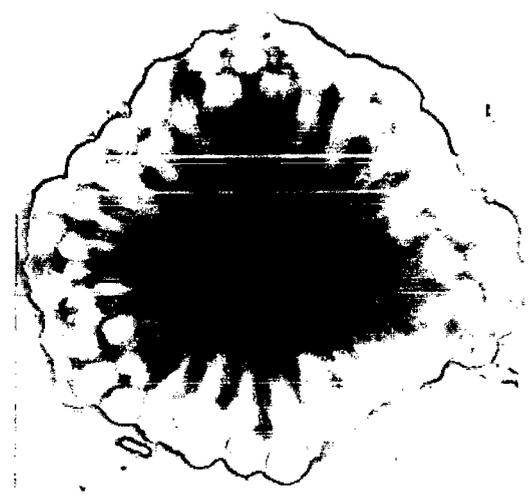
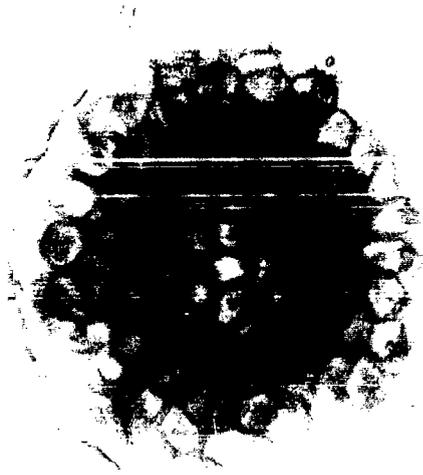
Spores hyaline, becoming slightly yellow with age or storage; usually of variable shape, more rarely globose; 70 - 90 μm . *Spore wall* consisting of three hyaline layers. The outermost layer is 1 - 1.5 μm thick when intact. The second layer is finely laminate, 0.5 - 1.2 μm thick, continuing into the wall of the subtending hypha without any noticeable thickening. The innermost layer also is finely laminate, 0.5 - 1.5 μm thick, thickening at the point of hyphal

PLATE XIX

Spores and sporocarps of *Glomus taiwanensis* (a-e)

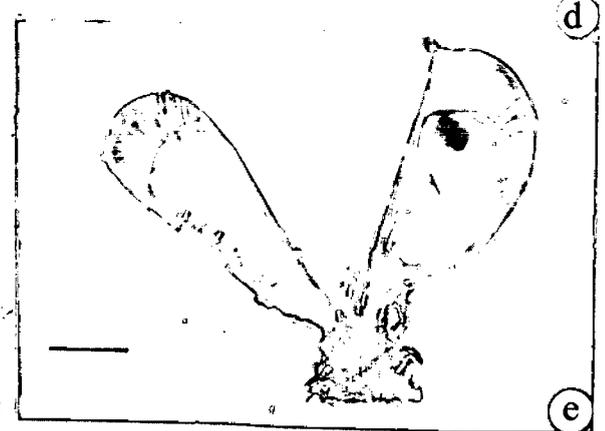
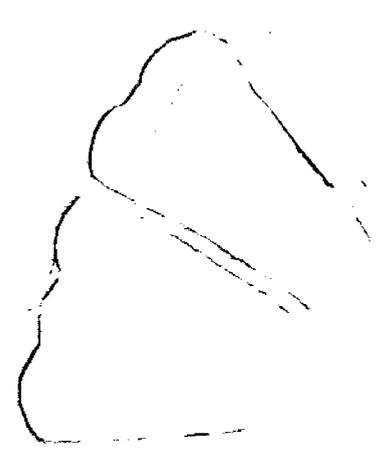
- (a) Cross section of sporocarp of *Glomus taiwanensis*.
- (b) The outer surface of sporocarp of *Glomus taiwanensis*.
- (c) Portion of sporocarp of *Glomus taiwanensis* showing chlamydospores tightly arranged.
- (d) Chlamydospores with a hyaline separate outer layer.
- (e) Spore wall thickening of *Glomus taiwanensis* at the apex.

(Scale bar: a, b= 50 μ m; c- e = 22 μ m).



a

b



c

d

e

PLATE - XIX

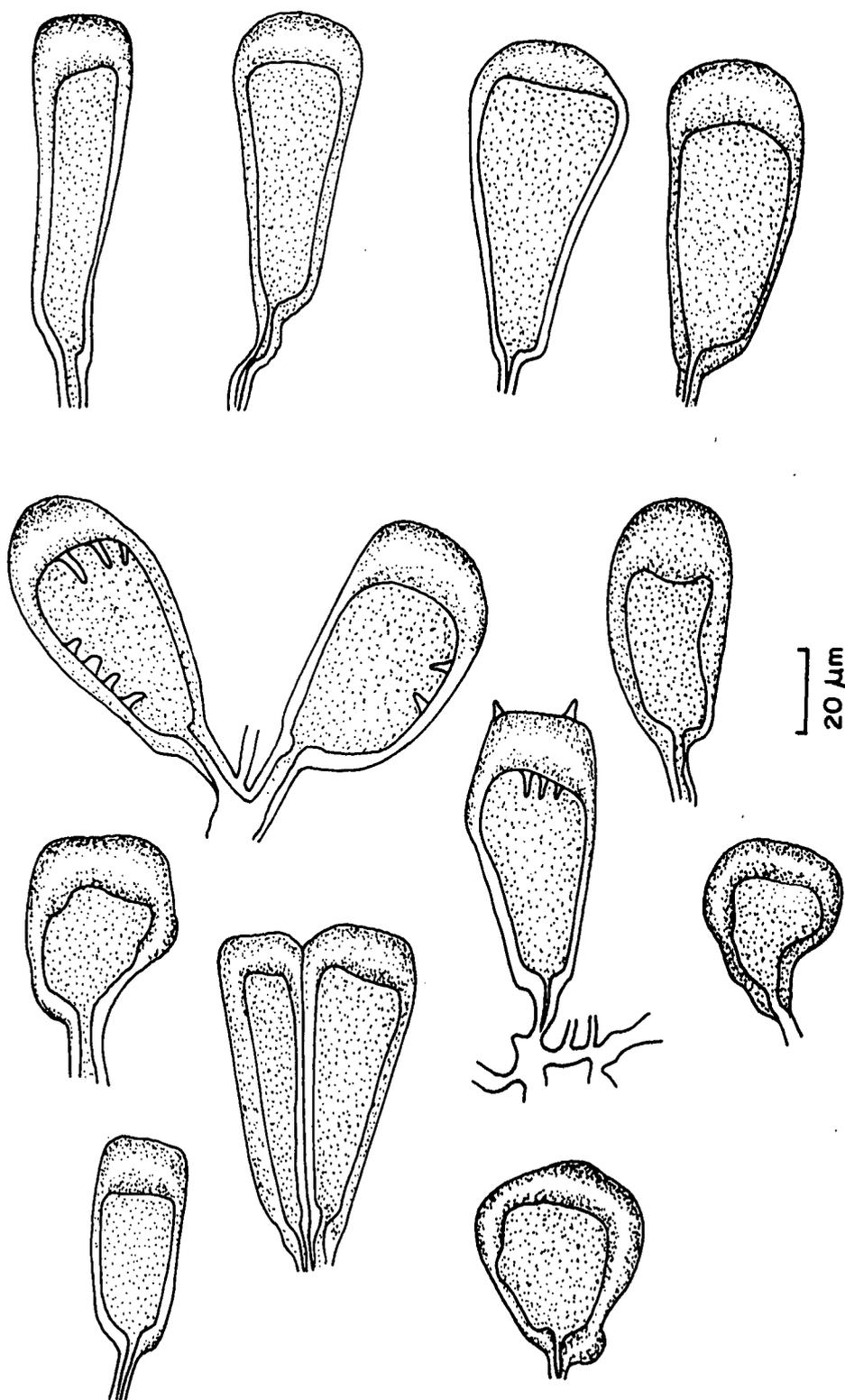


Fig. 27 Different spore shapes in *Gl. taiwanensis*

attachment, continuing into the wall of the subtending hyphae. Spore contents are occluded by thickening of the spore wall. *Subtending hyphae* is cylindrical to slightly flared, 3.5 - 4.5 μm in diam. and 4 - 8 μm wide at the point of attachment with the spore.

Scutellospora calospora (Nicolson & Gerdemann) Walker & Sanders, *Mycotaxon* **27**: 219-235, 1986 (Plate-XX a, b).

= *Endogone calospora* Nicol. & Gerd., *Mycologia* **60**: 322, 1968.

= *Gigaspora calospora* (Nicol. & Gerd.) Gerd & Trappe, *Mycologia. Mem.* **5**: 28, 1974.

Spores found singly in the soil, terminally on a bulbous sporogenous cell; hyaline to pale yellow; globose to subglobose; 200 - 480 x 230 - 490 μm diam. *Spore wall structure* of four walls (1-4) in two groups (A, B). Group A of a thin hyaline unit wall (Wall-1) 1 - 1.5 μm thick, tightly appressed to laminated wall, hyaline to pale yellow-brown brittle, (Wall-2) 3 - 4.5 μm thick. Group B of two hyaline membranous walls (Wall 3 and 4) each 0.5 to 1 μm thick. *Sporogenous cell* 35 - 45 μm broad; borne terminally on a septate subtending hyphae 10 - 12 μm broad.

Germination shield oval, or subcircular 40 - 70 x 50 - 85 μm , often with invaginations along the margins.

PLATE XX

Spores of *Scutellospora* (a-f)

- (a) *Scutellospora calospora* intact spore showing sporogenous cell (sc) and hyphal protrusion (hp).
- (b) Broken spore of *Scutellospora calospora* with germination shield (gs).
- (c) Intact spore of *Scutellospora gregaria*.
- (d) Broken spore of *Scutellospora gregaria* showing details of sporogenous cell (sc) and germination shield (gs).
- (e) A portion of crushed spore of *Scutellospora gregaria* showing germ tube (gt), sporogenous cell and warts (wts) on the spore surface.
- (f) Enlarged view of germination shield (gs) of *Scutellospora gregaria*.

(Scale bar: a = 100 μm ; b = 22 μm ; c, d = 100 μm ; e, f = 22 μm ;
f = 44 μm)

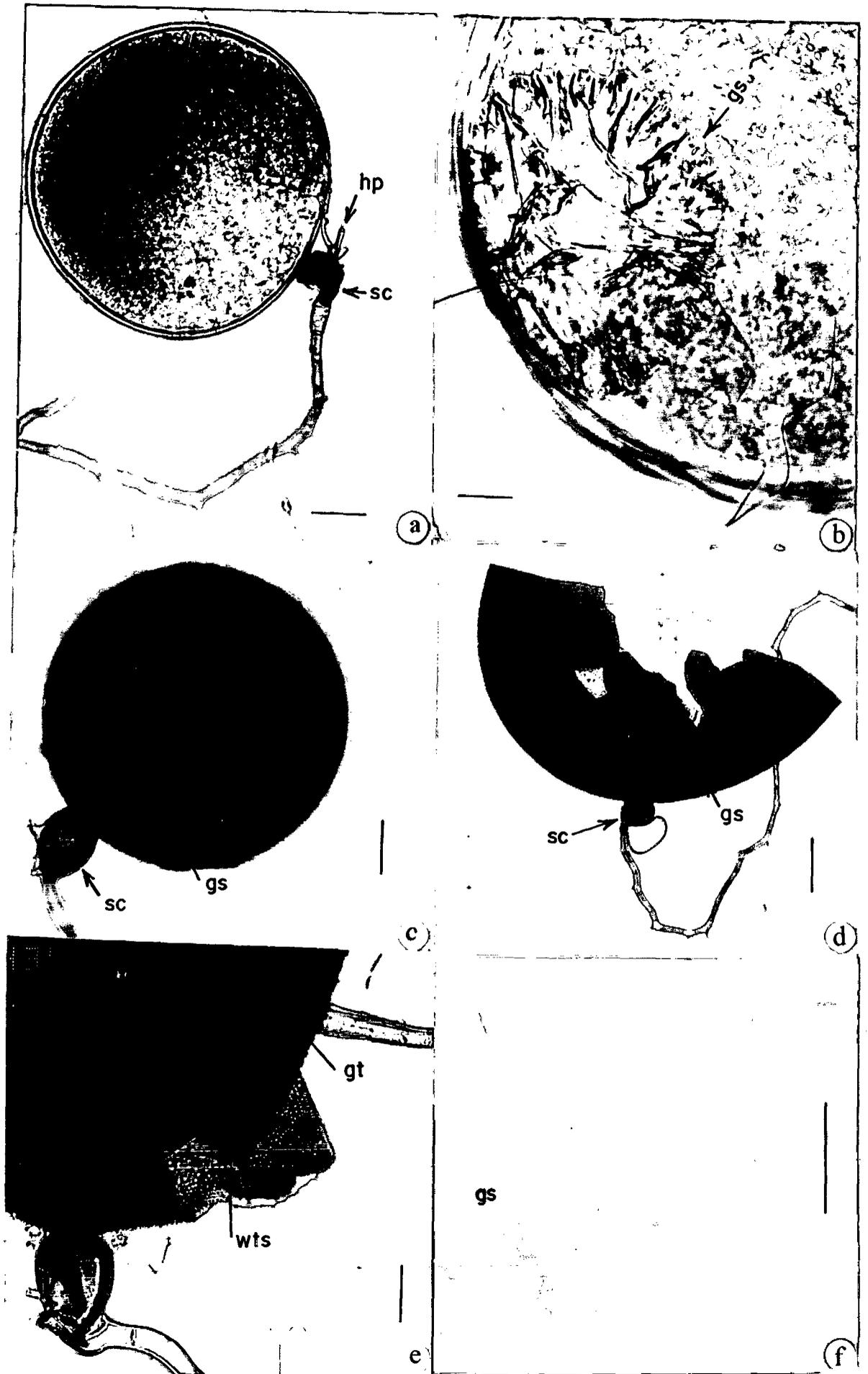


PLATE - XX

Scutellospora gregaria (Schenck & Nicolson) Walker & Sanders, *Mycologia* 77: 702720, 1985 (Plate-XX c-f).

Spores found singly in the soil, terminally on a bulbous sporogenous cell, red-brown to dark brown, globose to subglobose; 250 - 290 x 250 - 300 μm . *Spore wall structure* of four walls (1-4) in two groups (A & B). 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, 1.5 - 3 μm thick excluding the closely packed warts situated on its outer surface. Warts pale brown, 1 - 4 μm high with rounded tips, 2 - 5 μm diam., at the base, crowded together in-group, tightly adherent to two laminated walls (3 & 4). Wall-2 brittle, yellow 3 - 4 μm thick. Wall-3, brittle, pale yellow to nearly colourless, 5 - 10 μm thick. Group B of a single membranous hyaline wall (Wall-4) 1 - 3 μm thick.

Sporogenous cell 45 - 55 μm wide, pale brown, with 1 or 2 thick or thin walled hyaline projections, borne terminally on a septate subtending hyphae 13 - 16 μm wide with wall thickness 2 - 3 μm distally, thickening to 1.2 - 1.5 μm at the spore base.

Germination shield globose or oval 90 - 135 μm diam with inward folds (Fig-28 b).

Scutellospora nigra (Redhead) Walker & Sanders, *Mycologia* 71: 178-198, 1979 (Plate-XXI).

= *Gigaspora nigra* Old, Nicolson & Redhead, *New Phytol.* 72: 817, 1973.

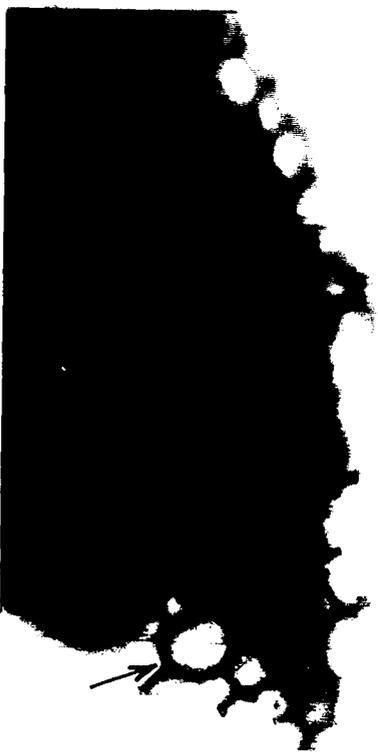
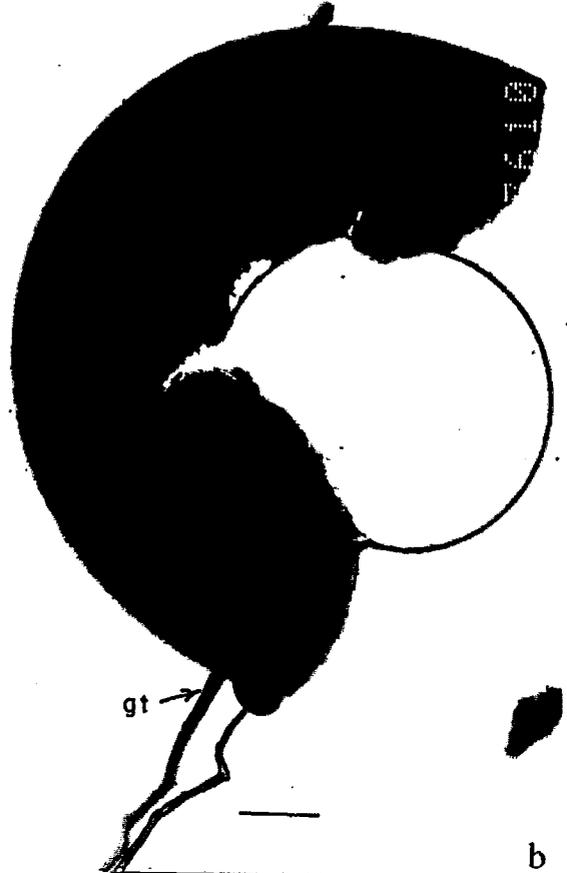
Spores found singly in the soil, laterally on a bulbous sporogenous cell; dark-brown to black, globose to subglobose 400 - 800 μm diam. *Spore wall*

PLATE XXI

Spores of *Scutellospora nigra* (a-d)

- (a) Intact spore with laterally attached sporogenous cell and adhering soil debris (arrowed).
- (b) Broken spore showing sporogenous cell (sc) and germ tube (gt).
- (c) A portion of crushed spore with reticulate ornamentation (arrowed).
- (d) A portion of crushed spore showing germination shield (gs).

(Scale bar: a, b= 100 μ m; c = 22 μ m; d =40 μ m).



structure of five walls (1-5) in two groups (A ,B). Group A consists of a hyaline, ornamented wall (wall-1) 1.5 - 3 μm thick with large reticulum 20-25 μm diam. Wall-2, laminated, 3 - 10 μm thick. Group B of three membranous walls (wall 3-5) tightly adherent to each other 1-5 μm thick.

Sporogenous cell 40 - 50 x 80 - 120 μm broad, brown, borne laterally on subtending hyphae 39 μm in width.

Germination shield spherical to sub circular, 150 - 170 μm in diam. (Fig- 28 a).

Scutellospora pellucida (Nicol. & Schenck) Walker & Sanders, *Mycotaxon* 27: 219-235, 1986 (Plate-XXII a, b).

= *Gigaspora pellucida* Nicol. & Schenck, *Mycologia* 71: 178-198, 1979.

Spores found singly in the soil, terminally on a bulbous sporogenous cell; hyaline to pale gray, glistening with oil droplets, globose-ellipsoid, or irregular 220 - 280 μm diam. *Spore wall structure* of six walls (1-6) in three groups (A,B,C). Group A of a brittle, hyaline smooth wall (Wall-1) 1 - 1.5 μm thick, closely appressed to an inner (wall-2) 2 - 10 μm thick laminated wall. Group B of a hyaline membranous (wall-3) less than 1 μm thick and two hyaline unit walls (wall 4 and 5) measuring 1 μm each respectively. Group C consists of a single amorphous wall (wall-6) 2 - 4.5 μm thick.

Sporogenous cell 30 - 40 μm broad; borne terminally on septate subtending hyphae.

PLATE XXII

Spores of *Scutellospora* (a-e)

- (a) Spore of *Scutellospora pellucida* with sporogenous cell.
- (b) Wall layers of spore of *Scutellospora pellucida*.
- (c) Intact spore of *Scutellospora reticulata* showing laterally attached sporogenous cell.
- (d) Lateral attachment of sporogenous cell of *Scutellospora reticulata*.
- (e) A portion of crushed spore of *Scutellospora reticulata* showing reticulate surface ornamentation.

(Scale bar: a=50 μm ; b= 22 μm ; c= 75 μm ; d, e= 22 μm).

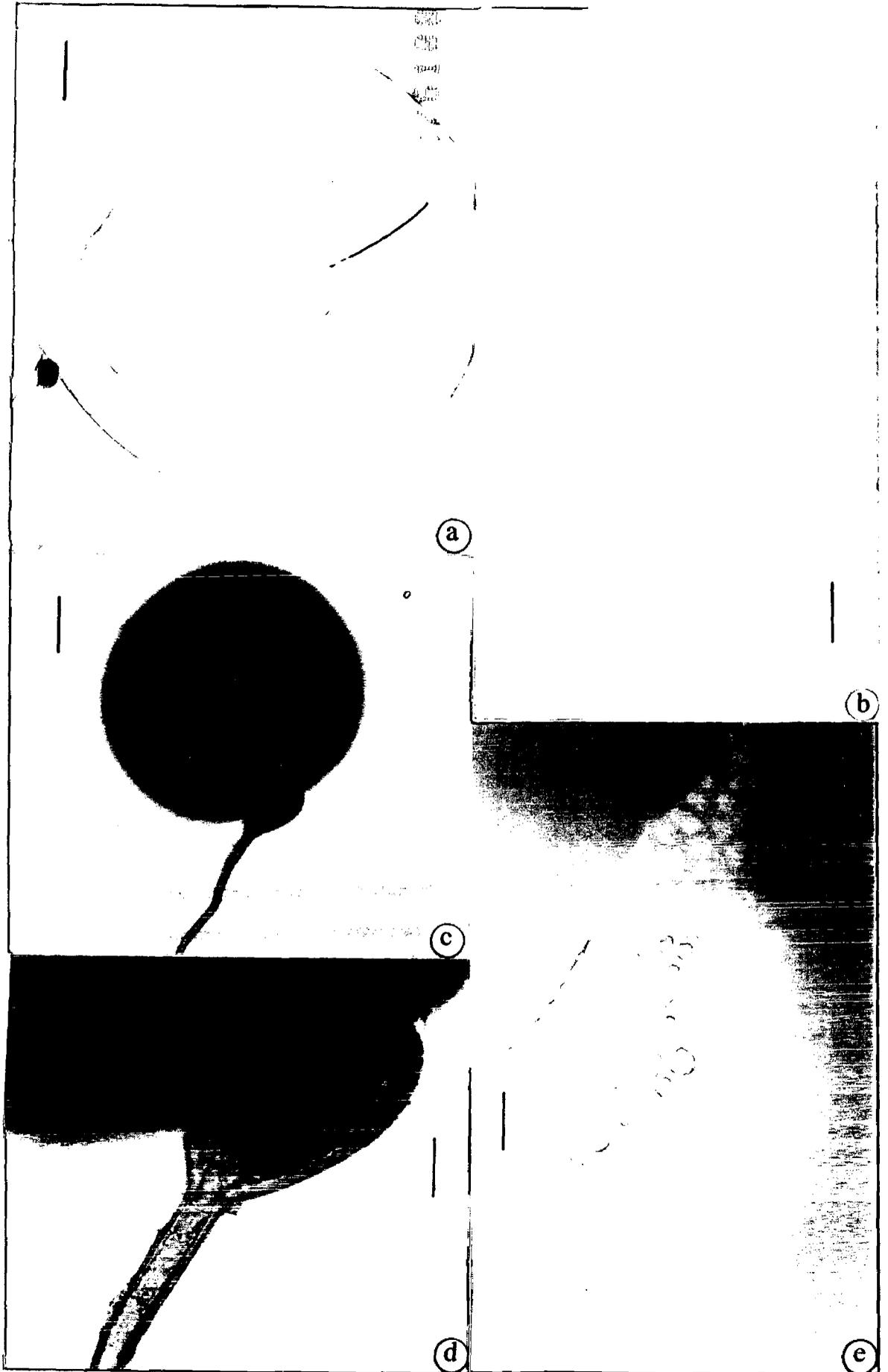


PLATE - XXII

Germination shield oval to spherical; 60 - 75 x 90 - 120 µm; margins with invaginations.

Scutellospora persica (Koske & Walker) Walker & Sanders, *Mycologia* 77: 702-720, 1985.

=*Gigaspora persica* Koske & Walker, *Mycologia* 77: 702-728, 1985.

Spores found singly in the soil, terminally on a bulbous sporogenous cell; brownish-orange; globose to subglobose to ellipsoid; 250 - 350 µm diam.

Spore wall structure of four walls (1-4) in two groups (A,B). Group A has an outer ornamented hyaline wall (Wall-1) 0.5 - 0.8 µm thick, with rounded warts 0.5 - 1 µm high and 0.5 µm diam. at the base, tightly adherent to (wall-2), 4 - 8 µm thick, laminated, brittle, pinkish-orange to brown. Group B of two tightly appressed hyaline membranous walls (wall 3 & 4) totaling less than 1 to 1.5 µm thick.

Sporogenous cell 40 - 44 µm wide borne terminally on a septate subtending hyphae.

Germination shield sub circular, 45 - 50 x 85 - 120 µm diam.

Scutellospora reticulata (Koske, Miller & Walker) Walker & Sanders, *Mycotaxon* 16: 429-435, 1983 (Plate-XXII c-e).

Spores found singly in the soil, laterally on a bulbous sporogenous cell; dark red-brown, globose to subglobose 200 x 350 µm diam. *Spore wall structure*

complex, consisting of two separate groups of wall layers overlaid by an alveolate reticulum. Outer wall group three layered. Outer layer 0.5 - 1 μm thick, orange brown to red-brown, supporting raised, straight to sinuous interconnecting ridges that form a reticulum 0.5 - 1 μm high with 4 - 8 sided meshes 2 - 24 x 2 - 30 μm across. Spore surface between ridges covered with polyhedral, conical or sub cylindrical spines, or narrow straight, curved, or angular ridges 0.5 - 1 μm high and 0.25 - 0.5 (<1 μm) apart; middle layer hyaline to pale yellow, 5 - 11 μm thick; inner layer hyaline 0.4 - 0.7 μm thick. Reticulate ridges on outer wall supporting a detachable alveolate reticulum 0.5 - 2 μm wide and 2 - 5 μm high. Inner wall group 3 layered, totaling to 3 μm thickness. *Sporogenous cell* 65 - 120 μm .

Germination shield, sub circular 55 - 65 x 90 - 110 μm in diam with inward projections.

Scutellospora verrucosa (Koske & Walker) Walker & Sanders, *Mycologia* 77: 702-720, 1985 (Plate-XXIII a-c).

Spores found singly in the soil, terminally or somewhat laterally on a bulbous sporogenous cell, pale-straw to yellow to orange-brown, globose to subglobose; 250 - 480 μm . *Spore wall structure* of three walls (1-3) in two groups (A,B) . Group A of hyaline to pale yellow brittle, ornamented unit wall (Wall-1) 2.5 - 3 μm thick including crowded, low rounded warts mostly 0.5 - 1.5 x 0.5 - 1.5 μm , 0.5 - 1 μm high, spaced 0.5 - 2 μm apart on the surface, tightly adherent to an inner pale yellow to orange-brown laminated

wall (Wall-2) 3.5 - 10.5 μm thick. Group B of a single membranous hyaline wall (Wall-3), 0.4 - 0.5 μm thick.

Sporogenous cell 55 - 75 x 35 - 70 μm diam, yellow-brown, with 1 or 2 peg like hyphal projections 22 - 40 x 6 - 10 μm , often darker than the spores, borne terminally on a coenocytic to sparsely septate subtending hyphae, with wall 2-3 μm thick, thickening to as much as 5 - 6 μm at the spore base .

Germination shield oval 60 - 80 x 100 - 150 μm wide, from which germ tube emerges, dark brown, often darker than the spores.

***Scutellospora weresubiae* Koske & Walker *Mycotaxon* 27: 219-235, 1986 (Plate-XXIII d, e).**

Spores found singly in the soil, terminally on a bulbous sporogenous cell, translucent, glistening, pale pink to deep pink, globose to subglobose; 225 - 390 μm diam. *Spore wall structure* of six walls (1-6) in three groups (A,B,C). Group A of a brittle, hyaline, smooth wall (Wall-1) upto 4 - 5 μm thick, tightly adherent to brittle, pink, laminated wall (Wall-2) 10 - 12 μm thick. Group B of two membranous walls (3 & 4), each 1 - 1.3 μm thick respectively. Group C of a thin hyaline coriaceous wall (Wall-5) 2 - 6 μm thick, surrounding a hyaline membranous innermost wall (Wall-6) 0.4 - 0.5 μm thick.

Sporogenous cell 35 - 45 μm wide, hyaline to pale brownish-yellow, with 1 or 2 hyphal pegs 12 - 15 μm long and 3 - 5 μm wide, borne terminally on a sparsely septate or aseptate subtending hyphae, with walls 4 - 4.5 μm thick distally, thickening to 2.5 μm at the spore base.

PLATE XXIII

Spores of *Scutellospora* (a-e)

- (a) Intact spore of *Scutellospora verrucosa* showing germination shield (gs).
- (b) Sporogenous cell (sc) of *Scutellospora verrucosa* with hyphal peg (hp).
- (c) Surface ornamentation of broken spore of *Scutellospora verrucosa*.
- (d) Broken spore of *Scutellospora weresubiae* showing sporogenous cell (sc) and hyphal peg (hp).
- (e) Broken spore of *Scutellospora weresubiae* showing germination shield (gs) with germ tube initials (gti).

(Scale bar: a = 100 μm ; b, c = 22 μm ; d, e = 100 μm).

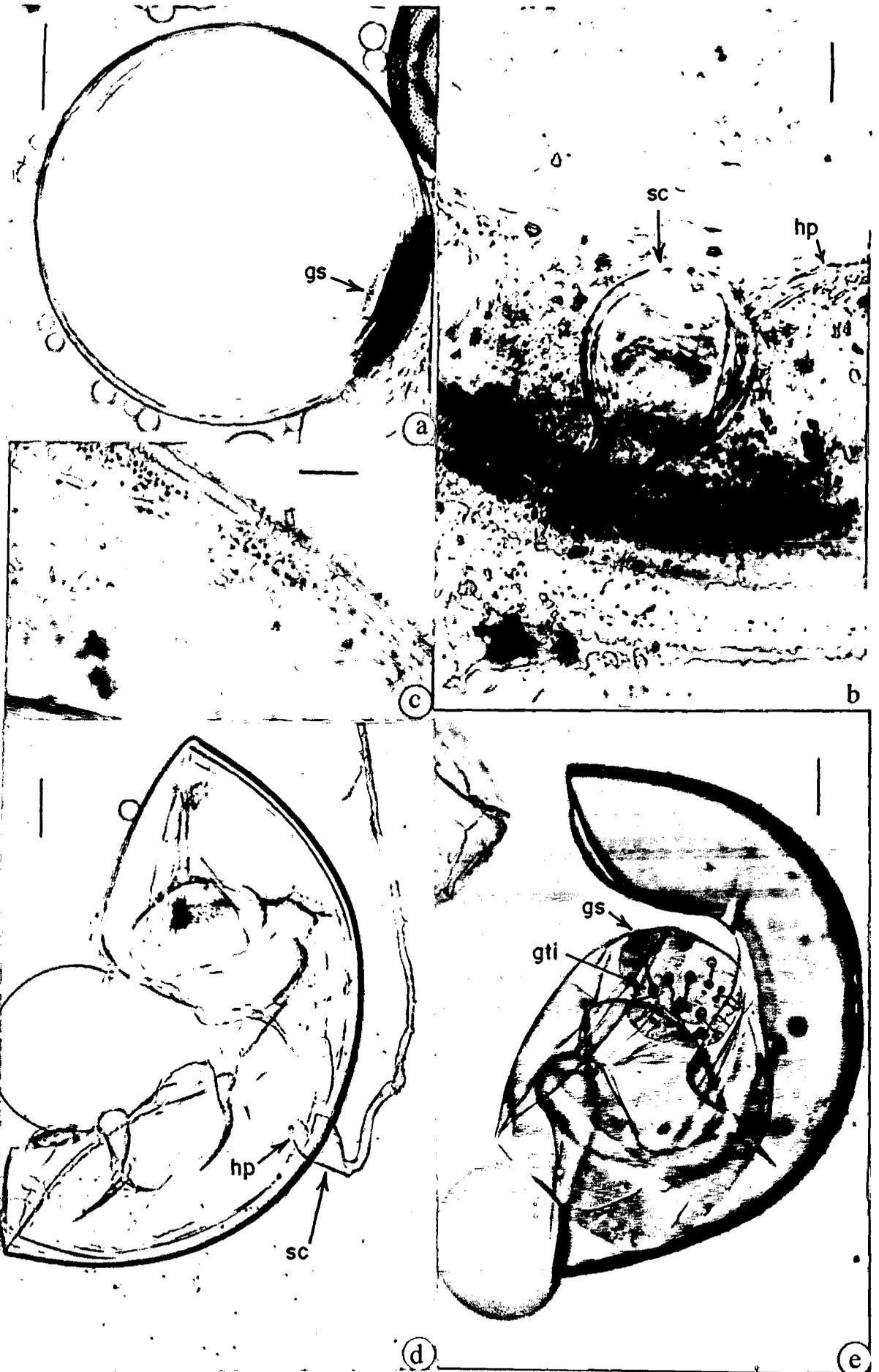


PLATE - XXIII

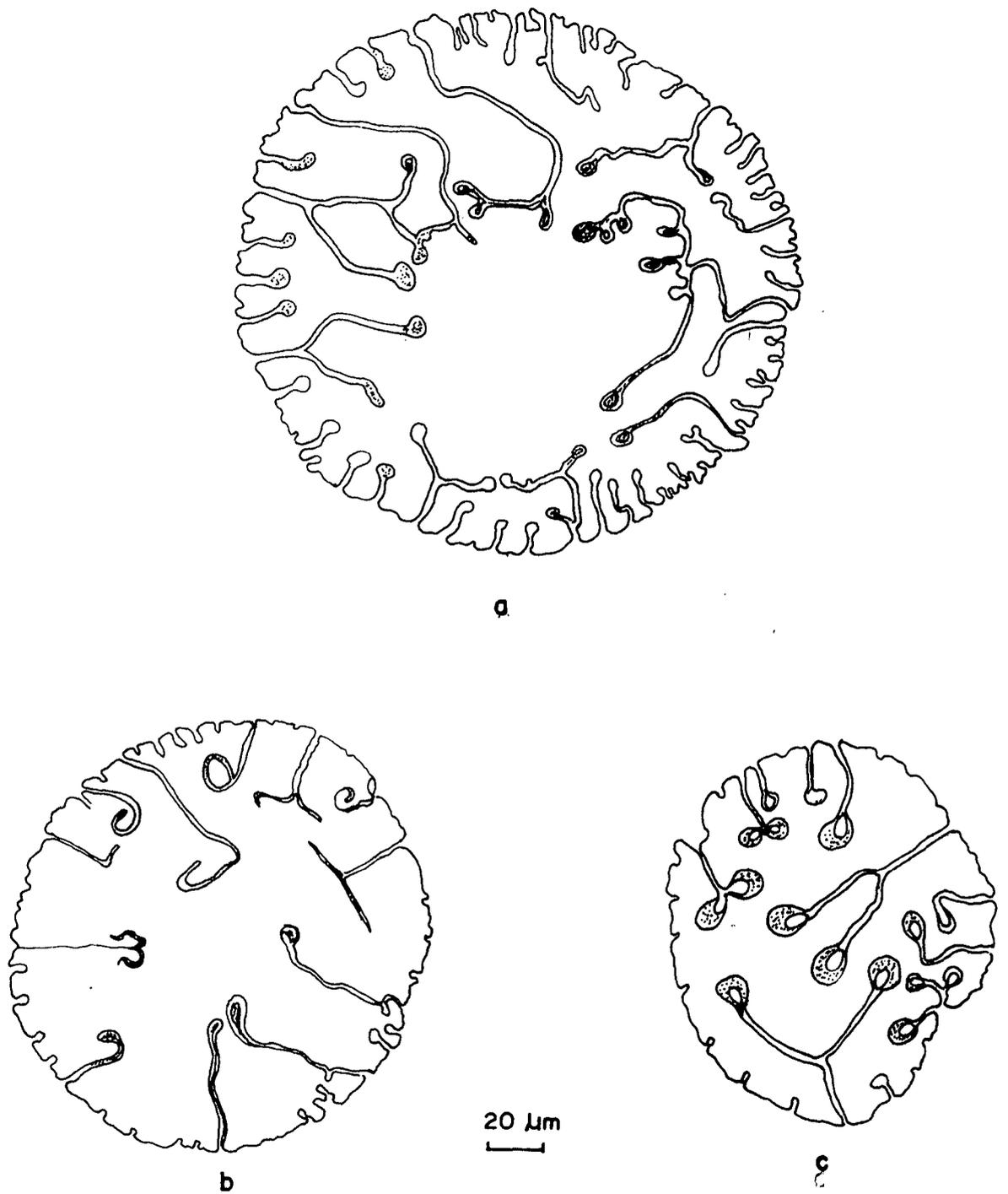


Fig. 28 Germination shields
a - S. nigra , *b - S. gregaria* , *c - S. weresubiae*

PLATE XXIV

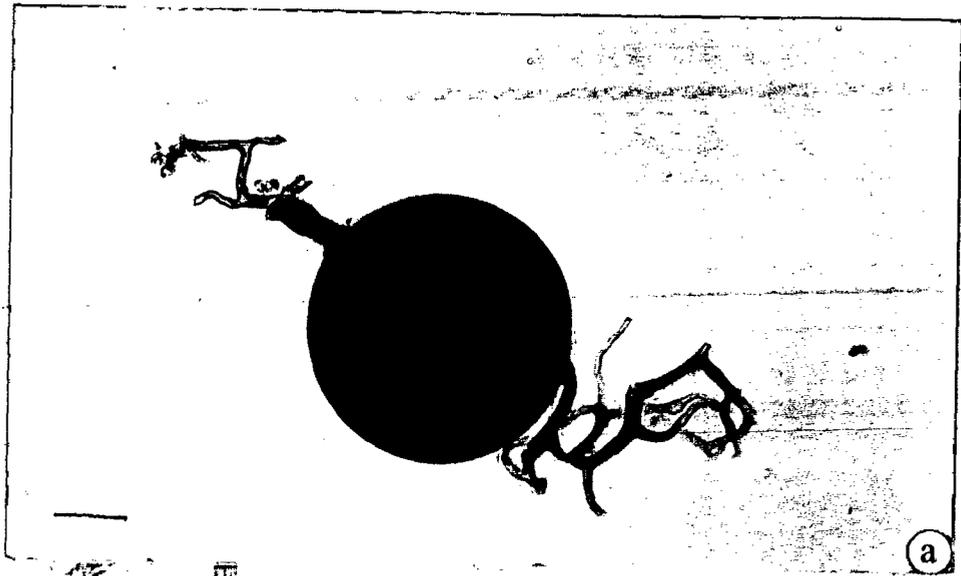
Unidentified spores

- (a) Intact spore of *Glomus sp.* with hypha arising at opposite ends.

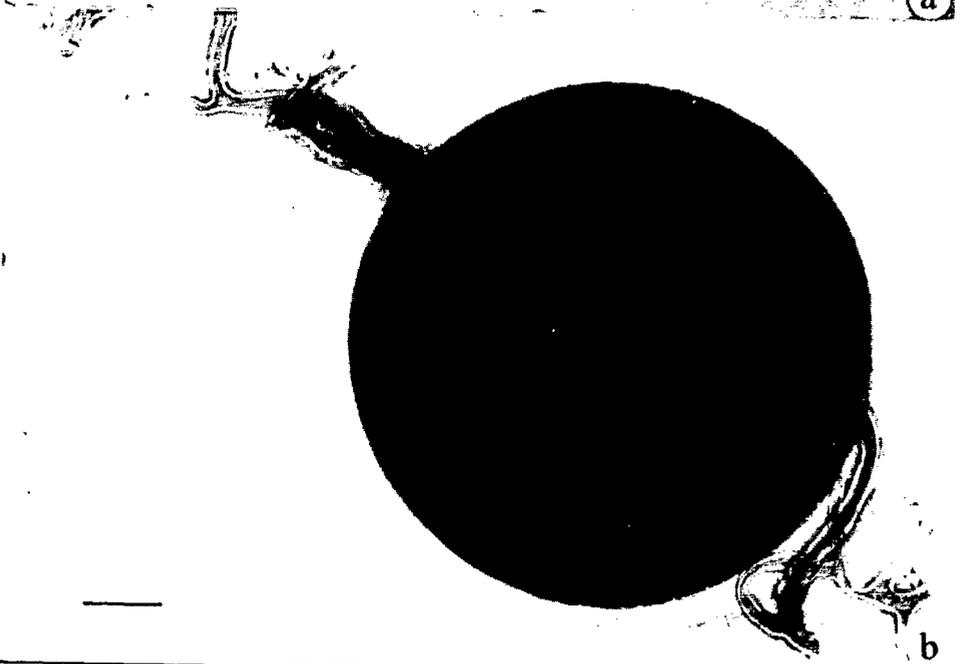
- (b) Enlarged view of above *Glomus sp.* showing details of wall layers.

- (c) Broken spore of *Scutellospora sp.* with sporogenous cell.

(Scale bar: a = 100 μm ; b, c = 50 μm).



a



b



c

PLATE XXV

Spore-in-spore syndrome (a, b)

- (a) Spores of *Glomus macrocarpum* occupied with spores of *Glomus microaggregatum* (arrowed).

- (b) Spores of *Glomus sinuosum* occupied with spores of *Glomus microaggregatum* (arrowed).

(Scale bar: a = 50 μm ; b = 44 μm).



PLATE - XXV

PLATE XXVI

Trap culture and multiplication of spores using host plants

(a-c)

(a) Host plant *coleus sp.*

(b) Host plant *Allium cepa* L.

(c) Host plant *Lycopersicum esculentum* Mill.

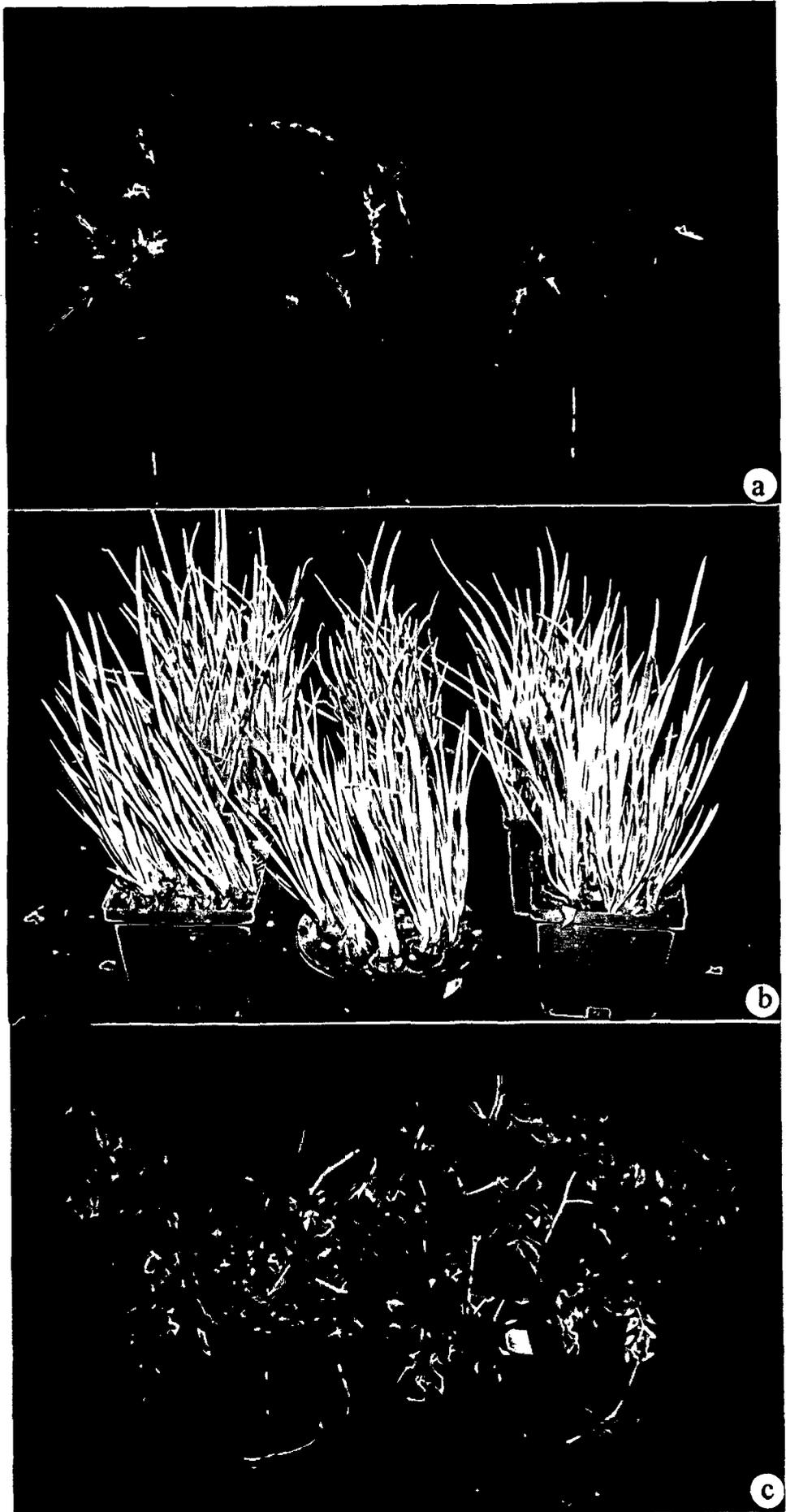


PLATE - XXVI

Germination shield, cardioid, 48 - 65 x 90 - 110 μm with smooth margins 1 μm thick and with a germ tube initial in each lobe (**Fig-28 c**).

Glomus species (**Plate- XXIV a, b**).

Spores formed singly in the soil, globose 250 - 288 μm diam., yellow with 2 hyphal attachments at opposite ends. *Spore wall structure* of two walls (1-2). Outer wall hyaline, 0.5 - 1.0 μm thick, inner wall 5 - 7 μm thick. Hyphae branched nonseptate.

Scutellospora species (**Plate- XXIV c**).

Spores found singly in the soil, terminally on a bulbous sporogenous cell; reddish-brown; globose to subglobose; 280 - 350 μm in diam. *Spore wall structure* of three walls (1-3). Outer wall hyaline (Wall-1) 1 - 1.5 μm thick tightly appressed to wall-2, brown 3 - 3.5 μm thick. Inner wall, 0.4 - 0.5 μm thick.

Sporogenous cell 35 - 45 x 30 - 40 μm broad; borne terminally on a septate subtending hyphae. Spore surface appears smooth but shows presence of warts about 0.5 - 1.0 μm wide.

DISCUSSION

Most of the *Acaulospora*, *Gigaspora*, *Glomus*, and *Scutellospora* species recovered during the study period from various study sites fitted well into the known description. Recently Walker and Vestberg, (1998) considered the

spores of *Glomus maculosum*, *Glomus multisubstenum* 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. multisubstenum* 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.

Morton and Redecker, (2001) reported two new families of Glomales, Archaeosporaceae and Paraglomaceae with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters.

Glomus sp. (Unidentified) closely resembles *Glomus mosseae* in size and wall characters but, differed from it in having oppositely attached hyphae, it also resembles *Glomus multicaule* in having oppositely attached hyphae but differed from the same in having smooth outer surface as against the rough surface of *Glomus multicaule*.

Almeida and Schenck, (1990) transferred most of the species of *Sclerocystis* (Except *Sclerosystis 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*. Recently Redecker *et al.*, (2000b) transferred *Sclerocystis*

coremioides the only species retained by Almeida and Schenck (1990) in the genus *Sclerocystis* to *Glomus* based on molecular studies.

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

The spores and sporocarps of *Glomus taiwanensis* in the present investigation fit well into descriptions of Wu and Chen (1987). Spores of *Glomus taiwanensis* resembles *Glomus clavisporem* and *Glomus microcapra* in lacking a peridium and the spore wall thickness at the apex. Almeida and Schenck (1990) considered *Glomus microcapra* to be a synonym of *Glomus clavisporem* while, Wu (1993b) reported the occurrence of smaller sporocarps of *Glomus clavisporem*. Various spore shapes in *Glomus taiwanensis* has been reported from India by Muthukumar *et al.*, (1997). However, the species of *Glomus taiwanensis* differs from the other species viz., *Glomus clavisporem* and *Glomus microcapra* in having the presence of outer hyaline wall.

The spores and sporocarps of *Glomus clavisporum* in the present investigation fitted well into the original description of Trappe (1977) and the spores of *Glomus rubiforme* fitted well with the type description. Almedia and Schenck (1990) reported the presence of an outer evanescent wall and occlusion of the pore by wall thickening by one or two septa in the subtending hyphae near the spore base, which are not given in the type descriptions and both these characters were observed in the present investigation.

The spores and sporocarps of *Glomus coremioides* in the present investigation fitted well with the type description. Almedia and Schenck (1990) reported the presence of septum at the spore base which is not given in the type description and both these characters were observed in the present study.

In the present study, spores of *Glomus microaggregatum* were found within the spores of *Glomus macrocarpum* and spores of *Glomus sinuosum*. Similar results have been reported by Koske *et al.*, (1986) who indicated the frequent occurrence of *Glomus microaggregatum* spores within the spores of other arbuscular mycorrhizal fungi. Later, Almeida and Schenck, (1990), and Muthukumar *et al.*, (1993) have reported the occurrence of *Glomus microaggregatum* and *Glomus aggregatum* in the spores of *Glomus sinuosum*. Similarly, presence of *Glomus*-like spores within spores of *Glomus sinuosum* has been reported from Taiwan (Wu and Chen, 1993). Muthukumar and Udaiyan, (1999) reported the occurrence of *Acaulospora*, *Glomus* and *Scutellospora* species within arbuscular mycorrhizal fungal species of

Gigaspora, *Glomus*, and *Scutellospora* in the Western Ghat regions, in Southern India.

Presence of arbuscular mycorrhizal fungal spores inside the dead spores of other arbuscular mycorrhizal fungal species suggests that spores of arbuscular mycorrhizal fungi act as a microhabitat when they are dead, apart from their normal role as propagules. This also suggests the ability of different arbuscular mycorrhizal fungal species to sporulate in close proximity with each other.

Thus, the study reveals a rich diversity of arbuscular mycorrhizal fungal species from mines of varying ages and, from the disturbed and undisturbed areas of mine wastelands from Goa, suggesting the screening of dominant arbuscular mycorrhizal fungal species and their multiplication for revegetation of degraded lands.

CHAPTER-VII

**GROWTH RESPONSES OF ARBUSCULAR
MYCORRHIZAL (AM) FUNGAL SPECIES ON SELECTED
TREE SPECIES.**

INTRODUCTION

Mining is one of the most degrading actions of man on the earth. During mining activity, mine rejects are dumped in the surrounding areas. As a result, the natural soil horizons get disturbed and the nutrient rich topsoil is usually buried (Benerjee *et al.*, 1999). Mine spoils are responsible for the destruction of soil fertility, vegetation and soil stability (Shetty *et al.*, 1987). Thus, revegetation of mined areas and waste rock dumps can reduce erosion and dust problems and give a more aesthetically pleasing landscape (Jasper *et al.*, 1988).

Degraded mine wastelands can be stabilized by physical means such as site preparation and overburden placement, soiling and mixing, amending with soil, mulch and fertilizers and, selection of suitable plant materials, but these techniques are very expensive and often short-lived (Smith and Bradshaw, 1979). An alternative is to cover the mine wastelands with vegetation (Street and Goodman, 1967). Revegetation of any site occurs naturally with time (Bradshaw, 1984) but, because mines are invariably poor in plant nutrients and tend to have physical shortcomings, natural colonization can be extremely slow process. The use of expensive inputs is inappropriate for developing countries where there is general reduction to increase mining costs because of limited financial resources. 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. Further, their constant use is known to degrade the soil quality. Hence, there is an urgent need of switching on to biofertilizers.

Arbuscular mycorrhizal fungi are beneficial symbiotic organisms that colonize plant roots. This symbiotic relationship allow the plants to efficiently absorb poorly mobile nutrients especially phosphorus from soil because external hyphae of the fungi greatly increase the effective absorbing surface area of the plant (Bolan, 1991). They constitute towards the development of resistance to water stress. It may be attributed to improved P nutrition or reduced stomatal resistant or its influence on the root/shoot hormonal balance (Cooper, 1984). 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 Whiletam, 1977).

Arbuscular mycorrhizal fungi have been reported to increase the growth of plant by enhancing nutrient uptake (Tinker, 1978) through a reduction of the distance that nutrient must diffuse to plant roots (Hattingh *et al.*, 1973; Rhodes and Gerdemann, 1975) by accelerating the rate of nutrient absorption and nutrient concentration at the absorbing surface (Cress *et al.*, 1979) and by chemically modifying the availability of nutrients for uptake by plants through hyphae. The increased nutrient uptake and better water utilization in mycorrhizal plants reduce the transplant shock, quick recovery after wilting and survival after temporary wilting and transplanting (Biermann and Linderman, 1983; Michelson and Rosendahl, 1990).

Land disturbances, such as mining 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; Daft *et al.*, 1975; Khan, 1978, Lambert and Cole, 1980; Khan, 1981).

The beneficial effect of inoculating forest trees with arbuscular mycorrhizal fungi to improve plant growth are well known (Kormanik *et al.*, 1976, Janos, 1983; Jeffries, 1987; Bagyaraj *et al.*, 1989). As there are too many problems associated with the mass production of arbuscular mycorrhizal fungi (Menge, 1984) it is not feasible to inoculate large tracts of land with these organisms. However, transplants colonized by arbuscular mycorrhizal fungi should be at a greater advantage on mine reclamation site, as limited nutrients in the soil would be more effectively extracted (Sylvia, 1990).

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, plants do respond to inoculation with efficient strains of arbuscular mycorrhizal fungi even in unsterile soil (Khan, 1972). A number of studies have reported the effects of pre-inoculation of plants with

arbuscular mycorrhizal fungi in unsterile soils (Daft and Hacskaylo, 1977; Khan, 1981; Bagyaraj and Manjunath, 1980; Reena and Bagyaraj, 1990; Vasanthakrishna *et al.*, 1994; Muthukumar *et al.*, 2001).

Artocarpus heterophyllus Lam. and *Syzygium cumini* (L.) Skeels are multipurpose tree species commonly found in Goa. They are fast growing species and can grow in nutrient deficient soils of mine wastelands. They are important sources of fruit, fuel, fodder and timber.

The present chapter deals with the study on growth responses of *Artocarpus heterophyllus* and *Syzygium cumini* to arbuscular mycorrhizal fungal inoculation in unsterilized iron ore mine rejects which had low indigenous arbuscular mycorrhizal fungal population and available phosphorus. Present study also determines the symbiotic efficiency of indigenous and introduced arbuscular mycorrhizal fungi in stimulating plant growth in the rejects

MATERIALS AND METHODS

Pure cultures of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Glomus intraradices* Schenck & Smith were procured from Tata Energy Research Institute (TERI), New Delhi, and were maintained as pot cultures using *Eleusine coracana* (L.) Gaertn, as host plant. The inoculum so produced consisted of mixture of soil, spores, and pieces of hyphae and colonized root pieces.

Sand was sterilized in an autoclave at 120° C for 2 hours for three continuous days to eliminate naturally occurring endophytes and other contaminants. Uniform seeds of *Artocarpus heterophyllus* and *Syzygium cumini* were surface sterilized for 20 minutes in 70% ethanol, soaked overnight in sterile water, then placed in moist sterile sand at room temperature until germinated.

Uniform seedlings were planted in trays filled with sterilized sand and 30g of inoculum containing approximately 600 arbuscular mycorrhizal fungal spores and colonized root bits of *Glomus mosseae* (GM), *Glomus intraradices* (GI), *Glomus intraradices* and *Glomus mosseae* (GI+GM), which were applied separately as a thin layer 2 cm below the soil surface to obtain mycorrhizal plants. Non mycorrhizal plants were obtained by growing uniform seedlings in sterilized sand without endophyte.

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). Presence of arbuscular mycorrhizal fungal structures viz., hyphae, arbuscules and/or vesicles in the inoculated plants confirmed the establishment of arbuscular mycorrhizal fungi. Seedlings of *Artocarpus heterophyllus* and *Syzygium cumini* were transplanted to polyethylene bags filled with unsterile mine reject. The experiment consisted of four treatments with eight replicates. The treatments were as follows.

- a) No addition of endophyte (Control).
- b) Mycorrhizal with *Glomus intraradices* (GI).
- c) Mycorrhizal with *Glomus mosseae* (GM).

d) Mycorrhizal with *Glomus intraradices* + *Glomus mosseae* (GI+GM).

After transplanting, the polyethylene bags were labeled as per treatments and were randomized in trays in polyhouse under optimal condition of humidity and temperature. Hogland's solution (Hogland and Arnon, 1950) minus phosphorus was applied every fifteen days after transplantation.

Ninety days after transplantation, the growth response of the two tree species in different treatments were analyzed by studying the various growth parameters (physical and chemical) viz., stem girth, leaf number, leaf length, leaf area, shoot and root length, shoot and root fresh weights, shoot and root dry weights, total biomass and shoot and root phosphorus.

ESTIMATION OF ARBUSCULAR MYCORRHIZAL COLONIZATION

At harvest, two seedlings from each treatment were uprooted, freed from the adhering soil, gently washed and cut into 1cm segments and later cleared with 10 % KOH, acidified with 1N HCl and stained overnight with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970). Percent root colonization was measured by using slide method (Giovannetti and Mosse, 1980).

ESTIMATION OF FRESH AND DRY WEIGHTS

At harvest, seedlings were uprooted without damaging the roots and washed in running water to remove the adhering soil. Root and shoot fresh weights were recorded separately after removing the external moisture from all the treated

plants. Later, the roots and shoots were separately oven dried to a constant weight at 70°C for 72 hours and their dry weights were recorded.

ESTIMATION OF PHOSPHORUS

Phosphorus estimation in root and shoot was carried out separately in all the four treatments in *Artocarpus heterophyllus*. Whereas, in the case of *Syzygium cumini*, entire plants were used to estimate phosphorus. The concentration of phosphorus in dried shoot and root was determined by using Ammonium molybdate method following triple acid digestion (Jackson, 1971).

STATISTICAL ANALYSIS

Data on seedling growth and phosphorus status were subjected to Analysis of Variance (ANOVA) to test the significance of the various treatments.

RESULTS

Results on growth responses to various arbuscular mycorrhizal fungal treatments in *Artocarpus heterophyllus* and *Syzygium cumini* are summarized in **Table- 36** and **Table-37**. Mycorrhizal seedlings showed distinct variations than the non-mycorrhizal seedlings for most of the growth parameters. Pre-inoculation with different arbuscular mycorrhizal fungal species had varied effects on the shoot and root growth, stem girth, leaf length, leaf area, leaf number, shoot and root fresh and dry weights and phosphorus content.

Table- 36. Response of various arbuscular mycorrhizal (AM) fungi on plant growth, biomass, and phosphorus uptake in *Artocarpus heterophyllus* Lam.

| Parameter | Treatments | | | | F Stats |
|---|---------------|--------------------------|---------------------------|--------------------------|---------|
| | Control | GI | GM | GI+GM | |
| Stem girth (cm) | 1.48 ± 0.19 | 1.68 ± 0.18 (13.51) | 2.12 ± 0.12 (43.24) | 2.03 ± 0.0.27 (37.16) | 13.62** |
| No. of leaves | 6.67 ± 0.52 | 5.50 ± 1.05 (-17.54) | 6.83 ± 1.17 (2.40) | 7.17 ± 1.17 (7.50) | 3.08* |
| Leaf length (cm) (3 rd leaf from top) | 14.33 ± 1.21 | 14.55 ± 1.44 (1.53) | 18.17 ± 2.48 (26.80) | 16.43 ± 1.31 (14.65) | 6.79** |
| Leaf area (cm ²) (3 rd leaf from top) | 86.83 ± 10.85 | 81.83 ± 15.10 (-5.76) | 116.83 ± 17.63 (34.55) | 91.07 ± 14.63 (4.88) | 6.70** |
| Shoot length (cm) | 32.00 ± 8.41 | 33.17 ± 5.53 (3.66) | 35.50 ± 3.39 (10.94) | 37.17 ± 4.35 (16.16) | NS |
| Shoot fresh wt. (g) | 6.42 ± 1.15 | 7.70 ± 2.70 (19.94) | 9.84 ± 2.08 (53.27) | 9.32 ± 2.21 (45.17) | 3.28* |
| Shoot dry wt. (g) | 2.13 ± 0.70 | 2.72 ± 0.66 (27.70) | 3.49 ± 0.67 (63.85) | 3.14 ± 0.88 (47.42) | 4.39* |
| Shoot phosphorus (µg 100 g ⁻¹ dry wt.) | 12.12 ± 1.09 | 14.74 ± 0.26 (21.62) | 18.07 ± 1.31 (49.09) | 17.30 ± 3.01 (42.74) | 14.45** |
| Root length (cm) | 18.83 ± 3.61 | 19.17 ± 3.97 (1.81) | 21.33 ± 3.50 (13.28) | 16.67 ± 3.67 (-11.47) | NS |
| Root fresh wt. (g) | 3.46 ± 1.01 | 4.52 ± 1.28 (30.64) | 7.54 ± 1.13 (117.92) | 5.17 ± 1.40 (49.42) | 14.09** |
| | | | | | Cont. |

| | | | | | |
|---|--------------|-------------------------|-------------------------|------------------------|--------|
| Root dry wt. (g) | 0.83 ± 0.302 | 1.08 ± 0.300 (30.12) | 1.61 ± 0.37 (93.98) | 1.34 ± 0.42 (61.45) | 6.23** |
| Root phosphorus (µg 100 mg⁻¹ dry wt.) | 12.26 ± 0.95 | 12.27 ± 0.98 (0.08) | 17.10 ± 4.20 (39.48) | 13.13 ± 1.59 (7.10) | 5.78** |
| Plant biomass dry wt. (g) | 2.96 ± 0.320 | 3.80 ± 0.95 (28.38) | 5.10 ± 0.83 (72.30) | 4.48 ± 1.27 (51.35) | 6.08** |

Significant at: *- P<0.05, **- P<0.01 and NS- Not significant.

C- Control; GI- *Glomus intraradices*; GM- *Glomus mosseae*;

GI+GM- *Glomus intraradices* + *Glomus mosseae*.

± - Standard deviation.

Values are mean of six replicates.

Values in parenthesis indicates percent increase over control.

Table- 37. Response of various arbuscular mycorrhizal (AM) fungi on plant growth, biomass, and phosphorus uptake in *Syzygium cumini* (L.) Skeels

| Parameter | Treatments | | | | F Stats |
|---|--------------|-------------------------|--------------------------|--------------------------|---------|
| | Control | GI | GM | GI+GM | |
| Stem girth (cm) | 0.41 ± 0.04 | 0.80 ± 0.09 (95.12) | 0.87 ± 0.186 (112.20) | 0.90 ± 0.06 (119.51) | 24.77** |
| No. of leaves | 9.33 ± 1.63 | 9.67 ± 1.50 (3.64) | 10.50 ± 1.76 (12.54) | 10.00 ± 1.26 (7.18) | NS |
| Leaf length (cm) (3 rd leaf from top) | 3.50 ± 0.44 | 4.93 ± 0.69 (40.86) | 5.50 ± 1.00 (57.14) | 6.00 ± 0.89 (71.43) | 11.34** |
| Leaf area (cm ²) (3 rd leaf from top) | 4.59 ± 0.94 | 8.32 ± 1.90 (81.26) | 10.17 ± 2.00 (121.57) | 9.02 ± 2.09 (96.51) | 10.88** |
| Shoot Length (cm) | 12.17 ± 1.47 | 11.58 ± 1.11 (-4.85) | 13.33 ± 1.63 (9.53) | 11.17 ± 2.64 (-8.22) | NS |
| Shoot fresh wt. (g) | 0.63 ± 0.11 | 1.15 ± 0.37 (82.54) | 1.16 ± 0.46 (84.13) | 1.12 ± 0.30 (77.78) | NS |
| Shoot dry wt. (g) | 0.16 ± 0.02 | 0.24 ± 0.11 (50.00) | 0.35 ± 0.18 (118.75) | 0.24 ± 0.10 (50.00) | NS |
| Root length (cm) | 8.83 ± 1.33 | 17.00 ± 2.37 (92.53) | 15.17 ± 3.49 (71.80) | 19.00 ± 3.03 (115.18) | 16.16** |
| Root fresh wt. (g) | 0.24 ± 0.09 | 0.84 ± 0.34 (250.00) | 0.75 ± 0.05 (212.5) | 0.71 ± 0.24 (195.83) | 7.029** |
| Root dry wt. (g) | 0.06 ± 0.02 | 0.18 ± 0.06 (200.00) | 0.20 ± 0.05 (233.33) | 0.19 ± 0.05 (216.67) | 9.540** |
| | | | | | Cont. |

| | | | | | |
|--|--------------|-------------------------|-------------------------|-------------------------|----------|
| Plant biomass dry wt. (g) | 0.22 ± 0.04 | 0.42 ± 0.16 (90.91) | 0.55 ± 0.23 (150.00) | 0.43 ± 0.14 (95.45) | 4.462* |
| Phosphorus (µg 100 mg⁻¹ dry wt.) | 10.71 ± 0.87 | 13.44 ± 1.65 (25.49) | 16.08 ± 1.71 (50.14) | 14.55 ± 0.69 (35.85) | 17.815** |

Significant at: *- P<0.05, **- P<0.01 and NS- Not significant.

C- Control; GI- *Glomus intraradices*; GM- *Glomus mosseae*;

GI+GM- *Glomus intraradices* + *Glomus mosseae*.

± - Standard deviation

Values are mean of six replicates.

Values in parenthesis indicates percent increase over control.

In seedlings of *Artocarpus heterophyllus* the stem girth, leaf length, root fresh and dry weights, total plant biomass, shoot and root phosphorus, leaf number, shoot fresh and dry weights varied significantly between the treatments at different levels of significance ($P < 0.05$, $P < 0.01$). However, the difference in shoot length and root length was not statistically significant between the treatments. Growth responses of arbuscular mycorrhizal fungal species on *Artocarpus heterophyllus* are represented in **Plate- XXVII a, b**.

In seedlings of *Artocarpus heterophyllus* maximum arbuscular mycorrhizal colonization (40%) was recorded on preinoculation with GM followed by GI+GM (35%) and GI (20%). Whereas, arbuscular mycorrhizal colonization was 10% in uninoculated seedlings. Similarly in the seedlings of *Syzygium cumini* maximum arbuscular mycorrhizal colonization was observed on inoculation with GM (60%) followed by GI+GM (55%) and GI (40%) while, natural colonization in uninoculated seedlings was found to be only 10%.

Inoculation of *Artocarpus heterophyllus* with GI, GM or GI+GM increased the stem girth from 13.51% to 43.24%, leaf length from 1.53% to 26.80%, shoot length from 3.66% to 16.16%, shoot fresh weight from 19.94% to 53.27%, shoot dry weight from 27.70% to 63.85%, root fresh weight from 30.64% to 117.92%, root dry weight from 30.12% to 93.98% and plant biomass from 28.38% to 72.30% over uninoculated control.

PLATE XXVII

Growth responses of arbuscular mycorrhizal fungal species on *Artocarpus heterophyllus*. Lam.

- (a) Response of various treatments viz., Control (no addition of endophyte), GI (Mycorrhizal with *Glomus intraradices*), GM (Mycorrhizal with *Glomus mosseae*) and, GI+ GM (Mycorrhizal with *Glomus intraradices* + *Glomus mosseae*) on plant growth.

- (b) Response of various treatments viz., Control (no addition of endophyte), GI (Mycorrhizal with *Glomus intraradices*), GM (Mycorrhizal with *Glomus mosseae*) and GI+ GM (Mycorrhizal with *Glomus intraradices* + *Glomus mosseae*) on root growth of the plant growth.

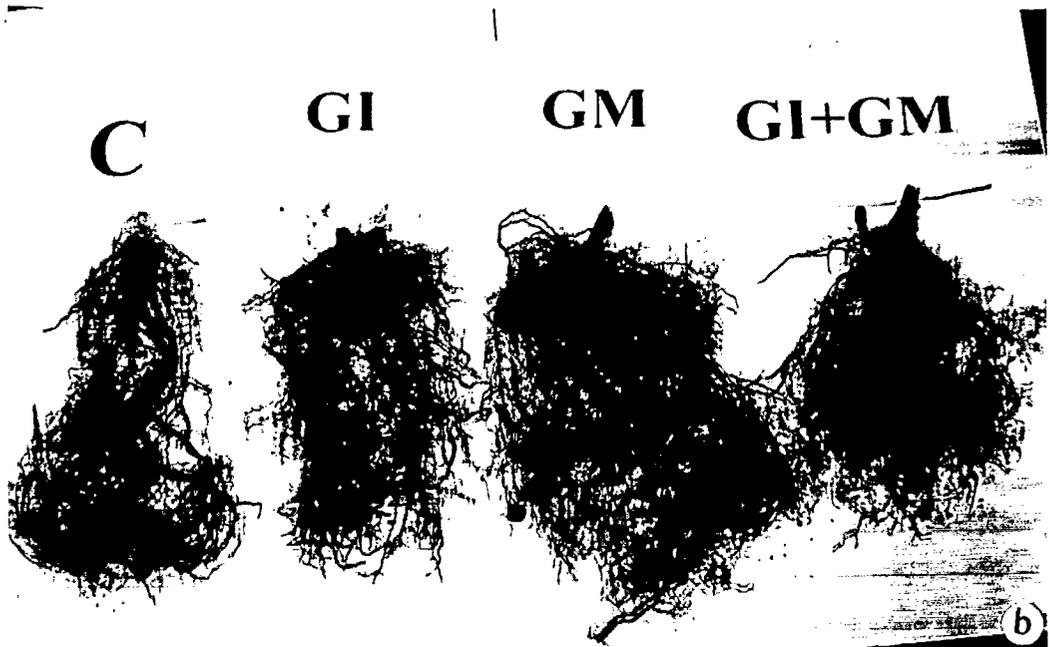
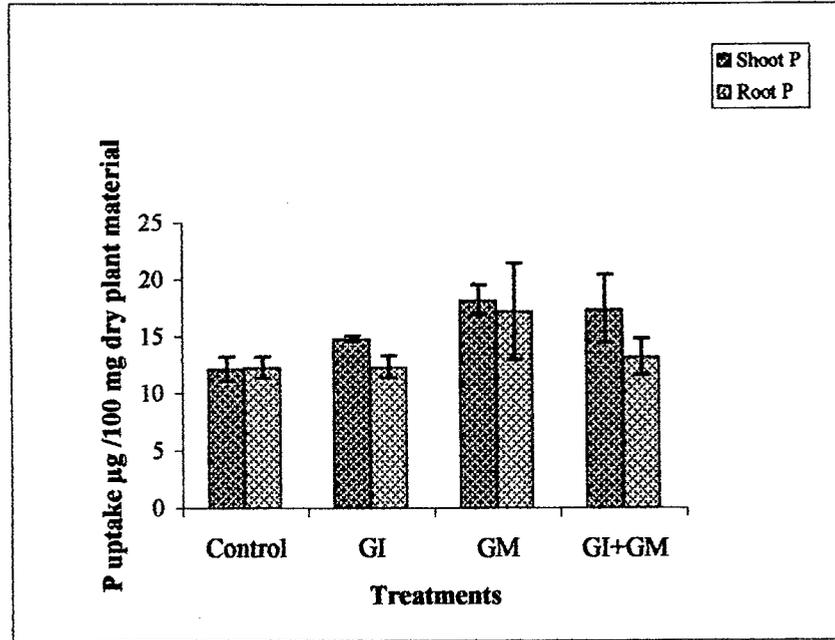


PLATE - XXVII

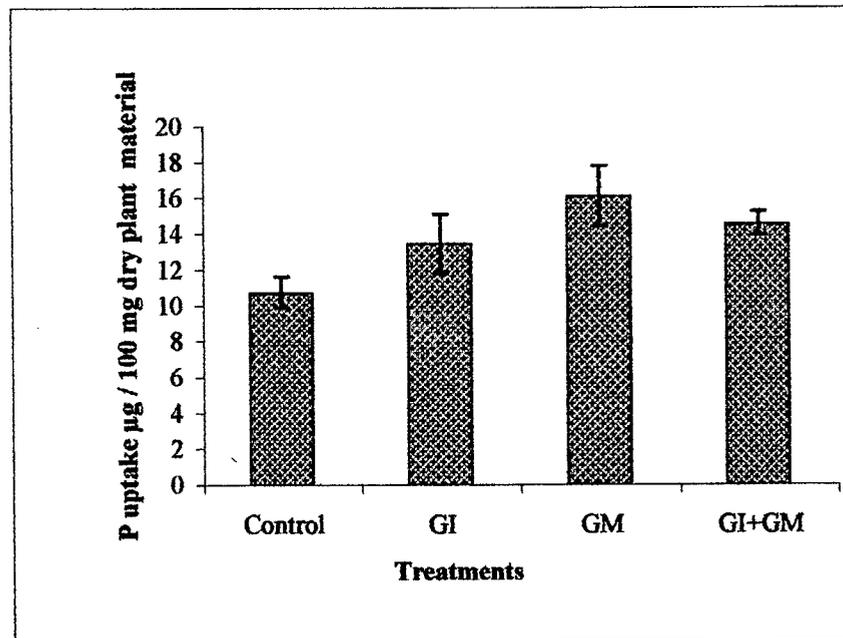
In addition arbuscular mycorrhizal inoculation had profound effect on the P uptake in *heterophyllus Artocarpus* (Table- 36; Fig.- 29a). Phosphorus concentration in shoot tissue of arbuscular mycorrhizal inoculated seedlings ranged from 14.74 μg to 18.07 μg 100 mg^{-1} dry material as compared to 12.12 μg 100 mg^{-1} material in the uninoculated control. This showed an increase, which ranged from 21.62% to 49.09% over control. Similarly, the P concentration in the root tissue in the arbuscular mycorrhizal inoculated seedlings ranged from 12.27 μg to 17.10 μg 100 mg^{-1} dry material as compared to 12.26 μg 100 mg^{-1} dry material which is an increase of 0.08% to 39.48% over control.

In *Syzygium cumini* seedlings the stem girth, leaf length and area, shoot and root fresh weights, root length, root dry weight, plant P and total seedling biomass varied significantly between treatments at different levels ($P < 0.05$, 0.01). However, the leaf number and shoot dry weight did not differ significantly between the treatments. Growth responses of arbuscular mycorrhizal fungal species on *Syzygium cumini* are represented in Plate- XXVIII a, b.

In *Syzygium cumini*, inoculation of arbuscular mycorrhizal fungi either individually (GI, GM) or dually (GI+GM) increased the stem girth ranging from 95.12% to 119.51%, number of leaves from 3.64% to 12.54%, leaf length from 40.86% to 71.43%, leaf area from 81.26% to 121.57%, shoot fresh weight from 77.78% to 84.13%, shoot dry weight from 50.00% to 118.75%,



(a)



(b)

Fig.- 29. Phosphorus uptake in (a) *Artocarpus heterophyllus* (b) *Syzygium cumini*, Control, GI = *Glomus intraradices*; GM = *Glomus mosseae*; GI+GM = *Glomus intraradices* + *Glomus mosseae*. Error bars indicate ± 1 SD

PLATE XXVIII

**Growth responses of arbuscular mycorrhizal fungal species
on *Syzygium cumini* (L.) Skeels.**

- (c) Response of various treatments *viz.*, Control (no addition of endophyte), GI (Mycorrhizal with *Glomus intraradices*), GM (Mycorrhizal with *Glomus mosseae*) and, GI+ GM (Mycorrhizal with *Glomus intraradices* + *Glomus mosseae*) on plant growth.
- (d) Response of various treatments *viz.*, Control (no addition of endophyte), GI (Mycorrhizal with *Glomus intraradices*), GM (Mycorrhizal with *Glomus mosseae*) and GI+ GM (Mycorrhizal with *Glomus intraradices* + *Glomus mosseae*) on root growth of the plant growth.

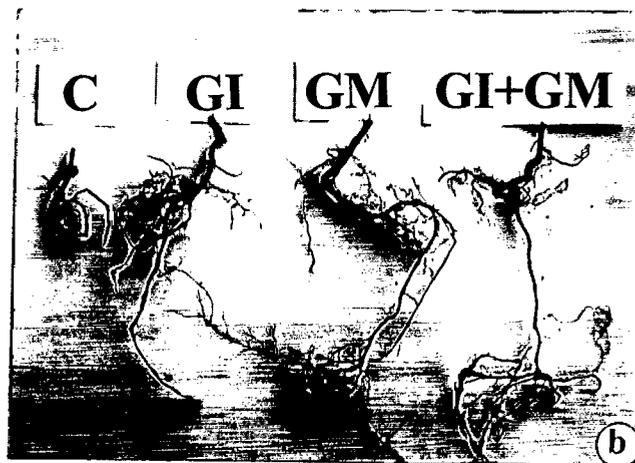
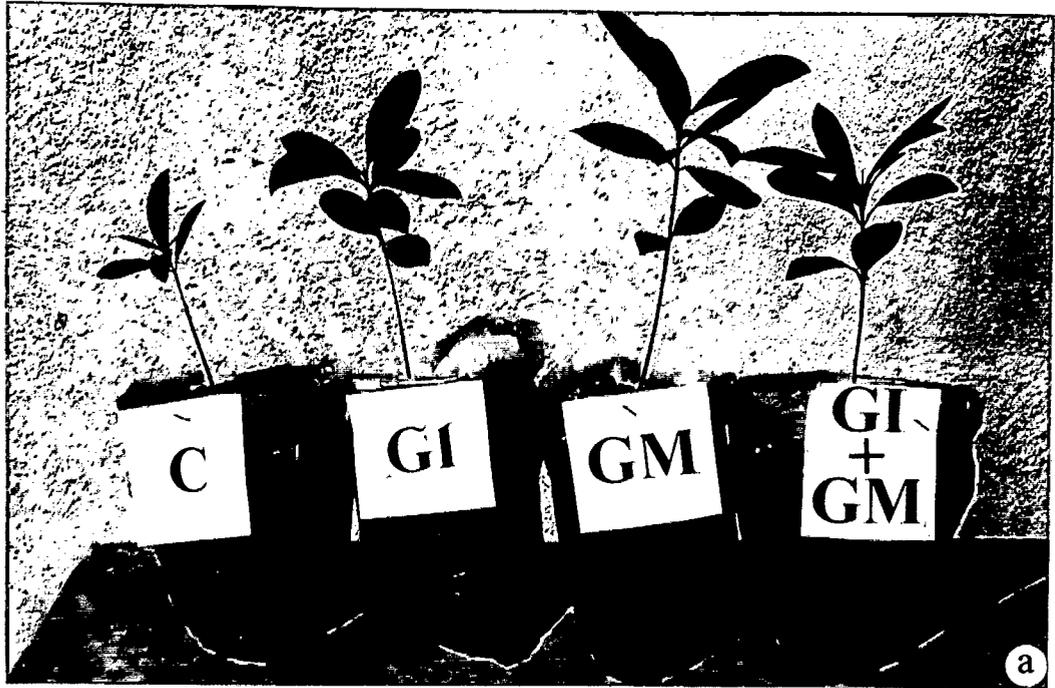


PLATE - XXVIII

root length from 71.80% to 115.18%, root fresh weight from, 195.83% to 250.00%, root dry weight from 200.00% to 233.33% and total plant biomass from 90.91% to 150.00% over uninoculated control.

Arbuscular mycorrhizal inoculation in *Syzygium cumini* seedlings had a profound effect on the P uptake (Table- 37; Fig.- 29 b). Maximum P uptake (16.08 $\mu\text{g } 100 \text{ mg}^{-1}$ dry plant material) was recorded in GM inoculated seedlings followed by GI+GM (14.55 $\mu\text{g } 100 \text{ mg}^{-1}$ plant material) and GI (13.44 $\mu\text{g } 100 \text{ mg}^{-1}$ dry plant material) inoculated seedlings. While, least P uptake was recorded in uninoculated control (10.71 $\mu\text{g } 100 \text{ mg}^{-1}$ dry plant material). The increase in P uptake in arbuscular mycorrhizal inoculated seedlings ranged from 25.49% to 50.14% over control.

Among the three arbuscular mycorrhizal fungal treatments pre-inoculation of GM followed by GI+GM and GI increased total seedling biomass and P uptake in both the tree species.

DISCUSSION

In the present investigation, a significant positive response to arbuscular mycorrhizal inoculation was observed in the growth of seedlings of *Artocarpus heterophyllus* and *Syzygium cumini*. The observations in the present study are in agreement with earlier workers (Barea *et al* 1990; Giri *et al*, 1999 & 2000) who made similar observations in woody legumes inoculated with arbuscular mycorrhizal fungi.

The significant increase in dry matter production through mycorrhizal inoculation could be attributed to formation of better root system leading to trapping of larger volumes of soil for water and nutrients. Increased root length associated with arbuscular mycorrhizal colonization may influence the drought resistance of the host plant (Kothari *et al.*, 1990; Giri *et al.*, 2000).

There was decrease in seedlings height in GI and GI+GM inoculated *Syzygium cumini*. Similarly, there was decrease in root length in GI+GM inoculated *Artocarpus heterophyllus* seedlings. The reduced shoot/root length can result from: (1) an increase in root diameter leading to more material per unit length of root axis, (2) a decrease in cell size leading to increased tissue density and thus weight per unit length, and (3) an increase in cellular or intracellular constituents leading to increased weight per unit length.

Larger leaf area in inoculated plants is known to support sufficient photosynthate to the symbiont. Increase in stem girth and leaf area in the present study are in accordance with the earlier work of Krishna *et al.*, (1981) who recorded that stem and leaves undergo noticeable anatomical modifications like increase in the leaf thickness, size of midrib vein, mesophyll cells and number of plastids following mycorrhizal inoculation. Similarly, Daft and Okasanya, (1973) demonstrated that colonization increased the amount of vascular tissue, lignification of xylem, and number of vascular bundles. Increased stem girth in arbuscular mycorrhizal inoculated plants could be due to the influence of arbuscular mycorrhizal fungi through

enhanced nutrient uptake (Barea, 1991; Koide, 1991) and increased production of phytohormones (Allen *et al.*, 1980).

Seedlings responded more to arbuscular mycorrhizal inoculation and subsequently showed increased root length. This is in accordance with the studies of Berta *et al.*, (1995) and Fidelibus *et al.*, (2001) where arbuscular mycorrhizal fungi are known to increase root growth. Improved host plant nutrients due to high arbuscular mycorrhizal root colonization and increased level of cytokinins (Allen *et al.*, 1980, Edriss *et al.*, 1984; Dixon *et al.*, 1988; Thiagrajan and Ahmad, 1994; Berta *et al.*, 1995) might have resulted in the increased root length. Since water flow through growing roots involve crossing of membrane barriers, water absorption can be improved by membrane permeability through improved phosphorus nutrition. (Cooper, 1984). Mycorrhizal roots have greater surface absorbing area because of the greater root length and diameter (Hardie and Leyton, 1981) and increased branching (Allen *et al.*, 1981; Berta *et al.*, 1995). Such alterations to root morphology are known to change hydraulic conductivity and water flow rates (Nye and Tinker, 1977; Fiscus and Markhardt, 1979; Fidelibus *et al.*, 2001).

Increased root biomass was recorded in plants inoculated with arbuscular mycorrhizal fungi. This could be possibly due to the influence of inoculation which might have enhanced the root initials through phytohormone production (Allen *et al.*, 1980; Barea and Azcon-Aguilar, 1982; Edriss *et al.*, 1984; Dixon *et al.*, 1988; Hooker *et al.*, 1992; Thiagrajan and Ahmad, 1994)

and enhancing nutrient uptake and accumulation (Harley and Smith, 1983; Barea, 1991; Tholkappian, *et al.*, 2000).

In both the tree species increased plant biomass and increased P uptake in arbuscular mycorrhizal treated seedlings could be linked with significant arbuscular mycorrhizal colonization of the root system. The limited P uptake as a result of poor development and poor arbuscular mycorrhizal colonization could be the probable cause for the reduced biomass in uninoculated seedlings. Similar observations have been recorded by Berta *et al.*, (1995) in their studies on arbuscular mycorrhizal induced changes to plant growth and root system in *Prunus cerasifera*.

The enhanced phosphorus uptake due to arbuscular mycorrhization may be attributed to better phosphorus nutrition in inoculated plants by introduced arbuscular mycorrhizal fungi rather than the native arbuscular mycorrhizal fungal population. Such increased uptake of phosphorus has been well documented in earlier studies (Hayman and Mosse, 1972; Mosse *et al.*, 1973; Powell and Daniel, 1978). The increase may be due to an increase in the number of uptake sites per unit area of roots and a greater ability of these roots to exploit the soil nutrients. (Bolan, 1991). According to Sanders and Tinker's (1971) theory, the higher influx of phosphorus from soil to root is governed by the action of electrical potential gradients leading to bulk flow of H_2PO_4^- into the protoplasmic streaming system of roots which is then probably is translocated into leaves through the xylem strands.

Marschner and Dell, (1994) have attributed the increase in phosphorus content in the arbuscular mycorrhizal inoculated plants to the external hyphae of arbuscular mycorrhizal fungi, which enhance the ability of the plants to retrieve phosphorus by increasing the additional absorbing surface area. The fungal mycelium which extends from the mycorrhizal roots forms a three dimensional network which links the roots and the soil environment. It constitutes an efficient system for nutrient uptake (particularly P). The mycelium also contributes to the formation of water-stable aggregates necessary for good soil tilth (Jeffries and Barea, 2000). Such an increase in P content has been reported in several agricultural and forestry species (Reena and Bagyaraj, 1990; Antones and Cardou, 1990; Durga and Gupta, 1995; Jeffries and Barea, 2000).

The present study revealed that there was difference in the growth promoting efficiency of different arbuscular mycorrhizal fungal species. *Glomus mosseae* was found to be more efficient than mixed inoculum and *Glomus intraradices* in increasing the growth rate, total biomass production and phosphorus uptake. A wide variation among and within different species of arbuscular mycorrhizal fungi in the ability for stimulating plant growth have been observed earlier (Abbott and Robson, 1978; Govinda Rao *et al.*, 1983, Bagyaraj *et al.*, 1989).

The variation in the extent of increase in seedling growth inoculated with various arbuscular mycorrhizal fungi could be due to the observed

differences in the uptake of phosphorus. Enhanced phosphorus nutrition can increase stomatal conductance and transpiration. Several investigations concluded that increase in stomatal conductance and transpiration of plants colonized by mycorrhizal fungi was related to high leaf phosphorus concentration (Nelsen and Safir, 1982 a & b; Koide, 1985). Khan (1978 & 1981) reported an increased growth of pre-inoculated onions with arbuscular mycorrhizal fungi in unsterilized, bituminous waste, which had an indigenous population of endophyte spores and contained little available phosphorus.

A positive response to arbuscular mycorrhizal inoculation was observed in *Artocarpus heterophyllus* and *Syzygium cumini* in unsterile mine spoil. Even though the spoil harboured native mycorrhizal fungal spores, the natural colonization was much lower as compared to the pre-inoculated plants. The observation on dry matter production and phosphorus content indicates that the native endomycorrhizae are less efficient in stimulating plant growth than the exotic *Glomus* species. Mosse (1974) pointed out that if the inoculated endophytes can be more beneficial to plant growth than the indigenous ones then this could have important practical application. The results further showed that inoculated plants took up more phosphorus from soil than uninoculated plants. Studies by Tinker, (1978) and Bagyaraj and Manjunath, (1980) also indicated that plants colonized with arbuscular mycorrhizal fungi take up more phosphate from P deficient soils.

Thus, increased growth of indigenous flora on mine reject dumps can be obtained by either increasing the population of the suitable arbuscular mycorrhizal fungal species and or/ transplanting plants pre-inoculated with suitable species instead of applying inorganic fertilizers and fertilizing the top soil cover. The evidences presented by Khan (1972, 1975a, b) in his field studies indicates that introduced arbuscular mycorrhizal fungi can become established in competition with the indigenous arbuscular mycorrhizal fungi and can improve plant growth. The growth of Maple was increased when the plants, grown in anthracite waste containing bonemeal were colonized with arbuscular mycorrhizal endophyte (Daft *et al.*, 1975). Similarly Bagyaraj and Manjunath, (1980) observed increased growth on inoculation with arbuscular mycorrhizal fungi in an unsterile Indian soil with low available phosphorus.

Studies of Daft *et al.*, (1975) and Daft and Hacskaylo (1976) suggests that symbiotic association can be exploited in revegetation schemes and accelerate the development of a suitable plant community.

From the present study it appears that further research on the value of arbuscular mycorrhizal fungi to survival and growth of plants on mine reject dumps should be concentrated on testing a variety of arbuscular mycorrhizal fungal species which are dominant and persisting on iron ore mine wastelands in order to find an ecologically adapted and efficient arbuscular mycorrhizal fungal species. The results clearly indicate that arbuscular mycorrhizal inoculation can substantially reduce fertilizer requirement in seedling production. Furthermore,

the results of the present study clearly suggest that application of efficient arbuscular mycorrhizal symbionts can be very effective in obtaining healthy seedlings which could be used in mine wastelands reclamation programmes.

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** - Originals not seen

SUMMARY

Iron ore mine wastelands are of great ecological significance. In mine wastelands acute deficiencies of macro- and micronutrients and organic matter together with low water retention capacity and other factors contribute to make the mine wastelands a harsh and unfavourable medium for plant growth. Arbuscular mycorrhizae are of great significance due to their ability for nutrient uptake, and are important for the survival of plants and in sustaining their growth in highly stressed environments.

Understanding the mycorrhizal association in mine wastelands and their distribution would be of great significance in reclamation of mine wastelands. The work carried out in the present study can be summarized as follows:

- Depending upon the age of the mine sites and the magnitude of disturbance, five sites of varying ages were surveyed for vegetation. The oldest mine *viz.*, Sanquelim exhibited a rich plant diversity with a total of 250 plant species while, the recently degraded mine *viz.*, Xelpi (10 year old) accounted only 29 plant species. This study indicated that the diversity of plant species increased with increase in age of mine site.
- A good number of legume species in all the mine sites is an indication of gradual improvement of the fertility status of the spoil as majorities of them are potential nitrogen fixers.

- The presence of tree species at different mine sites is an indication of gradual venture of invading the dumps and succession.
- A total of 55 plant species from Codli iron ore mine site were studied for arbuscular mycorrhizal colonization, spore density and diversity. The study revealed that the degree of root colonization varied between plant species. Mean root colonization levels also showed variations in herb, shrubs and tree species. Arbuscular mycorrhizal spore numbers also showed variations between the different plant species. Mean spore numbers were found to be maximum in herbs followed by shrubs and trees. Positive correlation was observed between spore number and root colonization. This study also reported the presence of 40 arbuscular mycorrhizal fungal species from the rhizosphere of 55 plant species. Arbuscular mycorrhizal fungal species *viz.*, *Acaulospora spinosa* Walker & Trappe, *Acaulospora scrobiculata* Trappe, *Gigaspora margarita* Becker & Hall, *Glomus constrictum* Trappe, *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker and Koske, *Glomus geosporum* (Nicolson & Gerdemann) Walker, *Glomus macrocarpum* Tul. & Tul., *Glomus taiwanensis* (Wu & Chen) Almeida & Schenck, *comb. nov.*, *Scutellospora gregaria* (Schenck & Nicolson) Walker & Sanders, *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders, *Scutellospora reticulata* (Koske, Miller & Walker) Walker & Sanders, and *Scutellospora weresubiae* Koske & Walker were the most frequently occurring species at Codli mine site.

- To study the impact of mining on soil properties, arbuscular mycorrhizal colonization, spore density and diversity of arbuscular mycorrhizal fungal species, disturbed and undisturbed areas of Xelpi (10 year old) and Bimbol (45year old) iron ore mine sites were taken up. Soil analysis results revealed that the soil at the disturbed area of both the sites was low in nutrients as compared to the surrounding undisturbed area. Results on analysis of variance (ANOVA) exhibited significant variation in edaphic factors between disturbed and undisturbed areas of both the sites. Analysis of variance on mycorrhizal colonization and spores number between disturbed and undisturbed areas of both the sites indicated significant variation in colonization levels and spore number between the species, areas and species and areas together. Shannon's diversity index was greater on disturbed area of Bimbol disturbed area of the site as compared to the surrounding undisturbed area while, it was very low on the disturbed area of Xelpi mine site as compared to the surrounding undisturbed area. Simposn's index of dominance was greater on Xelpi disturbed area then the surrounding undisturbed area.
- Studies on soil characteristics, arbuscular mycorrhizal colonization and spore density from mines of varying ages revealed significant differences in soil properties and arbuscular mycorrhizal fungal colonization and spore densities. Analysis of variance (ANOVA) exhibited significant variation in edaphic factors between mine sites of varying ages. Soil nitrogen, organic carbon and organic mater increased with increase in age of mine site. Analysis of variance on mycorrhizal colonization and spores number between mine sites of varying ages indicated significant variation in colonization levels and spore

number between the species, sites and species and sites together. As regards to species richness, maximum number of arbuscular mycorrhizal fungal species were reported from well established mine (Sanquelim) while, least number of arbuscular mycorrhizal species were reported from recently degraded mine (Xelpi). Shannon's index of diversity was highest in oldest and well established mine (Sanquelim) and was least in the recently degraded youngest mine(Xelpi). Simpson's index of dominance was greater in the youngest and recently degraded mine while it was very low for well established mine. Shannon's evenness ranged from 0.7457 to 0.7954 at the four iron ore mine sites.

- Seasonal studies conducted at Codli iron ore mine site on soil factors, arbuscular mycorrhizal colonization and spore density revealed that soil was low in nutrients and characterized by significant periodic differences in soil factors. Calculated correlation coefficient between edaphic factors revealed that soil moisture, N, and K were positively correlated to each other. Maximum colonization was recorded during monsoon followed by post monsoon and least was recorded in pre-monsoon. Spore density was maximum in pre-monsoon followed by post-monsoon and was least in monsoon season. Mean colonization levels which includes total root length colonization and root length colonized by hyphae, arbuscules and vesicles exhibited significant seasonal variations. Three way analysis of variance on mycorrhizal colonization and arbuscular mycorrhizal (AM) fungal structures indicated significant variations in the colonization levels and structures between species, seasons, and, species and seasons together.

- A total of 60 spore forming arbuscular mycorrhizal fungal species belonging to 6 genera viz., *Acaulospora*, *Archaeospora*, *Gigaspora*, *Glomus*, *Paraglomus* and *Scutellospora* were isolated during the study. Of these, 58 arbuscular mycorrhizal species have been identified.
- Studies on the growth responses of *Artocarpus heterophyllus* Lam. and *Syzygium cumini* (L.) Skeels. to inoculation with *Glomus intraradices* (GI), *Glomus mosseae* (GM), and *Glomus intraradices* + *Glomus mosseae* (GI + GM) revealed improved growth on inoculation as compared to the uninoculated control. Mycorrhizal seedlings showed distinct variations than the non-mycorrhizal seedlings. Pre-inoculation with different arbuscular mycorrhizal fungal species had varied effects on the shoot and root growth, stem girth, leaf length, leaf area, leaf number, shoot and, root fresh and dry weights and phosphorus content. In addition arbuscular mycorrhizal inoculation had profound effect on the P uptake in seedlings of *Artocarpus heterophyllus* and *Syzygium cumini*.

SYNOPSIS

INTRODUCTION

In terms of employment and foreign exchange earnings, mining industry plays an important role in the country's economy. The State of Goa alone accounts for nearly half of the total iron ore production in the country. However, the destruction of the native vegetation due to the mining can change the characteristics of the soil to such an extent that it can severely diminish its productivity. Indiscriminate mining since 1961 has destroyed over 50,000 hectares of natural forest in the state, and it has been estimated that during all these years as much as 900 to 1000 million tones of waste rock, low-grade ore and tailings have been accumulated near mining sites.

Changes in soil micro-flora contribute to the poor plant growth in disturbed mine soils. Land disturbances such as surface mining can disrupt mycorrhizal populations and often results in growth media with minimal levels of entophyte inoculums. This results in poor plant growth and survival. 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.

Mycorrhizal fungi play an important role in the rehabilitation of many disturbed lands. The occurrence of arbuscular mycorrhizal (AM) fungi in the mine spoils has been documented in studies by Ponder (1979) and

Kiernan *et al.*, (1983). In fact, understanding the role of mycorrhizal associations in relation to establishment and development of plant communities could offer solutions to many of the problems that are encountered in the mine wastelands. Arbuscular mycorrhizal fungi enables the plant to grow and survive better under stress condition through an increased uptake of nutrients especially phosphorus, zinc and copper, and water.

The present research work was taken up to study the diversity of arbuscular-mycorrhizal (AM) fungi in iron ore mine wastelands of Goa.

AIMS AND OBJECTIVES

The present research programme has the following objectives.

1. Survey of vegetation of various mine sites to identify the dominant plant species.
2. Assessment of arbuscular mycorrhizal colonization in the plants growing on the mine sites and the assessment of spore density in the rhizosphere soil.
3. To study the effect of severe land disturbances on fungal population due to iron ore mining.
4. Survey for the occurrence of native arbuscular mycorrhizal fungal spores and root colonization in the mine reject dumps of varying ages.
5. To study the seasonal variation in arbuscular mycorrhizal fungi with respect to root colonization, spore density and edaphic factors.

6. Taxonomic identification of the arbuscular mycorrhizal fungal species.
7. To study the response of selected arbuscular mycorrhizal species on selected plant species.

METHODOLOGY

1. Survey of the mining spoils to find out the dominant plant species.
2. Random sampling of the soil samples for physical and chemical analysis.
3. Collection of the rhizosphere soil samples and root samples.
4. Examination of the roots to find out percent infection using Phillips and Hayman method, (1970).
5. Isolation of native arbuscular mycorrhizal fungal spores from the rhizosphere soil samples by wet sieving and decanting method (Gerdemann and Nicolson, 1963).
6. Degree of root colonization by using Root slide method (Giovannetti and Mosse, 1980) and, Gridline Intersection Method (McGonigle *et al.*, 1990).
7. Quantification of spores and sporocarps of arbuscular mycorrhizal fungi in the rhizosphere soil samples (Gaur and Adholeya, 1994).
8. Identification of spores and sporocarps of arbuscular mycorrhizal fungi isolated was done using various bibliographies (Schenck and Perez, 1990; Almeida and Schenck, 1990; Walker and Vestberg, 1998; Redecker, *et al.*, 2000b; Morton and Redecker, 2001; Schubler *et al.*, 2001).

OBSERVATIONS

The present work recorded in the thesis is divided into seven chapters.

The **first chapter** deals with survey study of five iron ore mines of different age groups *viz.*, Sanquelim (50), Bimbol (45), Sonchi (35), Codli (30) and Xelpi (10) with age of the mines given in parenthesis. The objective of this work was to study the diversity of vegetation and to find out the dominant plant species on the various sites. It was observed that among all the sites surveyed, the recently degraded mine (Xelpi) had sparse vegetation whereas Sanquelim, Bimbol, Codli showed well-developed vegetation while, Sonchi had moderate vegetation cover. The mine wastelands were found to be covered with different plant species. The diversity of plant species increased with increase in age of mine sites. It was observed that a total of 250 plant species (both cultivated and naturally occurring) were reported from the mine wastelands of which 29 were common to all the five sites and were the most dominant species at all the sites.

The **second chapter** deals with the study of mycorrhizal status in naturally occurring plant species from Codli mine. The study revealed that all the plants *viz.*, Peridophytes, herbs, shrubs and trees were mycorrhizal. The extent of colonization varied among different groups of plants ranging from 8-99%. The study also represents a rich diversity of AM fungi. The spore density ranged from 9-396 spores 100g⁻¹ rhizosphere soils. The arbuscular mycorrhizal fungi recorded belonged to four genera *viz.*,

Acaulospora, *Gigaspora*, *Glomus*, and *Scutellospora*. Arbuscular mycorrhizal fungi viz., *Acaulospora spinosa* Walker & Trappe, *Acaulospora scrobiculata* Trappe, *Gigaspora margarita* Becker & Hall, *Glomus constrictum* Trappe, *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker and Kosk, *Glomus geosporum* (Nicolson & Gerdemann) Walker, *Glomus macrocarpum* Tul. & Tul., *Glomus taiwanensis* (Wu & Chen) Almeida & Schenck, *comb. nov.*, *Scutellospora gregaria* (Schenck & Nicolson) Walker & Sanders, *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders, *Scutellospora reticulata* (Koske, Miller & Walker) Walker & Sanders, and *Scutellospora weresubiae* Koske & Walker were the most frequently occurring species at Codli mine.

The **third chapter** deals with the occurrence and distribution of arbuscular mycorrhizal fungi in disturbed sites. The objective of this work was to compare the spore density and diversity of arbuscular mycorrhizal species of a recently degraded mine and its surrounding undisturbed vegetation with that of a well established mine and its surrounding undisturbed vegetation. The study reveals that propagules of arbuscular mycorrhizal fungi were poorly distributed in the recently degraded mine, where they were associated with sparse vegetation. Whereas, the arbuscular mycorrhizal fungal propagules showed marked increase with increased vegetation cover on older disturbed sites. Arbuscular mycorrhizal fungi were numerous from natural surrounding vegetation but the diversity of arbuscular mycorrhizal fungal species was higher in older disturbed site as compared to the surrounding undisturbed vegetation. Soil properties

The **seventh chapter** deals with the response of plant species to pre-inoculation with arbuscular mycorrhizal fungal species. The aim of this study was to evaluate the growth responses of *Artocarpus heterophyllus* and *Syzigium cumini* on pre-inoculation with selected arbuscular mycorrhizal fungal species. It was observed that the plants inoculated with arbuscular mycorrhizal fungi showed increased growth as compared to the uninoculated ones. Increase in fresh and dry weights was observed in the seedlings of both the plant species inoculated with arbuscular mycorrhizal fungi. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe responded well as compared to *Glomus intraradices* Schenck & Smith and mixed inoculum (*Glomus mosseae* + *Glomus intraradices*).

CONCLUSIONS

The present study represents a rich diversity of arbuscular mycorrhizal fungi in the native vegetation of iron ore mine wastelands of Goa. All the plant species studied were found to be mycorrhizal, thus, exhibiting a rich diversity of arbuscular mycorrhizal fungi in the mine wastelands. Variations in spore density, diversity and percent root colonization of arbuscular mycorrhizal fungi were observed in disturbed and undisturbed surrounding sites. Arbuscular mycorrhizal (AM) fungal population increased with increase in the age of mine dumps. Variations in the percent root colonization and spore density of arbuscular mycorrhizal fungi have been encountered in studies with changes in the seasons. Inoculation with selected species of arbuscular mycorrhizal (AM) fungi has shown improved plant growth as compared to the uninoculated ones. The present

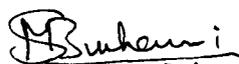
investigation forms a basis for further studies towards screening and multiplication of suitable arbuscular mycorrhizal fungal species, which can be used in reclamation of iron ore mine wastelands of Goa.

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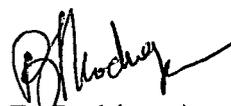
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Signature of the Candidate



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Signature of the Guide

APPENDIX

➤ LIST OF PUBLICATIONS

➤ PAPERS IN PRESS

➤ PAPERS PRESENTED AND ABSTRACTS
PUBLISHED

➤ AWARDS

RESEARCH PAPERS PUBLISHED

- (1) Rodrigues, B.F. and Bukhari, M.J. (1995). Occurrence of VAMF colonization in herbaceous plant species growing on iron ore mine wasteland in Goa". In: *Microbial Biotechnology*. (S.M. Reddy, H.P. Srivastava, D.K. Purohit, and Reddy, S.R. eds.). pp. 83-86.
- (2) Rodrigues, B.F. and Bukhari, M.J. (1996). Preliminary investigation into VAM colonization of plant species found growing in abandoned tailing pond of iron ore mines in Goa. *Proceedings of national seminar on Microorganisms in sustainable agriculture- Madurai*. pp. 25-28.
- (3) Kulkarni Rajendra Rao; Rita Sharma and Mehtab Bukhari(2002). "Diurnal variation of physico-chemical aspects of pollution in Kushawati river at Quepem". *J. Aqua. Biol.* **17(1)**: 27-28.
- (4) Sharda. W. Khade.; M. J. Bukhari; V. Jaiswal; U.C. Gaonkar and B.F. Rodrigues. (2002). Arbuscular mycorrhizal status of medicinal plants: A field survey of arbuscular mycorrhizal fungal association in shrubs and trees" *J. Taxo. Eco. Bot.* **26(3)**: 571-578.
- (5) Rodrigues, B.F., Khade Sharda W., Bukhari Mehtab, Jaiswal Varsha and Uday Gaonkar (2003). Prospects in reclamation of iron ore mine wastelands: Role of arbuscular mycorrhizal (AM) fungi and inoculation procedures. In: *Recent Advances in Environmental Science*. (K.G. Hiremath, ed.), Discovery Publishing House, New Delhi. PP. 66-85.

PAPERS IN PRESS

- (1) Bukhari, M. J., S. W. Khade, V. Jaiswal, U. C. Gaonkar and B.F. Rodrigues. Arbuscular mycorrhizal (AM) status of tropical medicinal plants: A field survey of arbuscular mycorrhizal fungal association in herbs. *Plant Archives* Vol 3(2), October 2003 (*In press*)

**PAPERS PRESENTATED AND ABSTRACTS
PUBLISHED**

- (1) **Mehtab. J. Bukhari** and B.F. Rodrigues
“Arbuscular mycorrhizal fungal diversity in some plant species of iron ore mine wastelands at Codli (Goa)” at 27th Annual Meeting of the Mycological society of India and the International Symposium on “Frontiers of Fungal Diversity and Diseases in S-E Asia” (MSI 2001), Gorukhpur, India February 9-11, 2001

- (2) **Mehtab. J. Bukhari** and B.F. Rodrigues
“Arbuscular mycorrhizal fungal diversity in disturbed and natural areas from mine wastelands of Goa” at the “Asian Congress of Mycology and Plant Pathology” 2002 (ACMPP), Mysore, October 1-4, 2002.

- (3) **Mehtab. J. Bukhari** and B.F. Rodrigues
“Arbuscular mycorrhizal fungal diversity from Iron ore mines of varying ages of Goa- India” at the National Symposium on Prospecting of Fungal Diversity and Emerging technologies and 29th annual meeting of the mycological society of India, held at Agarkar Research Institute (MACS), Pune on 6th and 7th Feb’2003.

- (4) **Mehtab. J. Bukhari** and B.F. Rodrigues
“Taxonomy of AM fungal species from iron ore mine wastelands of Goa- India” at the National Symposium on Prospecting of Fungal Diversity and Emerging technologies and 29th annual meeting of the mycological society of India, held at Agarkar Research Institute (MACS), Pune on 6th and 7th Feb’2003.

AWARDS

Awarded the **“Late Thirumalachar Memorial Prize for Best Poster Presentation”** for the paper entitled ‘Arbuscular mycorrhizal fungal diversity in some plant species of iron ore mine wastelands at Codli (Goa) during the 27th Annual Meeting of the Mycological society of India and the International Symposium on “Frontiers of Fungal Diversity and Diseases in S-E Asia” (MSI 2001), held at Gorukhpur, India from February 9-11, 2001.

Occurrence of VAMF Colonization in Herbaceous Plant Species Growing on Iron Ore Mine Wastelands in Goa.

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ABSTRACT : An assessment of mycorrhizal association in 29 naturally occurring herbaceous plant species belonging to 26 genera and distributed among 16 families collected from a 12 year old iron ore mine reject dump was made. It was observed that all the plant species showed mycorrhizal infection. The mycorrhizal infection varied from 10% to 98% in the different species. Plants exhibiting mycorrhizal infection had typical vesicular or arbuscular and inter- and intra-cellular hyphal infection.

Key Words : Vesicular-arbuscular mycorrhizal fungi, Mine wastelands, Herbaceous plants.

INTRODUCTION

Mine rejects are not true soils but are derived mostly from crushed bedrock and/or glacial deposits. They are very low in organic matter, and normally have low levels of available nitrogen (N), Phosphorus (P), and sometimes potassium (K) (Jeffrey *et al.*, 1975; Grant, 1978). The role of microorganisms in rehabilitation has received little attention then correction of nutritional deficiencies and imbalances, toxicity, moisture deficits and wind erosion (Hutnik & Davis, 1973).

VA mycorrhizae are sometimes reported as important associates of pioneers (Jehne and Thompson, 1981) as many plants may require endomycorrhizal infection in order to survive disturbed land (Reeves *et al.*, 1979). VAM seems to provide a primary mechanism of phosphorus up-

take from soil and may thus perform an important function in mineral cycling (Fogel, 1980).

Therefore, the present study was undertaken to find out whether the mycorrhizal fungi could colonize and infect the naturally growing herbaceous plant species of the iron ore mine wastelands.

MATERIALS AND METHODS

The root samples were collected from plants growing on a 12 year old reject dump at Sanquelim iron ore mines of M/s. Sesa Goa Limited. The roots were freed from adhering soil, gently washed and cut into 1 cm segments. The degree of colonization was calculated by using the slide method (Giovannetti and Mosse, 1980). Colonization of the roots was determined after clearing the roots with 10% KOH and

staining with Trypan blue (Philips and Hayman, 1970).

The soil pH was measured in 0.01 M CaCl₂. Electrical conductivity and cation exchange concentrations were determined in 1 : 1 water : waste extracts. Cation concentrations were measured by Atomic absorption spectrophotometry. Mineral (available) nitrogen was determined after extraction in 2M KCl (Bremner, 1965a) and total nitrogen determined after acid-Kjeldahl digestion (Bremner, 1965b). Phosphorus was determined by using Olsen and Dean's (1965) method. Total water soluble sulphate-sulphur was measured turbidimetrically (ADAS, 1981). All analyses were carried out on air-dried material but results are expressed on an oven-dry (105°C) weight basis after correction for moisture content.

RESULTS AND DISCUSSION

The pH of the reject dumps was found to be 6.02 (0.18) with an EC value of 0.005 (0.012) (mS cm⁻¹). All the plant macro- and micro- nutrients analyzed were in very low levels (Table 1).

Table 1. Some properties of iron ore mine rejects of Goa.

| Properties | Mean (S.D.) |
|---------------------------|---------------|
| pH | 6.02 (0.18) |
| EC (ms cm ⁻¹) | 0.051 (0.012) |
| Total N | 93.2* (NA) |
| Available N | 3.8* (NA) |
| P | 1.5 (NA) |
| SO ₄ -S | <0.1 (NA) |
| Ca | 1.76 (0.80) |
| Mg | 0.92 (0.55) |
| K | 0.76 (0.26) |
| Na | 2.60 (0.54) |
| Cu | < 0.05 (NA) |
| Fe | < 0.1 (NA) |

Concentrations in µg.g⁻¹ oven-dry spoil.

NA = Not applicable. S.D. = Standard deviation. EC = Electrical conductivity.

* = Mean of two replicates taken from bulked sample.

Vesicular-arbuscular mycorrhizal association was observed in all the herbaceous plant species studied (Table 2). Very heavy mycorrhizal association was observed in *Smilax sensitiva* (98%), *Merremia tridentata* (96%), *Striga asiatica* (93%), *Lindernia crustacea* (90%), *Blumea mollis* (87%), *Parthenium hysterophorus* (81%) and *Impatiens klenii* (81%); While low infection was observed in *Polygala elongata* (10%), *Vernonia cinerea* (11%) and *Atylosia scarabaeoides* (19%). A member of the pteridophyta viz., *Lygodium flexuosum* showed heavy infection (72%). Rest all the other species showed infection ranging from 23% to 79%.

Although, vesicles or arbuscules and inter- and intracellular hyphae were observed, it was seen that the frequency of occurrence of vesicles and inter- and intracellular hyphae was higher. The vesicles varied in size and shape in the different species and also within the same species.

Extensive infection of VAM fungi in plants colonizing the coal waste was recorded by Daft and Haeskylo (1976) who hypothesized that application of VAM fungi may ensure successful reclamation of coal mine areas. Heyler and Godden (1977) have estimated that the introduction of VAM fungi would decrease the amount of fertilizer required in the establishment phase.

CONCLUSIONS

VA mycorrhizal infection was recorded in all the 29 herbaceous species. It is well known that mycorrhizal fungi play a fundamental role in the rehabilitation of many disturbed lands. Thus, identification of the VAM species already thriving on the mine wastelands, their multiplication and proper utilization would help the reclamation of iron ore mine wastelands in the state.

Table 2. Degree of root colonization (%) in some naturally occurring herbaceous plant species of iron ore mine wastelands of Goa.

| Sr. No. | Plant Species | Family | Degree of root colonization (%) | Type of infection | |
|---------|---|------------------|---------------------------------|-------------------|-----|
| 1. | <i>Lygodium flexuosum</i> (L.) Swartz. | Schizacaceae | 72 | H | A |
| 2. | <i>Polygala elongata</i> Klein ex Willd. | Polygalaceae | 10 | H | A |
| 3. | <i>Impatiens Klenii</i> W.A. | Balsaminaceae | 81 | H | V |
| 4. | <i>Atylosia Scarabaeoides</i> Benth. | Fabaceae | 19 | H | A V |
| 5. | <i>Crotalaria pallida</i> Aiton | Fabaceae | 59 | H | V |
| 6. | <i>Smithia conferta</i> Sin. | Fabaceae | 79 | H | V |
| 7. | <i>Smithia sensitiva</i> Ait. | Fabaceae | 98 | H | V |
| 8. | <i>Smithia salsuginea</i> Hance | Fabaceae | 69 | H | A V |
| 9. | <i>Cassia tora</i> L. | Caesalpinaceae | 72 | H | A V |
| 10. | <i>Hydrocotyle asiatica</i> L. | Apiaceae | 42 | H | A V |
| 11. | <i>Neanotis foetida</i> Benth. & Hook. | Rubiaceae | 79 | H | V |
| 12. | <i>Spermacoce hispida</i> L. | Rubiaceae | 37 | H | V |
| 13. | <i>Blumea mollis</i> (D. Don) Merr. | Asteraceae | 87 | H | A |
| 14. | <i>Parthenium hysterophorus</i> L. | Asteraceae | 81 | H | V |
| 15. | <i>Vernonia cinerea</i> (L.) Less. | Asteraceae | 11 | H | V |
| 16. | <i>Canscora diffusa</i> (Vahl) R. Br. | Gentianaceae | 40 | H | V |
| 17. | <i>Merremia tridentata</i> (L.) Hallier f. | Convolvulaceae | 96 | H | A |
| 18. | <i>Lindernia crustacea</i> (L.) F. Muell. | Scrophulariaceae | 90 | H | A |
| 19. | <i>Lindernia parviflora</i> (Roxb.) Haines | Scrophulariaceae | 60 | H | A |
| 20. | <i>Ramphicarpa longiflora</i> Benth. | Scrophulariaceae | 36 | H | V |
| 21. | <i>Striga asiatica</i> (L.) Kuntze | Scrophulariaceae | 93 | H | V |
| 22. | <i>Centranthera hispida</i> R. Br. | Scrophulariaceae | 56 | H | V |
| 23. | <i>Justicia procumbens</i> L. | Acanthaceae | 23 | H | A V |
| 24. | <i>Gomphrena celosioides</i> C. Martius | Amaranthaceae | 62 | H | A |
| 25. | <i>Amorphophallus commutatus</i> Engler | Araceae | 74 | H | V |
| 26. | <i>Eriocaulon cinereum</i> R. Br. | Eriocaulaceae | 29 | H | V |
| 27. | <i>Eragrostis amabilis</i> W. & A. | Poaceae | 29 | H | V |
| 28. | <i>Heteropogon contortus</i> (L.) P. Beauv. ex Roemer & Schultes | Poaceae | 30 | H | A |
| 29. | <i>Ischaemum semisagittatum</i> Roxb. | Poaceae | 67 | H | V |

Legend :

H = Hyphal; A = Arbuscular; V = Vesicular.

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Preliminary Investigations into VAM Colonization of Plant Species found growing in Abandoned Tailing Pond of Iron Ore Mines in Goa.

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ABSTRACT

Mycorrhizal infection was assessed in 20 plant species belonging to 20 genera and distributed among 15 families found growing in abandoned tailing pond of iron ore mines in Goa.

All the plant species showed mycorrhizal infection that ranged from 5% to 99%. *Alternanthera sessilis* (Amaranthaceae) showed least mycorrhizal infection (5%), while maximum mycorrhizal infection (99%) was recorded in *Blumea wightiana* (Asteraceae). Plants exhibiting mycorrhizal infection had typical vesicular or arbuscular and inter- and intra-cellular hyphal infection.

Introduction

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

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

VAM fungi are ubiquitous in distribution. They hold tremendous potential for use in the reclamation industry. In many instances they should improve plant growth and establishment on disturbed lands and speed processes of ecosystem recovery (Wood, 1982).

The present study was undertaken to find out whether the VA mycorrhizal fungi could colonize and infect plant species growing in abandoned tailing pond of iron ore mines in Goa.

Materials And Methods

Root samples were collected from plant species growing in abandoned tailing pond at Sanquelim iron ore mines of M/S Sesa Goa Limited. The roots were freed from adhering soil, gently washed and cut into 1cm segments. The degree of colonization was calculated by using the slide method (Giovannetti & Mosse, 1980). Colonization of the roots was determined after clearing the roots with 10% KOH and staining with trypan blue (Philips & Hayman 1970).

The soil pH was measured in 0.01 M CaCl₂. Electrical conductivity and cation exchange concentrations were determined in 1:1 water : waste extracts. Cation concentrations were measured by Atomic absorption spectrophotometry. Mineral (available) nitrogen was determined after extraction in 2M KCl (Bremner, 1965a) and total nitrogen determined after acid-Kjeldahl digestion (Bremner 1965b). Phosphorus was determined by using Olsen and Dean's (1965) method. Total water soluble sulphate sulphur was measured turbidimetrically (ADAS, 1981). All analyses were carried out on air-dried material but results are expressed on an oven-dry (105°C) weight basis after correction for moisture content.

Results And Discussion

The pH of the tailings was found to be 6.48 (0.07) with an electrical conductivity (Ec) value of 0.065 (0.018) (ms cm⁻¹). All the plant macro- and micro-nutrients analyzed were in very low levels (Table 1)

Table 1. Some properties of iron ore mine tailings.

| Properties | Mean (S.D.) |
|---------------------------|---------------|
| pH | 6.48 (0.07) |
| EC (ms cm ⁻¹) | 0.065 (0.018) |
| Total N | 60.3* (NA) |
| Available N | 1.7* (NA) |
| P | 1.9* (NA) |
| SO ₄ -S | <0.1 (NA) |
| Ca | 2.34 (0.57) |
| Mg | 0.75 (0.17) |
| K | 0.71 (0.30) |
| Na | 4.85 (2.94) |
| Cu | <0.05 (NA) |
| Fe | <0.1 (NA) |

Concentrations in µg.g⁻¹ oven-dry soil.

NA = Not applicable

EC = Electrical conductivity

* = Mean of two replicates taken from bulked sample.

Vesicular-arbuscular mycorrhizal association was observed in all the plant species investigated. Very heavy infection was recorded in *Blumea wightiana* (99%) and *Vandellia*

crustacea (98%). Rest of the species showed moderate infection ranging from 75% in *Ludwigia parviflora* to 25% in *Ficus glomerata*. Low infection was recorded in *Cassia tora* (11%) and *Alternanthera sessilis* (5%) (Table 2.) Plants exhibiting mycorrhizal infection had typical vesicular or arbuscular and inter- and intra-cellular hyphal infection. The vesicles showed variation in size and shape in the different species and also with the same species.

Daft and Hacskaylo (1976) recorded extensive infection of VAM fungi in plants colonizing the coal waste. They hypothesized that application of VAM fungi may ensure successful reclamation of coal mine areas.

Table 2 : Degree of root colonization in plant species growing in abandoned tailing pond of iron ore mines.

| Sr. No | Plant species | Habit | Family | Degree of root colonization | Type of infection | | |
|--------|--|-------|------------------|-----------------------------|-------------------|---|---|
| 1 | <i>Psidium guajava</i> L. | Tree | Myrtaceae | 64 | H | A | V |
| 2 | <i>Leucaena glauca</i> Benth. | Tree | Mimosaceae | 50 | H | | V |
| 3 | <i>Crotalaria filipes</i> Benth. | Herb | Fabaceae | 56 | H | A | V |
| 4 | <i>Desmodium triflorum</i> (L.) DC. | Herb | Fabaceae | 46 | H | A | V |
| 5 | <i>Cassia tora</i> L. | Herb | Caesalpiniaceae | 11 | H | A | |
| 6 | <i>Melochia corchorifolia</i> L. | Herb | Sterculiaceae | 48 | H | | V |
| 7 | <i>Ludwigia parviflora</i> Roxb. | Herb | Onagraceae | 75 | H | A | |
| 8 | <i>Hydrocotyle asiatica</i> L. | Herb | Apiaceae | 54 | H | A | |
| 9 | <i>Oldenlandia corymbosa</i> L. | Herb | Rubiaceae | 45 | H | A | |
| 10 | <i>Blumea wightiana</i> DC. | Herb | Asteraceae | 99 | H | A | V |
| 11 | <i>Eupatorium odoratum</i> L. | Herb | Asteraceae | 57 | H | A | V |
| 12 | <i>Canscora diffusa</i> (Vahl.) R. Br. | Herb | Gentianaceae | 58 | H | A | |
| 13 | <i>Physalis minima</i> L. | Herb | Solanaceae | 60 | H | A | V |
| 14 | <i>Vandellia crustacea</i> (L.) Benth. | Herb | Scrophulariaceae | 98 | H | A | V |
| 15 | <i>Alternanthera sessilis</i> Br. | Herb | Amaranthaceae | 5 | H | A | |
| 16 | <i>Phyllanthus maderaspatensis</i> L. | Herb | Euphorbiaceae | 40 | H | A | V |
| 17 | <i>Ficus glomerata</i> Roxb | Tree | Moraceae | 25 | H | A | |
| 18 | <i>Dimeria woodrowii</i> Stapf. | Herb | Poaceae | 50 | H | A | |
| 19 | <i>Isachne elegans</i> Dalz. ex Hook. | Herb | Poaceae | 31 | H | | V |
| 20 | <i>Ischaemum semisagittatum</i> Roxb. | Herb | Poaceae | 42 | H | A | V |

Legend: H=Hyphal; A=Arbuscular; V=Vesicular.

Conclusions

VA mycorrhizal association was observed in all the 20 plants species growing in the mine tailings. Although ecosystems are self-sustaining and have capacity to develop, the process of natural succession on degraded areas like mine wastelands would be much slow. This could lead to further degradation of the already degraded land and can have serious effect on the surrounding land. Hence, identification of VAM species already thriving on the fairly established land and their application would help the reclamation of iron ore mine wastelands.

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DIURNAL VARIATIONS OF PHYSICO-CHEMICAL ASPECTS OF POLLUTION IN KHUSHAVATI RIVER AT QUEPEM, GOA

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Abstract :

The River Khushavati is a major source of water supply in the villages of Sanguem, Quepem and Saleete Talukas of Goa State. The river water has multi- farious uses, drinking, bathing, agriculture, horticulture, live stock and other Agro related activities. The present study deals with water Quality and pollution load of khushavati at Quepem based on short term study. The villagers are hard hit during the monsoon when the run off from the mining ore. dumps, washing of manganese ore along the banks and trucks in the river results in the contamination of river water making it unsafe for drinking and irrigation .The need to develop an action plan for river conservation is advocated.

Introduction :

Most of the perennial rivers and their tributaries are being used as a site for disposal of domestic and industrial water in India which impairs their water quantity River Khushavati as it speeds down from Nundem and join river Zuari after a winding run of nearly 40 km through south Goa is an important source of water for drinking, washing, irrigation and transport specially in Cazor, Cosmoi, Sulcoma, Colomba, Kevana, Usgalimol, Rivona and other villages of Sanguem and Quepem. There were reports of washing of manganese are at Vagotem near Cazor bridge, Colomba and Cosmoi. To understand the magnitude of this pollution problem and to test the suitability of such water at Quepem the present investigation was undertaken.

Material and methods :

Water samples were collected from five sampling stations located on both the right and left banks. The sampling station location was chosen to ascertain the impact of pollution. Temperature (air and water) pH, dissolved oxygen (Do) were estimated on the spot in the field while for estimation of other parameters water samples were brought to the laboratory in the

ice-box. Standard methods as prescribed APHA(1989) Saxena (1990) were followed for examination of various physical and chemical parameters of water.

Results and Discussion :

Temperature during the present investigation the water temperature of river stretch explored ranged from 25.2 to 28.8, which shows a clear correlation with atmospheric temperature pH is one of the most important abiotic factor that serves as an index for pollution. The factors like photosynthesis, exposure to air, disposal of industrial water and domestic sewage effect pH (Saxena 1990). It varied from 6.87 to 8.74 ISI (1983) indicate that the pH values below 5.0 and above 8.4 are detrimental. The pH of Khushavati river reaches 8.7 indicating its unsuitability.

Total solids ranged from 252 to 495.00 minimum at mid night at evening corresponding to mining activity. A high contents of total solids elevate the density of water and such a medium increases osmoregulatory stress on aquatic biota (Verma *et al*, 1978).

Dissolved oxygen in water at a given temperature depends on factors like temperature of water , the concentration of dissolved salts, biological activity and geology of basins of river (Gold man and Horne 1983). It ranged from 2.86 to 4.14. The critical requirement level for all fishes is 3- 6 mg L⁻¹ of DO₂. The low level of DO₂ in the Khushavati river is indicative of stress problem for aquatic organism and pollution.

Total alkalinity ranged from 189 to 450. The low alkalinity is at mid night and maximum at 6pm indicating the high pollution load. Total hardness

**Table 1. Diurnal Variations of Physico Chemical Parameters of Khushavati River at Quepem.
(Time 9 AM to 6 AM)**

| Parameter | 09.00 | 12.00 | 15.00 | 18.00 | 21.00 | 24.00 | 03.00 | 06.00 |
|------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Tempera-ture°C | 29.00 | 31.00 | 30.00 | 28.80 | 27.00 | 26.10 | 25.20 | 27.50 |
| Water Temp. °C | 27.00 | 28.80 | 27.90 | 26.10 | 26.10 | 25.20 | 26.10 | 27.00 |
| PH | 08.46 | 08.74 | 07.20 | 07.20 | 06.94 | 06.87 | 07.20 | 08.10 |
| Total Solids | 482.22 | 401.40 | 395.10 | 495.00 | 340.20 | 252.00 | 290.25 | 297.00 |
| DO | 03.60 | 04.14 | 03.60 | 03.42 | 03.42 | 02.86 | 02.90 | 03.60 |
| Total Alkalinity | 351.00 | 342.00 | 423.00 | 450.00 | 252.00 | 189.00 | 288.00 | 324.00 |
| Total Hardness | 108.00 | 142.00 | 142.00 | 153.00 | 131.00 | 131.00 | 126.00 | 126.00 |
| Calcium | 72.00 | 90.00 | 81.00 | 101.70 | 90.00 | 78.40 | 64.80 | 56.90 |
| Magnesium | 10.74 | 10.43 | 20.16 | 24.30 | 19.80 | 10.43 | 06.21 | 09.00 |
| Chlorides | 124.20 | 161.10 | 123.30 | 104.04 | 104.04 | 104.04 | 92.52 | 90.00 |
| Nitrates | 00.36 | 00.36 | 00.30 | 00.40 | 00.33 | 00.30 | 00.22 | 00.28 |
| Phosphates | 02.70 | 02.70 | 03.15 | 03.60 | 03.24 | 02.70 | 02.25 | 02.61 |

All values in mg/L, except temperature and pH

ranged from 108 to 153mg/L. Calcium levels varied from 56.9 to 101.7mg/L. The maximum levels were observed at 18.00hm similarly magnesium levels ranged from 6.21mg/L to 24.30 mg/L. High values of calcium, magnesium contents indicate the unplanned mining activity as their levels in increase corresponds with it.

High values and chlorides and calcium in drinking water are generally not harmful to human beings but high concentration of chloride may affect some persons who already suffer from diseases of heart or kidneys. In the present study chlorides ranged from 90.00 to 161.10 Excess concentration of nitrates and phosphates brings eutrophication and is considered sufficient to stimulate algal gloom (Bahura, C.K., 1998). In Khushavati river nitrate levels ranged from 0.22 to 0.40 phosphate levels ranged from 2.25 to 3.60 mg/L.

It was observed that 2.53 mg/L of PO₄ is the maximum limit of its concentration. Which could be accepted as the danger signal of evaluation of Eutrophication of Estuary. In the present investigation, nitrates and phosphates levels are observed above the danger signal limit.

To summarize, the present study indicates that Khushavati river is highly polluted due to the unplanned mining activity and there is an urgent need to take steps for the protection of river.

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

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ABSTRACT

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

INTRODUCTION

Arbuscular mycorrhizal fungi are ubiquitous in both natural and man-made ecosystems (Hayman, 1982). These fungi benefit the plant by improving the supply of nutrients, especially phosphorus and other minerals such as Zn, Cu, K and Ca (Cooper & Tinker, 1978). Besides direct nutritional advantage, arbuscular mycorrhizae are accredited with other benefits to the host plants such as ability of AM fungal roots to overcome water stress by stomatal regulation (Levy & Krukum, 1980), increasing disease resistance by depressing root penetration and larval development of nematodes (Sikora, 1978), tolerance to toxic heavy metals, drought, high soil

temperature, adverse pH etc. Mycorrhizal inoculation also stimulates rooting (Barrow *et al.*, 1977) growth and transplant survival (Bryan & Kormanik, 1977). Hence AM fungi are gaining importance in agro-forestry, agricultural, horticultural sectors and more recently in the field of ethnobotany for the commercial cultivation of medicinal plants in order to enhance plant growth and productivity.

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

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

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

MATERIALS AND METHODS

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

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

slope.

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

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

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

Identification of plant species was

Table IA. List of medicinal shrubs.

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

Table IB. List of medicinal trees.

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

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

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

transformations whereas data for spore density was subjected to log transformations.

RESULTS

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

Table IIA. List of medicinal shrubs.

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

*Mean value of three readings. ± Indicates Standard deviation.

H = Hyphal colonization; A = Arbuscular colonization; V = Vesicular colonization.

Table IIB. List of medicinal trees.

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

*Mean value of three readings. ± Indicates Standard deviation.

H = Hyphal colonization; A = Arbuscular colonization; V = Vesicular colonization.

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

rhizosphere and rhizoplanes of medicinal plants (Sen, 1998).

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

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

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INTRODUCTION

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

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

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

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

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

MINING AND ITS IMPACT ON ENVIRONMENT

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

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

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

With the present annual production of 15 million tons of iron ore, it is expected that 40-50 million tons of wastes have to be stored per year, and approximately 150 million cubic meters of water is to be discharged from pits to the drainage system. Mine waste dumps are biggest man-made hillocks, volume and height of such dumps increases every year. Most of the waste dumps

rise up to 50-60 meters high with 50° -55° angle of repose. These being unconsolidated are prone to slumps and slides due to heavy monsoon rains.

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

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

ECOSYSTEM, PLANT SUCCESSION AND MINING

The vegetation together with the soil in which it has its roots, the associated fauna, and the environment that surrounds them form a closely interrelated and interdependent to the ecosystem. Although ecosystems are sensitive to the outside influences, they are self-sustaining. Once properly established, they need no further support. This is because of natural cycling of accumulated materials, which maintain the vegetation and the other organisms with it. After a major disturbance, vegetation slowly and gradually develops over a period of time: a process termed plant succession.

If the above two properties (self-sustaining and capacity to develop) of the ecosystem are considered, then one may presume that after mining disturbance, there is no need of any revegetational efforts *i.e.*, a self-sustaining vegetation cover will develop naturally. But the process of natural succession will take many years.

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

CHARACTERISTICS OF MINE WASTES

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

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

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

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

Nutrient deficiencies are widely reported as a major limitation, particularly in terms of a low or a complete lack of organic matter and nitrogen in mining wastes. Smith and Bradshaw, (1970) stated that micro-nutrient deficiencies are frequently encountered in the mine wastes. Wong *et al.*, (1983) showed that the tailings were alkaline, lacking in organic matter and nitrogen, but were rich in metals such as Fe, Zn, Cu, Mn, Mg and Ca.

Table—1 Some properties of iron ore mine rejects in Goa. (Rodrigues and Bukhari, 1997).

| Properties | Mean (S.D.) |
|-------------------------------|---------------|
| pH | 6.02 (0.18) |
| EC (mS /cm) | 0.051 (0.012) |
| Total N | 93.2* (N.A.) |
| Available N | 3.8* (N.A.) |
| P | 1.5 (N.A.) |
| SO ₄ ^{-s} | <0.1 (N.A.) |
| Ca | 1.76 (0.80) |
| Mg | 0.92 (0.55) |
| K | 0.76 (0.26) |
| Na | 2.60 (0.54) |
| Cu | <0.05 (N.A.) |
| Fe | <0.1 (N.A.) |

Concentrations in $\mu\text{g.g}^{-1}$ oven dry spoil.

N.A. = Not applicable.

S.D. = Standard deviation.

EC = Electrical conductivity.

* = Mean of two replicates taken from bulked samples.

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

MYCORRHIZAL FUNGI

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

Table—2. Some properties of iron ore mine tailings in Goa. (Rodrigues and Bukhari, 1996).

| Properties | Mean (S.D.) |
|-------------------------------|---------------|
| pH | 6.48 (0.07) |
| EC (mS /cm) | 0.065 (0.018) |
| Total N | 60.3* (N.A.) |
| Available N | 1.7* (N.A.) |
| P | 1.9 (N.A.) |
| SO ₄ ²⁻ | <0.1 (N.A.) |
| Ca | 2.34 (0.57) |
| Mg | 0.75 (0.17) |
| K | 0.71 (0.30) |
| Na | 4.85 (2.94) |
| Cu | <0.05 (N.A.) |
| Fe | <0.1 (N.A.) |

Concentrations in $\mu\text{g.g}^{-1}$ oven dry spoil.

N.A. = Not applicable.

S.D. = Standard deviation.

EC = Electrical conductivity.

* = Mean of two replicates taken from bulked samples.

ADVANTAGES OF MYCORRHIZAL FUNGI

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

Absence of suitable mycorrhizal fungi is one of the main reasons for the failure of afforestation programmes. Tree species, especially exotics, fail to establish on afforestation sites in the absence of their fungal symbionts. Repeated attempts for the last twenty years to raise pine plantation in Puerto Rico failed due to lack of ectomycorrhizae on their roots. The pine seedlings would grow to 5-30 cm in height, then become chlorotic, and die. Transfer of ectomycorrhizal soil from successful plantations in the mainland to Puerto Rico nurseries helped in the successful establishment of pine plantations on this island. The inoculated plants of slash pine grew healthy and reached a height of 2.0- 2.5 m in three years. Similarly, success in raising pine plantations in South Africa was achieved only when ectomycorrhizal inoculum from Netherlands was introduced in South African nurseries. Soil from South African pine nurseries was then used as soil inoculum in Kenya and this resulted in large scale successful pine plantations in that country.

Normal growth occurs only when mycorrhizal fungi present in the site colonize and establish mycorrhiza on the roots. It may be possible to grow mycotrophs without its fungal symbiont if nursery plants are given a high dose of fertilizers. However, such seedlings when transplanted in the field where mycorrhiza is absent and nutrient is discontinued for economic reasons, the growth of plants is retarded appreciably in many cases.

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

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

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

BROAD CLASSIFICATION OF MYCORRHIZAE

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

Characteristics of Ectomycorrhizae

1. Prevalent in the temperate regions, in forest and ornamental tree species.
2. Characterized by the presence of a mantle of fungal tissue around the host rootlet and intercellular penetration of the rootlet cortex (Hartig net), and not intracellular.

3. Fungi belong to Basidiomycetes, which form sporophores and release air-borne spores. The inoculum thus is easily spread by wind from one location to another.
4. Host plant roots with ectomycorrhizal colonization are short, swollen, dichotomously branched with distinctive colours *viz.*, white, black, orange, yellow or olive green.
5. It is fairly easy to isolate a number of ectomycorrhizal fungi in pure culture by using routine microbiological techniques and grow them saprophytically. Thus, it is possible to produce inoculum on a large scale.

Characteristics of Endomycorrhizae

1. These fungi are prevalent in the tropics.
2. The colonization is both intercellular and intracellular. In this case, there is no 'Hartig net' and no 'mantle'.
3. Endomycorrhizal fungi cannot be cultured in vitro conditions. The production of endomycorrhizal inoculum requires the growth of a susceptible host plant. The inoculum has to be produced under sterile glasshouse conditions.

Classification of Endomycorrhizae

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

Arbuscular Mycorrhizae (AM) Fungi:

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

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

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

Arbutoid Mycorrhizae

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

Ericoid Mycorrhizae

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

Orchid Mycorrhizae

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

STAGES OF DEVELOPMENT OF AM FUNGI

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

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

CURRENT STATUS OF AM FUNGI

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

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

Table—3: Classification of AM fungi.

| Old Classification (Gerdemann & Trappe, 1974; Benjamin, 1979; Warcup, 1990) | Present Classification (Morton & Benny, 1990) |
|--|--|
| Order: Endogonales | 1. Order: Endogonales |
| Family: Endogonaceae | Family: Endogonaceae |
| Genera: <i>Endogone</i> <i>Sclerogone</i> <i>Glomus</i> <i>Sclerocystis</i> <i>Acaulospora</i> <i>Entrophospora</i> <i>Gigaspora</i> <i>Scutellospora</i> | Genera: <i>Endogone</i> <i>Sclerogone</i> |
| | 2. Order: Glomales |
| | Sub-order: Glomineae |
| | (i) Family: Glomaceae |
| | Genera: <i>Glomus</i> <i>Sclerocystis</i> |
| | (ii) Family: Acaulosporaceae |
| | Genera: <i>Acaulospora</i> <i>Entrophospora</i> |
| | Sub-order: Gigasporineae |
| | Family: Gigasporaceae |
| | Genera: <i>Gigaspora</i> <i>Scutellospora</i> |

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

TYPES OF ECTO- AND ENDO-MYCORRHIZAL INOCULA FOR NURSERIES

The following types of inocula being employed for introduction of ecto- and endo-mycorrhizal fungi in nurseries are described below:

I. SOIL INOCULUM

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

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

II. INOCULATION WITH SPORES

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

(a) Spore Soil Mix

In this, approximately 2 µg of spore powder (containing approximately 1×10^{12} spores) is thoroughly mixed with one kg of sterilized or fumigated soil and this spore mix is used as inoculum. For inoculation of nursery beds or potting mixture, 1 kg of soil spore mix is evenly spread over one square meter of nursery bed soil and the soil is then thoroughly raked up to uniformly distribute the spore inoculum in the soil.

(b) Seed Encapsulation with Spores

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

(c) Spore Pellets

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

(d) Mycorrhizal Beads

In Philippines, scientists have produced ecto-mycorrhizal beads, under the trade name "MYCORRHIZAL BEADS" by entrapping mycelia of *Pisolithus tinctorius* grown in liquid.

fermenters in calcium alginate beads. The entrapped mycelial inoculum has many advantages over solid medium inoculum. With this technique, the mycelium is produced in 1-2 weeks while in solid technique, several months are required to produce the inoculum.

(e) Pure Culture Inoculum

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

INOCULATION PROCEDURES FOR NURSERIES

I. BROADCAST INOCULATIONS

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

II. BANDING OF INOCULUM BELOW SEEDS

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

III. SLURRY DIPS

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

MYCORRHIZAL INOCULUM TECHNOLOGY

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

Seedlings can be inoculated at three different stages *viz.*, i. before seeds are sown, ii. When seeds are sown and, iii. After seedlings emerge. The most efficient stage would be to inoculate the seedlings before or when the seeds are sown. An efficient time to inoculate cuttings is at the time of propagation. It also depends upon the economic considerations and on the ecology of mycorrhizal fungi. As regards to economics, the stage when the seeds are sown or cuttings are

propagated requires least amount of inoculum per volume of growing medium. Again, the newly developed rootlets of seedlings or cuttings are receptive to mycorrhizal colonizations, as they are non-lignified. The mycorrhizal fungi are also known to increase the rooting in cuttings and increase the root developments during propagation. It is also cost efficient to develop a mechanical inoculation system for use when seeds are sown or cuttings are propagated than any other later stages. Inoculation at the time of transplantation is time consuming, requires more inoculum, and the introduced fungi must be compatible with the native microorganisms and climatic conditions of the planting site.

ROLE OF AM FUNGI IN RECOVERY OF MINE WASTELANDS

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

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

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

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

(Contd...)

| | | | | | | |
|-----|---|------------------|----|----|---|---|
| 5. | <i>Neanotis foetida</i> Benth. & Hook. | Rubiaceae | 79 | H | V | |
| 6. | <i>Spermacoce hispida</i> L. | Rubiaceae | 37 | H | V | |
| 7. | <i>Blumea mollis</i> (D. Don) Merr. | Asteraceae | 87 | H | A | |
| 8. | <i>Parthenium hysterophorus</i> L. | Asteraceae | 81 | H | V | |
| 9. | <i>Vernonia cinerea</i> (L.) Less. | Asteraceae | 11 | H | V | |
| 10. | <i>Canscora diffusa</i> (Vahl.) R.Br. | Gentianaceae | 40 | H | V | |
| 11. | <i>Merremia tridentata</i> (L.) Hallier f. | Convolvulaceae | 96 | H | A | |
| 12. | <i>Lindernia crustacea</i> (L.) F.Muell | Scrophulariaceae | 90 | H | A | |
| 13. | <i>Lindernia parviflora</i> (Roxb.) Haines | Scrophulariaceae | 60 | H | A | |
| 14. | <i>Ramphicarpa longiflora</i> Benth. | Scrophulariaceae | 36 | H | V | |
| 15. | <i>Striga asiatica</i> (L.) Kuntze | Scrophulariaceae | 93 | H | V | |
| 16. | <i>Centranthera hispida</i> R.Br. | Scrophulariaceae | 56 | H | V | |
| 17. | <i>Justicia procumbens</i> L. | Acanthaceae | 23 | H | A | V |
| 18. | <i>Gomphrena celosioides</i> C.Martius | Amaranthaceae | 62 | H | A | |
| 19. | <i>Amorphophallus commutatus</i> Engler | Araceae | 74 | H | V | |
| 20. | <i>Eriocaulon cinereum</i> R. Br. | Eriocaulaceae | | 29 | H | V |
| 21. | <i>Eragrostis amabilis</i> W & A. | Poaceae | 29 | H | V | |
| 22. | <i>Heteropogon contortus</i> (L.) P. Beauv. Ex Roeme & Schultes | Poaceae | 30 | H | A | |
| 23. | <i>Ischaemum semisagittatum</i> Roxb. | Poaceae | 67 | H | V | |

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

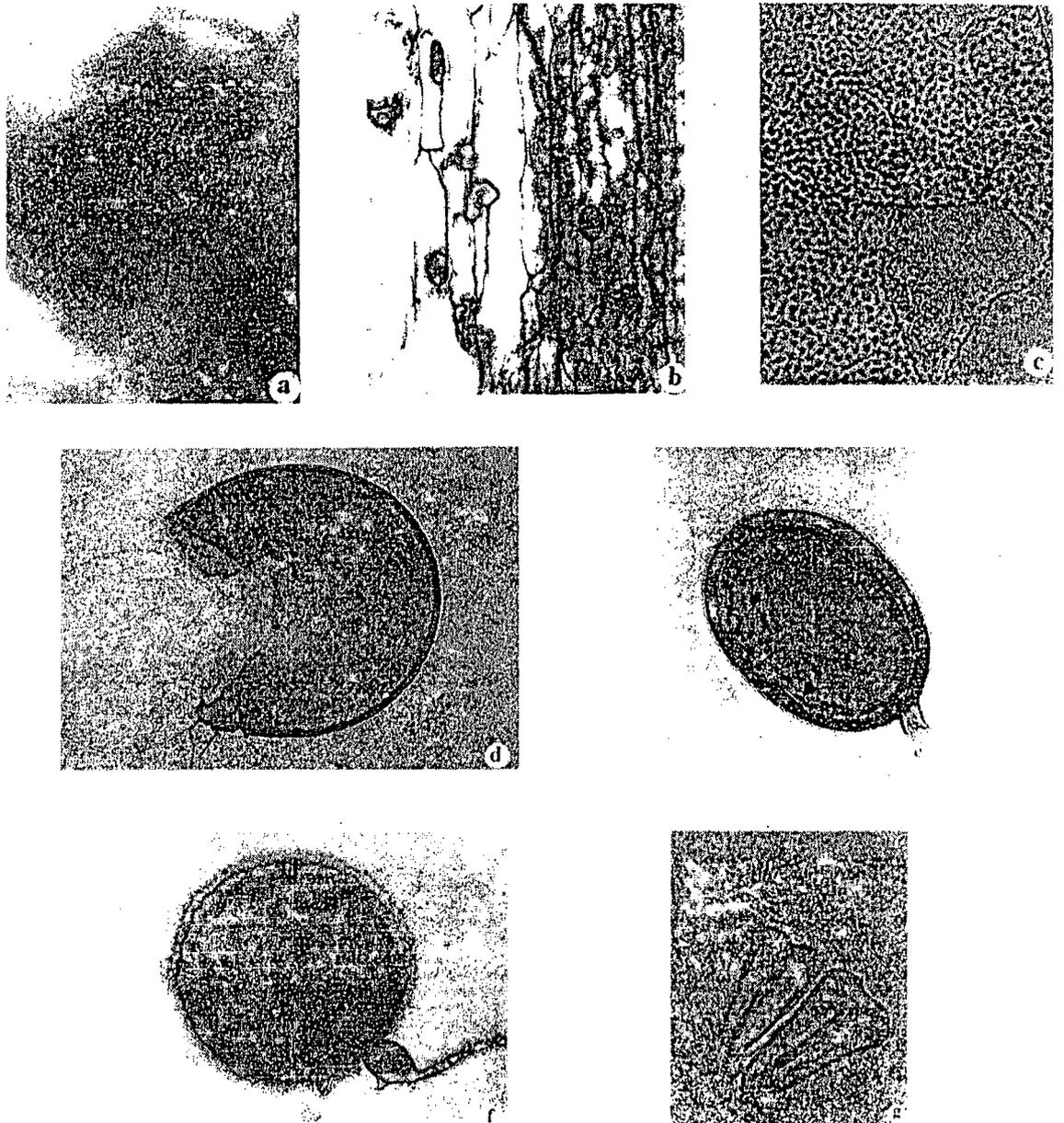


Plate I: a. Arbuscular Colonization (400x); b. Vesicular and hyphal Colonization (100x)
 c. Surface ornamentation in *Acaulospora spinosa* spore (400x);
 d. Spore of *Gigaspora margarita* (100x);
 e. Spore of *Glomus macrocarpum* (400x);
 f. Spore of *Scutellospora gregaria* (100x), and
 g. Chlamydospores of *Sclerocystis taiwanensis* (400x)

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

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

| Sr. No. | AM species | Sr. No. | AM species |
|---------|----------------------------|---------|---------------------------------|
| 1. | <i>Glomus geosporum</i> | 16. | <i>Glomus deserticola</i> |
| 2. | <i>Glomus mosseae</i> | 17. | <i>Gigaspora albida</i> |
| 3. | <i>Glomus fasciculatum</i> | 18. | <i>Gigaspora margarita</i> |
| 4. | <i>Glomus hoi</i> | 19. | <i>Gigaspora caudida</i> |
| 5. | <i>Glomus aggregatum</i> | 20. | <i>Acaulospora nicolsonii</i> |
| 6. | <i>Glomus australe</i> | 21. | <i>Acaulospora spinosa</i> |
| 7. | <i>Glomus reticulatum</i> | 22. | <i>Acaulospora bireticulata</i> |
| 8. | <i>Glomus clarum</i> | 23. | <i>Acaulospora laevis</i> |
| 9. | <i>Glomus constrictum</i> | 24. | <i>Acaulospora morrawae</i> |
| 10. | <i>Glomus caledonium</i> | 25. | <i>Acaulospora foveata</i> |
| 11. | <i>Glomus radiatum</i> | 26. | <i>Acaulospora mellea</i> |
| 12. | <i>Glomus etimucatum</i> | 27. | <i>Scutellospora gilmorei</i> |
| 13. | <i>Glomus albidum</i> | | |
| 14. | <i>Glomus monosporum</i> | | |
| 15. | <i>Glomus globiferum</i> | | |

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

| Plant species | Total dry wt. (g) | | |
|------------------------------|-------------------|------------|------------|
| | Control | G. F. | G. M. |
| <i>Delonix regia</i> | 1.48 (0.10) | 2.28(0.14) | 4.05(0.31) |
| <i>Tamarindus indica</i> | 1.68 (0.06) | 1.93(0.43) | 2.54(0.25) |
| <i>Acacia farnesiana</i> | 0.22 (0.03) | 0.64(0.06) | 1.00(0.13) |
| <i>Acacia mangium</i> | 0.12 (0.00) | 0.35(0.03) | 0.38(0.03) |
| <i>Adenantha pavonina</i> | 0.82 (0.09) | 1.37(0.10) | 1.56(0.06) |
| <i>Albizia lebbek</i> | 0.24 (0.02) | 0.84(0.17) | 0.86(0.12) |
| <i>Leucaena leucocephala</i> | 0.31 (0.03) | 0.74(0.05) | 0.75(0.09) |
| <i>Samanea saman</i> | 0.44 (0.07) | 0.89(0.10) | 2.36(0.26) |
| <i>Ziziphus jujuba</i> | 0.35 (0.04) | 0.78(0.07) | 0.43(0.07) |

Legend:

Control=Pure reject; G.F. = *Glomus fasciculatum* & G.M. = *Glomus mosseae* Mean of 5 replicates. Figures in the brackets denote Standard deviation values.

CONCLUSION

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

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

Future revegetational research has to be oriented towards:

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

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

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

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DECLARATION

I hereby declare that the Ph. D. thesis entitled “**ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY OF DEGRADED IRON ORE MINE WASTELANDS 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.

MEHTAB JAHAN BUKHARI

(Signature of the Candidate)

B. F. RODRIGUES

(Signature of the Guide)

