STUDIES ON ARBUSCULAR MYCORRHIZAL (AM) FUNGI IN ORNAMENTAL FLOWERING PLANTS COMMONLY FOUND IN GOA

A Thesis submitted to Goa University for the Award of the Degree of

DOCTOR OF PHILOSOPHY
in
BOTANY

By
Ms. Jyoti Damodar Vaingankar

Goa University,
Taleigao Goa

2012

STUDIES ON ARBUSCULAR MYCORRHIZAL (AM) FUNGI IN ORNAMENTAL FLOWERING PLANTS COMMONLY FOUND IN GOA

A Thesis submitted to Goa University for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

BOTANY

By

MS. JYOTI D. VAINGANKAR, M. Sc.

Research Guide

PROF. B.F. RODRIGUES, M.Sc., Ph.D.

Goa University,

Taleigao Goa

2012

DECLARATION

I hereby declare that the thesis entitled **"STUDIES ON ARBUSCULAR MYCORRHIZAL (AM) FUNGI IN ORNAMENTAL FLOWERING PLANTS COMMONLY FOUND IN GOA"** submitted to Goa University, for the award of the degree of **DOCTOR OF PHILOSOPHY IN BOTANY** is a record of original and independent work carried out by me during August 2007 - August 2012, in the **DEPARTMENT OF BOTANY, GOA UNIVERSITY** under the supervision of **Dr. B. F. RODRIGUES,** Professor, Department of Botany, Goa University and that it has not previously formed the basis for the award of any Degree, Diploma, Associate-ship or Fellowship or any other similar title to any candidate of this or any other University.

Signature of the Guide
(B. F. RODRIGUES)

Professor,

Department of Botany

Goa University

Signature of the student
(JYOTI D. VAINGANKAR)

CERTIFICATE

I hereby certify that the thesis entitled "**STUDIES ON ARBUSCULAR MYCORRHIZAL (AM) FUNGI IN ORNAMENTAL FLOWERING PLANTS COMMONLY FOUND IN GOA"** submitted to Goa University, for the award of the degree of **DOCTOR OF PHILOSOPHY IN BOTANY** is a record of original and independent work carried out by her in the **DEPARTMENT OF BOTANY, GOA UNIVERSITY** during the period of August 2007 – August 2012, under my supervision and that it has not previously formed the basis for the award of any Degree, Diploma, Associate-ship or Fellowship or any other similar title to any candidate of this or any other University.

I affirm that the thesis submitted by Ms. Jyoti D. Vaingankar is completely the independent research work carried out by her under my supervision.

Signature of the guide

(**B. F. RODRIGUES**)

Professor,

Department of Botany

Goa University


Dedicated to my father...

ACKNOWLEDGEMENT

My most sincere gratitude goes to my guide, **Dr. B. F. Rodrigues**, Professor, Department of Botany, Goa University for his invaluable support, guidance, encouragement and patience throughout the duration of this research.

My sincere thanks to **Prof. M. K. Janarthanam**, HOD, Department of Botany, Goa University for providing the facilities for carrying out my research work.

I am thankful to **Prof. Irene Furtado**, Professor, Department of Microbiology, for her valuable help, encouragement and suggestions in carrying out my research work.

My special thanks to Dr. P. K. Sharma, Professor, Dr. V. U. Kerkar, Associate Professor, Dr. S. Krishnan, Associate Professor and Dr. N. M. Kamat, Assistant Professor, Department of Botany for their valuable help.

My special thanks to Prof. D. J. Bhat, former HOD, Department of Botany, Goa University for his support and encouragement.

I am grateful to **Department of Science and Technology**, New Delhi for providing fellowship. I am also grateful to **Goa University** for providing research studentship.

I am also thankful to the Director, National Institute of Oceanography (NIO), Dona Paula, Goa for the Scanning Electron Microscopy facility and the Director, Ela Farm, Old Goa for providing the soil testing facilities.

My sincere thanks to non-teaching staff, Department of Botany, Goa University for their kind co-operation and help.

I also thank Dr. Emilia Mascarenhas and Mrs. Milinda N. Kamat for their help.

Special thanks to my labmates Dr. Radhika K. P. Nair, Mr. James D' Souza, Ms. Seema Dessai, Ms. Cassie Rodrigues and Ms. Kim Rodrigues for their help and reducing the lab stress with their good sense of humour.

I thank Ms. Sonashia Velho-Pereira, Mr. M. Bhaskaran, Ms. Shilpa Bhosle, Ms. Vera D'costa, Mr. Ashish Prabhugaonkar, Mr. Sidhesh Naik, Mr. Ravikiran Pagare, all my friends and well wishers for their valuable help and moral support.

I shall always remain extremely grateful to my Brother-in-law, **Mr. Amar S. Mayekar** and my sister **Mrs. Arpita A. Mayekar** for their encouragement and moral support. They have helped me immensely in many ways. Few complimentary words may not really justify their help.

I shall always be grateful to my brother, **Mr. Mahesh D. Vaingankar** for being kind, understanding, tolerant and helping during the course of my work.

I shall be always indebted to my mother for her moral support and encouragement. I am also grateful to each one of my family members who stood by me all through my academic and research career.

Jyoti Damodar Vaingankar

INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1

INTRODUCTION

Flowering plants are found in all habitats around the world and comprise of about 80-90% of all plant species. Ornamental flowering plants are grown for their decorative flowers, or as house plants, for cut flowers and most commonly for display of their flowers. Flowers have a prominent place in our daily life as they are used in almost every occasion right from birth to death, social functions, gatherings, etc. They are seen in myriad of colours, shapes and sizes and add beauty to the garden. The cultivation of these plants *i.e.* floriculture forms a major branch of horticulture. Floriculture in the form of cut flowers and bouquets is getting more and more attention.

India is an agricultural country and therefore floriculture as an industry can offer lot of opportunities for sustainable employment and economic growth in our country. In case of agriculture and floriculture in particular, India has favourable cultivable land available as vast and huge infrastructure with suitable climatic conditions and large manpower. In earlier days floriculture had only aesthetic value but now it has made its place in the market and is known as commercial floriculture. World floriculture market has developed many folds and has reached to the level of billions of dollars in last few decades and is growing. As on 2004, 74,000 hectares of land was under floriculture and annual production of loose flowers was estimated at 4.6 lakh tonnes and cut flowers were 11 crores in number. Presently world flower market is $ 4 billion. The world trade data of flowers shows that the developing countries produce more than 20% whereas Indian contribution in floriculture is not been more than 0.5% of the world transaction (http://www.iiem.com/em/floriculture/chapter2.html). Floriculture is considered

as the latest addition to the commercial economy of the agriculture sector and is developing with its entire potential world over and also in India. Proper planning can help the floriculture sector to become the biggest contributor for agro based economy of our country. It can contribute a lot in creating job opportunities for our qualified human resource and become a very popular commercial activity in the coming years. Therefore, besides plant growers or farmers, floriculture has attracted attention of several researchers. Realizing the potential of floriculture industry in the coming years, large investment in this sector has been done in India. It is essential to improve floriculture production technology and infrastructure with modern and latest technical practices. Biotechnological methods can significantly help farmers to improve quality, widen the assortment and decrease the price of cultivated flowering plants.

In nature great diversity of fungi form associations among which Arbuscular Mycorrhizal (AM) fungi of the phylum Glomeromycota are the important symbiotic organisms of most terrestrial plants with respect to agriculture and plant productivity (Parniske, 2008; Schűβler *et al*., 2001). In natural habitats and in an ecologically meaningful time span these associations have evolved to improve the fitness of both plant and fungal symbionts (Johnson *et al*., 1997). The AM fungi are an important component of the soil microbial biomass. The mutualistic relationship between AM fungi and host plants has traditionally been studied in terms of the benefits to individual plants and fungi (Francis and Read, 1995; Smith and Smith, 1996). These fungi aid the host plant in obtaining certain nutrients from the soil, and

are in turn provided with carbon and energy in the form of carbohydrates by the plant. These interactions depend on a fungal species successfully colonizing and living in or on the roots of a plant. The beneficial effects of AM fungal association are attributed to the growth promoting aspects in many situations particularly in infertile soils, where mycorrhizal plants grow better than non-mycorrhizal plants (Gerdeman, 1968; Mosse, 1973). When the soil conditions are suitable, the fungal spores germinate and through some signal communications which are somewhat similar to the signal exchange process between the rhizobia and legumes (Miransari and Smith, 2007, 2008, 2009) begin their symbiosis with the host plant (Boglárka *et al*., 2005) by entering the root tissue and forming special structures called vesicles and arbuscules. Due to their extramatrical hyphae, mycorrhizal roots can explore more soil volume than non-mycorrhizal roots (Malcova *et al*., 2003; Sawaki and Saito, 2001). These fungi play a significant role in improving absorption and translocation of nutrients, and therefore aids in increasing the supply of slowly diffusing ions such as phosphate (P) to the plant (Valentine *et al*., 2001; Ortas, 2003; Smith and Read, 2008). Though P uptake usually dominates consideration of the AM association, they also help in the uptake of other nutrients by the host plant. Zinc (Zn) nutrition is most commonly reported as being influenced by the AM association, though uptake of copper (Cu), iron (Fe), nitrogen (N), potassium (K), calcium (Ca) and magnesium (Mg) is also enhanced (Smith and Read, 1997; Clark and Zeto, 2000, Perner *et al*., 2007). In some cases, the availability of these nutrients controls the symbiosis initiation (Ryan and Angus, 2003). They also enhance plant uptake of N from

organic sources (Hodge *et al*., 2001) but still more work is required to fully understand the mechanisms involved (Read and Perez-Moreno, 2003).

Other benefits rendered by AM association to the host plant includes improved water relations (Allen *et al*., 1981; Allen and Allen, 1986; Augé, 2004), enhanced uptake of micro-nutrients (Faber *et al*., 1990; Kothari *et al*., 1991), increased resistance to foliar-feeding insects (Sharma *et al.,* 1992; Gange and West, 1994; Rabie, 1998; Gernns *et al*., 2001) improved drought resistance (Augé *et al*., 1994), increased resistance to soil pathogens (Dehne, 1982; Jalali and Chand, 1988; Abdel-Fattah and Mankarios, 1995; Newsham *et al*., 1995a; Abdalla and Abdel-Fattah, 2000; Lingua *et al*., 2002; Pozo *et al*., 2002) and increased tolerance of salinity and heavy metals (Shetty *et al*., 1995; Diaz *et al*., 1996; Al-Karaki *et al*., 2001; Feng *et al*., 2002; Mohammad *et al*., 2003). In addition AM fungi are also known to improve soil structure (Tisdall and Oades, 1979; Degens *et al*., 1996). The hyphae extend from root surfaces into the surrounding soil, binding particles and increasing micro- and macro-aggregation (Augé, 2001) and interact with other rhizosphere microorganisms including free living N fixing bacteria and general plant growth promoting rhizobacteria (PGPR) to the benefit of host plant (Requena *et al*., 1997; Biró *et al*., 2000; Requena *et al*., 2001; Bending, 2007; Richardson *et al*., 2009).

Modern agricultural practices such as fertilization, biocide application and monoculture affect the community composition and diversity of AM fungi (Douds and Millner 1999; Johnson, 1993; Oehl *et al*., 2004). In general, these

agricultural practices are known to have negative impacts on AM association. Management practices typical of conventional high input systems, particularly P fertilizer application and the use of biocides are known to be deleterious to AM fungal symbiosis (Bagyaraj *et al*., 1989; Kabir *et al*., 1998; Miller and Jackson, 1998; Thingstrup *et al*., 1998).

Organic farming systems help to improve sustainability of agricultural production by reducing external inputs to a minimum with high crop yields. Crop pests and diseases are controlled through diverse rotations, while crop nutrition is maintained through the inclusion of legumes in the rotation and recycling of nutrients via crop residues and animal manures (Watson *et al*., 2002; Lotter, 2003). Once the nutrients are used up from soil they cannot be recycled. This results in declining of soil fertility mainly in terms of soil P and K (Loes and Ogaard, 2001; Gosling and Shepherd, 2005). Arbuscular Mycorrhizal fungi are important for organic farming (Powell and Bagyaraj, 1984; Gosling *et al*., 2006). Few horticultural crops and flowers have been used as host plants in several experimental tests as potential target plants for practical use of mycorrhizal inoculation (Chang, 1994; Lovato *et al*., 1995; Šrámek *et al*., 2000). Strong AM colonization allow adequate crop P nutrition at a lower concentration of extractable soil P than would otherwise be the case, a useful attribute for organic systems with limited P inputs, but many other agricultural practices are detrimental to AM fungi (Gosling *et al*., 2006). As a result of which agricultural soils become impoverished with AM communities, resulting in low levels of AM colonization of hosts with potential impairment of function (Helgason *et al*., 1998). Practices which are

detrimental to AM fungi such as application of soluble P fertilizers are avoided in organic systems and as a result are likely to exhibit increased levels of mycorrhizal colonization in crops and increased numbers of propagules in the soil. However, so far the results published are contradictory, some suggesting a benefit from organic farming (Ryan *et al*., 1994; Eason *et al*., 1999; Mäder *et al*., 2000; Galvez *et al*., 2001; Oehl *et al*., 2003; Entz *et al*., 2004; Oehl *et al*.,2004; Galván *et al*., 2009), while some with no benefit (Galvan *et al*., 2009).

Although AM association is known to offer multiple benefits to the host plant it may not be obviously mutualistic at all times. The AM fungi may cheat their host plant by deriving carbon without any benefit to the host plant. This can cause a decline in growth under certain conditions (Lerat *et al*., 2003). Colonization by AM fungi may result in increased uptake of one nutrient, but a reduction in another (Kothari *et al*., 1990), an effect which may be mediated by the concentration of other soil nutrients (Liu *et al*., 2000). A reduction in host plant manganese (Mn) absorption following AM fungal colonization is especially common, even where uptake of other nutrients has increased (Kothari *et al*., 1990; Azaizeh *et al*., 1995). When colonization by AM fungi is disrupted, uptake of P, growth and in some cases yield is significantly reduced (Thompson, 1987, 1991, 1994) and crops fail to respond to colonization by native AM fungi (Ryan *et al*., 2002). This is due to high concentration of available soil P (Hetrick *et al*., 1996; Thingstrup *et al*., 1998; Sorensen *et al*., 2005) which is often known to decrease root colonization by AM fungi (Jensen and Jakobsen, 1980; Al-Karaki and Clark, 1999; Kahiluoto *et al*., 2001).

Where strong AM fungal colonization occurs under conditions of high soil P concentrations it may reduce crop growth (Kahiluoto *et al*., 2001). Even on soils low in available P plants sometimes fail to respond to native AM fungi (Ryan and Graham, 2002), or inoculation with AM fungi (Sainz *et al*., 1998), though colonization may be significantly increased, the reasons for which are not always clear.

Importance of AM fungi for plant development and health is now widely demonstrated. Because of their role as bioregulators, biofertilizers and biocontrol agents, they represent potentially important tools for new orientations in agriculture where there is increasing demand for development of new plant management techniques that are less dependent on chemical inputs (Gianinazzi *et al*., 1995). These fungi have the potential to play a significant role in low input sustainable systems through enhanced nutrient acquisition and protection from soil borne pests and diseases, as well as through more generalised positive interactions with beneficial rhizosphere microorganisms and indirectly through enhancing nitrogen fixation by legumes and improved soil structure (Gosling *et al*., 2006). There are evidences suggesting that many of these beneficial effects are dependent on appropriate combinations of AM fungi, host plants and other soil microorganisms (Piotrowski *et al*., 2004; Dwivedi *et al*., 2009; Sikes *et al*., 2009).

Adopting management practices to increase plant production with low fertilizer input will minimize adverse effects on the environment and keep production costs low, making it suitable for marginal farmers with low

incomes. Floriculture plants are mostly grown from seedlings and cuttings in disinfected soils or on inert substrates under controlled nursery conditions, to overcome the possibilities of microbial contamination. Arbuscular Mycorrhizal fungi are obligate endosymbionts and therefore, production of a large quantity of inoculum and inoculation of the soil under field conditions is difficult. The inoculation of seedlings with AM fungi under controlled nursery conditions presents an ideal opportunity to establish the symbiosis before transplanting, helping the plants in superior and stronger growth in the nursery, to survive the transplant and increasing plant performance after transplanting in field (Giananazzi *et al*., 2001; Ortas, 2008). Thus difficulty in producing a large amount of inoculum of AM fungi for agricultural practices can be easily overcome in floricultural crops, because inoculation can be carried out in seedlings or cutting beds, over a relatively small surface area.

In spite of several evidences of AM fungi being an integral part of many cultivated plants and an essential component of soil fertility, their rational use in flower production is still not well developed. Production of ornamental species in nutrient-deficient soils is more difficult because these plants generally have a high fertilizer requirement. Such high fertilizer requirement tend to eliminate AM fungi and create the most promising conditions for application of mycorrhizal biotechnology. Preferential application of AM fungi to floriculture plants is necessary and realistic for their low cost and high economic output (Azcón-Aguilar and Barea, 1997). Plant growth responses to AM fungi varies with the host plant, endophyte and soil (Entry *et al*., 2002; Hart and Reader, 2002) and may be related to the timing and extent of AM

fungal colonization (Graham *et al*., 1991; Abbott and Gazey, 1994; Wilson and Hartnett, 1998). Colonizing ability and growth promoting effect of different AM species or even strains for a given plant in terms of plant growth (Linderman and Davis, 2004; Sensoy *et al*., 2007) and P uptake (Graham *et al*., 1982) are variable, indicating that not all AM fungi are functionally equivalent (Trent *et al.,* 1993; Clark and Zeto, 1996; van der Heijden *et al.,* 1998a, b). Few reports emphasized that maximum benefits to plants might be achieved with a single, most efficient AM species, and indicated that mycorrhizal diversity would not bring further benefits (Daft and Hogarth, 1983; Edathil *et al*., 1996). Due to the beneficial effects of AM fungi to agricultural crops efforts are on throughout the world to exploit these microorganisms to increase the productivity. Screening of AM fungi for selecting suitable species is an important preliminary step for the use of AM fungi in the production of flowers.

The present study was carried out with the following aims and objectives:

1. To study AM fungal root colonization in ornamental flowering plants commonly found in Goa.
2. To isolate, multiply (using trap cultures) and identify spores of AM fungi from the rhizosphere soils of ornamental flowering plants.
3. To investigate mycorrhizal status of ornamental flowering plants selected for the study as influenced by its phenology.
4. To produce pure cultures of dominant AM fungal species and their mass multiplication.
5. To evaluate the effects of the dominant AM fungal species on growth and flower quality in selected ornamental flowering plants.

CHAPTER 2

REVIEW OF LITERATURE

Mycorrhizae are naturally widespread symbiotic associations between plants and soil borne fungi. They play a significant role in the land colonization by plants due to their ability to acquire nutrients unavailable to non-mycorrhizal individuals (Simon *et al*., 1993; Smith and Read, 2008). The term “mycorrhiza” was coined by Frank (1885) by recognizing special structures in tree roots. The term “myco” means fungus and “riza”, root, which was later characterized as ectomycorrhiza (Kirk *et al*., 2001). The AM fungi colonize the plant roots during active plant growth and efficiently acquire nutrients for the plant, while the plant in return supplies the fungal symbiont with carbohydrates and thus in nature this plant-fungus symbiosis is mandatory.

2.1 HISTORY OF MYCORRHIZA

Arbuscular Mycorrhizal associations are about 450 million years old and have led to adaptations in both partners *i.e.* plants and fungi that consolidate their symbiotic development and function (Smith *et al*., 2003). According to the fossil record and molecular data, the origin of the AM symbiosis goes back to the Ordovician, 450–500 million years ago (Remy *et al*., 1994; Redecker *et al*., 2000). Sequence data and fossils of spores and hyphae proves existence of AM fungi more than 460 mya, and it is assumed that AM symbiosis aided plants during their land colonization (Pirozynski, 1975; Simon *et al*., 1993; Redecker, 2000) in the acquisition of water and minerals, especially P (Simon *et al*., 1993). A number of bryophytes and pteridophytes are still capable of forming AM associations (Read *et al*., 2000; Schüβler, 2000). Fossils of the earliest land plants in which AM fungi have been observed were found in the Rhynie chert of the lower Devonian (Remy *et al*., 1994). The ancient phylogenetic origin of the Glomales is confirmed by fossil findings, with

symbiotic structures within fossil roots from the Devonian (Remy *et al*., 1994; Taylor *et al*., 1995) and fossilized glomalean spores from the Ordovician (Redecker *et al*., 2000). Intra-radical mycelium was observed in root intracellular spaces and arbuscules were observed in the layer thin wall cells resembling palisade parenchyma. The fossil arbuscules were similar to those of existing AM fungi. The cells containing arbuscules have thickened walls, which are also observed in extant colonized cells (Remy *et al*., 1994). The nature of the relationship between plants and the ancestors of AM fungi is contentious. Two hypotheses suggest that:

a. Mycorrhizal symbiosis evolved from a parasitic interaction that developed in to a mutually beneficial relationship.

b. Mycorrhizal fungi developed from saprobic fungi that became endosymbiotic.

Both saprotrophs and biotrophs were found in the Rhynie Chert, but there is little evidence to support either hypothesis. There is some fossil evidence to suggest that the parasitic fungi did not kill the host cells immediately upon invasion, although a response to the invasion was observed in the host cells. This response may have evolved into the chemical signaling processes required for symbiosis. In both cases, the symbiotic plant-fungi interaction is thought to have evolved from a relationship in which the fungi was taking nutrients from the plant into a symbiotic relationship where the plant and fungi exchange nutrients (Remy *et al*., 1994).

2.2 CLASSIFICATION OF MYCORRHIZA

Traditionally mycorrhizas were classified into two main types based on the formation of fungal structures in relation to the root tissues of the plant and referred as endomycorrhiza or ectomycorrhiza; "*ecto*" means outside the root, "*endo*" means inside. The classical endomycorrhiza is the Arbuscular Mycorrhiza (AM) which is the most common underground association and the role of these below-ground soil organisms interacting with plant roots has gained increased attention in recent few years (Reynolds *et al*., 2003; van der Putten, 2003; Callaway *et al*., 2004). This classification was then regarded as too simplistic and there is now a nomenclature identifying four major types with the seven mycorrhizal types (Harley and Smith, 1983; Smith and Read, 1997; Read, 1998) which are described as under:

2.2.1 Ericoid mycorrhiza: Are mainly found in *Erica*, *Calluna* and *Vaccinium* i.e. plants growing in moorlands and similar challenging environments. These fungi are members of the Ascomycota e.g. *Hymenoscyphus ericae*. The plant's rootlets are covered with a sparse network of hyphae; the fungus digests polypeptides saprotrophically and passes absorbed nitrogen to the plant host; under extremely harsh conditions the mycorrhiza may even provide the host with carbon sources (by metabolising polysaccharides and proteins for their carbon content). Two specialised subgroups may be separated out of the ericoid endomycorrhizal group:

2.2.1.1 Arbutoid endomycorrhiza: Are the mycorrhizal associations formed by the plants belonging to the genus *Arctostáphylos*, *Arbutus*, *Pyrola*.

2.2.1.2 Monotropoid endomycorrhizas: Are the mycorrhizal associations formed by the achlorophyllous plants of the Montropaceae.

2.2.2 Orchidaceous endomycorrhizas: Are similar to ericoid mycorrhizas but their carbon nutrition even is more dedicated to supporting the host plant as the young orchid seedling is non-photosynthetic and depends on the fungus partner utilising complex carbon sources in the soil and making carbohydrates available to the young orchid. All orchids are achlorophyllous in the early seedling stages, but usually chlorophyllous as adults, so in this case the seedling stage orchid can be interpreted as parasitising the fungus.

2.2.3 Ectomycorrhizas: Are the most advanced symbiotic association between higher plants and fungi, involving about 3% of seed plants including the majority of forest trees. In this association the plant root system is completely surrounded by a sheath of fungal tissue which can be more than 100 µm thick, though it is usually up to 50 µm thick. The hyphae penetrate between the outermost cell layers forming the Hartig net**.** From this a network of hyphal elements (hyphae, strands and rhizomorphs) extends out to explore the soil domain and interface with the fungal tissue of the root. They mainly belong to Basidiomycota and include common woodland mushrooms, such as *Amanita* spp., *Boletus* spp. and *Tricholoma* spp. Ectomycorrhizas can be highly specific eg. *Boletus elegans* associating with *Larix* and non-specific eg. *Amanita muscaria* associating with 20 or more tree species. In the other specificity direction, 40 fungal species are capable of forming mycorrhizas with pine. These fungi depend on the plant host for carbon sources, most

being uncompetitive as saprotrophs. They are unable to utilise cellulose and lignin except *Tricholoma fumosum*; but provides greatly enhanced mineral ion uptake for the plant and capture nutrients, particularly phosphate and ammonium ions, which the root cannot access. Host plants grow poorly when they lack ectomycorrhizas. This ectomycorrhizal group is reasonably homogenous thus a subgroup ectendomycorrhizas is been appended.

2.2.3.1 Ectendomycorrhiza is a purely descriptive name for mycorrhizal roots that exhibit characteristics of both ectomycorrhizas and endomycorrhizas. Ectendomycorrhizas are essentially restricted to the plant genera *Pinus*, *Picea* and to a lesser extent, *Larix*. Ectendomycorrhizas have the same characteristics as ectomycorrhizas but show extensive intracellular penetration of the fungal hyphae into living cells of the host root.

2.2.4 Endomycorrhizas: Are the one in which the fungal structure is present within the host root and are called as Arbuscular Mycorrhiza (AM). Plants which are able to form arbuscular mycorrhizas are taxonomically very diverse and belong to almost all phyla. The plant hosts of AM fungi are mostly angiosperms, some gymnosperms, pteridophytes, lycopods, and mosses (Smith and Read, 2008). The presence of AM in virtually all terrestrial habitats (Smith and Read, 1997; Brundrett, 2002, 2004) together with the hitherto comparatively small number of identified AM fungal taxa could indicate a high promiscuity among the fungal species, and it was long believed that most AM fungal species are able to form a successful symbiosis with most plant hosts. The symbiosis is characterized by a bidirectional nutrient transfer, the plant

supplies the fungus with carbon and in return the plant gets nutrients in particular phosphorus (P) from the fungal symbiont, which is required in large amounts for the biosynthesis of primary and secondary compounds, in the energy metabolism of the cells and as constituent of nucleic acids and phospholipids (Marschner, 2002). Arbuscular Mycorrhizal fungi are totally dependent on photosynthetic plants and thus cannot be cultured without their autotrophic partner (Smith and Read, 2008). Members of the Brassicaceae, Caryophyllaceae, Chenopodiaceae or Urticaceae do not show the presence of AM associations (Vierheilig *et al*., 1994, 1996; Smith and Read, 1997).

The AM consists of three important components the plant root itself, the fungal structures within and between the root cells and the huge extra-radical mycelium in the soil. Intra-radical structures are arbuscules which are highly branched, tree-like structures formed in root cortical cells, the so-called arbuscules from which the name ‘arbuscular’ is derived. Vesicles, are thick walled, mainly lipid-filled storage organs, inter- and intra-cellular hyphae, very rarely intra-cellular coils are formed. Arbuscules are the main structures where the carbohydrate transfer between the plant and the fungus takes place, but a carbon transfer may also occur from the intracellular hyphae (Smith and Read, 2008). The AM association is endotrophic, and has previously been referred to as vesicular-arbuscular mycorrhiza (VAM). This name has since been dropped in favour of AM since all of the fungi do not form vesicles.

2.3 CLASSIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI

Arbuscular Mycorrhizal fungi belong to the fungal phylum Glomeromycota, a sister group of Basidiomycota and Ascomycota (Schüβler *et al*., 2001), with three classes (Archaeosporomycetes, Glomeromycetes and Paraglomeromycetes), five orders (Archaeosporales, Diversisporales, Gigasporales, Glomerales and Paraglomerales), 14 families, 29 genera and approximately 230 species (Oehl *et al*., 2011a). Since molecular methods have been used to elucidate the phylogenetic relationships among these fungi, their classification has been in a rapid transition. Molecular field studies have also revealed a large number of putative new species. The classification of AM fungi given below is based on Schüβler *et al.* (2001); Oehl and Sieverding (2004); Walker and Schüβler (2004); Sieverding and Oehl (2006); Spain *et al.* (2006); Walker *et al.* (2007 a, b); Palenzuela *et al*. (2008); Oehl *et al*. (2008); Schüßler *et al*. (2010); Oehl *et al*. (2011a, b, c, d, e). The family Geosiphonaceae with a single species *Geosiphon pyriformis* is placed under the order Archaeosporales and does not form arbuscular mycorrhizae. It forms endocytosymbiosis with cyanobacteria (*Nostoc* sp.) and is placed under the phylum Glomeromycota due to its close molecular relationship (**Table 1**).

Table 1: Classification of AM fungi

GLOMEROMYCOTA C. Walker & Schuβler

- **Glomeromycetes** Cavalier-Smith, emend. Oehl, G.A. Silva, B.T. Goto & Sieverd.
  - **Diversisporales** C. Walker & Schuβler, emend. Oehl, G.A. Silva & Sieverd.
    - **Diversisporaceae** C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverd.
      - ***Tricispora*** Oehl, Sieverd., G.A. Silva & Palenz.
      - ***Otospora*** Oehl, J. Palenzuela & N. Ferrol
      - ***Diversispora*** C. Walker & A. Schüβler, emend. G.A. Silva, Oehl & Sieverd.
      - ***Redeckera*** C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverd.
    - **Acaulosporaceae** J.B. Morton & Benny
      - ***Kuklospora*** Oehl & Sieverd
      - ***Acaulospora*** Gerd. & Trappe emend. S.M. Berch
    - **Sacculosporaceae** Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz.
      - ***Sacculospora*** Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz.
    - **Pacisporaceae** C. Walker, Blaszk., Schuβler & Schwarzott
      - ***Pacispora*** Oehl & Sieverd.
  - **Gigasporales** Sieverd., G.A. Silva, B.T. Goto & Oehl
    - **Scutellosporaceae** Sieverd., F.A. Souza & Oehl
      - ***Orbispora*** Oehl, D.K.A. Silva, Maia, Sousa, Vieira & G.A. Silva
      - ***Scutellospora*** C. Walker & F.E. Sanders. emend. Oehl, F.A. Souza & Sieverd.
    - **Gigasporaceae** J.B. Morton & Benny emend. Sieverd., F.A. Souza & Oehl
      - ***Gigaspora*** Gerd. & Trappe emend. C. Walker & F.E. Sanders
    - **Dentiscutataceae** F.A. Souza, Oehl & Sieverd.
      - ***Dentiscutata*** Sieverd., F.A. Souza & Oehl
      - ***Quatunica*** F.A. Souza, Sieverd. & Oehl
      - ***Fuscutata*** Oehl, F.A. Souza & Sieverd.
    - **Racocetraceae** Oehl, Sieverd. & F.A. Souza
      - ***Cetraspora*** Oehl, F.A. Souza & Sieverd.
      - ***Racocetra*** Oehl, F.A. Souza & Sieverd.
  - **Glomerales** J.B. Morton & Benny, emend. Oehl, Palenz., G.A. Silva & Sieverd.
    - **Claroideoglomeraceae** C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverd.
      - ***Viscospora*** Sieverd., Oehl & G.A. Silva
      - ***Claroideoglomus*** C. Walker & A. Schüβler, emend. Oehl, Sieverd., B.T. Goto & G.A. Silva
      - ***Entrophosphora*** R.N. Ames & R.W. Schneid., emend. Oehl, Sieverd., Palenz. & G.A. Silva
      - ***Albahypha*** Oehl, G.A. Silva, B.T. Goto & Sieverd.
    - **Glomeraceae** Piroz. & Dalpé emend. Oehl, G.A. Silva & Sieverd.
      - ***Simiglomus*** Sieverd., G.A. Silva & Oehl
      - ***Funneliformis*** C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverd.
      - ***Rhizophagus*** P.A. Dang.
      - ***Septoglomus*** Sieverd., G.A. Silva & Oehl
      - ***Glomus*** Tul. & C. Tul. emend. Oehl, G.A. Silva & Sieverd.
- **Archaeosporomycetes** Sieverd., G.A. Silva, B.T. Goto & Oehl
  - **Archaeosporales** C. Walker & Schuβler, emend. Sieverd., G.A. Silva, B.T. Goto & Oehl
    - **Ambisporaceae** C. Walker, Vestberg & Schuβler
      - ***Ambispora*** (= *Appendicispora*) Spain, Oehl & Sieverd.
    - **Geosiphonaceae** Engler. & E. Gilg emend. Schuβler
      - ***Geosiphon*** (Kütz.) F. Wettst.
    - **Archaeosporaceae** J.B. Morton & D. Redecker emend. Oehl & Sieverd.
      - ***Intraspora*** Oehl & Sieverd.
      - ***Archaeospora*** J.B. Morton & D. Redecker
- **Paraglomeromycetes** Oehl, G.A. Silva, B.T. Goto & Sieverd.
  - **Paraglomerales** C. Walker & Schuβler
    - **Paraglomeraceae** J.B. Morton & D. Redecker
      - ***Paraglomus*** J.B. Morton & D. Redecker

2.3.1 Morphological characters used for identification of AM fungi: Various morphological characters important in establishing the taxonomic identity and relationships of AM species used for the identification of AM fungi are described below:

2.3.1.1 Sporocarp morphology: The sporocarps may be formed in soil, roots, empty seed coats, insect caracaces or rhizomes. Peridium may be present around the sporocarps in the form of loosely or compact interwoven hyphae, a patchy covering over the sporocarps or as hyphal network covering single or small clusters of spores. The presence or absence of peridium accounts for much of the variation observed in size of sporocarps. External sporocarp colour range from white to brown, while the internal sporocarp colour range from white to black and brown.

2.3.1.2 Spore morphology: Spores in the soil may be produced terminally, laterally on subtending hyphae or on a single suspensor-like cell. Characters such as spore colour, shape and size may vary considerably depending on the developmental stage and environmental conditions. Spore colour varies from hyaline to white to yellow, red, brown and black with all intermediate shades. The difference in colour may be due to pigmentation in spore wall or in the spore content (Morton, 1988). Shape of spores is mainly governed by the genotype of the fungus and the substrate in which the spores are formed. Intra-radical spores are mainly globose, sub-globose to ellipsoidal, while the extra-radical spores may be globose, sub globose, ellipsoidal, oblong, ovate to highly irregular shaped.

2.3.1.3 Subtending hyphae: The subtending hyphae may be simple to recurved or sometimes swollen or constricted at the point of attachment to the spore in *Glomus* species. The width of the hyphae varies considerably within different genera and species of AM fungi. The mechanism of pore occlusion at the point of attachment of the subtending hypha to the spore has some taxonomic significance. Walker (1992) suggested three distinct lines with regard to the occlusion of the spore content in *Glomus viz.*, spores possessing a complete endospore formed by more or less flexible inner wall group, spores sealed by the ingrowths and thickening of the wall layer of the subtending hypha and occlusion by the septum usually somewhat distal to the spore base.

2.3.1.4 Auxiliary cells: The auxiliary cells are encountered in both roots and soil. The size and the shape of the auxiliary cells have been found to be important in differentiating species of *Gigaspora* from *Scutellospora*. In *Gigaspora*, the auxiliary cells are echinulate with spines that are forked dichotomously (Bentivenga and Morton, 1995), whereas in *Scutellospora*, the projections on the surface of the auxiliary cells are knobby and highly variable in shape and size (Morton, 1995).

2.3.1.5 Mycorrhizal anatomy: Generally, fungal anatomy in roots is not used in taxonomic descriptions to separate taxa below the generic level. Colonization of the root with AM fungi initiates a series of developmental processes culminating in a morphologically and functionally unique symbiosis. It is possible to differentiate among certain AM fungi, using visual differences

in morphology of fungal hyphae and vesicles within roots (Abbott and Gazey, 1994). Biermann and Linderman (1983) thought that intra-radical vesicles in some species of AM fungi act as propagules and contribute significantly to the colonization of other roots. Abbott (1982) developed a key for 10 AM fungal species using 20 characteristics (based entirely on the morphological anatomy of hyphal development) such as hyphal diameter, mode of branching, vesicles, arbuscules, staining reactions, *etc.* and concluded that these characteristics are stable in different hosts and soil environments.

2.3.1.6 Spore wall structure: The spore wall of AM fungi exhibit a unique array of wall layers which are taxonomically important as they are highly conserved and phenotypically stable in almost any environment. Spore wall characteristics have been universally accepted as more stable and reliable criteria than other spore features (Mehrotra, 1997). Every spore, irrespective of species, forms a spore wall (Morton, 2002). In most juvenile spores, the spore wall may be 1 to 2 layered. However, the spore wall of most AM fungal species usually consist of at least two wall layers, while some species the spore wall differentiates upto 4 wall layers. Walker (1983) defined a wall group as "an aggregation of walls that are either adherent, or that remain close together when a spore is crushed". The different wall types and the muronym codes encountered in AM fungal spores are as follows:

a. Amorphous (A): A formless, flexible wall whose elasticity is affected by the mountant. It appears rigid in water or glycerol; in acidic mountants (< pH 2) it is plastic and tends to collapse partially. The shape is maintained when attached to a more rigid wall.

b. Coriaceous (C): a robust, tough but flexible inner walls which turns leathery in appearance in hypertonic solutions.

c. Germinal (G): Innermost layer of *Gigaspora* species from which germ tube arises. Frequently bears papillae which project distally from innermost surface.

d. Evanescent (E): An outermost ephemeral one to multilayered wall, which is sloughed off as spore matures. Seen only in pot culture and rarely in field.

e. Laminated (L): Generally outer many layered wall, layers increasing as spore ages.

f. Membranous (M): Generally inner, very thin, frequently wrinkled, flexible walls that frequently collapse in hypertonic solutions.

g. Hyphal peridium (P): Tightly adherent hyphal layers around the spore or spores.

h. Unit (U): Outer, single, rigid non-layered walls sheathing like brittle plastic on crushing.

i. Expanding (X): A unit wall which expands when placed in lactic acid or polyvinyl alcohol.

The number, width and position of wall layers differ among species and they have been increasingly relied upon for identification purposes. Ornamentation on the spore wall layer appears to be an important taxonomic criterion in identification of species, especially when other morphological characters are overlapping.

2.3.1.7 Spore germination: Ultrastructural studies of spore germination processes may play a role in the identification of AM fungal species. Spores

of glomalean fungi have all the necessary metabolic constituents and genetic information to germinate and produce new hyphae (Sequeira *et al*.,1985), although they cannot continue to grow without a host. However, spore germination in AM fungi has been studied in only a few species.

2.4 CHARACTERISTIC FEATURES OF ORDERS

2.4.1 Diversisporales: Diversisporales members form mycorrhizae with arbuscules, frequently failing to produce vesicles, may or may not produce auxiliary cells. They produce spores which develop either inside (entrophosporioid) or laterally on the neck of a sporiferous saccule (acaulosporioid), from a bulbous base (gigasporioid) or blastically at the tip of a sporogenous hypha (glomoid). The distinct feature of the members belonging to this order is the possession of the rRNA SSU gene sequence signature YV**R**RYW/1-5/NGY**Y**YGB, corresponding to homologous position 658 of *S. cerevisiae* SSU rRNA sequence J01353 SSU rRNA, GTYARDYH**M**HYY/2-4/GRADRKKYGWCRAC, corresponding to homologous position of *S. cerevisiae* SSU rRNA sequence position 1346 of *S. cerevisiae* SSU rRNA sequence J01353, TTATCGGTTR**A**ATC, corresponding to homologous position 650 of *S. cerevisiae* rRNA SSU sequence J01353, and A**CT**GAGTTMATYT, corresponding to homologous position 1481 of *S. cerevisiae* rRNA SSU sequence J01353 with the nucleotides in bold being specific for the taxon.

2.4.2 Gigasporales: Gigasporales members do not form intra-radical vesicles but auxiliary cells in soils, which clearly distinguish them from Glomerales and

Diversisporales. Gigasporales exhibit gigasporoid or scutellosporoid spore formation (Oehl *et al.*, 2011b), i.e. spores formed terminally on sporogenous cells and with either germ warts on the inner surface of the mono-walled spore wall (gigasporoid; Gigasporaceae), or a discrete germination shield on the innermost (= 'germinal wall') of 2–4 walls (scutellosporoid). There are three families with scutellosporoid spore formation (*sensu lato*): Dentiscutataceae, Racocetraceae and Scutellosporaceae (Oehl *et al*., 2008). Scutellosporaceae form mono-lobed (*Orbispora*) or bi-lobed (*Scutellospora*), hyaline germination shields. Racocetraceae species form wavy-like, multiply lobed, hyaline germination shields and have either two (*Racocetra*) or three (*Cetraspora*) spore walls. Dentiscutataceae species form yellow-brown to brown germ shields that are bi-lobed (*Fuscutata*) or with multiple compartments (*Dentiscutata*, triple-walled; *Quatunica*, four-walled).

2.4.3 Glomerales: Spores form terminally on or intercalary in hyphae or within the necks of sporiferous saccules in soil (or sometimes roots) singly or (when glomoid) also in spore clusters or multi-spored loose to compact sporocarps; when in compact sporocarps (with or without peridium), spores randomly distributed or organized around a central hyphal plexus. Glomoid spores with one single or multiple-layered wall. Entrophosporoid spores with two walls: outer structural wall and inner (germinal) wall. In glomoid spores, wall of the subtending hyphae conspicuously continuous with the spore wall, subtending hyphae funnel-shaped, cylindrical, or constricted and concolourous with spore, slightly paler, or (sub)hyaline. In entrophosporoid spores, structural pigmented outer wall layer discontinuous with the hyphal

wall distal to the saccule. The distinct feature of the members belonging to this order is the possession of the rRNA SSU gene sequence signature **YTRRY**/2-5/R**YYA**RGTYGNCARCTTCTTAGAGG GACTATCGG**T**GTYTA**A**CCGRTGG, corresponding to homologous position 1353 of *S. cerevisiae* SSU rRNA sequence J J01353, with the nucleotides in bold being specific for the taxon.

2.4.4 Archaeosporales: The members of the order Archaeosporales form endocytosymbioses with photoautotrophic prokaryotes or produce mycorrhizae with arbuscules, with or without vesicles. The members produce colourless spores which do not react in Melzer's reagent. They produce both glomoid and acaulosporoid spores. The distinct feature of the members belonging to this order is the possession of the rRNA SSU gene signature YCT**AT**CYKYCTGGTGAKRCG, corresponding to homologous position 691 of *Saccharomyces cerevisiae* SSU rRNA sequence J01353, with the nucleotides in bold being specific for the taxon.

2.4.5 Paraglomerales: Paraglomerales members form arbuscular mycorrhizae, rarely with vesicles. They form glomoid spores that may all germinate directly through the spore wall instead of through the subtending hyphae as in *Glomus* and *Diversispora* (Spain and Miranda, 1996) and colourless. The distinct feature of the members belonging to this order is the possession of rRNA SSU gene sequence signature G**C**GAA**G**CGTCATG**G**CCTTAAC**C**GG**C**CGT, corresponding to homologous position 703 of *S. cerevisiae* SSU rRNA sequence J01353, with the

nucleotides in bold being specific for the taxon. The order Paraglomerales species are monogeneric.

2.5 CHARACTERISTIC FEATURES OF GENERA

***Acaulospora* Gerdemann & Trappe emend. Berch.** *Mycologia Memoir*, 5: 1–76. (1974).

Etyomolgy: Greek, *a* = without; *caulos* = stem; *spora* = spore; referring to the sessile spores.

Spores develop laterally from the neck of a sporiferous saccule (Morton and Benny, 1990; Morton, 2000). The spores are sessile, i.e. no pedicel is formed. The wall of the most juvenile spores consists of only one layer continuous with the wall of a sporiferous saccule hypha. Spores produced singly in soil, generally globose to sub-globose with oily contents. Spore composed of two distinct, separable wall groups; outer wall is continuous, laminated; variously ornamented, inner wall composed of one or more walls that are membranous, hyaline, laminated and ornamented. Spore walls are continuous except for a small-occluded pore. Spores germinate by germ tubes emerging from a plate-like germination orb formed by centrifugally rolled hyphae. The germ tubes penetrate through the spore wall. The mycorrhizae of *Acaulospora* species consist of (a) arbuscules with cylindrical or slightly flared trunks (b) irregular and knobby vesicles, and (c) straight and coiled intra-radical hyphae with coils mostly concentrated at entry points (Morton, 2000).

***Albahypha* Oehl, G.A. Silva, B.T. Goto & Sieverding.** *Mycotaxon*, 117: 297–316 (2011e).

Etymology: Latin, *alba* = white; *hypha* = hypha; referring to the white, slightly funnel-shaped subtending hypha which is characteristic for species of this genus. Spores formed generally singly in soil or rarely in roots; subtending hyphae white, rarely sub-hyaline, 1.2–2.0 times wider at spore base than their width 10–20 µm distance from the spore, giving a slightly funnel-shaped or cylindrical appearance. Spores with one wall of 1–4 layers; spore base pore closure often with a septum that may arise from the structural layer, an adherent innermost, (semi-) flexible layer, or both innermost layers.

***Ambispora* (= *Appendicispora*) Spain, Oehl & Sieverding.** *Mycotaxon*, 97: 163–182 (2006).

Etymology: Latin, '*ambispora'* referring to the capability to produce two kinds of propagules, acaulosporoid and glomoid.

Species of the genus *Ambispora* are dimorphic producing both acaulosporoid and glomoid spores *i.e.* spores originating similarly to those of *Acaulospora* and *Glomus* species (Morton and Redecker, 2001; Spain *et al*., 2006). The acaulosporoid spores occur singly in the soil and the glomoid ones are formed singly or in loose clusters in the soil and develop terminally from the thin walled hyphae grown from either the wall of a pedicel or branched germ tubes (Spain *et al*., 2006). In contrast to the sessile acaulosporoid spores of the genus *Acaulospora* and *Archaeospora,* those of *Ambispora* species develop blastically at the tip of a short branch formed at the distal end of the neck of a sporiferous saccule. This branch is called the appendix or pedicel. The

sporiferous saccule orginates terminally from mycorrhizal extra-radical hyphae by their swelling. The spores are globose to sub-globose and coloured. The sub cellular structure consists of three layered, coloured spore wall with two inner colourless germination walls. The outer spore wall completes development subsequent to the formation of the outer layer of the first inner germination wall. The spore wall and the outer layer of the first inner germination of the spores of *Ambispora* species are continuous with the pedicel wall layer. The mycorrhiza of the species *Ambispora* consists of arbuscules, vesicles as well as intra- and extra-radical hyphae. All these structures stain faintly in trypan blue (Spain *et al*., 2006).

***Archaeospora* J.B. Morton & D. Redecker emend. Oehl and Sieverding.** *Mycologia*, 93: 181–195 (2001).

Entymology: Greek, *archaios* = ancient; *spora* = seed; referring to the ancestral position of this genus within Glomerales.

Archaeospora is dimorphic, forming both acaulosporioid and glomoid spores (Morton and Redecker, 2001; Sieverding and Oehl, 2006; Spain *et al*., 2006). Acaulosporoid spores develop laterally on the neck of a sporiferous saccule and are sessile, similarly to most spores of the genus *Acaulospora.* Two layered glomoid spores originate blastically at the tip of intercalary in fertile hyphae as spores of *Glomus* species. The sub cellular structure of acaulosporoid spores *Archaeospora trappei* comprises of a spore wall and one inner germination wall, each consisting of 2-3 layers. Mycorrhizae of *Archaeospora* (1) do not contain intra-radical vesicles or they form rarely, (2) have intra-radical hyphae with many coils located within and between cortical

cells, (3) stain lightly or not at all in trypan blue and other stains, and (4) are patchily distributed along roots (Morton, 2002).

***Cetraspora* Oehl, F.A. Souza & Sieverding.** *Mycotaxon*, 106: 311–360 (2008).

Etymology: Latin, *cetra* = light shield; spora = spore; refers to the light coloured germination shield which often is difficult to observe.

Spores formed singly in soil and rarely in roots on bulbous sporogenous cells arising terminally on a subtending hypha (Oehl *et al*., 2008). Outer spore wall 3 layered and continuous with the wall of the sporogenous cell. Outer layer of the outer spore wall generally semi-persistent to persistent, rigid; second layer laminate; third layer thin, often membranous, tightly adherent to the laminate layer and thus, often difficult to observe. Pore between spore and sporogenous cell is narrow and usually closed by a plug formed by spore wall material. Two hyaline walls form *de novo* during spore formation and have 1–2 and 2–3 layers, respectively. A germination shield arises on the outer surface of the inner wall or beneath a thin outer layer of the inner wall. Germination shield hyaline to sub-hyaline seldom light yellow, generally oval to ellipsoid or sub-globose, with several (4–12) wave-like lobed projections forming the outer surface of the shield; large folds separate the lobes on the shield, and each lobe may have an germ tube initiation (~ 2–5 µm in diam.). Subtending hyphae form one to several septa in some distance to the sporogenous cells. Auxiliary cells knobby. Forming typical arbuscular mycorrhizae but lacking vesicles in roots.

***Claroideoglomus* C. Walker & A. Schüssler, emend. Oehl, Sieverd., B.T. Goto & G.A. Silva.** The *Glomeromycota.* A species list with new families and genera: 44 (2010).

Etymology: *Claroideoglomus*, referring to the glomoid spores sharing the developmental pattern and wall structure of *Glomus claroideum*.

Spores formed on subtending hyphae, generally singly in soil or rarely in roots; subtending hypha hyaline to white, rarely sub-hyaline, and funnel- or bill-shaped with widths >2.5 times greater at the spore base than at 10–20 µm from the spore. Spores with one wall of 1–4 layers; pore closure at spore base often with a septum that arises species-specifically from the structural layer, an adherent thin innermost layer, or both innermost layers.

***Dentiscutata* Sieverding, F.A. Souza & Oehl.** *Mycotaxon*, 106: 311–360 (2008). **Etymology:** Latin, *denti* (*culata*) = dentate; *scutata* = shielded; referring to the dentate nature of the brown germination shield periphery.

Spores formed singly formed on bulbous sporogenous cells that are formed terminally on subtending hypha (Oehl *et al*., 2008). Outer spore wall 3-5 layered and continuous with the wall of the sporogenous cell. Pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by outer spore wall material. A hyaline middle wall and a hyaline inner wall form *de novo* during spore formation; middle wall 1–2 layered; inner wall 2–3 layered. Germination shield formed on the outer surface of the inner wall or beneath a thin outer layer of the inner wall, yellow-brown to brown, generally ellipsoid (to oval), or rarely reniforme to cardioforme, with many (8–30) large folds separating the shield into 8–30 ‘small compartments’. Each

compartment has one circular germ tube initiation. The periphery of the germination shield generally appears dentate in planar view. Septa often formed in subtending hypha in some distance to the sporogenous cells. Auxiliary cells knobby. Forming typical arbuscular mycorrhizae without vesicle formation in roots.

Diversispora **C. Walker & A. Schüβler, emend. G.A. Silva, Oehl & Sieverding.** *Mycotaxon*, 116: 75–120 (2011d).

Entymolgy: Referring to the diverse nature of the spores found within the order the genus lends its name.

Spores either formed singularly, in small open clusters or in large multi-spored clusters or sporocarps where spores are not organized. In pigmented spores subtending hyphae conspicuously change colour, becoming hyaline to white behind the septum; subtending hyphae generally straight, cylindrical, sometimes species specifically constricted, often hyphal attachment looks like inserted in spore wall. Spores with 1–3 wall layers; pore closure often with a septum that may species-specifically arise from the innermost wall layer; rarely (species specifically) pore of attachment open.

Entrophospora **R.N. Ames & R.W. Schneider, emend. Oehl, Sieverding, Palenz. & G.A. Silva.** *Journal of Applied Botany and Food Quality*, 80: 69–81 (2006).

Entymolgy: Greek, *en* = within; *trophos* = nourished; *spora* = seed; referring to the spores reared within the vesicular stalk.

Sporocarps unknown. Entrophosporoid spores form within the hyphal neck of tightly attached terminal or intercalary sporiferous saccules, singly in soils, or (rarely) in roots. Sporiferous saccules generally are larger in size than the underlying spores. Entrophosporoid spores are globose to sub-globose and have two walls: an outer and an inner. Outer, semi-persistent to evanescent layers of the outer spore wall are the wall layers of the hyphal stalk and the sporiferous saccule. The structural, pigmented layer beneath does not continue within the hyphal wall but only for a short distance within the saccule terminus. Thus, spores have only one persistent cicatrix, which is proximal to the globose saccule terminus. A plug closes the pore towards the saccule. The inner wall is thick, finely laminated wall and forms *de novo*. No inner wall layers have a beaded appearance. Fungal structures in roots stain blue with trypan blue; forming vesicular-arbuscular mycorrhizae.

***Funneliformis* C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverding.** The *Glomeromycota.* A species list with new families and genera: 44 (2010).

Entymolgy: *Funneliformis* referring the often funnel-shaped spore base of species in the genus.

Spores formed within soil or rarely roots, singly or sometimes in sporocarps with a few to several spores per sporocarp only; the conspicuous subtending hyphae is concolourous with spore wall colour (or slightly lighter in colour), subtending hyphae is species-specific and generally funnel-shaped to cylindrical. Wall differentiation and pigmentation may continue over long distances from the spore base (often > 50–250 µm), then mycelium may

become hyaline. Pore regularly closed by a conspicuous septum that species-specifically arises from the structural wall layer, from an additional adherent innermost, (semi-)flexible lamina, or from both but not by introverted wall thickening, which is lacking. Forming typical vesicular arbuscular mycorrhiza, with mycorrhizal structures that stain blue to dark blue in trypan blue.

***Fuscutata* Oehl, F.A. Souza & Sieverding.** *Mycotaxon*, 106: 311–360 (2008).

Etymology: Latin *fu*(*scus*) = brown; *scutata* = shielded; referring to the brown colour of the germination shield.

Spores formed singly on bulbous sporogenous cells which arise terminally on the subtending hypha. Outer spore wall 3-4 layered. Pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by spore wall material. Two hyaline walls (middle wall and inner wall) form *de novo* during spore formation and have 1–2 and 2–3 layers, respectively. Germination shield generally formed on the outer surface of the innermost wall or beneath a thin outer layer of the inner wall, yellow-brown to brown, ovoid to violin-shaped to heart-shaped, consisting of 2 lobes and folds; both lobes with a germ tube initiation (‘gti’, about 3–6 µm in diam). Spore germination generally from one gti. In the subtending hypha one to several septa are formed in some distance to the sporogenous cell. Auxiliary cells knobby. Forming typical arbuscular mycorrhizae without vesicles in roots.

***Gigaspora* Gerdmann & Trappe emend. C. Walker & F. E. Sanders.** *Mycologia Memoir*, 5: 1–76 (1974).

Etymology: Greek, *giga* = giant; *spora* = spore; referring to the exceptionally large spores typically produced by the members of the genus.

Azygospores produced singly in soil, generally globose to sub-globose, with oily contents, usually with a narrow hypha extending from the suspensor cell to the pore. Spores of *Gigaspora* develop blastically from a bulbous sporogenous cell formed at the end of a fertile hypha connected with mycorrhizal roots (Walker and Sanders, 1986; Bentivenga and Morton, 1995). The wall of the most juvenile, expanding spores consists of two layers of equal thickness. The inner layer thickens due to the synthesis of new sub layers (laminae). At the end of ontogeny, a warty or knobby one-layered germination wall is formed, from which germ tubes arise. This wall tightly adheres to the inner surface of the laminate spore wall layer. The outermost spore wall layer of all the *Gigaspora* species is smooth. Apart from spores, *Gigaspora* species also form clusters of auxiliary cells. They are echinulate with spines. The mycorrhizae of *Gigaspora* species consist of only arbuscules and hyphae staining darkly in trypan blue; no vesicles are produced (Bentivenga and Morton, 1995). Arbuscules generally form fine branches directly from a swollen basal hypha. Intra-radical hyphae are straight to coiled and vary in diameter because of the presence of knob-like projections and inflated areas.

***Glomus* Tul. & C. Tul. emend. Oehl, G.A. Silva & Sieverding.** *Mycotaxon*, 116: 365–379 (2011c).

Etymology: Latin, *glomus* = a ball of yarn; referring to the sometimes rounded and cottony appearance of the species.

Spores formed singly within soil or sometimes roots, in disorganized, multiple-spored loose spore clusters or in compact sporocarps; compact sporocarps without or with peridium, spores are either not organized in sporocarp, or organized around a central hyphal plexus. Spores with a mono-to-multiple layered wall. Wall of the subtending hyphae conspicuously continuous and concolourous with the spore wall, or slightly lighter in colour than the spore wall. Spore pore closure often by introverted wall thickening, sometimes supported by a short bridging septum, rarely open. Forming typical vesicular-arbuscular mycorrhiza, with mycorrhizal structures that stain blue to dark blue in trypan blue.

***Intraspora* Oehl & Sieverding.** *Journal of Applied Botany and Food Quality*, 80: 69–81 (2006).

Etymology: Latin, *intra* = inside, within; *spora* = seed; referring to the spore formation with in the hyphal stock of the sporiferrous saccule.

Spores occur singly in the soil or in roots. The spores develop within the neck of a sporiferous saccule at some distance from the saccule. The sporiferous saccule originates terminally or intercalarously in extra- and intra-radical hyphae. The spores are globose to sub-globose and frequently pyriform. Their sub cellular structure consists of two walls, a spore wall and an inner germination wall. The spore wall is composed of two layers, of which the outer

layer sloughs with age and is continuous with the wall of the neck of the sporiferous saccule. The inner layer of this wall is persistent, semi-flexible and closes two opposite pores of spores. The inner germination wall is semi-flexible and laminate. The mycorrhizae comprise of arbuscules, vesicles as well as intra- and extra-radical hyphae. Vesicles rarely formed and all the mycorrhizal structures stain faintly in trypan blue.

***Kuklospora* Oehl & Sieverding.** *Journal of Applied Botany and Food Quality*, 80: 69–81 (2006).

Etymology: Greek, *kuklo* = ring; *spora* = seed; refers to the two cicatrices, which resemble circular depressions and the borders of them wedding ring on the spore surface when the hyphal connection have detached from the young spore.

The spores develop inside the neck of a sporiferous saccule at some distance from this saccule and originate from the neck and saccule contents. The sporiferous saccules originate terminally or intercalarously inside mycorrhizal extra-radical hyphae by their swelling and are globose to sub-globose. The sub-cellular structure consists of a 3-layered, coloured spore wall and two inner colourless germination walls. The outermost spore wall layer is colourless, and is continuous with the wall of the sporiferous saccule neck. The second structural layer of this wall consists of coloured, tightly adherent, thin sub layers (laminae). This layer occasionally develops towards the saccule, forming a stalk supporting the wall of the sporiferous saccule neck. The first inner germination wall consists of two adherent flexible to semi-

flexible layers. The second germination wall is composed of three layers, of which the outermost one is ornamented with small granules.

Orbispora **Oehl, D.K.A. Silva, Maia, Sousa, Vieira & G.A. Silva.** *Mycotaxon*, 116: 161–169 (2011b).

Etymology: Latin, *orbis* = circle, orb; *spora* = spore; referring to the monolobed, coiled, orb like germination shield of the spores.

Sporocarps unknown. Spores formed on sporogenous cells that form terminally on hyphae arising from mycelia in soil. Outer spore wall generally 2–3 layered and continuous with the wall of the sporogenous cell. Two hyaline walls (middle wall 'mw' and inner wall 'iw') form *de novo* during spore formation and have 1–2 and 2–3 layers, respectively. A germination orb is formed on the outer iw surface or between the outer and the subsequent layer of iw. Germination orb is transparent, or hyaline to sub-hyaline, seldom light yellow, mono-lobed; coiled and then, either circular or apparently broad ellipsoid to rarely irregular; with one rounded germ tube initiation in the outer periphery of the lobe. One (rarely two) germ tube arises from this gti to penetrate the outer spore wall layers. Forming typical arbuscular mycorrhizae.

Otospora **Oehl, J. Palenzuela & N. Ferrol.** *Mycologia*, 100(2): 296–305 (2008).

Etymology: Greek, *oto* = ear; *spora* = seed; referring to the persistent lateral ear-like stalk at the spore base after the detachment of sporiferous saccule.

Sporocarps unknown. Spores formed at a short distance to a terminal or intercalary formed sporiferous saccule by swelling laterally on the hyphal stalk

of the saccule with outer and an inner wall. Layers of the outer wall are continuous with the wall of the sporiferous saccule. The inner layers of the outer wall are persistent. One to several septa formed in the hyphal stalk during spore formation. During initial stages of sporogenesis, the content of the sporiferous saccule separated from the hypha by septa at some distance of the terminus and the not yet developed spore. In the later developmental stages, additional septa in the stalk, positioned between the saccule terminus and the developing spore, may separate the collapsing saccule terminus from the spore. A final plug-like septum usually closes the pore at the spore base. The inner wall forms *de novo* during spore formation and consists of a thick, finely laminate layer that might have one thin layer each adherent on its outer and its inner surface. None of the layers of the inner wall has beaded appearance. Formation of vesicular arbuscular mycorrhizae unknown.

***Pacispora* Oehl & Sieverding.** *Journal of Applied Botany and Food Quality*, 78(1): 72–82 (2004).

Etymology: Latin, *paci* = peace or peaceful; *spora* = seed. Dedicated to the peace in the world.

Spores develop blastically at the end of cylindrical sporogenous hyphae (subtending hyphae) continuous with extra-radical hyphae of AM fungi. Spore wall consist of three layers. Spore development is by the formation of a uniform, plate-like germination shield on the surface layer of the inner germination wall. A germ tube grows from this shield and penetrates through the spore wall. The mycorrhizae consist of arbuscules, vesicles, intra- and extra-radical hyphae, as well as of auxiliary cells. The arbuscules, vesicles

and hyphae morphologically resembled those of *Glomus* species and stained intensively in trypan blue. Auxiliary cells occur both outside and inside roots and are knobby.

***Paraglomus* J.B. Morton & D. Redecker.** *Mycologia*, 93(1): 181–195 (2001).

Etymology: Resembling "*Glomus*" with identical spore morphotypes.

Spores develop blastically at the tip of extra-radical hyphae. Spores occur singly in the soil, globose to irregular and colourless to pale-coloured. Spores sub-cellular structure consists of a spore wall comprising 2-3 layers continuous with those of their subtending hyphae. Spore germinates by germ tubes emerging from both the lumen of the subtending hypha and the spore wall (Morton and Redecker, 2001). Arbuscules of are cylindrical or slightly flared trunks with branches progressively tapering in width towards the tips (Morton and Redecker, 2001; Morton, 2002). They do not form vesicles and their intra-radical hyphae are frequently coiled within and between cortical cells. The main visible evidence of mycorrhizae is their light staining or the lack of any staining reaction in trypan blue or other stains.

***Quatunica* F.A. Souza, Sieverding & Oehl.** *Mycotaxon*, 106: 311–360 (2008).

Etymology: Latin *qua* = abbrevation from *quartuor* (four); *tunica* = wall; referring to the four spore walls.

Spores singly formed on bulbous sporogenous cells that are formed terminally on a subtending hypha. Spores have 4 walls. Outer spore wall 3-4 layered and continuous at least in part with the wall of the sporogenous cell. Pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by outer spore wall material. Two hyaline middle walls and a hyaline inner wall form *de novo* during spore formation. Germination shield generally arising on the outer surface of the inner wall or beneath a thin outer

layer of the inner wall, yellow-brown to brown, generally ellipsoid to oval, or rarely reniforme to cardioforme, usually with many (8–30) large folds separating the shield into 8–30 small compartments. Each compartment generally with one germ tube initiation. From one or few gti germ tubes may arise penetrating the middle walls and the outer wall; periphery of shield wall conspicuously dentate in planar view. One to several septa often formed in the subtending hypha in some distance to the sporogenous cell. Auxiliary cells knobby. Typical arbuscular mycorrhizal formation without vesicles is seen in roots.

***Racocetra* Oehl, F.A. Souza & Sieverding.** *Mycotaxon*, 106: 311–360 (2008).

Etymology: Greek, *ρακος*, *racos* = cloth, lobe; Latin, *cetra* = light shield; referring to the wavy-lobed surface of the germination shield in planar view.

Spores formed singly in soil and rarely in roots, on bulbous sporogenous cells arising terminally on mycelia hyphae. Outer spore wall 3 layered and continuous with the wall of the sporogenous cell. Outer layer of the outer spore wall generally semi-persistent to persistent, rigid; second layer laminated; third layer thin, often membranous, tightly adherent to the laminate layer. Pore between spore and sporogenous cell is narrow and usually closed by a plug formed by spore wall material. A single inner wall forms *de novo* during spore formation and has 2-3 layers. A germination shield arises on the outer surface or beneath a thin outer layer of the inner wall. Germination shield hyaline to sub-hyaline seldom light yellow, generally oval to ellipsoid or sub-globose, with several (4–12) wave-like lobed projections forming the outer

surface of the shield; folds separate the lobes on the shield, and each lobe have a germ tube initiation (2–5 µm in diam.). In the subtending hypha of the sporogenous cell one to several septa formed in some distance to the sporogenous cells. Auxiliary cells knobby. Forming typical arbuscular mycorrhizae without vesicle formation in roots.

***Redeckera* C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverding.** The *Glomeromycota.* A species list with new families and genera: 44 (2010).

Etymology: Named in recognition of the pioneering work of Dirk Redecker in molecular phylogeny of the Glomeromycota.

Spore formation disorganized in large and compact sporocarps, containing hundreds to thousands of spores per sporocarp; spores with 2 to rarely 3 wall layers; subtending hyphae generally broad at spore base and with a conspicuous, thick and broad septum that arises from the inner lamina of the generally bi-laminated, structural wall layer; structural spore wall layer 2 generally continue over very short distances (2–10 µm) into subtending hyphae; spore wall layer 1 fragile, usually inflating in a short distance to the spore base where swl2 becomes invisible in the subtending hyphae.

***Rhizophagus* P.A. Dang.** *Botaniste*, 5: 43 (1896).

Spores formed singly within soil or sometimes roots, in disorganized, forming abundant spores in the roots of vascular plants. Spores with a mono-to-multiple layered wall. Wall of the subtending hyphae conspicuously continuous and concolourous with the spore wall, or slightly lighter in colour

than the spore wall. Spore pore closure often by introverted wall thickening, sometimes supported by a short bridging septum, rarely open. Forming typical vesicular-arbuscular mycorrhiza, with mycorrhizal structures that stain blue to dark blue in trypan blue.

Sacculospora **Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz.** *Mycotaxon*, 117: 297–316 (2011e).

Etymology: Latin, *sacculus* = saccule; *spora* = spore; referring to the spore formation within the neck of sporiferous saccules.

Sporocarps unknown. Spores formed within the hyphal neck of closely adherent, terminal or intercalary sporiferous saccules. Spores have three walls: outer, middle and inner. At least two layers (including the outer wall structural layer) are continuous with the sporiferous saccule wall. Inner layers of the outer spore wall are permanent. After the hyphal neck connections break off, spores show two, often opposite, cicatrices that are closed by the permanent sublayers of the outer wall structural layer. Middle and inner wall form *de novo*. Middle wall is 1–2-layered. Inner wall consists of several layers, none of which have a granular (‘beaded’) appearance, and does not stain in Melzer’s reagent. The inner wall may be germinal in function, but a germination structure has not yet been found.

Scutellospora **C. Walker & F.E. Sanders. emend. Oehl, F.A. Souza & Sieverding.** *Mycotaxon*, 106: 311–360 (2008).

Etymology: Latin, *scutellum* = small shield; *spora* = spore; referring to the production of germination shield in spores of members of the genus.

Spores formed singly on sporogenous cells formed terminally on an extra-radical hypha. Outer spore wall is 3 layered and continuous with the wall of the sporogenous cell. Outer layer of the outer spore wall generally rigid, second layer laminate and third layer thin, often membranous, tightly adherent to the laminate layer and thus, often difficult to observe. Pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by spore wall material. Two hyaline walls having 1–2 and 2–3 layers, respectively are formed *de novo* during sporogenesis. The inner wall forms a germination shield on its outer surface or between the outer and the subsequent layer of inner wall (Oehl *et al*., 2008). Germination shield transparent, or hyaline to sub-hyaline, seldom light yellow, bi- to mono-lobed; often violin-shaped to oval to ovoid to more rarely cardioids or coiled and then, either circular or apparently broad ellipsoid to irregular; only a few folds cover the shield surface where 1–2 rounded germ tube initiations (~2–4 µm in diam.). Mycelial hyphae form one to several septa in some distance to the sporogenous cells. Auxiliary cells are knobby without spines on the surface. Forming typical arbuscular mycorrhizae without intra-radical vesicles.

Septoglomus **Sieverding, G.A. Silva & Oehl.** *Mycotaxon*, 116: 75–120 (2011d).

Etymology: Latin, *septum* = septum; *glomus* = cluster; referring to the relation with *Glomus,* to which species of the new genus previously belonged.

Spores formed singly or in very loose, small clusters. Spores with a mono-to-multiple layered spore wall. Wall of the subtending hyphae conspicuously continuous and concolourous with the spore wall, or slightly lighter in colour

than the spore wall. Subtending hyphae are cylindrical to constricted or slightly funnel shaped at spore base. Pore at spore base or at some distance from spore base closed by a septum. Forming typical vesicular-arbuscular mycorrhiza, with mycorrhizal structures that stain blue to dark blue in trypan blue.

Simiglomus **Sieverding, G.A. Silva & Oehl.** *Mycotaxon*, 116: 75–120 (2011d).

Etymology: Latin, *simi(laris)* = similar; *glomus* = cluster; referring to the relation with *Glomus,* to which species of the new genus previously belonged.

Spores formed singly or in very loose, small clusters. Spores with a mono-to-multiple layered spore wall. Wall of the subtending hyphae conspicuously continuous and concolourous with the spore wall, or slightly lighter in colour than the spore wall. Subtending hyphae are funnel-shaped to cylindrical. Wall at spore attachment not with introverted wall thickening. Pore at spore base open but several septa in hyphae in some distance from spore base can separate spore contents from mycelia contents. Walls of subtending hyphae thickened over very long distances from the spore base (up to > 1000 µm). Forming typical vesicular-arbuscular mycorrhiza, with mycorrhizal structures that stain blue to dark blue in trypan blue.

Tricispora **Oehl, Sieverd., G.A. Silva & Palenz.** *Mycotaxon*, 117: 297–316 (2011e).

Etymology: Latin, *(cica-)trix* = cicatrix; *spora* = spore; referring to the two conspicuous cicatrices left on the structural wall layer of the spores, even

when the sporiferous saccules and the hyphal neck distal to the saccule have detached completely from the spores.

Sporocarps unknown. Spores formed within the hyphal neck of closely adherent terminal or intercalary sporiferous saccules. The globose saccule terminus generally is substantially smaller than the attached mature spore. Spores have an outer and an inner wall. At least two layers (including the outer wall structural layer) are continuous with the sporiferous saccule wall. The outer layer of the outer wall is evanescent, the inner layers are permanent. After the hyphal neck connections break off, spores show two, often opposite, cicatrices that are closed by the permanent sublayers of the outer wall structural layer. The inner wall forms de novo, consists of several layers without granular ('beaded') appearance and does not stain with Melzer's reagent. The fungal structures in the roots stain blue to dark blue with trypan blue; forming vesicular-arbuscular mycorrhiza.

Viscospora **Sieverding, Oehl & G.A. Silva.** *Mycotaxon*, 116: 75–120 (2011d).

Etymology: Latin, *viscosus* = sticky; *spora* = spore; referring to the adhesive nature of the spore surface of the type species of the genus.

Spores generally formed in loose clusters; subtending hyphae hyaline to white, rarely sub-hyaline, often thick-walled. Spores with 1–4 wall layers; outer wall layer exuding a mucigel-like substance. Pore closure at spore base often open, or semi-closed by wall thickening.

2.6 SPORE IN SPORE SYNDROME

Various studies have reported the presence of AM fungal spores inside the dead spores of other AM fungal species (Koske *et al*., 1986; Muthukumar and Udaiyan, 1999). This suggests that spores of AM fungi act as a micro-habitat when they are dead, apart from their normal role as propagules and the ability of different AM fungal species to sporulate in close proximity to each other (Rodrigues and Muthukumar, 2009).

2.7 DEVELOPMENT OF AM SYMBIOSIS

Arbuscular Mycorrhizal fungi exist in the soil as resting spores, which in some species are large enough to be visible with the naked eye (Harrison, 2005). These AM spores and are able to germinate and grow in response to different edaphic and environmental conditions, but are unable to produce extensive mycelia and to complete their life cycle without establishing a functional symbiosis with a host plant (Mosse, 1959; Hepper and Smith, 1976). The key developmental switches occurring in the fungal symbiont involve a sequence of morphogenetic events such as spore germination and pre-symbiotic mycelial growth, differential hyphal branching pattern in the presence of host roots, appressorium formation, root colonization, arbuscule development, extra-radical mycelial growth and spore production (Giovannetti *et al*, 2000).

2.8 HOST-FUNGAL INTERACTIONS: PRE-PENETRATION

2.8.1 Signaling prior to physical interaction between the symbionts: The genetic composition of the coenocytic extra-radical mycelium is an important factor in the recognition process (Croll *et al*., 2009). Important initiators

secreted by the host plants (Giovannetti and Sbrana, 1998; Buée *et al*., 2000) have been identified as Strigolactones (Akiyama *et al*., 2005; López-Ráez *et al*., 2008). They stimulate fungal metabolic activity (Tamasloukht *et al*., 2003; Besserer *et al*., 2006; Bűcking *et al*., 2008) and also stimulate the hyphae of germinating spores to produce a fine and highly branched mycelium (Buée *et al*., 2000). Branched fungal hyphae then secrete a diffusible signal to the roots that leads to initiation of the symbiosis in roots that are in contact with the fungus, including the expression of symbiosis related genes (Chabaud *et al*., 2002; Kosuta *et al*., 2003). Only specific cells support the formation of an appressorium and the prepenetration apparatus (Genre *et al*., 2005) to allow the fungus to enter the root through the epidermis. The AM fungal hyphae grow intercellularly within the root, reach the inner cortex, penetrate cortical cell walls and form characteristic intracellular hyphal structures (Genre *et al*., 2008). Significant changes to both symbionts occur with the formation of arbuscules and a carbon gradient is involved in signaling arbuscles development (Blee and Anderson, 1998). Currently, nothing is known about the signaling pathways in the fungus that induce the repeated dichotomous branching and thus results in arbuscule formation (Harrison, 2005).

2.8.2 Root exudates from host plants and their relevance to AM fungal growth: Metabolites present in root exudates of the host plant contain compounds that stimulate the growth and hyphal branching of AM fungi (Bécard and Piché, 1989; Giovannetti *et al*., 1993; Buée *et al*., 2000; Nagahashi and Douds, 2000) and are called as branching factors. These compounds are most effective near or at the root surface and increase the

chances for the fungus to come in physical contact to form appressoria in the cell wall grooves between epidermal cells. Several branching factor have been identified in *Lotus japonicus* as 5-deoxy-strigol (Akiyama *et al*., 2005), *Menispermum dauricum* root culture as strigol (Yasuda *et al*., 2003) and *Sorghum* exudates as sorgolactone (Besserer *et al*., 2006). The increased exudation of such compounds can increase appressoria formation and thereby enhance the colonization of the host root by AM fungi (Tawaraya *et al*., 1998). These exuded compounds stimulate hyphal growth (Nair *et al*., 1991; Bécard *et al*., 1992; Scervino *et al*., 2005; Nagahashi and Douds, 2007) which probably helps the fungus to explore the soil at farther distances from the germinated spore.

2.9 HOST-FUNGAL INTERACTIONS: POST-PENETRATION

2.9.1 Root Colonization and morphological changes: The process of root colonization process beginns with germination (hyphal growth) of AM spore. Arbuscular Mycorrhizal fungi exist in the soil as spores, and following germination, the hyphal germ tube grows through the soil in search of a host root. Appressorium is formed from which the fungal hyphae penetrate the cell walls and develop within the cortex cells a highly branched tree-like structures called arbuscules, responsible for nutrient exchange (Cavagnaro, 2001; Harrison, 2005). The pattern of growth within the root varies depending on the species involved and based on this growth pattern two morphological types, the *Paris*-type (heavily curled ‘‘coils’’) and the *Arum*-type (highly branched arbuscules) named after the plant species in which they were first observed

(Smith and Read, 1997) are developed along with other intermediate structures (Dickson, 2004).

The colonization morphology depends on the combination of the plant and fungal species and is not necessarily consistent within plant or AM fungal genera. Members of Glomeraceae usually form the *Arum*-type of mycorrhiza (Cavagnaro *et al*., 2001; Burleigh *et al*., 2002; Dickson, 2004; Feddermann *et al*., 2008) while other genera, e.g. Gigasporaceae, form *Arum*-type or intermediate types of arbuscules with *Paris*-type hyphal coils (Cavagnaro *et al*., 2001; Karandashov *et al*., 2004; Smith *et al*., 2004; Dickson *et al*., 2007). Hyphal coils are involved in P transport to the plant similar to the proposed function of arbuscules (Karandashov *et al*., 2004; Smith *et al*., 2004). Intra-radical structures are short lived and the intra-radical mycelium is constantly under development and reorganization within the roots (Dickson and Smith, 2001).

The arbuscules represent a dead-end in the growth of AM fungi (Bonfante and Perotto, 1995) and they senesce and collapse after 4–10 days of symbiosis (Sanders et al., 1977; Strack *et al*., 2003). When the arbuscule begins to senesce, the fibrillar material encapsulates the collapsed fungal structures that are then degraded completely by the plant cell and the plant cells regain their original morphology (Jacquelinet-Jeanmougin *et al*., 1987) to allow another new arbuscule formation.

Apart from arbuscules certain AM fungi develop intercellular lipid-rich storage organs, termed as vesicles. Except for species from the genera *Scutellospora* and *Gigaspora*, all AM fungi form intra- or inter-cellular storage organs, lipid-rich vesicles, to varying degrees in late phases of the symbiosis (Smith and Read, 1997). To complete its life cycle the fungus exits the root giving rise to extensive growth of external hyphae and finally extra-radical spores are formed, which may enter another colonization process. Fungal root colonization is under control of the plant aiming at a morphological and functional compatibility of the two partners (Bonfante and Perotto, 1995).

2.9.2 Cytological features of AM plant roots: Colonization of root cortex cells by AM fungi and formation of arbuscules leads to changes in the cytoplasmic organization and morphology of the host root cells. The fungal arbuscule occupies a major portion of the plant cell volume separated from the cell protoplast by the host plasma membrane. This membrane completely surrounds the arbuscule and forms a periarbuscular membrane, leading to a two-to fourfold increase in the plasma membrane's surface. The resulting space between the plant protoplast and the fungus develops into an apoplastic compartment that represents the symbiotic interface (Bonfante and Perotto, 1995). The central vacuole becomes fragmented, the volume of cytoplasm and number of cell organelles increase significantly, and the nucleus moves into a central position and undergoes hypertrophy (Balestrini *et al*., 1994). The nucleus of arbusculated cells is characterized by enhanced fluorochrome accessibility, increased nuclease sensitivity and chromatin dispersion reflecting a greater transcriptional activity of the plant genome in

the colonized cells (Gianinazzi-Pearson, 1996). The number of plastids in colonized cortex cells increases (Bonfante and Perotto, 1995) and networks are formed covering the arbuscules (Fester *et al*., 2001). The formation of dense plastid network-covering arbuscules indicates an intensified metabolism in the host cells. Microtubules are involved in changes of host cell morphology and cytoplasmic architecture. Plant cytoskeletal components respond to the penetration of a symbiotic fungus with the reorganization of microtubules and microfilaments. Besides reorganizing the cell for the accommodation of the arbuscule, the cytoskeleton is also involved in developing the periarbuscular membrane. High activities of HC-ATPases and phosphate transporters are located specifically in this membrane (Gianinazzi-Pearson *et al*., 1991; Rausch *et al*., 2001; Harrison *et al*., 2002; Paszkowski *et al*., 2002) and shows differences in some of its properties relative to the membrane around the periphery of the cell.

2.10 BENEFITS FOR THE AM SYMBIONTS

Arbuscular Mycorrhiza fungal symbiosis is an symbiotic association wherein both the fungal and plant symbionts are benefited. The benefit derived by the fungal symbiont is supply of carbohydrates as a source of carbon. The major benefits of AM fungi to plant symbionts includes enhanced uptake of nutrients (macro- and micro-nutrients) and also non-nutritional benefits such as increased tolerance to root pathogens, drought resistance, tolerance to toxic heavy metals and improved soil aggregation and structure.

2.10.1 Carbon: Arbuscular Mycorrhizal fungi receive 100% of their carbon from the plant and this increase in carbon flow to the roots in exchange for

mineral nutrients is provided by the microsymbiont. A significant proportion of the photosynthesis products is delivered to the fungus (Jakobsen and Rosendahl 1990; Wright *et al*., 1998; Feddermann, 2010), increasing the sink strength of the root. The transfer of carbohydrates is the main benefit for the fungal symbiotic partner. Fungal H^+-ATPases, involved in the fungal uptake of carbohydrates from the apoplast, are located on arbuscular trunks and intercellular hyphae, supporting an uptake of carbohydrate by these structures (Gianinazzi-Pearson *et al*., 1991). Glucose is mainly transformed to trehalose or glycogen (Douds *et al*., 2000). After longer incubation periods, glucose is either used directly for lipid biosynthesis or enter the pentose phosphate pathway, thus providing the reduction equivalents necessary for lipid biosynthesis (Pfeffer *et al*., 1999). Lipids and glycogen are then transferred to the extra-radical mycelium (Bago *et al*., 2003) where the bidirectional movement of lipid bodies can be observed in vivo (Bago *et al*., 2002).

2.10.2 Phosphorus: One of the plant's main benefits from the AM symbiosis is improvement of P uptake. It is an essential mineral nutrient that constitutes up to 0.2% (dry weight) of each plant cell and is thus required in significant quantities (Schachtman *et al*., 1998). Plants require large quantities of P, which they obtain as phosphate (Pi) from the solution phase of the soil. Phosphorus is often not freely accessible in the soil and its availability to plants varies between different soils (Holford, 1997). The diffusion of Pi through the soil is slow thus Pi depletion zones develop rapidly around the roots. In the AM symbiosis, the additional volume of soil which can be explored by the fungal extra-radical hyphae is significant and therfore the

nutritional advantage conferred by symbiotic Pi transfer is considerable (Schachtman *et al*., 1998; Smith *et al*., 2003). Plants have specialized, high affinity phosphate transporters, located at the root epidermis, that are responsible for uptake of solubilised P directly from the soil into the roots (Javot *et al*., 2007).

2.10.2.1 Phosphate Transporters in AM Symbiosis: Symbiotic Pi transfer, i.e. the acquisition of Pi from the soil by the extra-radical hyphae and subsequent transfer to the plant cell requires transport proteins to move Pi across the membrane of the AM fungus and plant. In recent years, there has been tremendous progress in the identification of plant and fungal Pi transporter proteins, and coupled with the physiological data a greater understanding of symbiotic Pi transfer has emerged (Harrison *et al*., 2010). In AM the epidermal P transporters are downregulated and P is delivered into the root cortex through fungal hyphae as polyphosphate, resolubilised and offered to the plants. It is taken up in cells hosting arbuscules through high affinity P-transporters, which are located at the periarbuscular membrane (Harrison *et al*., 2002; Balestrini and Bonfante, 2005; Javot *et al*., 2007) which account for most P acquisition in AM plants (Pearson and Jakobsen, 1993; Smith *et al*., 2003; Poulsen *et al*., 2005).

2.10.3 Nitrogen: Nitrogen is a major nutrient that frequently limits growth of plants (Chapin *et al*., 2004). Arbuscular Mycorrhizal fungi play an active role in the liberation of nitrate from complex organic material within the soil (Hodge *et al*., 2001; Hause and Fester, 2005). Modification of soil aggregation by AM

fungi affects aeration of soil, and this could have an impact on nitrification and denitrification, the two N-cycling processes that are affected by oxygen concentration in the soil air (Veresoglou, 2011). Arbuscular Mycorrhizal presence affect soil pH (Li *et al*., 1991; Bago *et al*., 1996; Marschner and Baumann, 2003), and thus modify availability of N-compounds (De Boer and Kowalchuk, 2001) which may reduce availability of nitrates. Modification of carbon content of the soil mediates changes in soil properties such as water holding capacity (Bouyoucos, 1939) and thus influence moisture-sensitive N-cycling processes such as nitrification (Avrahami and Bohannan, 2007), denitrification (Davidson *et al*., 1993) and leaching (Currie and Aber, 1997).

2.11 ECOLOGICAL ROLES OF ARBUSCULAR MYCORRHIZAL FUNGI

2.11.1 Plant pests and pathogen protection: Arbuscular Mycorrhizal fungi play an important role in the suppression of crop pests and diseases, particularly soil-borne infection by pathogenic fungi (Paulitz and Linderman, 1991; Linderman, 1994; Borowicz, 2001; Harrier and Watson, 2004; Whipps, 2004; Tabin *et al*., 2009), above ground fungal diseases (West, 1995; Feldmann and Boyle, 1998) and herbivores (Gange and West, 1994; Gange *et al*., 2002). This increased resistance of a mycorrhizal plant to a pest or disease is mainly due to improved nutrition by their AM fungal symbiont (Cordier *et al*., 1996; Karagiannidis *et al*., 2002), changes in root exudates (Filion *et al*., 1999; Norman and Hooker, 2000) resulting in changes in the rhizosphere microbial community (Dar *et al*., 1997), changes in host root architecture (Yano *et al*., 1996; Vigo *et al*., 2000) or changes in root biochemistry connected with plant defence mechanisms (Azcón-Aguilar and

Barea, 1996; Gianinazzi- Pearson, 1996). An enhanced P uptake due to AM symbiosis may increase plant development, but not decrease pathogen infections (Trotta *et al*., 1996). Newsham *et al*. (1995a) found no effects of AM fungi on the P level of host plants but only pathogen protection against *Fusarium oxysporum*. Studies unrelated to pathogen protection have shown wide variation in terms of AM fungal-mediated nutrient uptake among AM fungal species (Jakobsen *et al*.,1992; van der Heijden *et al*., 1998, Smith *et al*., 2000; van Aarle *et al*., 2002; van der Heijden *et al*., 2003). This can be explained to a certain extent through the variation of traits such as mycelium development both in soil and roots and P uptake efficiency (Jansa *et al*., 2005). Inoculation with a multi-species AM fungal assemblage from a field soil increased the intensity of AM fungal colonization of date palm roots, when compared with *Glomus monosporus*, *Glomus clarum*, or *Glomus deserticola* in isolation, but this did not result in enhanced amelioration of the negative effects of *F. oxysporum* on plant growth (Jaiti *et al*., 2007). The majority of variation in root colonization by AM fungi is explained by the divergence of the two most species-rich fungal clades: the extensively colonizing Glomerales and the poorly colonizing Diversisporales (Powell *et al*., 2009; Hart and Reader, 2002). Arbuscular Mycorrhizal fungal colonization influences root architecture of the host plant in most studies by causing a more profusely branched root system (Price *et al*., 1989; Yano *et al*., 1996; Paszkowski *et al*., 2002; Olah *et al*., 2005; Gutjahr *et al*., 2009). Abundant lateral root tips and developing meristems produce highly branched root systems more susceptible for pathogen attack and result in an increasing demand for AM fungi to protect them (Newsham *et al*., 1995b).

2.11.2 Arbuscular mycorrhiza and heavy metal tolerance: Colonization with AM fungi helps alleviate heavy metal-induced stress (Gildon and Tinker, 1981; Dehn and Schűepp, 1989; Diaz *et al*., 1996; Hall, 2002) and the extent of alleviation can vary depending on the heavy metal involved, its concentration in the soil, the fungal symbiosis partner and the conditions of plant growth (Leyval *et al*., 1997; Hildebrandt *et al*., 1999; Turnau and Mesjasz-Przybylowicz, 2003). Arbuscular Mycorrhizal colonization of the roots has a significant impact on the expression of several plant genes coding for proteins involved in heavy metal tolerance and detoxification (Repetto *et al*., 2003; Rivera-Becerril *et al*., 2005; Hildebrandt *et al*., 2007). Enhanced tolerance to specific heavy metals of fungi isolated from soils contaminated with Pb, Zn, Cd or Cu has been observed by González-Chávez *et al*. (2004); González-Guerrero *et al*. (2005). Arbuscular Mycorrhizal fungi tolerant to increased heavy metals application readily colonize host roots despite low spore counts (Del Val. *et al*., 1999; Jacquot- Plumey *et al*., 2001). They produce an insoluble glycoprotein glomalin which binds heavy metals in the soil (González-Chávez *et al*., 2004; Wright and Upadhyaya, 1996, 1998). Due to the large surface area explored by fungi in the soil hyphal binding is an important sink for heavy metals. Since hyphae of heavy metal tolerant AM fungi display a higher affinity to heavy metals than plant cells (Joner *et al*., 2000) they gets immobilized within the fungus. The uptake of Pb and its immobilization were higher in roots of mycorrhizal than non-mycorrhizal plants (Chen *et al*., 2005). Under Zn limitation, mobilization of Zn and transfer to the shoot is improved by the AM symbiosis (Chen *et al*., 2003), reflecting the role

of Zn as a micronutrient and the beneficial role of the symbiosis on nutrient supply.

2.11.3 Interaction with other soil microorganisms: Bacterial communities and specific bacterial strains promote germination of AM fungal spores and can increase the rate and extent of AM root colonization (Johansson *et al*., 2004). Arbuscular Mycorrhizal hyphae influence mycorrhizosphere as AM symbiosis begins resulting in the development of distinct microbial communities relative to the rhizosphere and bulk soil (Andrade *et al*., 1997). Within the mycorrhizosphere AM fungi interact with beneficial rhizosphere microorganisms including free living N fixing bacteria and general plant growth promoting rhizobacteria (Requena *et al*., 1997; Biro *et al*., 2000; Galleguillos *et al*., 2000; Tsimilli-Michael *et al*., 2000). According to Scheublin *et al*. (2004), the legume-*Rhizobium* symbiosis is influenced by AM fungi. The *Rhizobium* symbiosis requires high concentrations of P and the enhanced P nutrition arising from the AM colonization result in an increased nodulation and N_2 fixation (Vázquez *et al*., 2002).

2.11.4 Crop water relations: Smith and Read (1997); Davies *et al*. (1992, 2002); Augé (2001, 2004) showed that AM fungi help to increase the tolerance of host plants against water stress. This tolerance is mainly due to an increased root hydraulic conductivity, improved stomatal regulation, osmotic adjustment of the host and improved contact with soil particles through the binding effect of hyphae, enabling water to be extracted from smaller pores (Augé 2001, 2004). Water and nutrient uptake are higher in drought stressed mycorrhizal plants than in non-mycorrhizal plants (Al-Karaki

and Clark, 1999; Subramanian and Charest, 1997, 1999; Srivastava *et al*., 2002). Ryan and Ash (1996) and Bryla and Duniway (1997) suggested that AM fungi can only alleviate moderate drought stress and in more severe drought conditions they are not effective.

2.11.5 Soil structure: Arbuscular Mycorrhizal fungi bind soil micro-aggregates into larger macro-aggregates through their hyphal enmeshing effects (Tisdall *et al*., 1997). Their exists a direct relationship between the development of extra-radical hyphae and soil aggregation (Bethlenfalvay *et al*.,1999). They produce an extracellular insoluble glycoprotein called glomalin which sticks mycorrhizal hyphae to soil. Glomalin accumulation in soils (Rillig *et al*., 2001) exerts a strong influence on soil aggregate stability (Wright and Upadhyaya, 1998; Franzluebbers *et al*., 2000; Wright and Anderson, 2000; Rillig *et al*., 2003; Rillig, 2004). Also, the exudates from AM fungal hyphae and rapid hyphal turnover (Johnson *et al*., 2002; Staddon *et al*., 2003) provide C to other soil microorganisms promoting aggregate stability (Jastrow *et al*., 1998).

2.12 DISTRIBUTION OF AM FUNGI

Arbuscular Mycorrhizal fungi have a large geographical distribution (Malloch *et al.*, 1980) and are found in almost all ecosystems such as tropical rainforests (Brundrett *et al*., 1999; Guadarrama and Álvarez-Sánchez, 1999; Siqueira and Saggin-Júnior, 2001; Zhao *et al*., 2001; Gaur and Adholeya, 2002), aquatic habitats (Khan, 1993; Radhika and Rodrigues, 2007), deserts (Corkidi and Rincön, 1997; Dalpe *et al*., 2000; Titus *et al*., 2002; Pezzani *et al*., 2006; Shi *et al*., 2006, Zhang *et al*., 2012) and also in ecosystems with

extreme environments such as strong saline soils (Carvalho *et al*., 2001; Sengupta and Chaudhuri, 2002; Karaarslan and Uyanöz 2011; Khare, 2011), sodic or gypsum soils (Landwehr *et al*., 2002), Arctic tundras and the Antarctic region (DeMars and Boerner, 1995; Allen, 1996; Gardes and Dahlberg, 1996). Interactions between plant and fungal communities is based on the preference of a given plant or fungus to the specific symbiotic partners from the populations which is to maintain diversity within plant communities (Bever, 2002). Sanders *et al*. (1996) have stressed the importance of fungal diversity for the ecological impact of the AM symbiosis, and van der Heijden *et al*. (1998b) have shown that fungal and plant population diversity are directly correlated to each other. In general, ecology of AM symbiosis are highly dependent on the local situation prevailing in the environment (Hartnett and Wilson, 2002).

2.13 ARBUSCULAR MYCORRHIZAL FUNGI AND ORNAMENTAL FLOWERING PLANTS

Arbuscular Mycorrhizal fungi do not exhibit host plant specificity and they can associate with a wide range of host plant species. There are fewer studies on the association and diversity of AM fungi in ornamental flowering plants. Ranganayaki and Manoharachary (2001) studied AM colonization in *Tagetes erecta* L. plants under natural field conditions and found 72% of AM fungal association with the rhizosphere soil harbouring *Acaulospora foveata*, *Entrophospora* sp., *Glomus constrictum*, *G. fasciculatum*, *G. heterosporum*, *G. hoi*, *Sclerocystis pakistanika* and *Scutellospora nigra* among which *G. fasciculatum* was predominant.

Muthukumar *et al*. (2006) studied mycorrhizal status in *Acalypha indica* L., *Lantana camara* L., *Rosa indica* L., *Clitoria ternatea* L., *Crotalaria verrucosa* L., *Jasminum sambac* L., *Nyctanthes arbor-tristis* L., *Hibiscus rosa-sinensis* L., *Michelia champaca* L., *Gloriosa superba* L., *Tagetes erecta* L., *Chrysanthemum cinerariifolium* (Trev.) Vis., *Catharanthus roseus* (L.) G. Don., *Justicia adhatoda* L., *Crossandra infundibuliformis* (L.) Nees. while studying mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. Yaseen *et al*. (2006) studied AM status of *in-vitro* raised plants of *Chrysanthemum cinerariifolium* (Trevir.) Vis. and observed 80% roots with AM colonization and therefore had more efficient root system with better nutrient absorption capability.

Radhika and Rodrigues (2010) found *Glomus maculosum*, *G. glomerulatum* and *Acaulospora scrobiculata* associated with *Hibiscus rosa-sinensis* while carrying out survey of AM fungal diversity in some commonly occurring medicinal plants of Western Ghats, Goa region. Panna and Highland (2010) studied mycorrhizal colonization and distribution of AM fungi associated with *Michelia champaca* under plantation system in northeast India. They reported significantly higher AM colonization than dark septate endophyte colonization and spore density varied significantly in all the sites. The distribution, abundance and principal component analysis plot suggested that *Glomus macrocarpum*, *G. multicaulis*, *G. constrictum* and *Acaulospora* sp. were the most host preferred species which possibly favour the host with proper nutrient acquisition and growth.

Yang *et al*. (2011) studied root colonization and the diversity of spore populations of AM fungi in rhizosphere soil samples of *Magnolia cylindrica* in Huangshan of Anhui Province, East-Central China and reported the presence of AM fungal colonization with hyphae, hyphal coils and vesicles in all root samples and rich spore density and diversity of AM fungi.

A positive vegetative response to AM colonization results in positive reproductive response due to inherent architectural constraints on flower production since more flowers require more branches, which contain more leaves and require more roots (Koide, 2010). The effects of AM colonization on vegetative growth may differ quantitatively from their effects on reproduction (Bryla and Koide, 1990; Stanley *et al*., 1993; Nakatsubo, 1997; Karagiannidis and Hadjisavva-Zinoviadi, 1998) because the extent of P deficit (Koide, 1991) for vegetative growth and reproduction may differ, and some of the nutrient requirement for reproduction may be met by reallocation from vegetative structures. Thus it is not possible to determine the extent of AM colonization effect of plant reproduction based on vegetative growth (Koide, 2010). Relatively little is known about the effects of colonization by AM fungi on the male function of plants. Apart from vegetative growth AM fungi can enhance a number of plant traits. These include total plant size, flower number, flower size and amount of pollen produced. Johnson *et al*. (1982) studied the effect of flower bud development in chrysanthemum on AM formation and showed that different carbon and nutrient allocation patterns between mycorrhizal and non-mycorrhizal plants can influence flowering.

Kandasamy *et al*. (1986) studied the influence of AM inoculation on the growth of *Pyrethrum* in the nursery and reported that AM inoculated plants flowered 7-10 days earlier than non-mycorrhizal plants. Davies *et al*. (1987) evaluated the effect of AM, soil amendments and water relations on growth of *Rosa multiflora* under reduced water regimes and showed that AM inoculated plants had higher resistance to water stress.

Wen (1991) evaluated effects of temperature and *Glomus* sp. on growth and cut flower quality of micropropagated *Gerbera jamesonii* and reported colonization by AM fungi increased the vase life of cut flowers. Naik *et al*. (1995) studied the effects of different AM fungi at different P levels on growth, yield and P content of *Callistephus chinensis* and concluded that interactions between AM fungi and P levels were positively significant in improving shoot dry weight production, P nutrition and flower yield.

Aboul-Nasr (1996) studied effects of AM on *Tagetes erecta* and *Zinnia elegans* and reported an increase in number of flowers after mycorrhization, while dry weight as well as K and P concentrations were unaffected. Chen and Chang (1996) while studying effect of AM fungi on growth and flowering of two cultivars of *Cineraria* spp. reported AM inoculation shortened flowering time compared to non AM plants.

Gaur and Adholeya (2000) evaluated effects of mixed AM inocula and chemical fertilizers in a soil with low P fertility on growth and flowering in *Petunia hybrida*, *Callistephus chinensis* and *Impatiens balsamina*. An

increase in P and K concentration in shoots of AM-inoculated plants along with an improvement in both flower number and vegetative phase of plants was reported. Ranganayaki and Manoharachary (2001) studied the impact of AM fungi on the growth of *Tagetes erecta* with native AM inoculum and *Gl. fasciculatum* was also studied and reported positive effect by both the mycorrhizal treatments on plant height, root length, early flowering, number of flower heads, flower head diameter and, shoot and root dry weights and plant tissue N, P, K levels in mycorrhizal treated plants over non-mycorrhizal control plants.

Scagel (2003a) studied the effect of AM fungal inoculation on flower and corm production in *Freesia* spp. grown in sterilized or non-sterilized soil. They observed AM fungi had no influence on flower opening in the first growth cycle, but inoculated plants flowered approximately 20 days earlier than non-inoculated plants in the second growth cycle. When grown in non-sterilized soil, inoculated plants produced more leaves, flowers, inflorescences and flowers per inflorescence than non-inoculated plants. Mycorrhizal plants produced heavier daughter corms with increased number of cormlets than non-inoculated plants. In *Zephyranthes* spp. soil pasteurization and inoculation with *Glomus intraradices* altered flower production and bulb composition (Scagel, 2003b).

Sohn *et al*. (2003) evaluated the effect of different timing of AM inoculation on rooting rate, colonization percentage and early plantlet growth at transplanting stage and successive plant growth, nutrient uptake and flower

quality of *Chrysanthemum morifolium* Ramat var. Baekgwang. A significant difference in plant growth, nutrient uptake and flower quality was observed in AM inoculated compared to non-inoculated plants. Linderman and Davies (2004) demonstrated varied response of different genotypes of *Tagetes* spp. inoculated with different AM fungi for colonization and responsiveness under low soil P conditions. Cultivars varied in their pattern of partitioning biomass into roots or shoots, with some partitioning more into roots than others with similar shoot biomass. Flower number or plant height did not vary between AM and non AM plants. Intra-radical colonization intensity ratings (arbuscules, vesicles and internal hyphae) varied significantly among cultivars and AM fungal inoculants, as did extra-radical hyphal development.

Nowak (2004) evaluated effects of lead (Pb) concentration and AM fungi on growth, flowering and Pb accumulation in shoots of *Pelargonium hortorum* L.H. Bailey and demonstrated that on a peat substrate with organic NPK fertilizer mycorrhizal plants flowered earlier and showed increased N, P and K concentrations at low nutrient supply as well as increased P concentrations at high nutrient supply, while the number of flowers and the leaf dry weights were unaffected by AM colonization.

Soil pasteurization and inoculation with AM fungi can alter plant characteristics that affect the quality and composition of corms and cut flower production in *Brodiaea laxa* (Scagel, 2004a). Also inoculation of *Sparaxis tricolour* with AM fungi and rhizobacteria influenced several aspects of plant development such as shoot emergence, leaf and flower production *via*

changes in mineral uptake, resource storage and biomass partitioning (Scagel, 2004b).

Gange and Smith (2005) studied three species of annual plants *viz*., *Centaurea cyanus*, *Tagetes erecta* and *T. patula* to evaluate the effect of AM inoculation and showed that inoculation with AM fungi influence visitation rates of pollinating insects to these plants due to increase in total plant size, flower number and size and, amount of pollen produced over un-inoculated control. Gaur and Adholeya (2005) studied the response of five ornamental plant species *viz. Petunia hybrida, Tagetes erecta, Callistephus chinensis, Papaver rhoeas* and *Dianthus caryophyllus* to mixed indigenous and single isolate AM inocula in marginal soils amended with organic matter and observed that AM inoculation increased flowering only in *C. chinensis*, whereas in *P. hybrida* and *T. erecta* fewer flowers were recorded in AM inoculated plants.

Scagel and Schreiner (2006) demonstrated the effect of AM inoculation on plant development, reproduction and tuber quality in *Zantedeschia* sp. by growing plants with or without mycorrhizal inoculum at different rates of P supply in order to separate P mediated effects from any non P mediated effects of the mycosymbiont. It was observed that AM inoculation had organspecific effects on tuber and flower quality and productivity.

D'Amelio *et al.* (2007) carried out an experiment to study the effects of combined inoculum of a *Rhizobacterium* and an AM fungus on plant

responses to phytoplasma infection, and on phytoplasma multiplication and viability in *Chrysanthemum carinatum* Schousboe. infected by chrysanthemum yellows phytoplasma. It was observed that combined inoculation with *Glomus mosseae* and *Pseudomonas putida* resulted in some resistance to phytoplasma infection, delayed symptom expression in non resistant plants, improved growth of the aerial parts of the infected plants and altered root morphology. Perner *et al*. (2007) evaluated the effect of AM colonization and two levels of compost supply on nutrient uptake and flowering of *Pelargonium peltatum* L' Her. and reported that addition of compost in combination with AM inoculation can improve nutrient status and flower development of plants grown on peat based substrates.

Flores *et al*. (2007) studied the effect of inoculation of *Bacillus subtilis* and *Glomus fasciculatum* at sowing and transplanting time on yield and quality enhancement of *Tagetes erecta* flowers. Number of inflorescences per plant, flower diameter, fresh weight, xanthophyll content and colour were evaluated at the end of the crop production cycle. It was observed that *Bacillus* and/or *Glomus* treated plants produced 14-24% more inflorescences than untreated plants. Although, the treated flowers had significantly higher fresh weight than control, they did not differ in size. *Bacillus* improved flower colour properties and yellow colour but not xanthophyll content and *Glomus* enhanced xanthophyll content but not colour properties.

Long *et al.* (2010) evaluated effects of AM fungi on *Zinnia elegans* and the difference in colonization between *Gigaspora* and *Glomus*. They showed

that mixed inoculations are not much effective in the growth promotion than the corresponding inoculation with *Glomus* alone. Matysiak and Falkowski (2010) evaluated mycorrhizal colonization, nutrient acquisition, and growth response of three ornamental plant species *viz*., *Physocarpus opulifolius*, *Spiraea japonica* and *Potentilla fruticosa* to AM inoculation, compost addition to peat substrate and controlled release fertilizer. It was found that AM fungal inoculation affected P content in plant tissue of all species, with a higher P content in AM than in non AM plants, regardless of the rate of controlled release fertilizer. However, the increased level of colonization caused by AM inoculum and higher P content in the leaves of all tested species did not correspond to higher biomass of plants.

Asar and Elhindi (2011) studied the effect *Glomus constrictum* on growth, pigments and P content of *Tagetes erecta* plant grown under different levels of drought stress and observed that AM inoculation positively stimulated all growth parameters such as plant growth, pigments, P content and flower quality compared to un-inoculated plants. Bharathiraja and Tholkappian (2011) observed AM fungal interaction and its beneficial effects on *Crossandra infundibuliformis*. It was observed that application of both AM fungi (*G. fasciculatum*) and phosphobacteria in combination and the recommended dose of fertilizers had a significant effect in improving the plant height.

Karishma *et al*. (2011) compared the efficacy of two AM fungi *viz., Glomus mosseae* and *Acaulospora laevis* along and in combination with

Pseudomonas fluorescens, *Trichoderma viride* and growth regulators like salicylic acid and kinetin and nutrients like sucrose and NaCl in prolonging the vase life of *Chrysanthemum indicum* L. It was reported that vase life of flowers was increased by inoculation with different bioinoculants as compared to the treatments with different growth regulators and nutrients. Schmidt (2011) studied the influence of AM fungi on the development of ornamental characters of *Tagetes patula* L. and found significant differences between the nutritional treatments and also between inoculated and un-inoculated plants having enhanced ornamental features.

CHAPTER 3

ARBUSCULAR MYCORRHIZAL FUNGAL (AMF) ASSOCIATION IN ORNAMENTAL FLOWERING PLANTS OF GOA

3.1 INTRODUCTION

Nature has given a wealth of wild flowers and ornamental plants and these plants have served for human adornment for millennia (Arora, 1993). Their use as ornaments is not only ancient but survives to the present time. In addition to decoration, adornment is often regarded as having amuletic powers or is used as social diacritical marks. Over 165 plant species used for human adornment in India have been identified from the literature (Francis, 1984).

Arbuscular Mycorrhizal fungi have been recognized as biological agents that contribute significantly to mineral nutrition, water uptake, pest control and pathogen suppression (Declerck *et al*., 1995; Schűβler *et al*., 2001; Declerck *et al*., 2002; Elsen *et al*., 2003; Johansson *et al*., 2004; Barea *et al*., 2005; Smith and Read, 2008) and heavy metal tolerance (Khan *et al*., 2000). They increase root surface area and enhance ability to explore for nutrients beyond the nutrient depletion zone (Smith and Read, 1997), facilitate the formation and stabilization of soil aggregates and soil dynamics (Smith and Read, 2008; Gianinazzi *et al*., 2010), and water infiltration (Rillig, 2004), prevent soil erosion (Schmid *et al*., 2008) and facilitate primary succession (Schram, 1966; Miller, 1987). In return they receive up to 30% of the hosts' photosynthate that is essential for the completion of its life cycle (Drigo *et al*., 2010).

Many AM fungal species are ubiquitous, occurring worldwide in quite different terrestrial ecosystems (Öpik *et al*., 2006), while others appear to be

restricted to specific ecosystems, land uses types, vegetations or climates (Castillo *et al*., 2006; Oehl and Sieverding, 2004; Oehl *et al*., 2010). Occurrence of specific AM species is related to soil physical and chemical characteristics such as soil texture, organic matter content and nutrient contents, and in particular to the availability of P (Uhlmann *et al*., 2004; Landis *et al*., 2004; Bashan *et al*., 2007; Lekberg *et al*., 2007). The fungi benefit from increased plant diversity due to the higher number of possible host-fungal pairings and increased density of plant roots available for colonization (Burrows and Pfleger, 2002). A higher diversity of AM fungi has been shown to increase plant productivity (van der Heijden *et al*., 1998b).

The biogeography of AM fungi remains relatively unknown at the global scale despite recent advances in understanding global distributions of other micro-organisms (Horner-Devine *et al*., 2004; Martiny *et al*., 2006) particularly in ornamental flowering plants. The general beneficial effects of AM colonization on nutrient acquisition and vegetative growth have been demonstrated (Gerdemann, 1968; Abbott and Robson, 1984; Cooper, 1984; Smith and Gianinazzi-Pearson, 1988; Bolan, 1991; Koide, 1991; Read, 1991; Read and Perez-Moreno, 2003). Colonization ability of AM fungi *i.e.* the rate and extent of colonization are commonly used measures of AM fungal activity (Hart and Reader, 2002). A positive vegetative response to AM colonization will generally lead to a positive reproductive response. This relationship exists because of the inherent architectural constraints on flower production; more flowers generally require more branches, which contain more leaves and require more roots. Therefore the studies on AM fungal association in this

community of plants are very important (Koide, 2010). Arbuscular Mycorrhizal fungal association was observed in ornamental plants species such as *Tagetes erecta* L. (Ranganayaki and Manoharachary, 2001), *Acalypha indica* L., *Lantana camara* L., *Rosa indica* L., *Clitoria ternatea* L., *Crotalaria verrucosa L.*, *Jasminum sambac* L., *Nyctanthes arbor-tristis* L., *Hibiscus rosa-sinensis* L., *Michelia champaca* L., *Gloriosa superba* L., *Tagetes erecta* L., *Chrysanthemum cinerariifolium* (Trev.) Vis., *Catharanthus roseus* (L.) G. Don., *Justica adhatoda* L., *Crossandra infundibuliformis* (L.) Nees. in Western Ghats, Southern India (Muthukumar *et al*., 2006), *Michelia champaca* L. in northeast India (Panna and Highland, 2010) and *Hibiscus rosa-sinensis* L. in Western Ghats, Goa (Radhika and Rodrigues, 2010). Due to the beneficial effects of AM fungi to floricultural crops effort is on throughout the world to exploit these micro-organisms to increase the productivity. Prior to exploiting the biofertilizer potential of AM fungi it is necessary to study their occurrence, distribution and colonization ability in their natural habitats. The present chapter deals with the study of AM fungal association in ornamental flowering plants of Goa.

3.2 MATERIALS AND METHODS

3.2.1 Study sites and collection of root samples: The study was conducted in Goa state which is located on the west coast of India and lies between 14°53′54″ and 15°40′00″ N latitude and between 73°40′33″ E and 74°20′13″ E longitude. Rhizosphere root samples were collected from 43 different plant species from garden and wild habitat, during the flowering stage, from a depth of 10–20 cm from 35 different localities of Goa. For seasonal and

geographical variation studies on AM fungal diversity, root samples were collected from 12 plant species *viz*., *Chrysantheumum morifolium*, *Crossandra infundibuliformis*, *Delonix regia*, *Erythrina indica*, *Gardenia jasminoides*, *Hedychium coronarium*, *Hibiscus rosa-sinensis*, *Jasminum nitidum*, *Mammea suriga*, *Michelia champaca*, *Nyctanthes arbor-tristis* and *Rosa* sp. from three different sites *viz*. Coastal (Arambol: 15°68′65″ N, 73°70′29″ E), Plateau (Bethora: 15°29′93″ N, 74°12′39″ E) and Western Ghats (Talauli: 15°22′74″ N, 74°15′75″ E) during pre-monsoon (April), monsoon (August) and post-monsoon season (December) for the period from 2008–2010 (**Fig. 1**). Samples were placed in polyethylene bags, labeled and transferred to the laboratory.

3.2.2 Processing of roots: Root colonization was assessed by using the method described by Phillips and Hayman, (1970). The root samples were first washed with water and cut into 1 cm pieces. These root pieces were cleared with 10% KOH at 90°C in an oven for 1–2 hrs, acidified in 2N HCl and then stained in 0.05% trypan blue in lactoglycerol. The stained roots were examined under a compound microscope (x 400) for the presence of hyphae, arbuscules and vesicles.

3.2.3 Estimation of root colonization: One hundred root segments from each sample were selected for microscopic observation and estimation of the degree of colonization was carried out using slide method (Giovannetti and Mosse, 1980). A segment was considered mycorrhizal when it showed the presence of hyphae and/or arbuscles and/or vesicles. Total root colonization

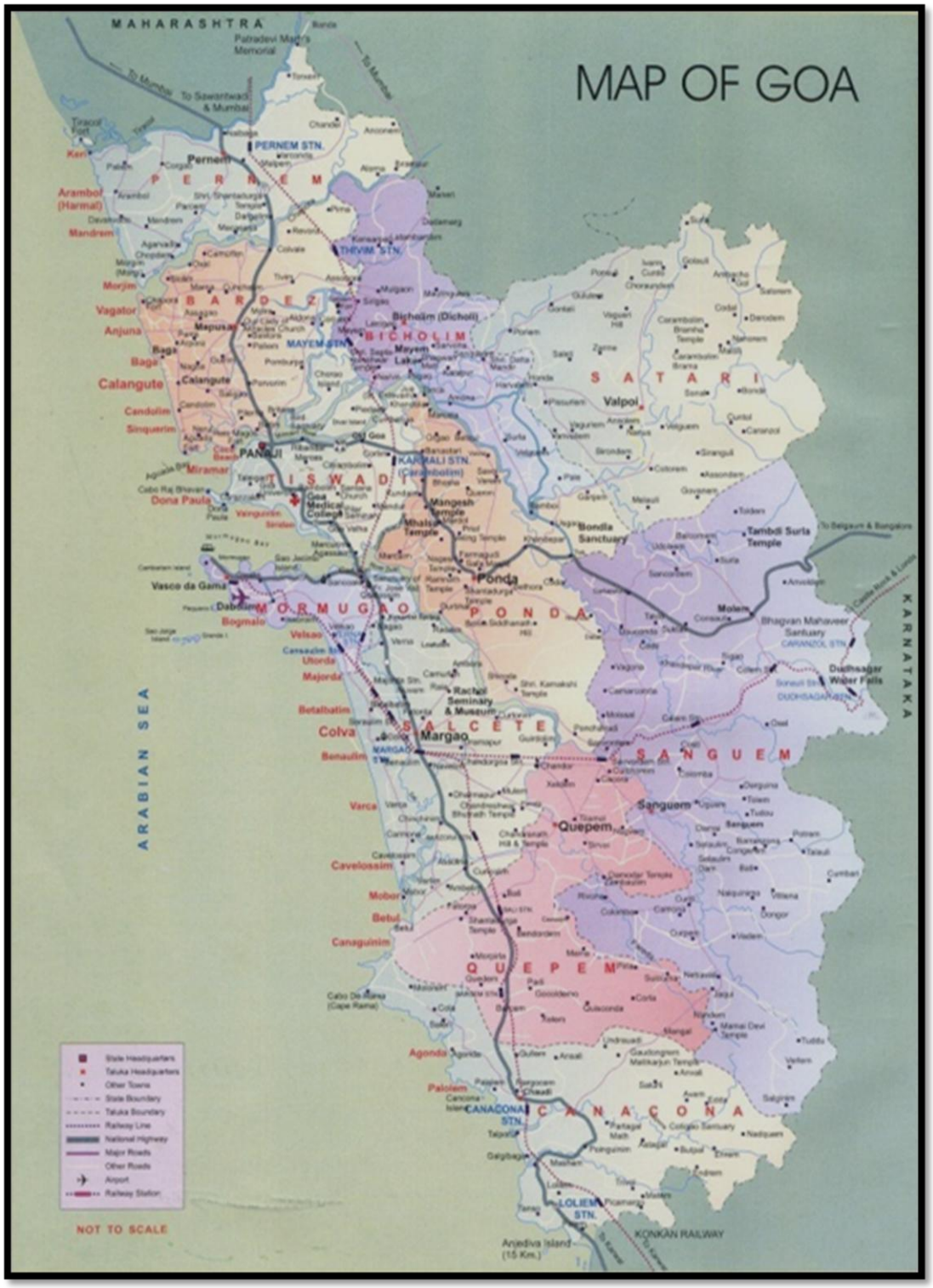


Figure 1: Map of Goa showing the study sites.

was expressed as percent AM colonization and was calculated using the formula,

Root colonization (%) = (Number of root segments colonized/Total number of root segments observed) x 100

3.2.4 Soil analyses: Soil pH was measured after dilution with distilled water (1:2 v/v) using pH meter (LI 120 Elicio, India). Electrical Conductivity (EC) was measured at room temperature in 1:5 soil suspension using Conductivity Meter (CM-180 Elico, India). The organic carbon and available P were analysed according to the methods outlined by Walkley and Black (1934) and rapid titration method (Bray and Kurtz, 1945), respectively. Available K was determined using ammonium acetate method (Hanway and Heidel, 1952) using Flame photometer (Systronic 3292).

3.2.5 Statistical analysis: Pearson's correlation coefficient test was performed to assess the relationships between mycorrhizal parameters such as root colonization and spore density and edaphic factors using WASP (Web Based Agricultural package, version 1.0 at $P \leq 0.05$).

3.3 RESULTS

A study of the association between AM fungi and roots of ornamental flowering plants commonly found in Goa state was carried out. In all 129 rhizosphere root samples were collected from 43 plant species **(Plates 1–6)** from 35 localities of Goa **(Table 2)**. Soil pH varied significantly between the sampling habitats and sampling seasons ($P \leq 0.05$). Soil pH in the rhizosphere of garden plants was lower than in the rhizosphere of wild plants

Table 2: Ornamental flowering plant species from Goa selected for study.

Sr. No.	Host plant	Family	Location	Status
1.	*Acalypha hispida* Burm. F.	Euphorbiaceae	Advalpal	Garden
2.	*Allamanda cathartica* L.	Apocyanaceae	Korgao	Wild
3.	*Bauhinia purpurea* L.	Caesalpiniaceae	Netravali	Wild
4.	*Bombax ceiba* L.	Bombacaceae	Mangal	Wild
5.	*Bougainvillea spectabilis* Willd.	Nyctaginaceae	Fatorda	Wild
6.	*Butea monosperma* Roxb. Ex Willd	Caesalpiniaceae	Honda	Wild
7.	*Caesalpinia pulcheriima* (L.) Swartz	Caesalpiniaceae	Valpoi	Wild
8.	*Callistemon lanceolatus* DC.	Myrtaceae	Corlim	Garden
9.	*Canna indica* L.	Cannaceae	Talauli	Garden
10.	*Cassia fistula* L.	Caesalpiniaceae	Potrem	Wild
11.	*Chrysanthemum morifolium* Ramat.	Asteraceae	Parsem	Garden
12.	*Clarodendron paniculatum* L.	Verbenaceae	Velpe	Wild
13.	*Crossandra infundibuliformis* (L.) Nees.	Acanthaceae	Chopdem	Garden
14.	*Delonix regia* (Hook.) Raf.	Caesalpiniaceae	Netravali	Wild
15.	*Erythrina indica* Lam.	Fabaceae	Arambol	Wild
16.	*Ethalidium barlerioides* (Roth) Nees	Acanthaceae	Dhargal	Wild
17.	*Gardenia jasminoides* Ellis.	Rubiaceae	Siolim	Garden
18.	*Hedychium coronarium* Koenig.	Zingiberaceae	Palyem	Garden
19.	*Hibiscus rosa-sinensis* L.	Malvaceae	Bethora	Garden
20.	*Ixora duffi* T. Moore	Rubiaceae	Advalpal	Garden
21.	*Jasminum auriculatum* Vahl.	Oleaceae	Velus	Garden
22.	*Jasminum nitidum* Skan.	Oleaceae	Mavlinge	Garden
23.	*Lagerstroemia purpurea* L.	Lythraceae	Colomb	Wild
24.	*Mammea suriga* Kosterm.	Clusiaceae	Pernem	Wild
25.	*Melastoma malabathricum* L.	Melastomaceae	Kudchire	Wild
26.	*Michelia champaca* L.	Magnoliaceae	Velus	Garden
27.	*Mimusops elengi* L.	Sapotaceae	Chandel	Wild
28.	*Murraya paniculata* (L.) Jack	Rutaceae	Arambol	Garden
29.	*Mussaenda frondosa* L.	Rubiaceae	Gaodongarem	Garden
30.	*Nerium indicum* Mill.	Apocyanaceae	Sancordem	Wild
31.	*Nyctanthus arbor-tristis* L.	Oleaceae	Uguem	Garden
32.	*Pachystachys spicata* (Ruiz & Pav.) Wassh.	Acanthaceae	Volvoi	Garden
33.	*Peltophorum pterocarpum* (DC.) K. Heyne.	Fabaceae	Paryem	Wild
34.	*Pithecellobium saman* (Jacq.) Benth.	Fabaceae	Valpoi	Wild

35.	*Plumeria rubra* L.	Apocyanaceae	Mulgaon	Wild
36.	*Pseudoeranthemum bicolor* (Sims) Radlk.	Acanthaceae	Kumbari	Garden
37.	*Rosa* sp.	Rosaceae	Velus	Garden
38.	*Spathodea campanulata* P. Beauv.	Bignoniaceae	Dabal	Wild
39.	*Tabernaemontana divericata* L.	Apocyanaceae	Barazan	Garden
40.	*Tagetes erecta* L.	Asteraceae	Korgao	Garden
41.	*Tecoma stans* L. Juss. Ex. Kunth.	Bignoniaceae	Assonora	Garden
42.	*Thevetia nerifolia* Juss.	Apocyanaceae	Nanorem	Garden
43.	*Thunbergia grandiflora* Roxb.	Acanthaceae	Honda	Wild

Plate 1: Ornamental plant species

A. ***Acalypha hispida*** Burm. F.

B. ***Allamanda cathartica*** L.

C. ***Bougainvillea spectabilis*** Willd.

D. ***Caesalpinia pulcheriima*** (L.) Swartz

E. ***Callistemon lanceolatus*** DC.

F. ***Canna indica*** L.

Plate 1

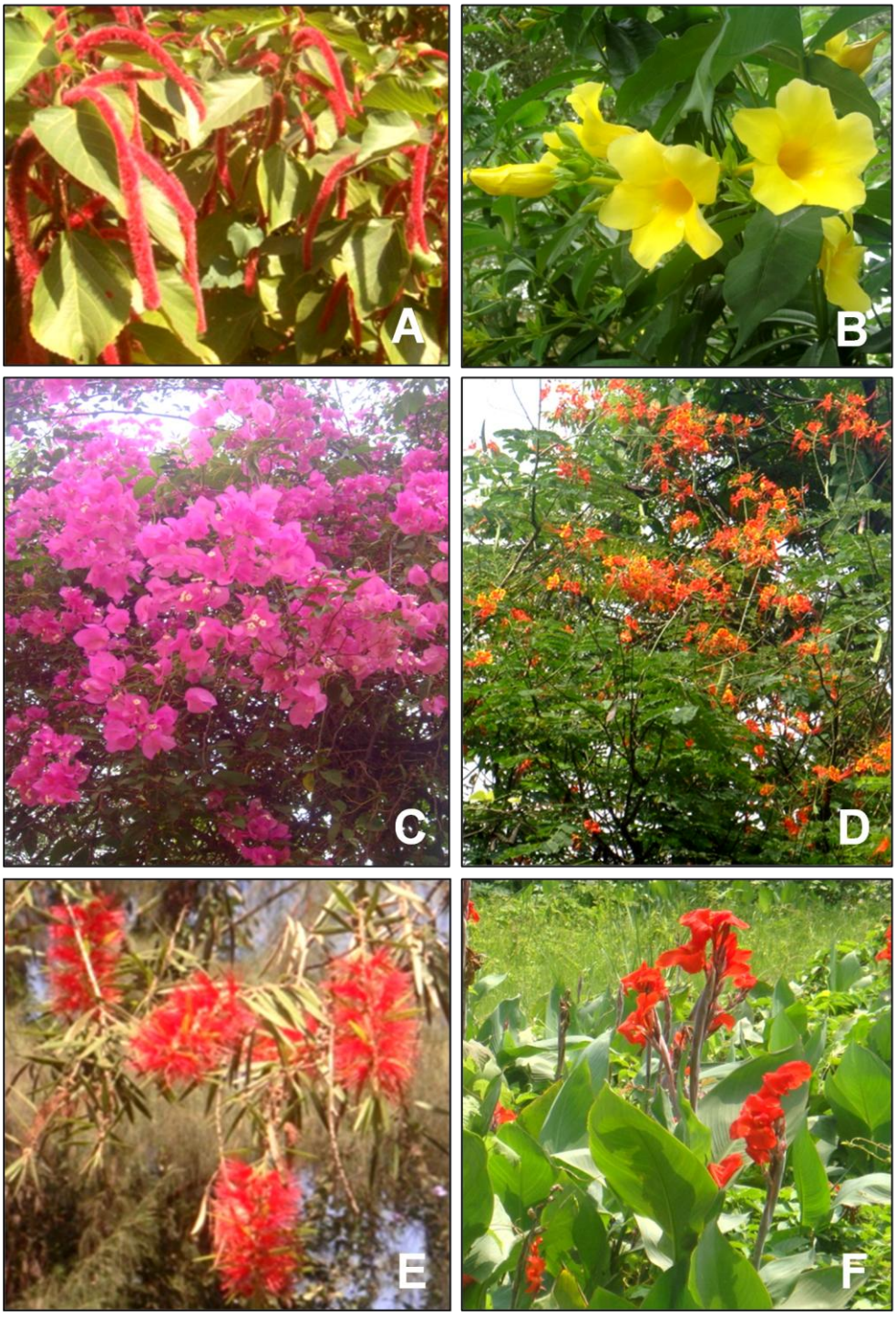


Plate 2: Ornamental plant species

A. ***Cassia fistula*** L.

B. ***Chrysanthemum morifolium*** Ramat.

C. ***Clarodendron paniculatum*** L.

D. ***Crossandra infundibuliformis*** (L.) Nees.

E. ***Delonix regia*** (Hook.) Raf.

F. ***Ethalidium barlerioides*** (Roth) Nees

Plate 2

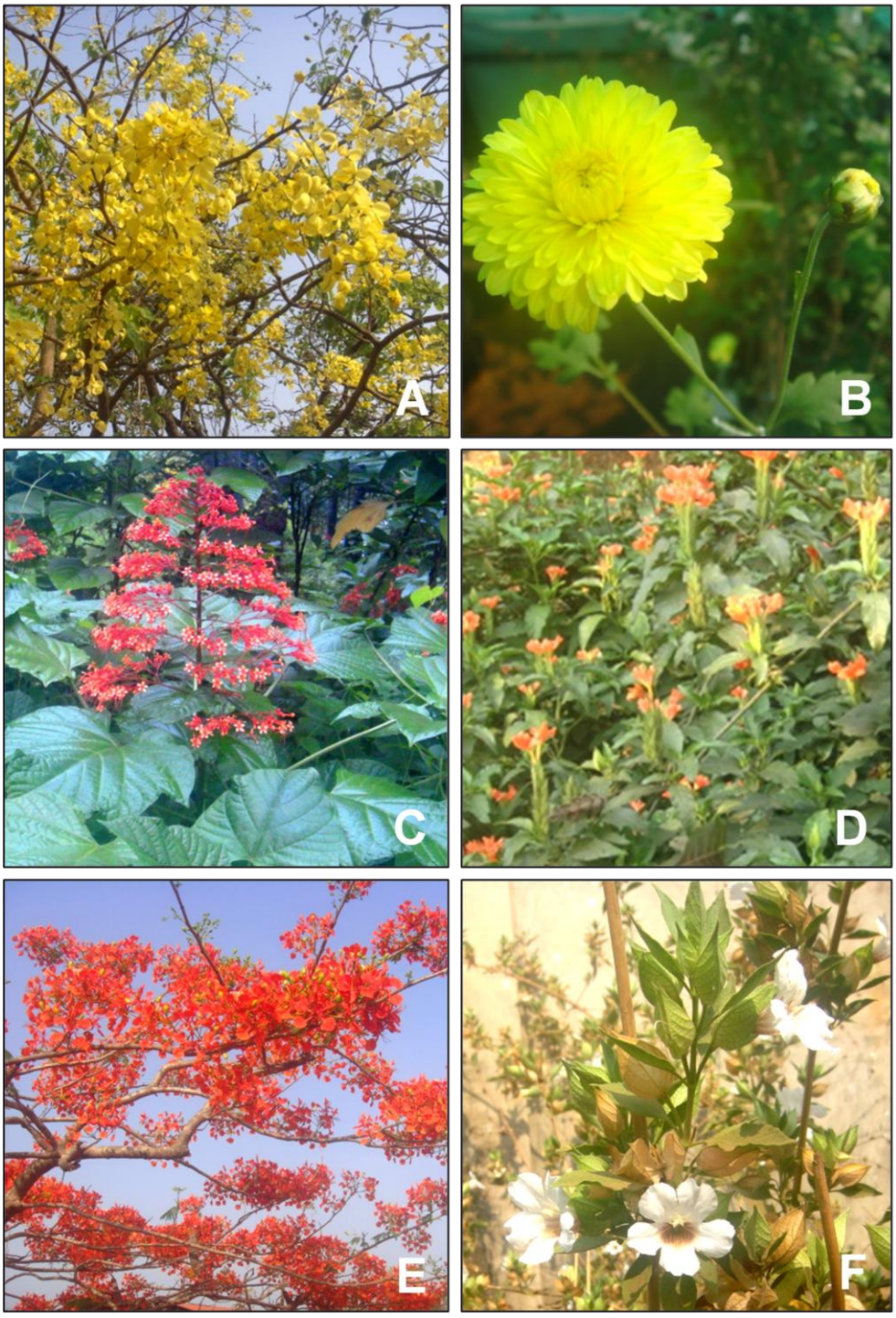


Plate 3: Ornamental plant species

A. ***Gardenia jasminoides*** Ellis.

B. ***Hedychium coronarium*** Koenig.

C. ***Hibiscus rosa-sinensis*** L.

D. ***Ixora duffi*** T. Moore

E. ***Jasminum nitidum*** Skan.

F. ***Lagerstroemia purpurea*** L.

Plate 3

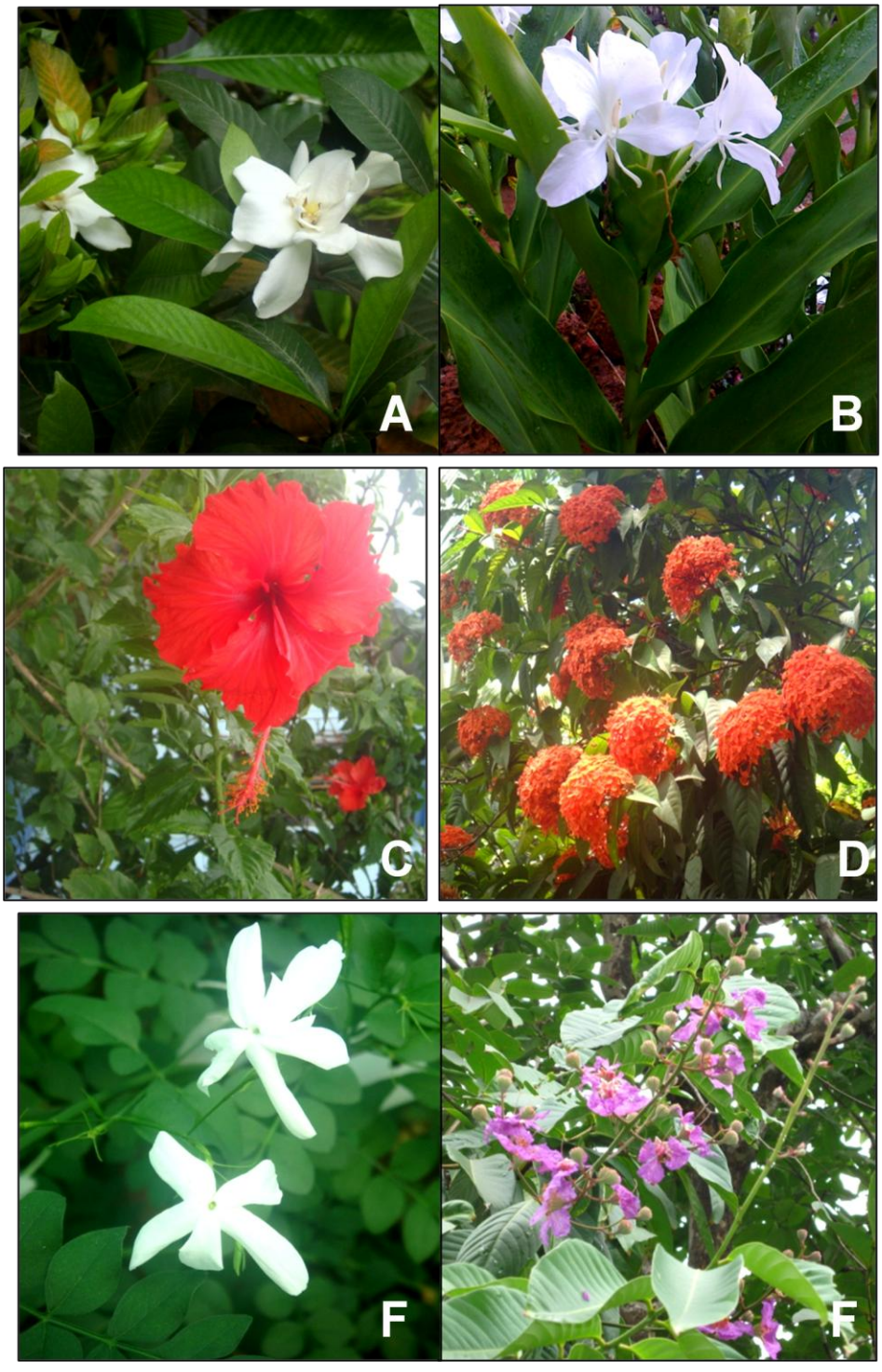


Plate 4: Ornamental plant species

A. ***Melastoma malabathricum*** L.

B. ***Michelia champaca*** L.

C. ***Murraya paniculata*** (L.) Jack

D. ***Mussaenda frondosa*** L.

E. ***Nerium indicum*** Mill.

F. ***Nyctanthus arbor-tristis*** L.

Plate 4

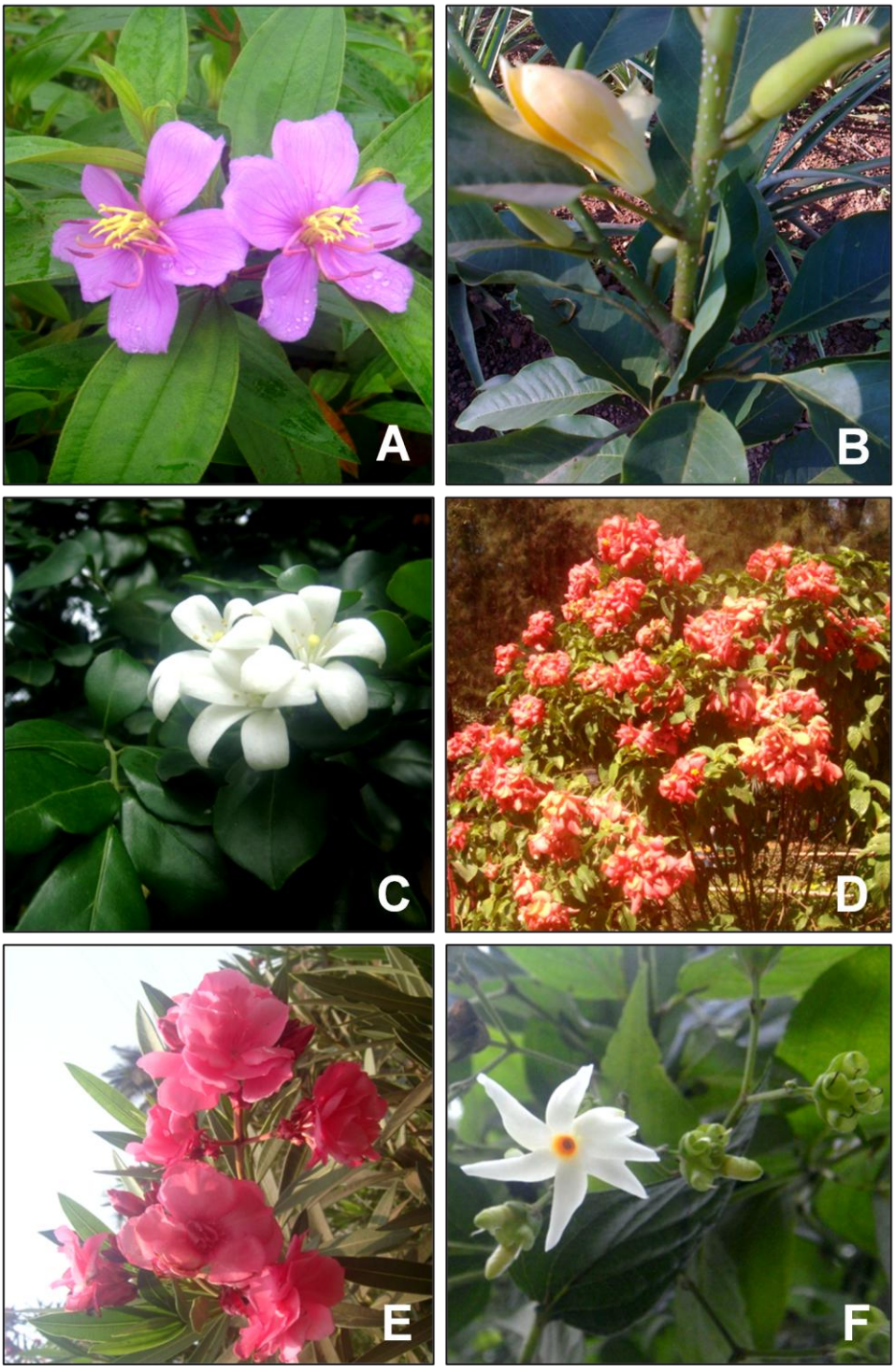


Plate 4

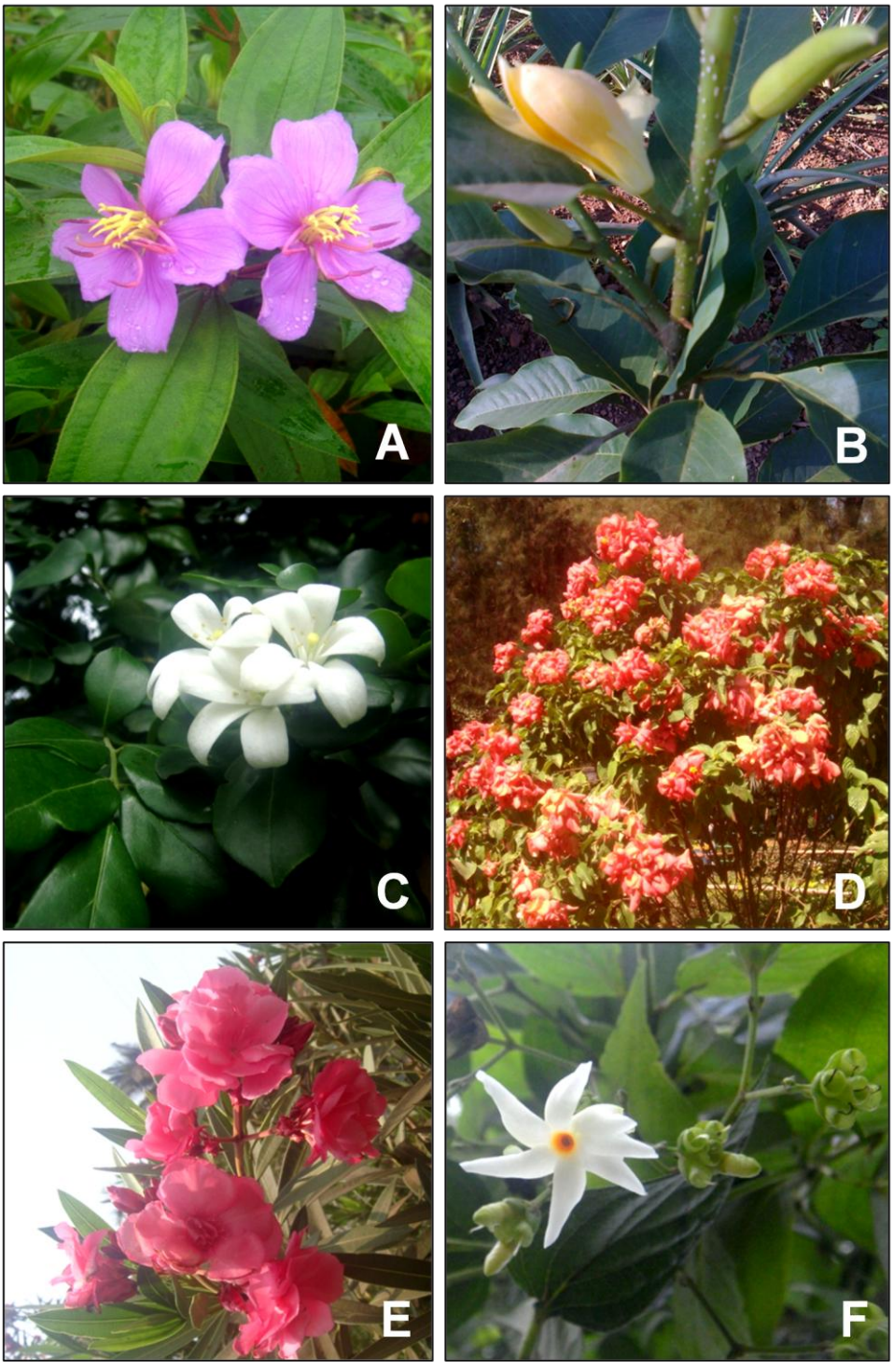


Plate 4

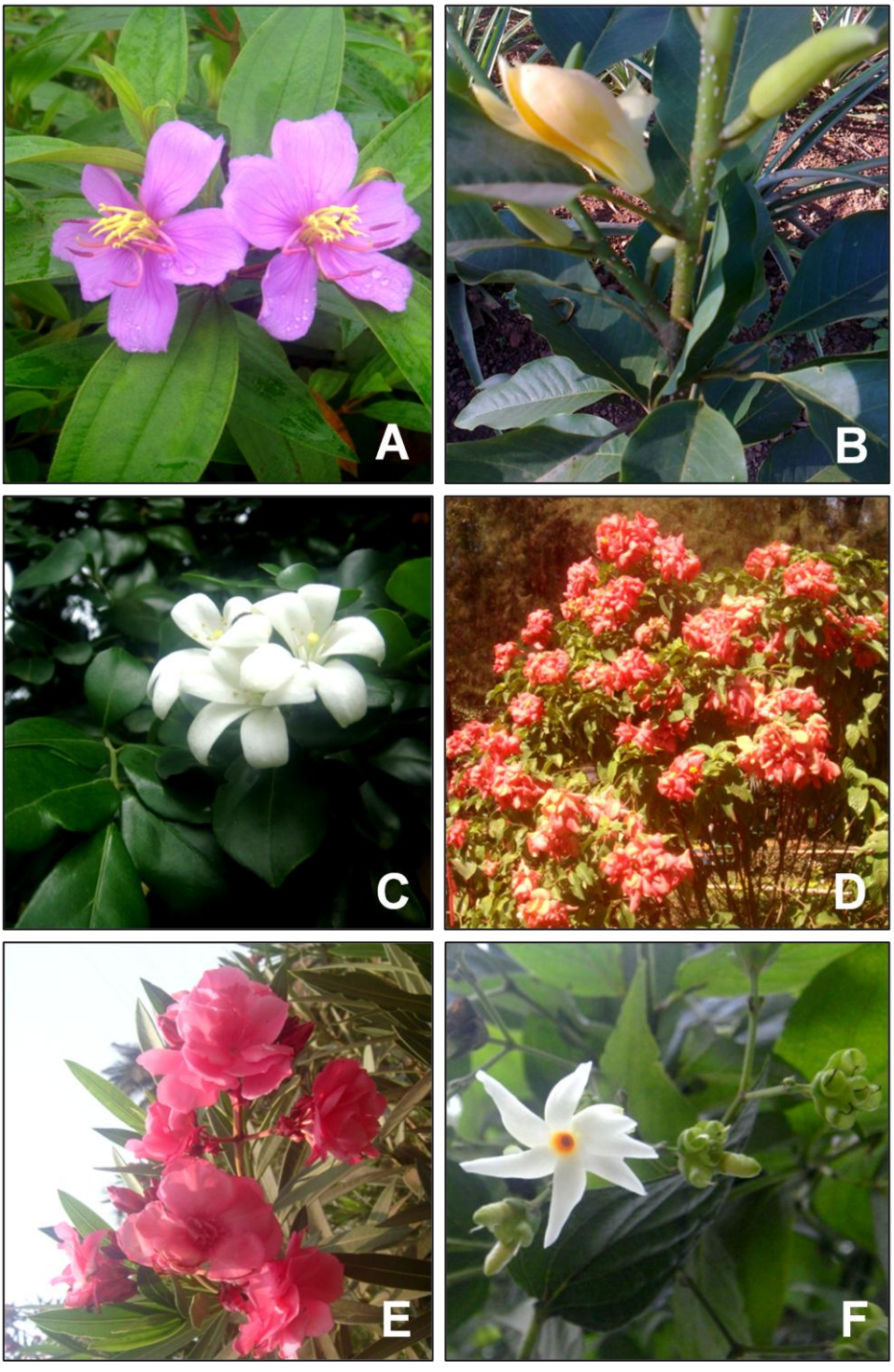


Plate 4

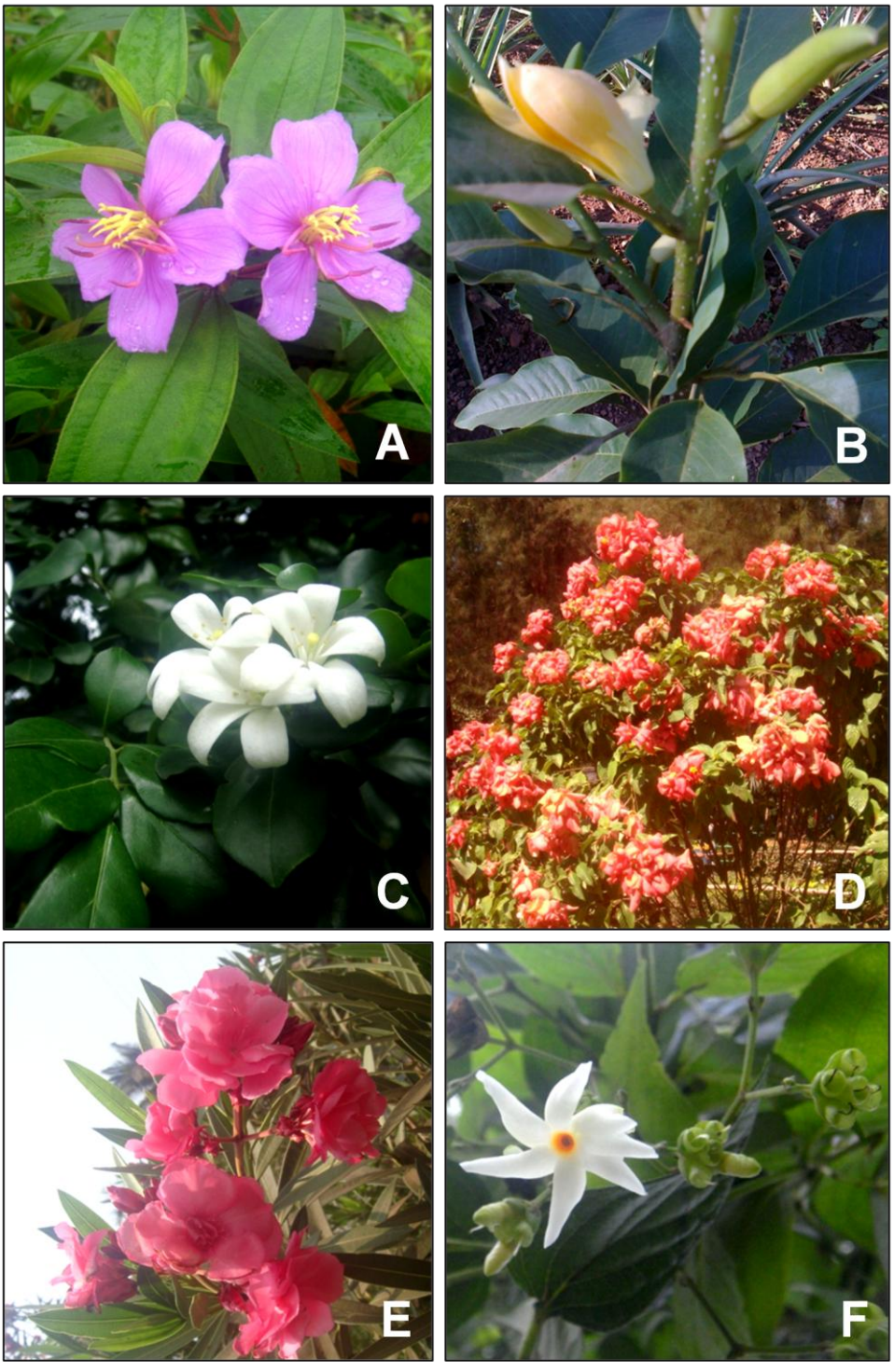


Plate 5: Ornamental plant species

A. ***Pachystachys spicata*** (Ruiz & Pav.) Wassh.

B. ***Peltophorum pterocarpum*** (DC.) K. Heyne.

C. ***Pithecellobium saman*** (Jacq.) Benth.

D. ***Plumeria rubra*** L.

E. ***Pseudoeranthemum bicolor*** (Sims) Radlk.

F. ***Rosa* sp.**

Plate 5

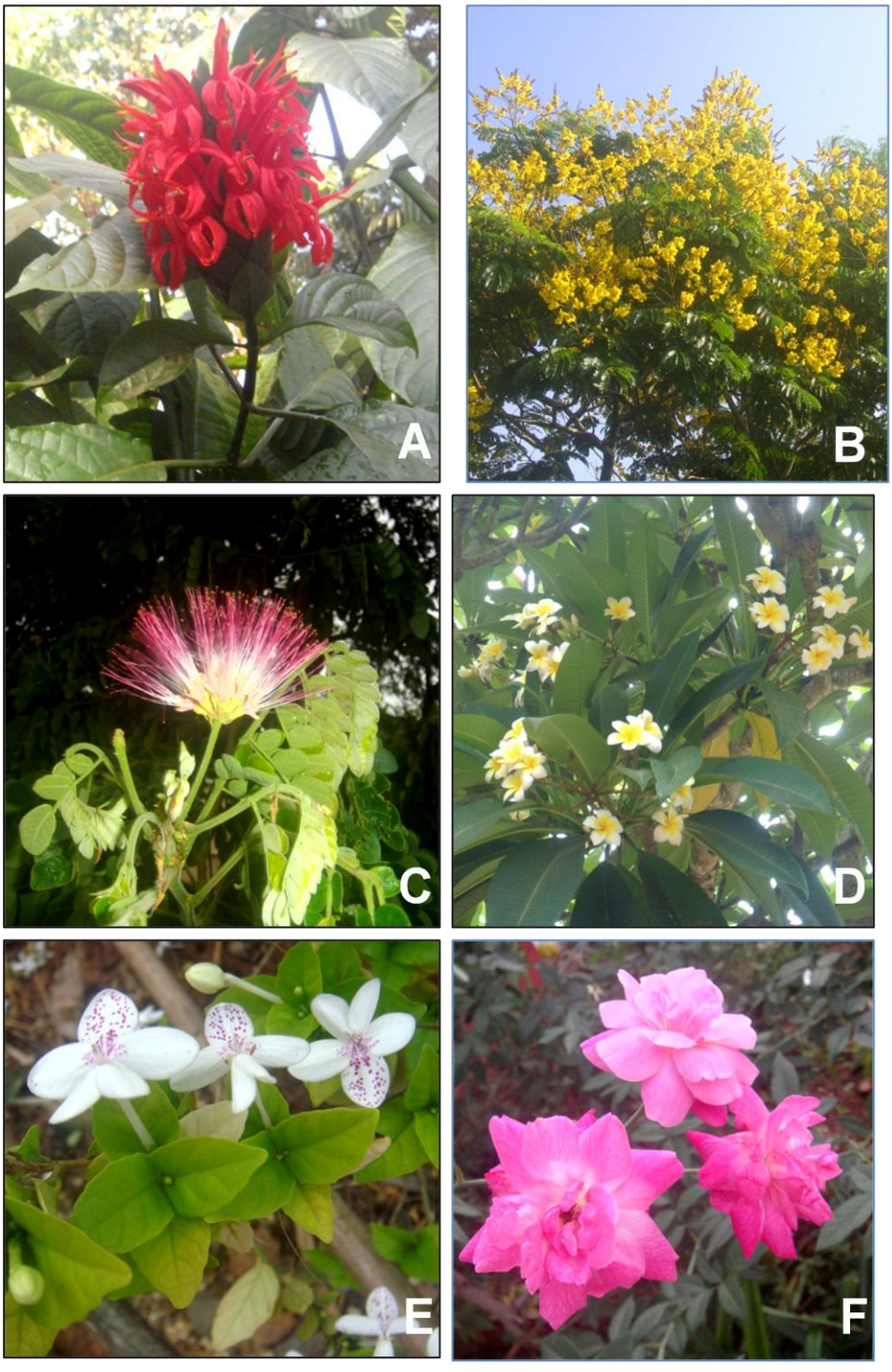


Plate 6: Ornamental plant species

A. ***Spathodea campanulata*** P. Beauv.

B. ***Tabernaemontana divericata*** L.

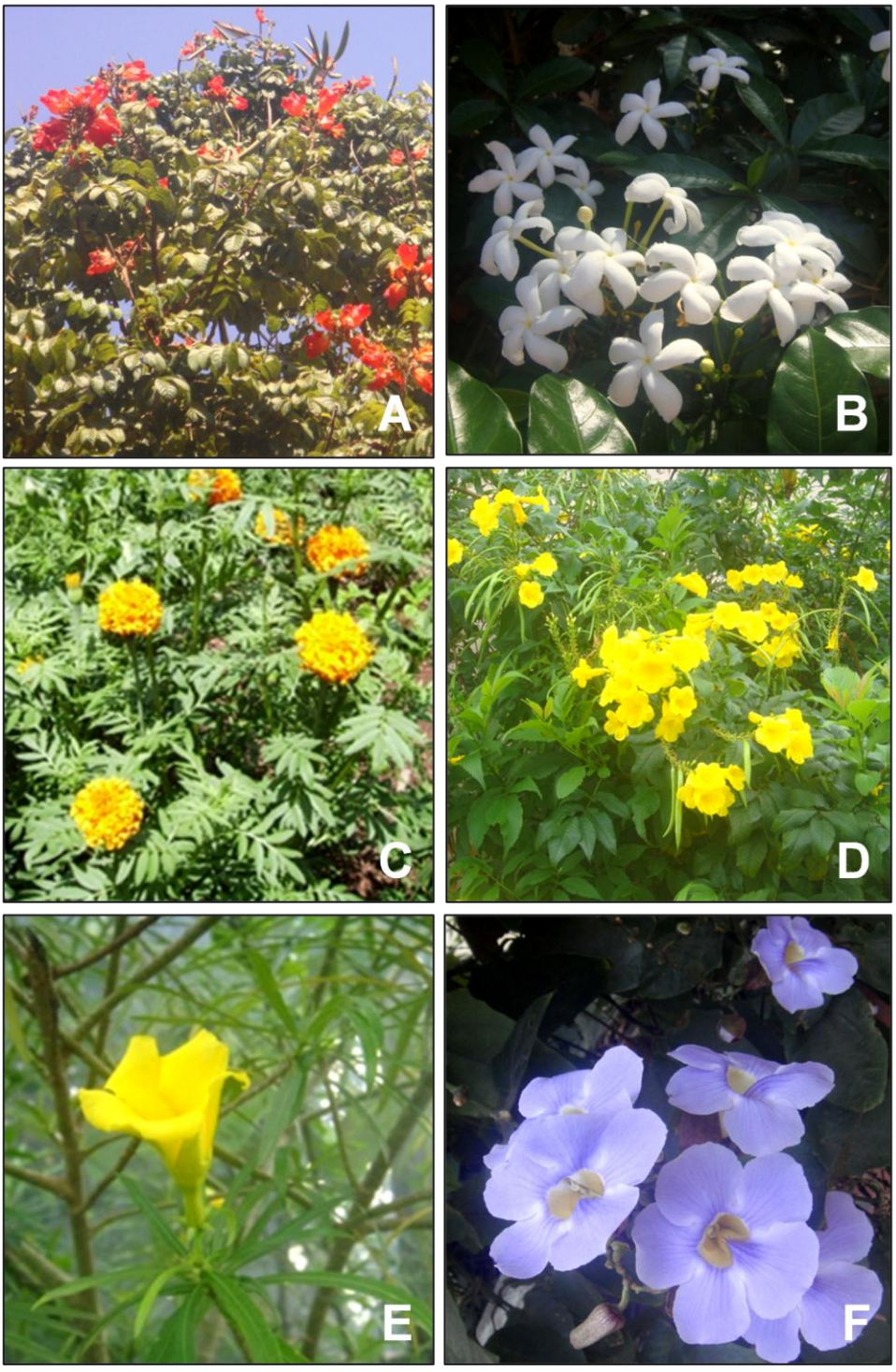
C. ***Tagetes erecta*** L.

D. ***Tecoma stans*** L. Juss. Ex. Kunth.

E. ***Thevetia nerifolia*** Juss.

F. ***Thunbergia grandiflora*** Roxb.

Plate 6



(**Table 3**). Similarly, significant differences were observed in the soil nutrient levels during pre-monsoon, monsoon and post-monsoon seasons (**Table 4**). Soil pH in monsoon season was significantly lower than in dry seasons in all the three sites. Soil Electrical conductivity, Organic carbon, P and K varied significantly ($P \leq 0.05$) between seasons and the sites. All the 43 plant species studied showed AM colonization. The results show that although the extent of AM colonization was lower, roots of all the selected plants showed the presence of mycorrhizal structures necessary in AM symbiosis. The mycorrhizal structures present included abundant intraradical hyphae (**Plate 7A–C**), arbuscules (**Plate 7D–F**), vesicles (**Plate 8A–D**), hyphal coils and/or sporadic internal spores in roots of investigated plants (**Plate 8E & F**).

Intensity of AM colonization in all replicates of the plant species studied ranged from 4 to 99%. Maximum percentage of root colonization was recorded in *C*. *indica* (99%) and minimum in *M*. *paniculata* (4%). Of the 43 plant species, 20 species showed vesicular, arbuscular as well as hyphal colonization (**Table 5 & 6**). Average root colonization was higher in garden plants (52.63%) than in wild plants (44.47%). In garden plants *C*. *indica* exhibited highest colonization (99%) and the lowest was recorded in *M*. *paniculata* (4%) (**Fig. 2**). Among the wild plants, *P*. *saman* exhibited highest root colonization (72%) and was least in *C*. *pulcherrima and M*. *malabathricum* (28%) (**Fig. 3**). In garden plants a positive significant correlation was found between root colonization and soil P ($r = 0.815$) and a negative significant correlation between root colonization and pH ($r = -0.996$). Colonization and organic carbon ($r = -0.579$) and colonization and K ($r =$ -

Table 3: Physical and chemical characteristics of soils from garden and wild habitats.

Sr. No.	Soil parameter	Habitat	
		Garden	Wild
1.	pH	6.00 ±0.10	6.70 ±0.10
2.	Electrical conductivity (m/mhos)	0.12 ±0.01	0.19 ±0.01
3.	Organic Carbon (%)	1.74 ±0.02	1.05 ±0.02
4.	Available P (Kg/Ha)	8.90 ±0.06	104.30 ±0.32
5.	Available K (Kg/Ha)	224 ±4.00	436.80 ±0.12

Legend: Values are mean of 3 replicates, ± indicates standard deviation.

Table 4: Seasonal variation on soil characteristics in selected study sites.

Sr. No.	Study site	Season	Soil characteristics				
			pH	EC	Organic carbon %	Available P (Kg Ha^{-1})	Available K (Kg Ha^{-1})
1.	Coastal	Monsoon	6.1^{d} ±0.06	0.016^{g} ±0.00	0.32^{h} ±0.01	1.05^{d} ±0.01	89.60^{h} ±0.10
		Post-monsoon	6.2^{cd} ±0.06	0.073^{f} ±0.00	0.35^{g} ±0.01	5.96^{b} ±0.02	89.60^{h} ±0.10
		Pre-monsoon	7.2^{a} ±0.10	0.727f ±0.00	0.97^{f} ±0.01	2.98^{c} ±0.01	380.80^{c} ±0.12
2.	Plateau	Monsoon	5.1^{f} ±0.06	0.143^{d} ±0.00	1.37^{d} ±0.01	0.99^{e} ±0.00	336.00^{e} ±0.06
		Post-monsoon	5.4^{e} ±0.06	0.141^{d} ±0.00	1.55^{c} ±0.02	1.00^{e} ±0.00	201.60^{g} ±0.06
		Pre-monsoon	5.6^{e} ±0.06	0.097^{e} ±0.00	1.67^{b} ±0.02	1.01^{de} ±0.00	246.40^{f} ±0.15
3.	Western Ghats	Monsoon	6.4^{c} ±0.10	0.175^{c} ±0.00	1.05^{e} ±0.02	2.98^{c} ±0.01	358.40^{d} ±0.15
		Post-monsoon	6.7^{b} ±0.06	0.318^{b} ±0.00	1.74^{a} ±0.01	1.49^{a} ±0.07	425.60^{b} ±0.72
		Pre-monsoon	6.9^{b} ±0.10	0.419^{a} ±0.00	1.67^{b} ±0.02	1.05^{d} ±0.00	660.80^{a} ±0.10

Legend: All values are mean of 3 replicates, ± indicates standard deviation.
Data with different letters for each season are significantly different at $P \leq 0.05$.

Table 5: Percent root colonization in garden plants.

Sr. No.	Host plant	Type of AM colonization	Root colonization (%)
1.	*Acalypha hispida*	A, H	52.3 ±1.53
2.	*Callistemon lanceolatus*	V, H	68.3 ±3.06
3.	*Canna indica*	V, A, H	99.3 ±0.58
4.	*Chrysanthemum morifolium*	V, A, H	38.0 ±2.00
5.	*Crossandra infundibuliformis*	V, A, H	66.0 ±2.00
6.	*Gardenia jasminoides*	V, H	58.3 ±1.53
7.	*Hedychium coronarium*	V, H	64.3 ±1.53
8.	*Hibiscus rosa-sinensis*	V, H	58.3 ±1.53
9.	*Ixora duffii*	V, H	34.3 ±2.08
10.	*Jasminum auriculatum*	A, H	10.3 ±1.15
11.	*Jasminum nitidum*	A, H	48.3 ±2.52
12.	*Michelia champaca*	V, A, H	24.3 ±1.15
13.	*Murraya paniculata*	A, H	04.3 ±0.58
14.	*Mussaenda frondosa*	V, A, H	52.0 ±1.00
15.	*Nyctanthus arbor-tristis*	V, H	56.3 ±0.58
16.	*Pachystachys spicata*	V, A, H	60.3 ±0.58
17.	*Pseudoeranthemum bicolor*	V, A, H	58.0 ±1.00
18.	*Rosa* sp.	V, A, H	68.0 ±1.00
19.	*Tabernaemontana divericata*	V, A, H	52.3 ±1.15
20.	*Tagetes erecta*	V, H	70.3 ±1.15
21.	*Tecoma stans*	V, H	68.3 ±2.52
22.	*Thevetia nerifolia*	A, H	50.3 ±1.53

Legend: V=Vesicular colonization, A= Arbuscular colonization, H= Hyphal colonization. Values are means of three replicates, ± indicates standard deviation.

Table 6: Percent root colonization in wild plants.

Sr. No.	Host plant	Type of AM colonization	Root colonization (%)
1.	*Allamanda cathartica*	V, H	50.3 ±1.15
2.	*Bauhinia purpurea*	V, A, H	46.3 ±1.53
3.	*Bombax ceiba*	V, H	40.3 ±2.08
4.	*Bougainvillea spectabilis*	V, A, H	54.3 ±1.53
5.	*Butea monosperma*	V, A, H	40.3 ±2.08
6.	*Caesalpinia pulcheriima*	V, A, H	28.3 ±2.08
7.	*Cassia fistula*	V, A, H	30.0 ±2.00
8.	*Clarodendron paniculatum*	V, H	62.3 ±1.53
9.	*Delonix regia*	V, A, H	42.6 ±1.15
10.	*Erythrina indica*	V, H	32.0 ±1.00
11.	*Ethalidium barlerioides*	A, H	38.3 ±2.08
12.	*Lagerstroemia purpurea*	V, A, H	38.0 ±2.00
13.	*Mammea suriga*	V, H	48.3 ±1.53
14.	*Melastoma malabathricum*	V, A, H	28.3 ±1.53
15.	*Mimusops elengi*	V, H	42.0 ±1.53
16.	*Nerium indicum*	V, A, H	60.0 ±1.00
17.	*Peltophorum pterocarpum*	V, H	32.3 ±2.08
18.	*Pithecellobium saman*	V, A, H	72.3 ±2.08
19.	*Plumeria rubra* L.	V, A, H	48.0 ±1.00
20.	*Spathodea campanulata*	V, H	48.0 ±1.00
21.	*Thunbergia grandiflora*	V, H	56.0 ±2.65

Legend: V= Vesicular colonization, A= Arbuscular colonization, H= Hyphal colonization. Values are means of three replicates, ± indicates standard deviation.

Plate 7: Root colonization by Arbuscular Mycorrhizal fungi

A. Hyphal colonization in *Canna indica* (x 100).

B. Hyphal colonization in *Hibiscus rosa-sinensis* (x 400).

C. Hyphal colonization in *Rosa* sp. (x 100).

D. Arbuscular colonization in *Rosa* sp. (x 100).

E. Arbuscular colonization in *Tagetes erecta*. (x 100).

F. Arbuscular colonization in *Canna indica* (x 400).

Plate 7

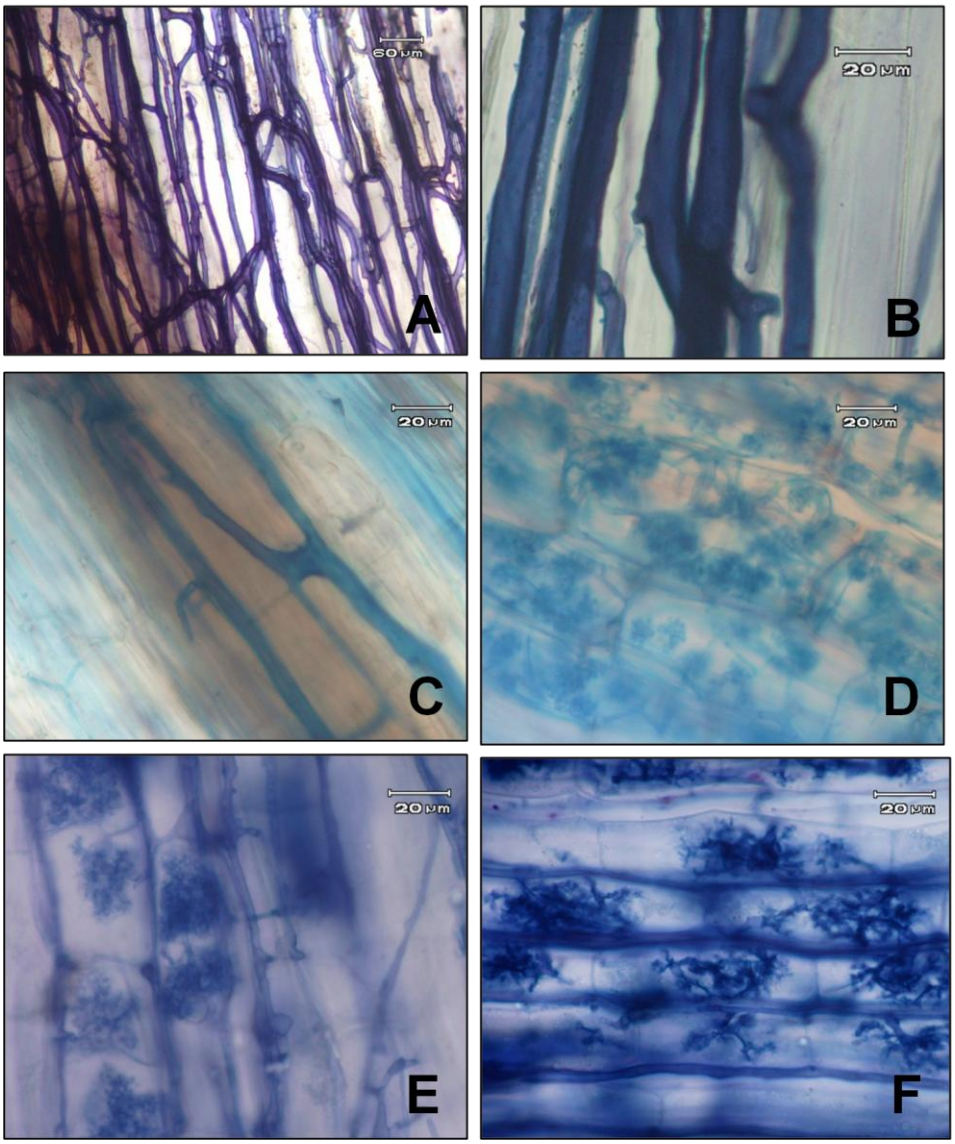


Plate 8: Root colonization and intraradical spores

A. Vesicular colonization in *Canna indica* (x 100).

B. Vesicular colonization in *Canna indica* (x 400).

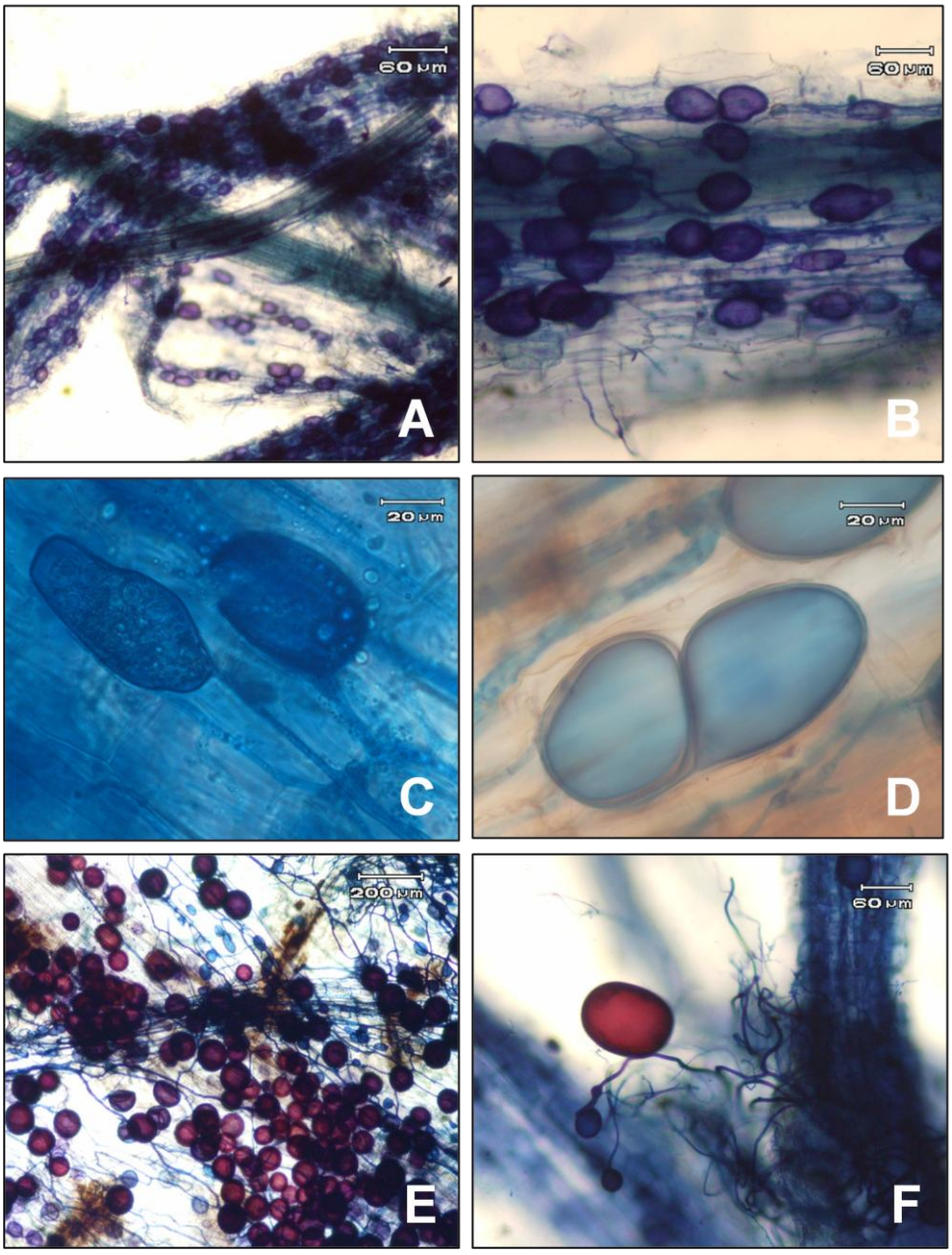
C. Vesicular colonization in *Clarodendron paniculatum* (x 400).

D. Vesicular colonization in *Rosa* sp. (x 400).

E. Intraradical spores in *Canna indica* (x 100).

F. Intraradical spores in *Canna indica* (x 400).

Plate 8



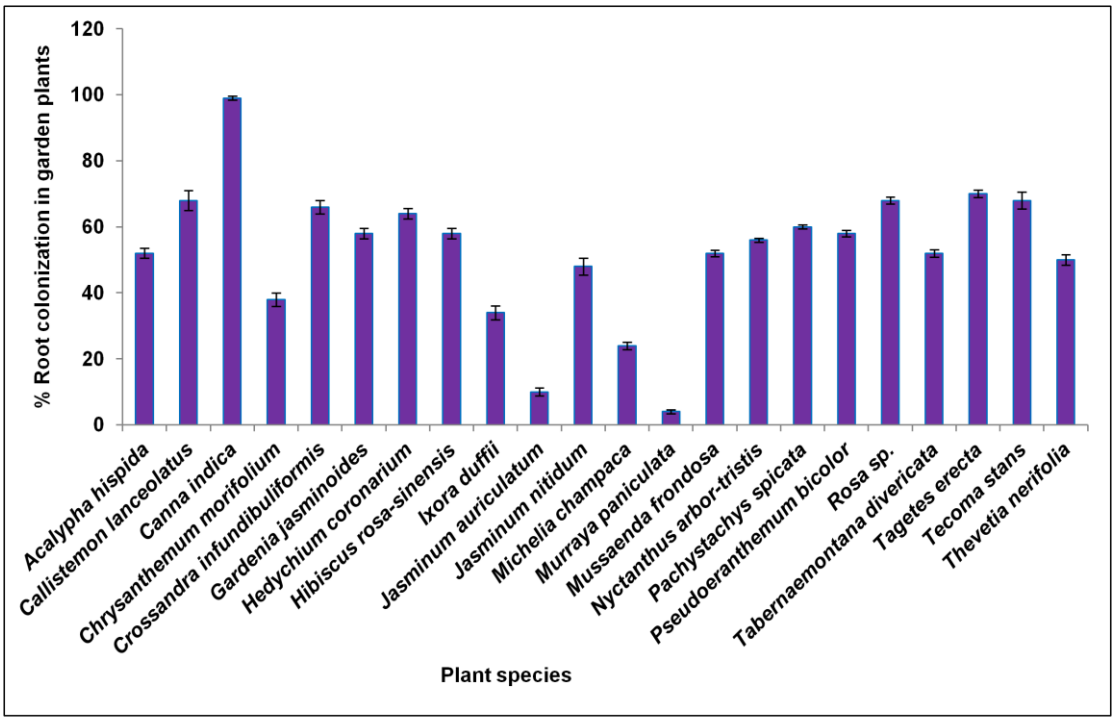


Figure 2: Arbuscular Mycorrhizal colonization in selected garden plants.

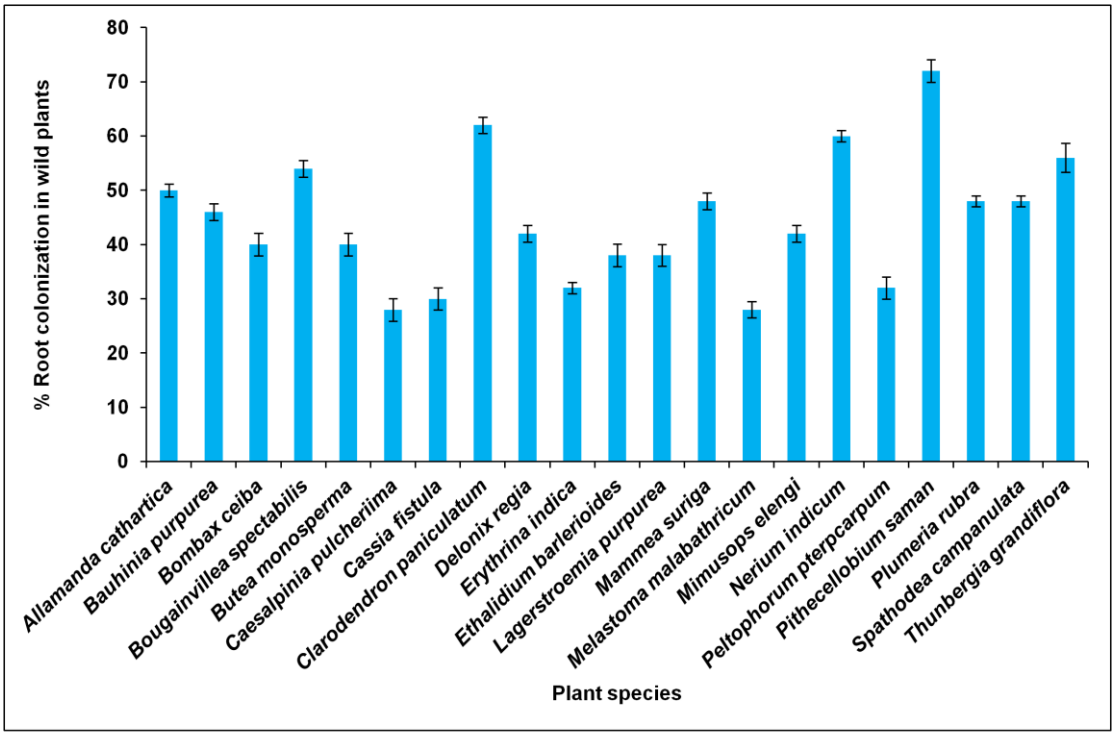


Figure 3: Arbuscular Mycorrhizal colonization in selected wild plants.

Table 7: seasonal variation in percentage root colonization in selected flowering plants from 3 distinct regions in Goa.

Sr. No.	Season	Average root colonization (%)	Study site	Root colonization (%)
1.	Monsoon	$39.45^{ab} \pm 4.88$	Coastal	$42.66^{a} \pm 1.66$
			Plateau	$33.83^{b} \pm 3.99$
			Western Ghats	$41.88^{a} \pm 2.20$
2.	Post-monsoon	$46.96^{a} \pm 6.42$	Coastal	$47.47^{a} \pm 3.81$
			Plateau	$40.50^{b} \pm 2.91$
			Western Ghats	$52.83^{a} \pm 1.83$
3.	Pre-monsoon	$31.30^{b} \pm 4.97$	Coastal	$31.91^{ab} \pm 3.36$
			Plateau	$26.05^{b} \pm 4.10$
			Western Ghats	$35.94^{a} \pm 1.83$

Legend: Values are mean of 3 replicates, ± indicate standard deviation.
Data with different letters for each season are significantly different at $P \leq 0.05$.

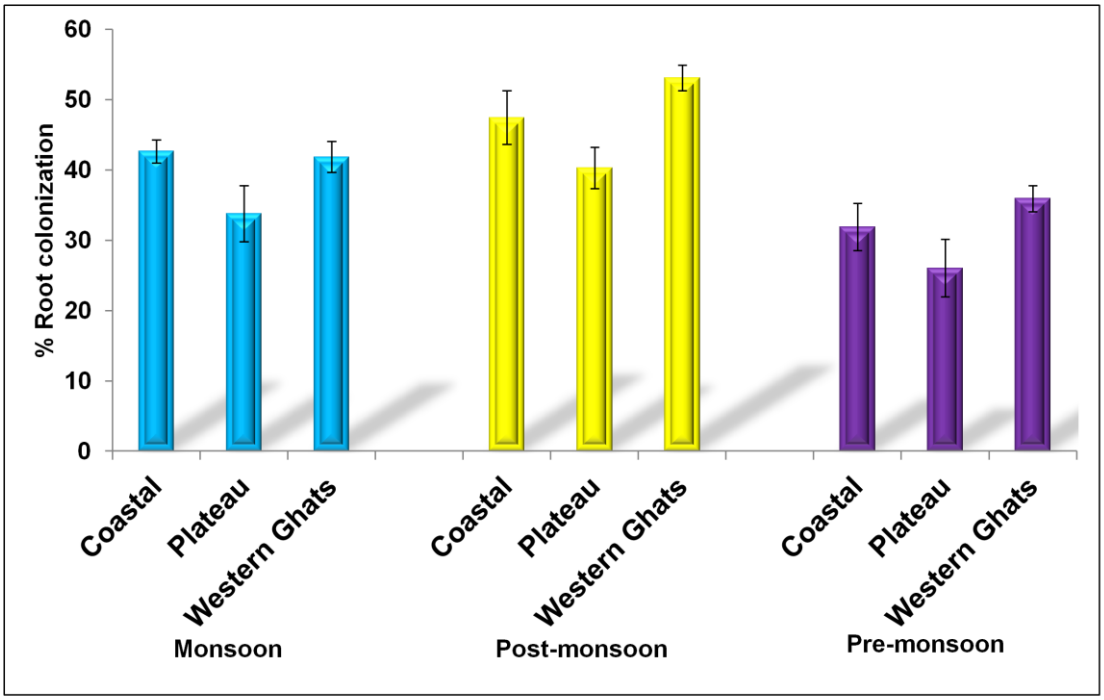


Figure 4: Influence of seasonal variation on percent root colonization in the selected study sites.

0.417) were found to be non-significant. In wild plants, a positive correlation between root colonization and soil pH (r = 0.129), P(r = 0.959) and K (r = 0.129) and negative correlation between root colonization and organic carbon (r = -0.625) was observed.

Root colonization varied significantly between the seasons and between the study sites. The statistical analyses revealed a seasonal and sampling site effect on the extent of root colonization (**Table 7**). Seasonal studies showed that the degree of root colonization was least during pre-monsoon season (31.3%), increased during monsoon (39.45%) and reached maximum during post-monsoon season (46.96%). Plants species growing in Western Ghats region showed maximum root colonization followed by plant species growing in coastal and plateau regions (**Fig. 4**).

3.4 DISCUSSION

In the present study a survey of ornamental flowering plant species commonly found in Goa was undertaken to examine symbiosis between AM fungi and roots. The presence of AM roots at all the study sites and root colonization intensity reveals that the AM association is naturally established as reported in earlier studies (Khaliel and Abou-Heilah, 1985; Al-Whaibi and Khaliel, 1994; Bouamri *et al*., 2006). Variation in the intensity of AM colonization was recorded in the plants species surveyed. Of the two habitats examined, the extent of AM colonization was higher in garden plants than in wild plants. An increase in the colonization levels in garden plants may possibly be due to watering that assists germination of AM spores in the rhizosphere. Soil water content can have variable effects on AM spore germination and thus

colonization. Soil wetting and drying cycles are the most important parameters known to affect survival, germination and thus colonizing ability of AM fungi in nature (Braunberger *et al*., 1996). Soil moisture is positively correlated with AM fungal colonization (He *et al*., 2002; Bohrer *et al*., 2004; Lingfei *et al*., 2005, Oliveira and Oliveira, 2005). The present study revealed variation in AM root colonization extent among the plant species. This variation in colonization extent may be due to the fact root sampling was carried out only in the blooming stage that varied for each plant species. Lugo *et al*. (2003) suggested that physiology, growth rate and turnover of plant roots are among the key factors contributing to variation in AM fungal colonization.

In the present study a positive significant correlation between the root colonization and soil P was reported in garden plants. High levels of colonization in soils rich in available P and the apparent insensitivity of AM fungal colonization to the incorporation of P fertilizers have been reported earlier (Vosátka, 1995; Hamel *et al*., 1996; Ryan and Ash, 1999). Contrasting findings however, where a reduction in AM colonization levels in roots was observed due to increased nutrient inputs to soil especially P, decreasing the AM fungal potential in the soil, have been reported (Schwab, 1983; Guillemin, 1995; Smith and Read, 1997; Kaushal, 2000; Mohammad *et al*., 2003; Smith and Read, 2008). Long-term P fertilization, even at low levels, can reduce mycorrhiza formation (Mäder *et al*., 2000; Bending *et al*., 2004). The application of P fertilizer decreases the rate of root colonization and the density of AM fungi in soil (Isobe and Tsuboki, 1998; Mohammed *et al*., 1998; Lekberg and Koide, 2005; Duan *et al*., 2010). Moreover, the use of other

readily soluble fertilizers, particularly nitrogen fertilizers, has similarly been found to have a negative impact on AM colonization in some plant species (Liu *et al*., 2000; Burrows and Pfleger, 2002; Treseder and Allen, 2002), though not in others (Ryan and Ash, 1999; Jumpponen *et al*., 2005).

A negative significant correlation between pH and percent colonization in garden habitat indicated that root colonization increased with increase in pH in fairly acidic soils which is in accordance with the studies of Wiseman and Wells (2005) who recorded a greater colonization in acid forest than less acidic landscape sites. However, Sunilkumar and Garampalli (2010) found significant positive correlation between soil pH and percentage colonization. pH and temperature differences in spore germination of AM fungal species are related to the moisture conditions of the environment to which they are ecologically adapted. Therefore it is not possible to derive conclusions without knowing germination responses of several isolates of a species, each from environments with widely different moisture regimes to different soil matrix potentials (Giovannetti *et al*., 2010).

The positive correlation between soil K and root colonization in wild plants observed in the present study suggest that the slow diffusion of K ions in the soil may favour spore germination and thus increase root colonization. Soil K is reported to have a stimulatory effect on AM variables (Furlan *et al*., 1989; Ouimet *et al*., 1996) and a minimum soil K is often a pre-requisite of mycorrhizal colonization in some plant species (Ouimet *et al*., 1996; Gamage *et al*., 2004).

In the seasonal variations study a significant difference in the soil nutrient levels was recorded in all the seasons. It was observed that soil acidity decreases in the monsoon season. Similarly electrical conductivity, organic carbon, P and K concentration also decreased after the dry season in most of the cases. Earlier studies suggest that soil nutrient concentrations vary spatially and temporally in all ecosystems (Lodge *et al*., 1997; Oliveira *et al*., 2001; Oliveira and Oliveira, 2010). Consistent seasonal patterns of AM colonization in several plant species have been reported earlier (Sanders and Fitter, 1992; Wright *et al*., 1998; Muthukumar and Udaiyan, 2002; Oleiveira and Oleiveira, 2005). Extent of root colonization varied significantly between the seasons and also between the study sites. Plant species growing in Western Ghats region showed greater root colonization than those growing in coastal and plateau regions. The extent of root colonization is known to vary with soil and climatic factors (Rajan *et al*., 2000). Root colonization was least during pre-monsoon season, increased during monsoon and reached maximum during post-monsoon. Sivakumar (2012) also recorded least colonization in the pre-monsoon season. However Oleiveira and Oleiveira (2010) recorded maximum root colonization during monsoon season. The present study showed negative correlation only in garden plants between pH and AM colonization indicating a possibe increase in the extent of AM association in fairly acidic soils.

Seasonal variation in root colonization extent can be due to soil moisture which is positively correlated with AM fungal colonization (He *et al*., 2002; Bohrer *et al*., 2004; Lingfei *et al*., 2005, Oliveira and Oliveira, 2005),

exudation of toxic metabolites and the production of easily oxidizable compounds (St. John and Coleman, 1983; Koske, 1987). The quality and source of exudates is known to play an important role in triggering spore germination. After germination, the spores must find a host root in their vicinity, to trigger the subsequent colonization stages. Evidence suggest that roots emit a volatile signal that stimulates the directional growth of the AM fungus toward them (Koske, 1982). Although these factors play a decisive role in colonization, several edaphic and climatic factors are also essential to influence the root colonization process (Giovannetti, 1985). It has also been reported that the community structure of AM fungi may determine host plant community's association and production (van der Heijden *et al*., 1998a). The influence of climatic factors on AM fungal colonization, development and spore numbers in natural ecosystems has been described earlier (Saito and Kato, 1994; Udaiyan *et al*., 1996; Muthukumar and Udaiyan, 2002; Staddon *et al*., 2003; Lingfei *et al*., 2005). It may be concluded that seasonal variations influence the extent of AM association in ornamental flowering plants.

CHAPTER 4

ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY IN THE RHIZOSPHERE OF ORNAMENTAL FLOWERING PLANTS OF GOA

4. 1 INTRODUCTION

Arbuscular Mycorrhizal fungi are known for their relationships with plants and AM fungal biogeography is primarily defined by the global distribution of known host plants and plant-defined biomes (Allen *et al*., 1995; Öpik *et al*., 2010). The knowledge about the roles of factors such as spatial, environmental and biological mechanisms that limit the distributions in microbial biogeography is scarce (Martiny *et al*., 2006). Some micro-organisms such as algae, fungi, bacteria, etc. exhibit species-area relationships and co-occurrence patterns that are equivalent to those of macro-organisms such as arthropods, birds, etc. (Horner-Devine *et al*., 2004, 2007; Peay *et al*., 2007), while other microbial taxa (<1 mm in size) have more cosmopolitan distributions (Finlay, 2002; Fenchel and Finlay, 2004). This was based primarily on the assumption that the high local abundance of microbes increases the probability that individual microbes may travel a long distance and successfully colonize a remote location simply by chance. In particular, the biogeography of AM fungi remains relatively unknown at the global scale despite recent advances in understanding global distributions of other microorganisms (Horner-Devine *et al*., 2004; Martiny *et al*., 2006). Arbuscular Mycorrhizal associations are the most frequent symbiosis found in nature because of their broad association with plants and cosmopolitan distribution (Harley and Smith, 1983). The biomass and community compositions of AM fungi differ with respect to biome, invasive plants and plant species richness (Helgason *et al*., 2002; Hawkes *et al*., 2006; *Ö*pik *et al*., 2006; Treseder and Cross, 2006; Kivlin and Hawkes, 2011) thus indicating that the spatial variation in plant community structure at many scales may influence the

distribution of AM fungal species. Dispersal limitation, environmental filtering, and biotic interactions between AM fungal species also contribute to their biogeography (Lekberg *et al*., 2007; Dumbrell *et al*., 2010). Some AM fungi produce relatively large spores that are dispersed over intermediate ranges and their hyphae can be dispersed over smaller areas (Warner *et al*., 1987; Mangan and Adler, 2000). The human-mediated introduction of microorganisms in soil and plant inoculum also results in large-scale dispersal of AM fungi (Schwartz *et al*., 2006; Vellinga *et al*., 2009).

Agricultural intensification, including conventional use of pesticides has resulted in biodiversity losses worldwide (Butler *et al*., 2007). Distribution and composition of AM fungi is also affected by many environmental parameters such as soil type and texture, disturbance, moisture, temperature and nutrient availability (Pringle and Bever, 2002; Rillig *et al*., 2002; Hawkes *et al*., 2006; Lekberg *et al*., 2007). Studies on AM fungal diversity in plants with respect to garden and wild habitats is thus essential. Plants growing in garden habitat usually get sufficient moisture and nutrients in the form of organic manures or fertilizers. However the plants from wild habitats rely solely on rain water. Arable fields have a low taxonomic diversity of AM fungi (Helgason *et al*., 1998), as the high use of chemical fertilizers, pesticides and intensive land management practices common in conventional agriculture result in decreased AM fungal diversity and abundance (Douds and Millner, 1999). Burrows and Pfleger (2002) found that increasing plant diversity had a positive effect on AM fungal sporulation and community composition due to the higher number of possible host-fungal pairings, and increased density of

plant roots available for colonization. In addition, Helgason *et al*. (1998) found that woodlands show much higher AM fungal species richness and diversity when compared to agricultural fields.

The interaction between tree and crop roots can also have an effect on AM fungi and therefore play an important role in the functioning and productivity of agroecosystems (Plenchette *et al*., 2005). Arbuscular Mycorrhizal fungi influence plant diversity and community structure (Grime *et al*., 1987; van der Heijden *et al*., 1998a, Bever *et al*., 2001; Klironomos, 2003; van der Heijden *et al*., 2007) and can induce different growth response in plants and thus play a potential role to determine plant biodiversity, ecosystem variability. The fungi have a significant effect in improving crop growth and productivity. Therefore AM fungi are considered one of the most important components of various ecosystems and play a crucial role in regulating the response of ecosystems to changing biotic and abiotic conditions (Landis *et al*., 2004).

Information on AM fungal diversity associated with ornamental flowering plants in India is scarce. High diversity of AM fungi was observed in *Tagetes erecta* L. (Ranganayaki and Manoharachary, 2001), *Rhododendron* spp., (Chaurasia *et al*., 2005) and *Hibiscus rosa-sinensis* L. (Radhika and Rodrigues, 2010). In the past few years, interest in the application of AM fungi to grow floricultural plants has been increasing all over the world and so in India. Production of large quantities of AM spores for nursery inoculation and collection of various AM fungi for inoculum production are key steps in the

development of AM biofertilizers. For this, a survey of the rhizosphere soils is an important pre-requisite. Diversity studies also help to understand the ecology of the habitat and to develop conservation strategies. The present work was carried out to determine AM fungal diversity, species richness, frequency of isolation and relative abundance in various ornamental flowering plant species commonly found in Goa and to study whether host plant species, soil conditions and seasonal variations influence the AM fungal species composition.

4.2 MATERIALS AND METHODS

4.2.1 Study sites and collection of rhizosphere samples: Rhizosphere soil samples from 43 ornamental plant species growing in garden and wild habitat were collected during the flowering stage, from a depth of 10–20 cm from 35 different localities of Goa. To study the effect of seasonal variation on AM spore density with respect to different sites, soil samples from 12 plant species *viz*., *Chrysantheumum morifolium*, *Crossandra infundibuliformis*, *Delonix regia*, *Erythrina indica*, *Gardenia jasminoides*, *Hedychium coronarium*, *Hibiscus rosa-sinensis*, *Jasminum nitidum*, *Mammea suriga*, *Michelia champaca*, *Nyctanthes arbor-tristis* and a *Rosa* sp. from three different sites *viz*. Coastal (Arambol), Plateau (Bethora) and Western Ghats (Talauli) were collected during pre-monsoon (April), monsoon (August) and post-monsoon season (December) for the period from 2008–2010. Soil samples were placed in polyethylene bags, labeled and brought to the laboratory and stored at 4°C until processing.

4.2.2 Extraction of AM fungal spores: Extraction of AM fungal spores and sporocarps from the rhizosphere soil samples was carried out using wet sieving and decanting procedure (Gerdemann and Nicolson, 1963). Rhizosphere soil (100g) was suspended in 1000 ml of tap water, stirred for 10–15 seconds and the coarse particles were allowed to settle at bottom for 1–2 minutes. The suspension was then poured through sieves arranged in descending order of mesh size ranging from 60 μm to 240 μm. The above steps were repeated thrice to ensure maximum extraction of spores from the soil sample. Debris containing spores from each sieve was collected separately in a beaker and filtered through Whatman paper. The contents of the filter paper were used for isolation of AM spores and sporocarps under a stereo-microscope.

Spores were also isolated from the soil sample using a combination of wet sieving and sucrose density gradient techniques (Daniels and Skipper, 1982). The sievings collected by wet sieving and decanting technique were re-suspended in saturated sucrose solution (50%) and centrifuged at 1000 rpm for 4 min. The layer of sucrose containing spores was then pipetted out using an autopipette and filtered through a Whatman filter paper, washed and placed in a Petri-plate for further isolation.

4.2.3 Quantification of AM fungal spores from soil: Quantification of AM fungal spores from soil was carried out using the method described by Gaur and Adolheya (1994). Whatmann filter paper No. 1 was folded dividing it into two equal halves. This was followed by a second fold resulting into four equal parts. The filter paper was re-opened and two lines were drawn to divide it

into four equal quadrants. Vertical lines were drawn on one half of the filter paper so as to divide it into approximately ten columns with each column about 1 mm apart. Each column was numbered and the direction of counting marked with arrows. The filter paper was then folded in such a way that the marked portion is the receiving surface for the sample during filtration. Thus the spores are collected only on the marked surface of the filter paper, the unexposed portion remain devoid of the spores. This filter paper with the spores and other debris was then spread on a Petri-plate and observed under the stereo microscope. The spores in every column between the numbered lines were counted by moving the Petri-plate. Thus spore density/100g soil sample was calculated.

The spores were recovered under the dissecting microscope, separated according to their morphotype and evaluated for their respective relative abundance and frequency of occurrence using the formulae by Beena *et al*., (2000) as given below:

Frequency of Occurrence (%) = (Number of soil samples that possessed spores of particular AM species/Total number of soil samples screened) x 100

Relative Abundance (%) = (Number of spores of particular AM species/Total number of spores of all the AM species) x 100

4.2.4 Establishment of pot cultures: Establishment of pot cultures using rhizosphere soil samples was carried out using the method by Gilmore (1968). *Solenostemon scutellarioides* (L.) Codd was used as the trap plant. Healthy cuttings were sterilized with calcium hypochlorite (1%) for 10 minutes and rinsed three times in sterile distilled water. Pot cultures were established in a

polyhouse using 100g of rhizosphere soil along with plant root bits mixed with an equal quantity of sterilized sand. The roots of host plants were checked for AM colonization after 45 days. Pot cultures were grown during two successive cycles of 4 months each under controlled growing conditions of 25–27°C temperature, 50% humidity and day/night period of 16/8h. Plants were watered 2–3 times a week and Hoagland's solution (Hoagland, 1939) without P was added once every two weeks. Phosphorus was added only if signs of deficiency symptoms appeared in the leaves. The most abundant AM fungal spore morphotypes were recovered and used to establish monospecific cultures.

4.2.5 Identification of AM fungal species: Spores with similar morphotypes were separated and diagnostic slides containing intact and crushed spores and sporocarps were prepared separately in Polyvinyl alcohol lactoglycerol - PVLG (Koske and Tessier, 1983), in a mixture of PVLG and Melzer's reagent (Brundrett *et al*., 1994) and in water. Wall descriptions and terminology were based on those suggested by Walker (1983) and Walker and Vestberg (1998). Spore morphology and wall characteristics were considered for the identification of AM fungi. These characteristics were ascertained using a compound microscope (Olympus BX41). Identification of spores was carried out using various bibliographies (Schenck and Perez, 1990; Bentivenga and Morton, 1995; Walker and Vestberg, 1998; Redecker *et al*., 2000; Morton and Redecker, 2001, Rodrigues and Muthukumar, 2009) and INVAM (International culture collection of Vesicular Arbuscular Mycorrhizal Fungi) http://invam.caf.wvu.edu.

4.2.6 Photomicrography and Scanning Electron Microscopy: Photomicrographs of AM fungal spores on microslides were taken on an Olympus BX41 compound microscope fitted with a digital camera DP12. Spores were prepared for Scanning Electron Microscopy (SEM) by removing any debris adhering to the spore surface and mounting clean dry spores on an aluminium stub with double-sided transparent tape. The spores were then coated with gold and observed for SEM.

4.2.7 Soil analyses: Were carried out as described under **3.2.4**.

4.2.8 Diversity studies: Arbuscular Mycorrhizal fungal species diversity, frequency of occurrence and relative abundance in ornamental flowering plants was studied.

Simpson's Index of Diversity: 1–D

Simpson`s Index of Dominance: $D = \Sigma (P_i)^2$

Where $P_i = n_i/N$, (n_i) is the relative abundance of species.

Relative abundance was calculated as (n_i/N)100, where 'ni' is the number of individuals of given species and N is the total number of individuals in each examined sample (Simpson, 1949).

Shannon Wiener Index (H): Is commonly used to characterize species diversity in a community, which accounts for both abundance and evenness of the species present and is calculated as,

$H = -\Sigma (P_i \, ln \, (P_i)$,

Where P_i is the proportion of individuals of species *i* which contribute to the total (Shannon and Weaver, 1949).

Species richness: The number of species present in an ecosystem and is calculated by counting the number of species.

Species Evenness (E') indicates the distribution of individuals within species and is calculated as,

Σ (*H*) = H'/H'_{max} where H'_{max} = *ln S*, *S*= total number of species in the community (richness).

Frequency of occurrence (%) of each species was calculated as (si/S) 100, where si is the number of soil samples containing spores of the i^{th} species and S is the total number of soil samples examined.

4.2.9 Statistical analysis: Pearson's correlation coefficient was performed to assess the relationships between root colonization and spore density, using WASP (Web Based Agricultural Package, version 1.0 at $P \leq 0.05$).

4.3 RESULTS

The diversity of AM fungi in rhizosphere soils of ornamental plants from Goa state was investigated. A total of 129 soil samples were collected from 43 different plant species from a depth of 10–20 cm from 35 localities of Goa. In all, 44 AM fungal species belonging to ten different genera *viz*., *Acaulospora*, *Ambispora, Claroideoglomus*, *Dentiscutata*, *Funneliformis*, *Gigaspora*,

Glomus, *Racocetra*, *Rhizophagus* and *Simiglomus* were recovered (**Table 8**). Diversity of AM fungal species increased with increase in the number of host plant species sampled (**Fig. 5**). *Glomus* (13) was the dominant genus followed by *Acaulospora* (12), *Gigaspora* (5), *Rhizophagus* (4), *Dentiscutata* (3), *Funneliformis* (2), *Racocetra* (2), *Ambispora* (1), *Claroideoglomus* (1) and *Simiglomus* (1) with species number given in the parenthesis (**Fig. 6**).

The AM fungal spore density in the rhizosphere soils ranged from 23–350 spores/100g. Weak negative correlation was found between percent colonization and spore density (r = –0.1, $P \leq 0.05$). Highest spore density was recorded in *Ixora duffii* (350) and lowest spore density was found in *Murraya paniculata* (23) with the spore numbers given in parenthesis. Species richness of AM fungi ranged from 2–8 with highest species richness recorded in *I. duffii* (8) and lowest in *Caesalpinia pulcherrima* and *M. paniculata* (2) (**Table 8**). Relative abundance recorded maximum in *Rhizophagus intraradices* (14.44%) and the minimum in *Racocetra gregaria*, *R. weresubiae* and *Rhizophagus diaphanus* (0.17%). The frequency of occurrence was maximum in *R. intraradices* (55.81%) followed by *Acaulospora scrobiculata* (48.84%) and *R. fasciculatus* (41.86%), and was least in *Acaulospora soloidea*, *A. elegans*, *A. tuberculata*, *Ambispora leptoticha*, *Gigaspora rosea*, *Glomus pachycaule*, *Glomus* sp., *G. tortuosum*, *Racocetra gregaria*, *R. weresubiae* and *Rhizophagus diaphanus* (2.33%) (**Table 9**).

Average spore density/100g rhizosphere soil was higher in garden plants (84) than in wild plants (52) with the spore number given in parenthesis.

Table 8: AM fungal species and spore density in the rhizosphere of ornamental flowering plants.

Sr. No.	Host plant	AM species	Spore density $100g^{-1}$
1.	*Acalypha hispida*	*Acaulospora polonica*, *A. scrobiculata*, *Dentiscutata heterogama*, *Rhizophagus intraradices*.	43.3 ±2.08
2.	*Allamanda cathartica*	*Acaulospora scrobiculata, Claroideoglomus claroideum*, *Glomus multicaule*, *G. pachycaule*, *Rhizophagus intraradices*.	55.0 ±4.58
3.	*Bauhinia purpurea*	*Acaulospora rehmii*, *A. scrobiculata*, *Glomus pubescens*, *Rhizophagus intraradices*, *Simiglomus hoi*.	48.3 ±4.36
4.	*Bombax ceiba*	*Acaulospora foveata*, *A. myriocarpa*, *A. scrobiculata*, *Glomus sinuosum*, *Rhizophagus fasciculatus.*	72.3 ±4.04
5.	*Bougainvillea spectabilis*	*Acaulospora laevis*, *Glomus multicaule*, *Rhizophagus intraradices.*	38.0 ±3.61
6.	*Butea monosperma*	*Acaulospora foveata*, *Funneliformis mosseae*, *Glomus taiwanense*, *Rhizophagus fasciculatus*, *R. intraradices*.	36.0 ±1.00
7.	*Caesalpinia pulcheriima*	*Acaulospora foveata*, *Funneliformis geosporus*.	32.0 ±2.52
8.	*Callistemon lanceolatus*	*Acaulospora mellea*, *A. scrobiculata*, *Gigaspora albida*, *Glomus radiatum*.	88 ±4.58
9.	*Canna indica*	*Acaulospora foveata*, *Rhizophagus clarus*, *R. fasciculatus*, *R. intraradices*.	158.3 ±6.51
10.	*Cassia fistula*	*Acaulospora laevis*, *A. scrobiculata*, *A. elegans*, *Glomus aggregatum*, *G. tortuosum*, *Rhizophagus intraradices*.	86.3 ±4.04
11.	*Chrysanthemum morifolium*	*Acaulospora laevis*, *Gigaspora albida*, *Rhizophagus fasciculatus*, *R. intraradices*.	56.3 ±3.51
12.	*Clarodendron paniculatum*	*Acaulospora laevis*, *A. myriocarpa*, *Glomus aggregatum*, *G. glomerulatum*, *Rhizophagus intraradices*.	83.3 ±4.16
13.	*Crossandra infundibuliformis*	*Acaulospora foveata*, *Claroideoglomus claroideum*, *Dentiscutata heterogama Funneliformis geosporus*, *Gigaspora albida*, *Glomus multicaule*, *Rhizophagus intraradices*.	87.3 ±3.06
14.	*Delonix regia*	*Acaulospora mellea*, *A. nicolsonii*, *Gigaspora albida*, *Glomus coremioides*, *Rhizophagus fasciculatus*, *Simiglomus hoi*.	38.0 ±2.08

15.	*Erythrina indica*	*Acaulospora rehmii*, *Dentiscutata reticulata*, *Gigaspora albida*, *Gi. margarita*, *Rhizophagus fasciculatus*, *R. intraradices*.	43.3 ±4.16
16.	*Ethalidium barlerioides*	*Acaulospora foveata*, *Glomus glomerulatum*, *Racocetra weresubiae*, *Rhizophagus fasciculatus*, *R. intraradices*.	36.0 ±4.00
17.	*Gardenia jasminoides*	*Acaulospora myriocarpa*, *A. scrobiculata*, *Gigaspora albida*, *Glomus heterosporum*, *G. multicaule*.	38.0 ±3.60
18.	*Hedychium coronarium*	*Dentiscutata reticulata*, *Gigaspora albida*, *Gi. margarita*, *Glomus pubescens*, *Rhizophagus intraradices*.	130.0 ±6.00
19.	*Hibiscus rosa-sinensis*	*Acaulospora delicata*, *A. scrobiculata*, *Claroideoglomus claroideum*, *Dentiscutata nigra*, *Funneliformis mosseae*, *Glomus radiatum*, *Rhizophagus intraradices*.	60.3 ±5.13
20.	*Ixora duffii*	*Acaulospora rehmii*, *Claroideoglomus claroideum*, *Funneliformis geosporus*, *Glomus coremioides*, *G. heterosporum*, *G. multicaule*, *Rhizophagus fasciculatus*, *R. intraradices*.	350.3 ±7.09
21.	*Jasminum auriculatum*	*Acaulospora foveata*, *A. scrobiculata*, *Dentiscutata heterogama*, *Gigaspora candida*, *Glomus multicaule*.	55.3 ±3.79
22.	*Jasminum nitidum*	*Acaulospora foveata*, *A. scrobiculata*, *Claroideoglomus claroideum*, *Gigaspora albida*, *Glomus microcarpum*.	80.0 ±5.13
23.	*Lagerstroemia purpurea*	*Acaulospora polonica*, *A. scrobiculata*, *Dentiscutata nigra*, *Glomus coremioides*, *Rhizophagus fasciculatus*.	32.0 ±3.21
24.	*Mammea suriga*	*Acaulospora foveata*, *Gigaspora albida*, *Gi. rosea*, *Glomus multicaule*, *Racocetra gregaria*, *Rhizophagus diaphanus*, *R. fasciculatus*.	55.0 ±6.66
25.	*Melastoma malabathricum*	*Acaulospora delicata*, *Dentiscutata nigra*, *Funneliformis geosporus*.	30.3 ±2.08
26.	*Michelia champaca*	*Acaulospora foveata*, *A. laevis*, *A. scrobiculata*, *Gigaspora albida*, *Rhizophagus clarus*.	75.3 ±5.03
27.	*Mimusops elengi*	*Acaulospora rehmii*, *Glomus aggregatum*, *Rhizophagus fasciculatus*, *R. intraradices*.	42.0 ±6.56
28.	*Murraya paniculata*	*Acaulospora soloidea*, *Dentiscutata nigra*.	23.3 ±2.56
29.	*Mussaenda frondosa*	*Acaulospora foveata*, *A. scrobiculata*, *A. tuberculata*.	120.0 ±5.00
30.	*Nerium indicum*	*Acaulospora rehmii*, *A. scrobiculata*, *Glomus microcarpum*, *G. multicaule*,	52.0 ±3.61

		Rhizophagus fasciculatus.	
31.	*Nyctanthus arbor-tristis*	*Acaulospora polonica*, *Gigaspora candida*, *Glomus sinuosum*, *Rhizophagus fasciculatus*.	92.3 ±4.36
32.	*Pachystachys spicata*	*Acaulospora foveata*, *A. scrobiculata*, *Ambispora leptoticha*, *Glomus multicaule*.	48.3 ±0.58
33.	*Peltophorum pterocarpum*	*Acaulospora scrobiculata*, *Funneliformis mosseae*, *Glomus taiwanense*, *Rhizophagus fasciculatus*, *R. intraradices*.	50.3 ±2.08
34.	*Pithecellobium saman*	*Acaulospora mellea*, *Dentiscutata heterogama*, *Glomus multicaule*.	35.3 ±2.65
35.	*Plumeria rubra*	*Acaulospora foveata*, *A. scrobiculata*, *Gigaspora albida*, *Glomus* sp., *Rhizophagus intraradices*.	105 ±3.00
36.	*Pseudoeranthemum bicolor*	*Claroideoglomus claroideum*, *Dentiscutata nigra*, *Gigaspora ramisporophora*, *Glomus heterosporum*, *G. multicaule*.	54.3 ±4.16
37.	*Rosa s*p.	*Acaulospora foveata*, *Claroideoglomus claroideum*, *Glomus glomerulatum*, *G. multicaule*.	32.0 ±3.79
38.	*Spathodea campanulata*	*Acaulospora rehmii*, *Glomus microcarpum*, *G. sinuosum*, *Rhizophagus fasciculatus*, *R. intraradices*.	38.3 ±1.54
39.	*Tabernaemontana divericata*	*Acaulospora scrobiculata*, *A. nicolsonii*, *Rhizophagus fasciculatus*, *R. intraradices*.	74.3 ±3.51
40.	*Tagetes erecta*	*Acaulospora scrobiculata*, *Funneliformis geosporus*, *Gigaspora albida*, *Gi. ramisporophora*, *Glomus heterosporum*, *G. multicaule*, *Rhizophagus intraradices*.	78.3 ±0.58
41.	*Tecoma stans*	*Acaulospora scrobiculata*, *Gigaspora albida*, *Gi. candida*, *Glomus sinuosum*.	82.0 ±9.07
42.	*Thevetia nerifolia*	*Acaulospora foveata*, *A. scrobiculata*, *Funneliformis geosporus*, *Rhizophagus fasciculatus*, *R. intraradices*.	39.3 ±3.21
43.	*Thunbergia grandiflora*	*Acaulospora foveata*, *Funneliformis geosporus*, *Rhizophagus clarus*, *R. fasciculatus*.	72.3 ±3.06

Legend: Values are mean of three replicates, ± indicates standard deviation.

Table 9: Frequency of Occurrence and Relative Abundance of AM fungal spores in 43 ornamental flowering plants.

Sr. No.	AM species	Frequency of Occurrence (%)	Relative Abundance (%)
1.	*Acaulospora delicata* Walker, Pfeiffer & Bloss	4.65	0.51
2.	*Acaulospora elegans* J.W. Trappe & Gerdemann	2.33	0.81
3.	*Acaulospora foveata* Trappe & Janos	37.21	7.07
4.	*Acaulospora laevis* Gerdemann & Trappe	11.63	1.69
5.	*Acaulospora mellea* Spain & Schenck	6.98	0.85
6.	*Acaulospora myriocarpa* Spain, Sieverding & N.C. Schenck	6.98	1.29
7.	*Acaulospora nicolsonii* C. Walker, L.E. Reed & F.E. Sanders	4.65	0.54
8.	*Acaulospora polonica* Blaszk.	6.98	0.88
9.	*Acaulospora rehmii* Sieverding & S. Toro	13.95	2.03
10.	*Acaulospora scrobiculata* Trappe	48.84	11.33
11.	*Acaulospora soloidea* Vaingankar & B.F. Rodrigues sp. nov.	2.33	0.30
12.	*Acaulospora tuberculata* Janos & Trappe	2.33	0.51
13.	*Ambispora leptoticha* (Schenck and Smith) Morton & Redecker	2.33	0.24
14.	*Claroideoglomus claroideum* (N.C. Schenck & G.S. Smith) C. Walker & A. Schüβler	16.28	2.87
15.	*Dentiscutata heterogama* (Gerdemann & Trappe) Almeida & Schenck	9.30	1.08
16.	*Dentiscutata nigra* (J.F. Redhead) Sieverding, F.A. Souza & Oehl	11.63	1.25
17.	*Dentiscutata reticulata* (Koske, D.D. Miller & C. Walker) Sieverding, F.A. Souza & Oehl	4.65	0.58
18.	*Funneliformis geosporus* (T.H. Nicolson & Gerdemann) C. Walker & A. Schüβler	13.95	3.58
19.	*Funneliformis mosseae* (T.H. Nicolson & Gerdemann) C. Walker & A. Schüβler	6.98	0.64
20.	*Gigaspora albida* Schenck & Smith	30.23	7.85
21.	*Gigaspora candida* Bhattacharjee, Mukerji, Tewari & Skoropad	6.98	1.49
22.	*Gigaspora margarita* Becker & Hall	4.65	1.29
23.	*Gigaspora ramisporophora* Spain, Sieverding & Schenck	4.65	0.61
24.	*Gigaspora rosea* Nicolson & Schenck	2.33	0.44
25.	*Glomus aggregatum* Schenck & Smith emend. Koske	6.98	1.01
26.	*Glomus coremioides* (Berk. & Broome) Redecker & Morton	6.98	3.45
27.	*Glomus glomerulatum* Sieverding	6.98	1.18
28.	*Glomus heterosporum* Smith & Schenck	9.30	2.43
29.	*Glomus microcarpum* Tulasne & Tulasne	9.30	1.62

30.	*Glomus multicaule* Gerdemann & Bakshi	30.23	6.15
31.	*Glomus pachycaule* (C.G. Wu & Z.C. Chen) Sieverding & Oehl	2.33	0.44
32.	*Glomus pubescens* (Saccardo & Ellis) Trappe & Gerdemann	4.65	0.47
33.	*Glomus radiatum* (Thaxter) Trappe & Gerdemann	4.65	0.68
34.	*Glomus sinuosum* (Gerdemann & Bakshi) Almeida & Schenck	9.30	1.62
35.	*Glomus* sp.	2.33	0.01
36.	*Glomus taiwanense* (Wu & Chen) Almeida & Schenck	9.30	0.51
37.	*Glomus tortuosum* Schenck & Smith	2.33	0.51
38.	*Racocetra gregaria* (N.C. Schenck & T.H. Nicolson) Oehl, F.A. Souza & Sieverding	2.33	0.17
39.	*Racocetra weresubiae* (Koske & C. Walker) Oehl, F.A. Souza & Sieverding	2.33	0.17
40.	*Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüβler	6.98	2.77
41.	*Rhizophagus diaphanus* (J.B. Morton & C. Walker) C. Walker & A. Schüβler	2.33	0.17
42.	*Rhizophagus fasciculatus* (Thaxter) C. Walker & A. Schüβler	41.86	10.92
43.	*Rhizophagus intraradices* (N.C. Schenck & G.S. Smith) C. Walker & A. Schüβler	55.81	14.44
44.	*Simiglomus hoi* (S.M. Berch & Trappe) G.A. Silva, Oehl & Sieverding	9.30	0.54

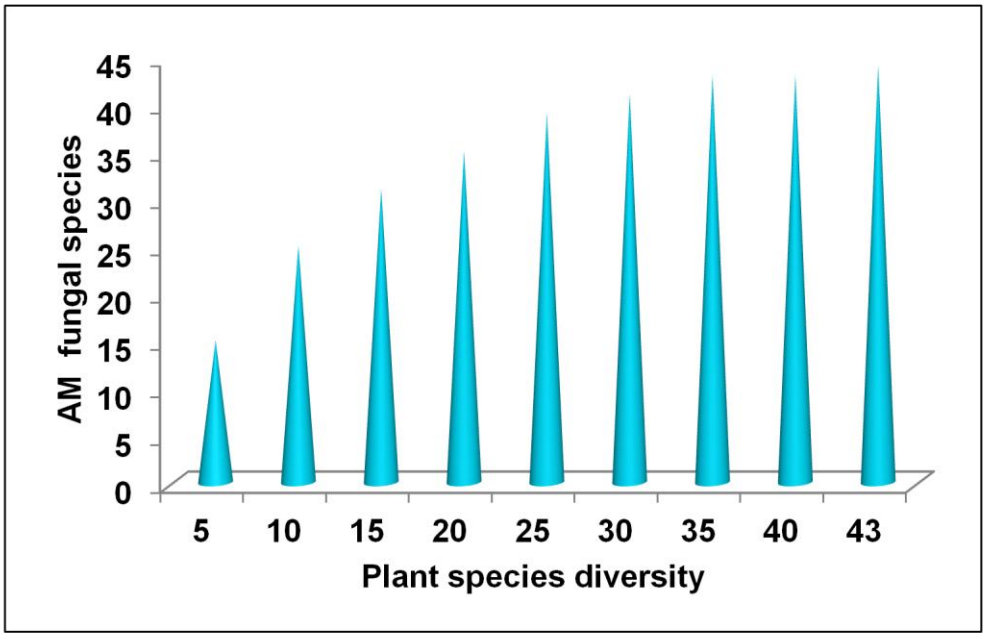


Figure 5: Effect of plant diversity on AM fungal species.

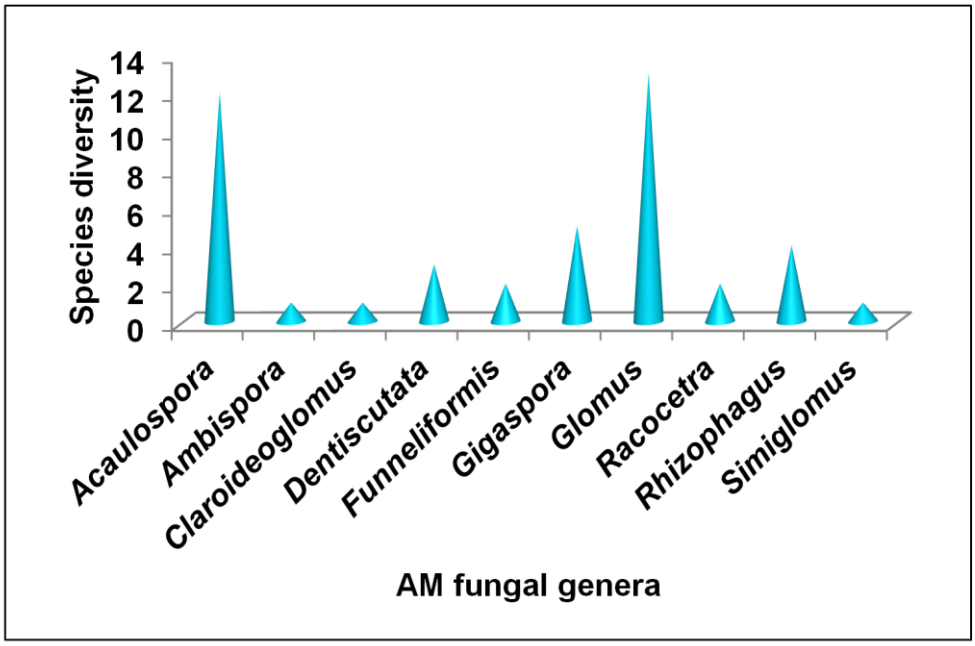


Figure 6: Variation in number of AM species in ornamental flowering plants.

In garden plants *I. duffii* showed highest spore density (**Fig. 7**) while in wild plants spore density was maximum in *Plumeria rubra* (**Fig. 8**). *Rhizophagus intraradices* was dominant in both garden and wild habitats. In garden plants *R. intraradices* exhibited the highest relative abundance (14.54%) and the lowest was recorded in *A. delicata* and *Ambispora leptoticha* (0.37%) (**Fig. 9**). The frequency of occurrence was maximum in *A. scrobiculata* (59.09%) followed by *R. intraradices* (54.54%) in garden plants (**Fig. 10**). In wild plants *R. intraradices* exhibited highest relative abundance (14.25%) followed by *R. fasciculatus* (13.43%) and was least in *A. nicolsonii* and *Claroideoglomus claroideum* (0.36%) (**Fig. 11**). The frequency of occurrence was highest in *R. fasciculatus* along with *R. intraradices* (57.14%) and *A. scrobiculata* (38.09%) among the wild plants (**Fig. 12**). Species richness was greater in wild plants (37) compared to garden plants (33) with the number of species given in parenthesis (**Fig. 13**). Species evenness was also higher in wild (0.1381) than in garden plants (0.0899) (**Fig. 14**). Shannon Wiener index was higher in wild (0.49) and lower in garden plants (0.31) while Simpson`s Index of Dominance (D) for AM fungi was equal (0.99) in both wild and in garden plants (**Fig. 15**).

A significant difference was observed in the soil nutrient levels during pre-monsoon, monsoon and post-monsoon seasons (**Table 4**). Similarly, significant difference was observed in spore density between the seasons and also between the sites. Mean spore density/100g soil was maximum during pre-monsoon (62.72) followed by post-monsoon (51.06) and was least during the monsoon season (39.31) (**Table 10**). Spore density varied at different

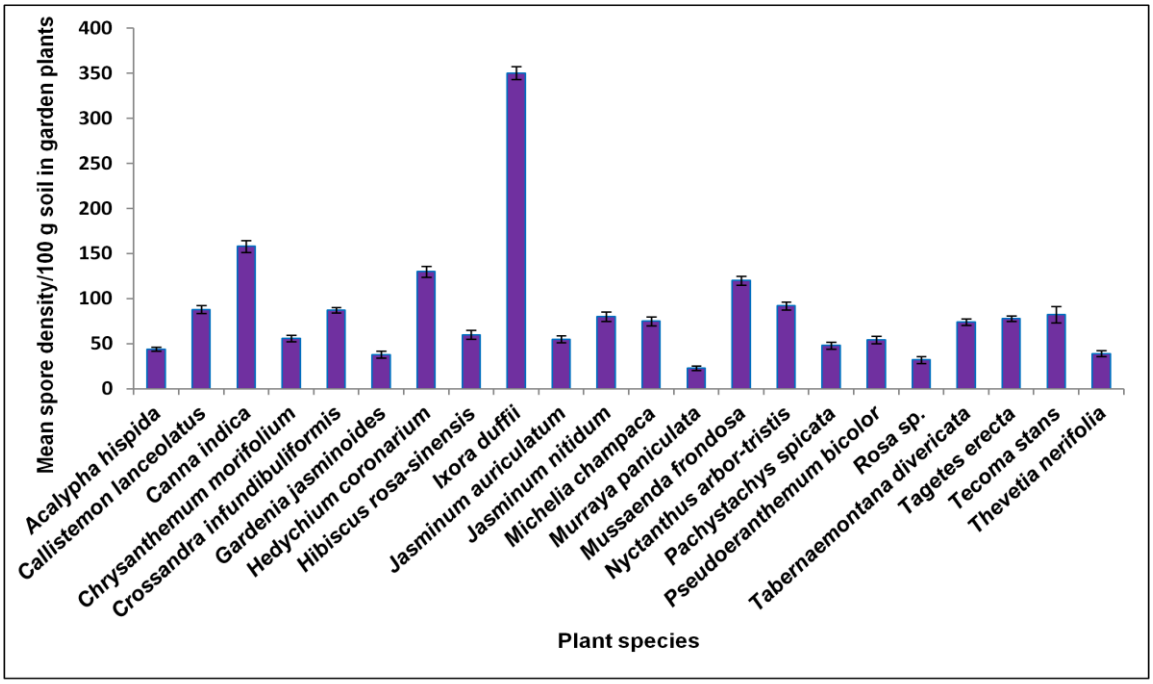


Figure 7: AM fungal spore density 100g^{-1} soil in garden plant species.

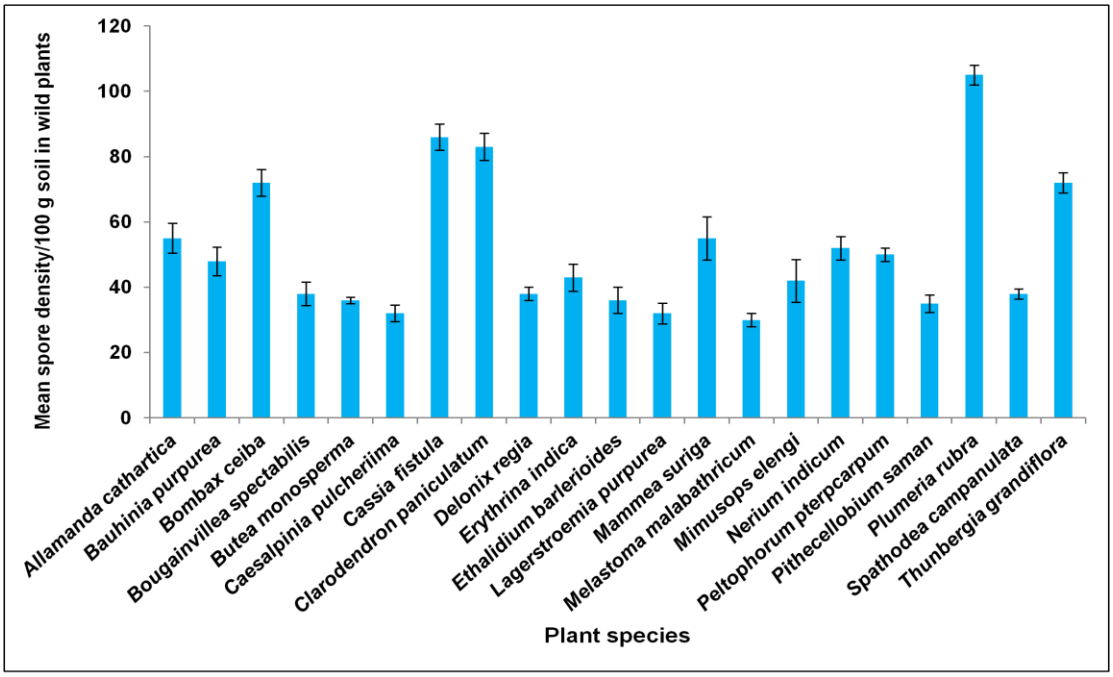


Figure 8: AM fungal spore density 100g^{-1} soil in wild plant species.

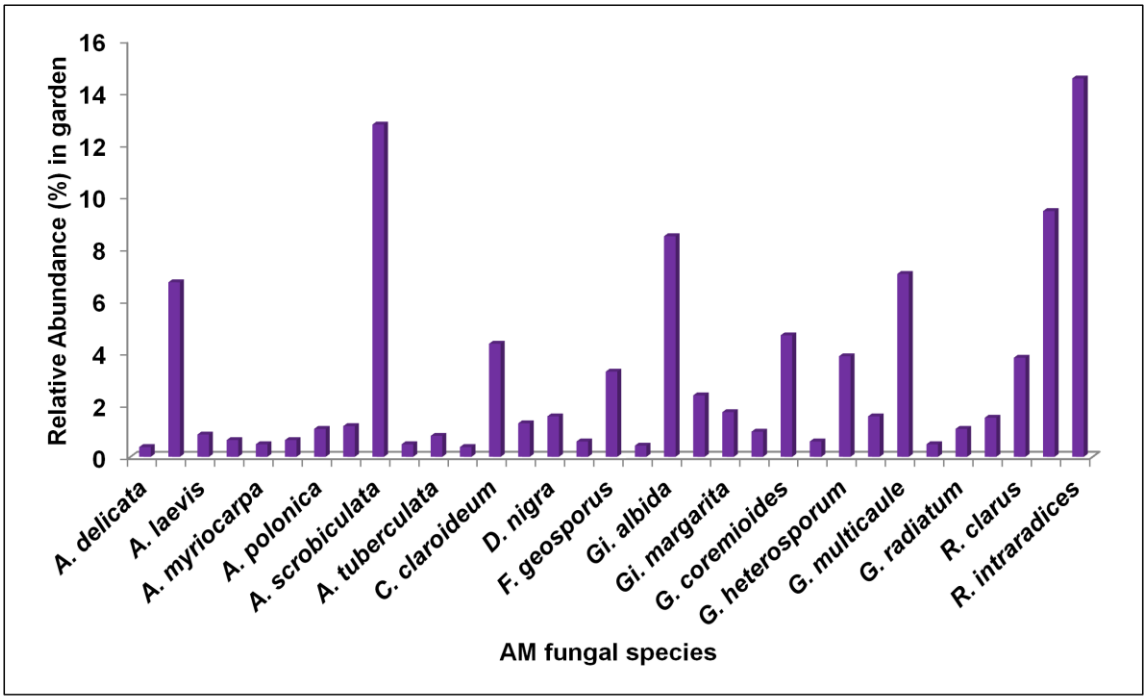


Figure 9: Relative abundance of AM fungi in garden plant species.

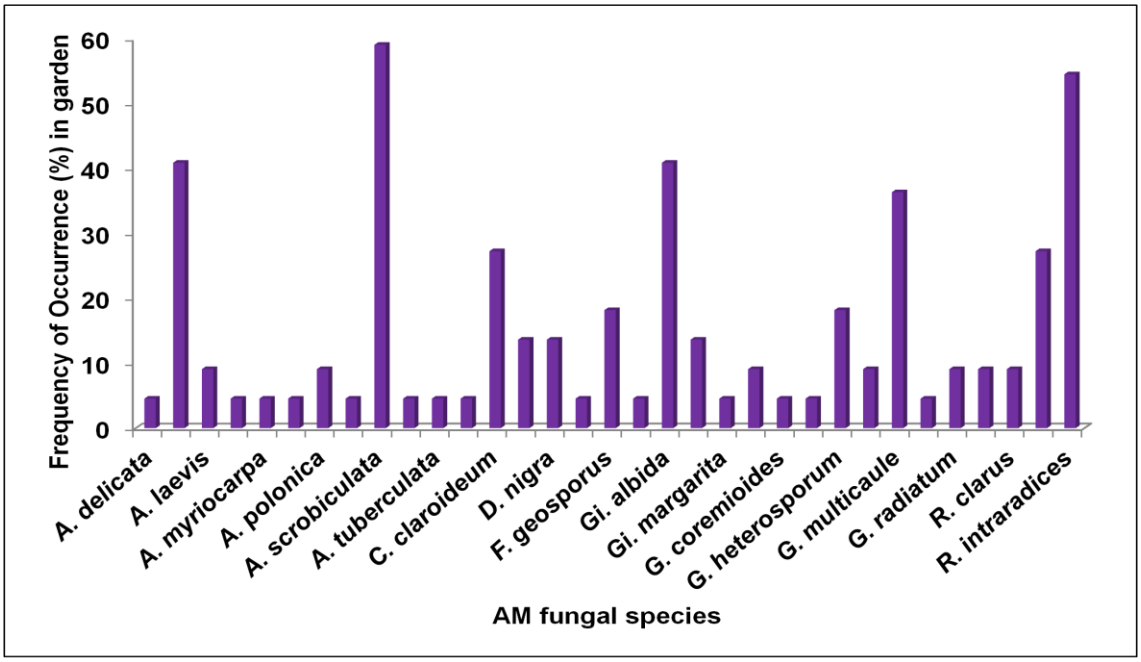


Figure 10: Frequency of occurrence of AM fungi in garden plant species.

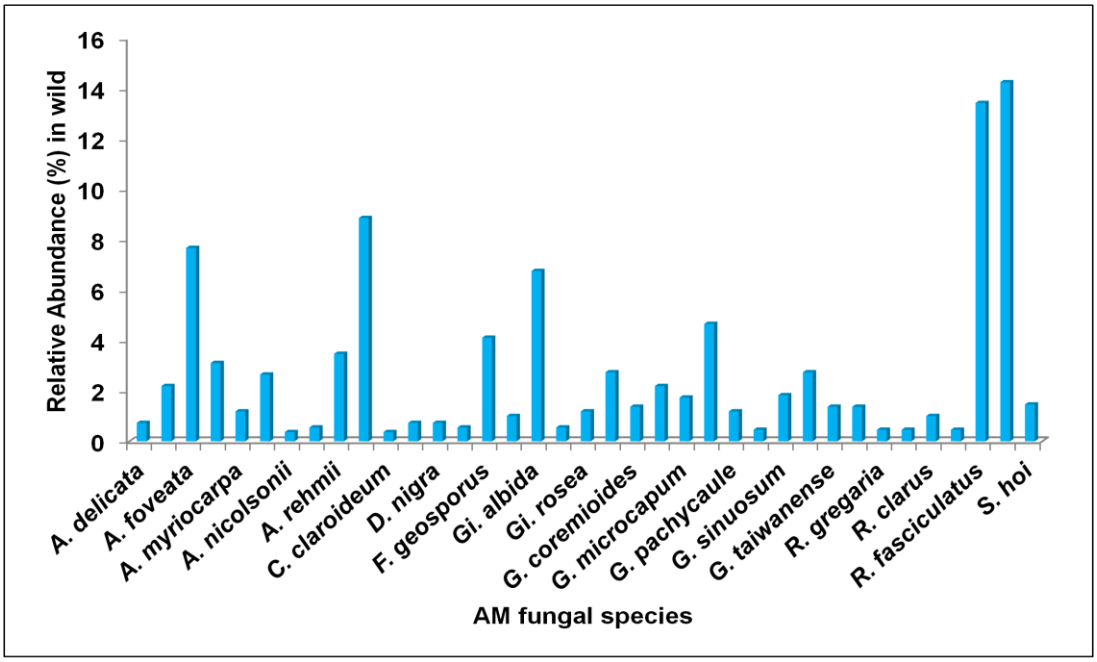


Figure 11: Relative abundance of AM fungi in wild plant species.

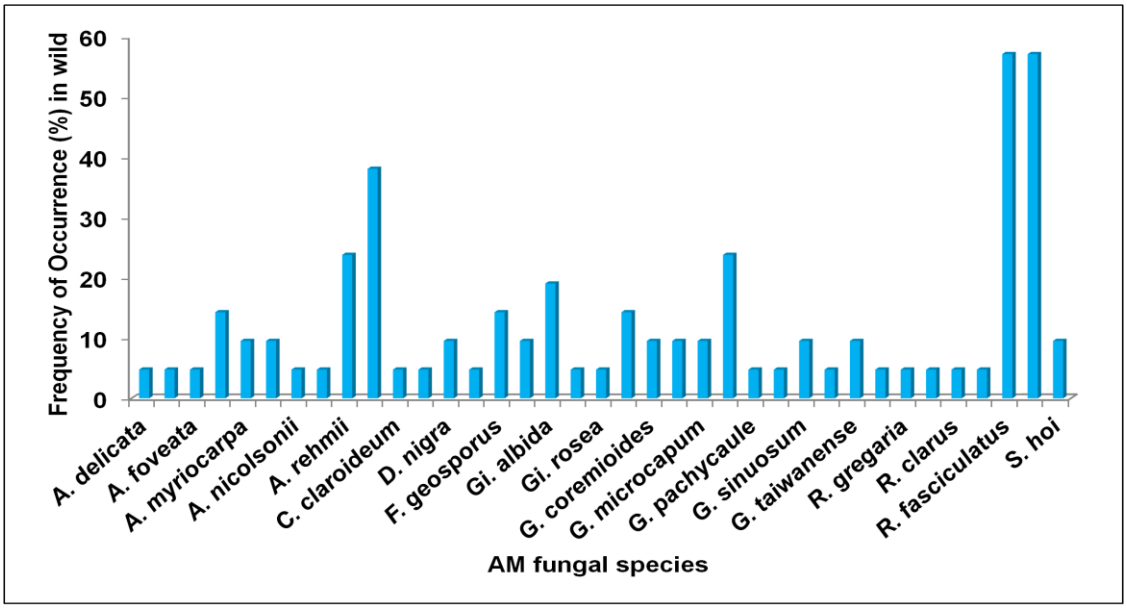


Figure 12: Frequency of occurrence of AM fungi in wild plant species.

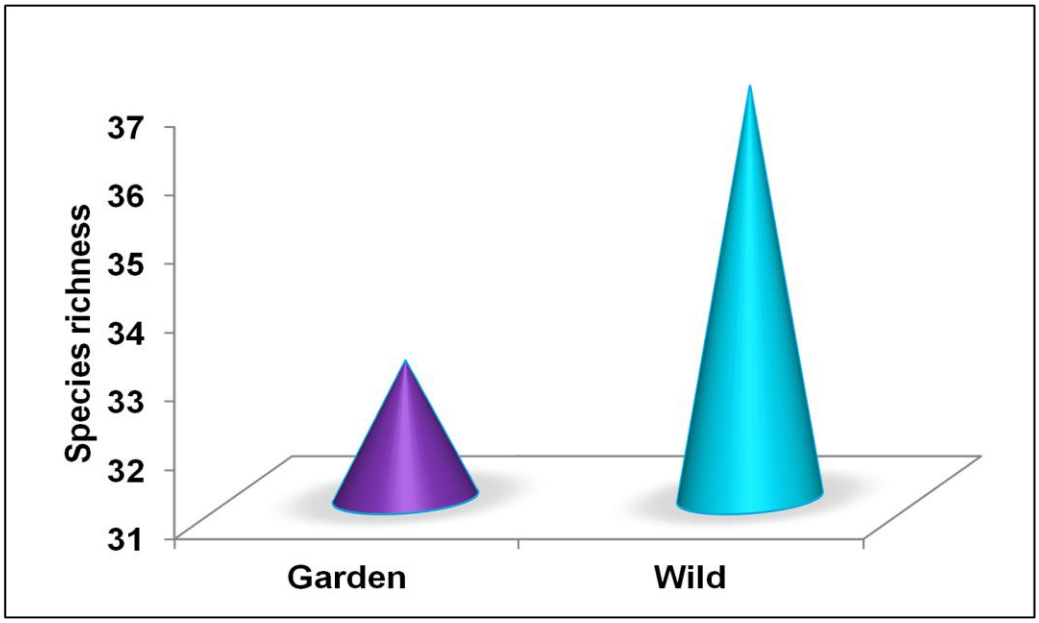


Figure 13: Species richness in garden and wild ornamental plant species.

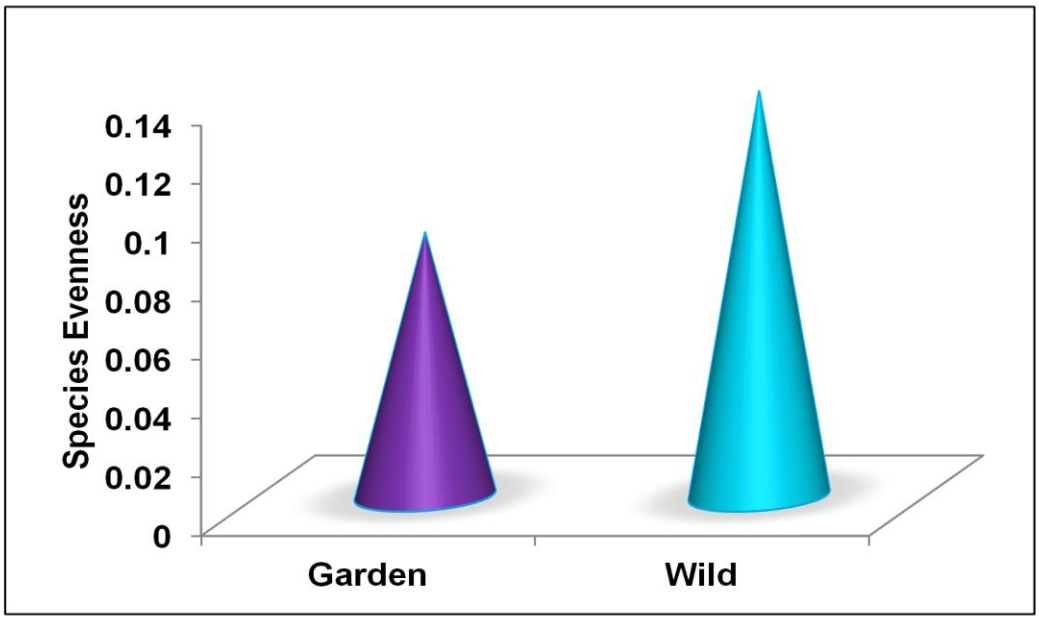


Figure 14: Species evenness in garden and wild ornamental plant species.

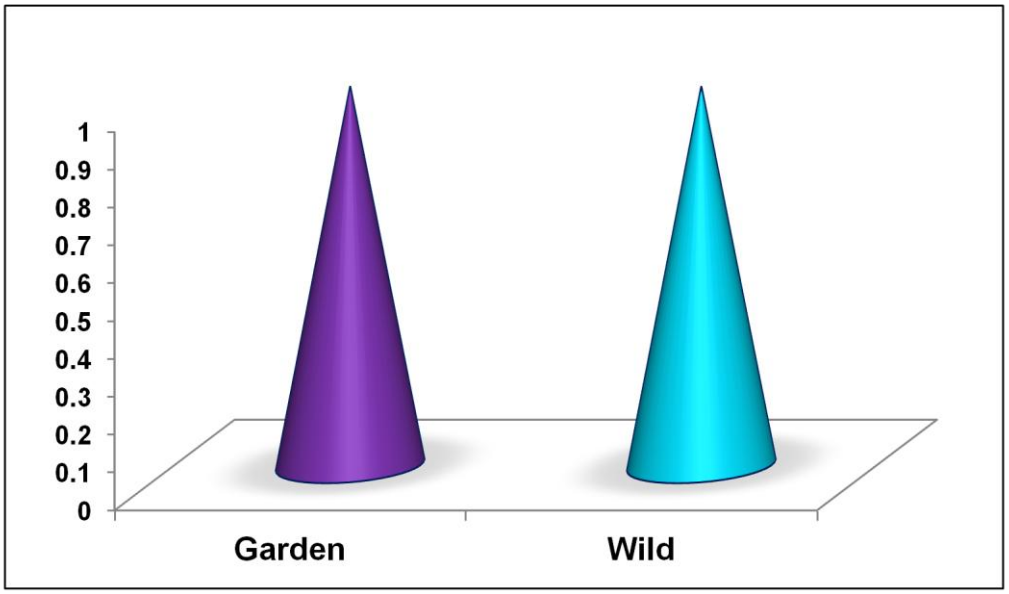


Figure 15: Simpson's index of dominance for garden and wild ornamental flowering plants.

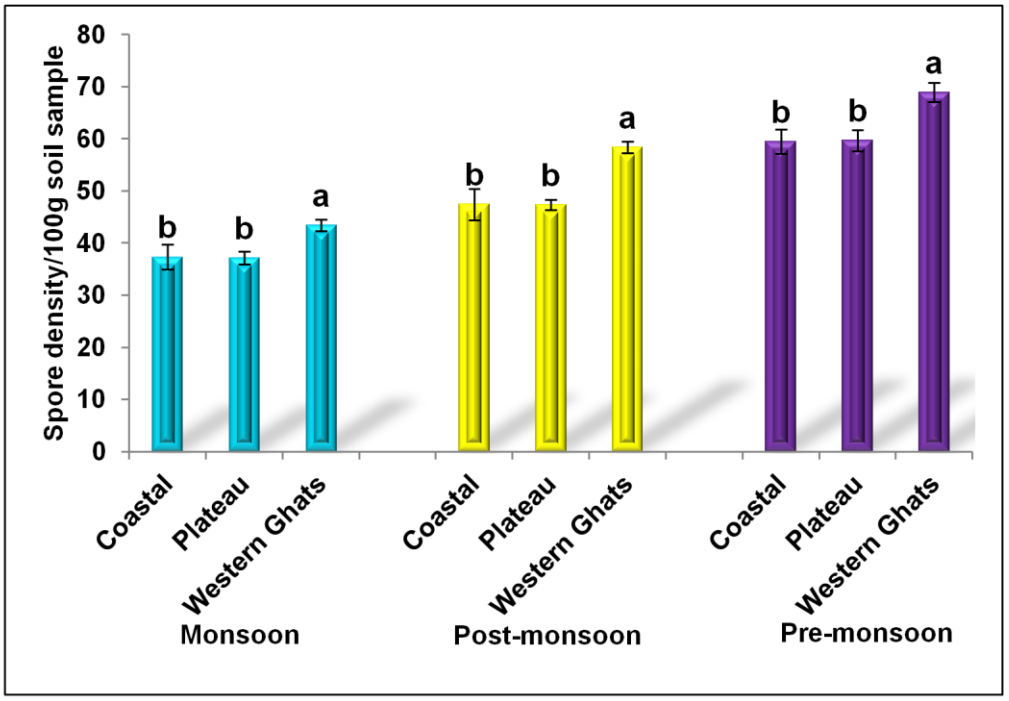


Figure 16: Influence of seasonal variation on mean spore density in the selected study sites.

Table 10: Influence of seasonal variation on mean spore density in the selected study sites.

Sr. No.	Season	Spore density $100g^{-1}$	Site	Spore density $100g^{-1}$
1.	**Monsoon**	$39.31^{c} \pm 3.54$	Coastal	$37.38^{b} \pm 2.37$
			Plateau	$37.16^{b} \pm 1.26$
			Western Ghats	$43.08^{a} \pm 0.60$
2.	**Post-monsoon**	$51.06^{b} \pm 6.33$	Coastal	$47.47^{b} \pm 3.00$
			Plateau	$47.35^{b} \pm 0.93$
			Western Ghats	$59.05^{a} \pm 0.13$
3.	**Pre-monsoon**	$62.72^{a} \pm 5.38$	Coastal	$59.52^{b} \pm 2.33$
			Plateau	$59.72^{b} \pm 2.02$
			Western Ghats	$68.94^{a} \pm 1.78$

Legend: Values are mean of 3 replicates, ± indicates standard deviation.
Data with different letters for each season are significantly different at $P \leq 0.05$.

study sites with highest in Western Ghats followed by coastal and plateau region (**Fig. 16**).

4.4 DISCUSSION

A survey of AM fungal diversity in ornamental flowering plant species from Goa region was undertaken with the objective of establishing data on AM fungal diversity and distribution, and also to examine variation in AM spore density with respect to seasons and sites. The results indicate that a rich diversity of AM fungi is involved in symbiosis with ornamental flowering plants of Goa. Forty four AM fungal species belonging to ten different genera *viz*., *Acaulospora*, *Ambispora*, *Claroideoglomus*, *Dentiscutata*, *Funneliformis*, *Gigaspora*, *Glomus*, *Racocetra*, *Rhizophagus* and *Simiglomus* were recovered from 43 ornamental flowering plants. The study revealed that the number of AM fungal species increased with in increase in number of host plant species. This observation is in conformity with earlier study (Stürmer and Bellei, 1994). Such a trend is known to be associated with increased benefits resulting from higher number of possible host-fungal pairings and increased density of plant roots available for colonization (Burrows and Pfleger, 2002). In return, a higher diversity of AM fungi helps in increasing plant productivity (van der Heijden *et al*., 1998b).

Of the ten genera, *Glomus* was the most dominant genus followed by *Acaulospora* and *Gigaspora*. Similar observations were reported earlier by Ferrer *et al*. (1987); Khalil *et al*. (1992); Kim and Kim (1992), Muthukumar and Udaiyan (2000); Bhattacharya and Bagyaraj (2002); Sannazzaro *et al*. (2004);

Facelli *et al*. (2009); Binet *et al*. (2011); Hindumathi and Reddy (2011). This is mainly due to their ability to sporulate abundantly irrespective of geographical region. Das and Kayang (2010) showed the dominance of *Glomus* under varied soil conditions which is due to a wider adaptation to varied soil conditions. The tolerance of *Glomus* to a wide range of temperature and pH is also responsible for its dominance (Wang *et al*., 1997). The growth in clusters and frequent sporulation pattern of *Glomus* is the main factor responsible for its dominance (Dhar and Mridha, 2006). The dominance of AM fungal genera in ornamental flowering plants is related to their sporogenous characteristics (Yang *et al*., 2011). *Glomus* and *Acaulospora* possess the smallest size spores in AM fungi taxa and thus these small spores are easy to distribute and produce a large number of spores in a short time (Hepper, 1984). The dominant AM fungal species such as *R*. *intraradices*, *A*. *scrobiculata*, *R*. *fasciculatus* and *A*. *foveata* therefore may play important roles in constructing the stable symbiotic relationship between AM fungi and ornamental flowering plants thereby increasing the floral productivity under natural conditions. Negative non-significant correlation was found between root colonization and spore density, which is in agreement with earlier study by Kalita *et al*. (2002). The negative correlation can be explained on the basis that when soil conditions are suitable for spore germination, the spore germinates producing hyphal network and AM colonization increases thus resulting in decreased spore number (Ragupathy and Mahadevan, 1993; He *et al*., 2002).

Arbuscular Mycorrhizal fungal spore density in the rhizosphere varied (23–350 spores/100g) between host plant species. It was highest in *I*. *duffii*

and lowest in *M*. *paniculata*. This variability can be due to microclimate (Koske, 1987), ecological factors which include soil fertility, the plant species (Tommerup, 1983; Kurle and Pfleger, 1994; Miller and Jackson, 1998), physico-chemical and microbiological properties (Anderson *et al*., 1984; Johnson *et al*., 1991) and to the sampling season (Gemma *et al*., 1989). In the present study the collection of rhizosphere samples carried out during the flowering stage and each plant species flowering in different seasons may also be responsible for the variation in spore density. Such seasonal variation in spore numbers between the plant species has been reported earlier (Haymann, 1975; Kruckelmann, 1975; Sparling and Tinker, 1975; Bhaskaran and Selvaraj, 1997; Muthukumar and Udaiyan, 2000). Interspecific competition due to the occurrence of several species of AM fungi in the same host plant and environmental factors also influence spore production in natural communities (Gemma and Koske, 1989). AM fungal spore production is also limited by moisture and nutrient availability (Augé, 2001; Johnson *et al*., 2003).

Species richness from 2–8 AM species belonging to 2–4 genera from each host plant species is in accordance with earlier studies (Bhattacharya and Bagyaraj, 2002; Mohan *et al*., 2005; Dubey *et al*., 2008; Facelli *et al*., 2009; Bhattacharjee and Sharma, 2011; Sundar *et al*., 2011; Bansal *et al*., 2012). This is because an individual plant may harbour a number of species of AM fungi, and their diversity and species composition can vary within host plant species and even within the same plant family (Vandenkoornhuyse *et al*., 2003; Santos González *et al*., 2007). However, studies by Helgason *et al*.

(2002); Husband *et al*. (2002); Smith and Read (2008); Öpik *et al*. (2009) reported that some AM fungal species are more host specific than others. Maximum species richness was recorded in *I. duffii* and least in *C. pulcherrima* and *M. paniculata*. Diversity and species composition of AM fungi is a major factor contributing to plant and productivity, and influences the structure and functioning of plant communities (van der Heijden *et al*., 1998a; Eom *et al*., 2000; Vogelsang *et al*., 2006).

Although garden and wild plants are subjected to the same climatic conditions the average spore density was higher in garden plants than in wild plants. The disturbances in garden plants include interference by humans, watering and addition of organic fertilizers. The results indicate that soil disturbance activities in garden may play an essential role in creating a stressful environment which cause AM fungi to sporulate and thus higher spore densities were recorded when compared to the wild habitat. The species richness of AM fungi was greater in wild plants in comparison to garden plants. The shift in AM fungal community composition is due to a number of factors including disturbance of AM fungal hyphal networks, changes in soil nutrient content and altered microbial activity in garden plants (Jansa *et al*., 2003) as there is an inverse relationship between management intensity and AM fungal species richness (Oehl *et al*., 2003; Hijri *et al*., 2006). Therefore the diversity of AM fungi in wild plants is greater due to undisturbed natural soil conditions and less chemical inputs. As to whether human interference of soil can affect AM fungal species, it can be concluded that the degree of disturbance varies resulting in varied AM fungal species

composition/densities as evidenced in the two habitats undertaken for the study.

Significant differences were observed in soil characteristics and soil nutrient levels during pre-monsoon, monsoon and post-monsoon seasons in the different sites. Mean spore density varied significantly between different seasons. Similar observations have been recorded earlier (Siguenza *et al*., 1996; Beena *et al*., 2000; Rodriguez-Echeverria *et al*., 2008). Mean spore density also varied between the different study sites. Such variation in the population of AM fungi and their symbiosis with plant roots is related to both soil properties and host plants (Hayman, 1982). Higher spore density during pre-monsoon may be attributed to soil temperatures because high soil temperature is known to enhance sporulation (Haymann, 1970; Furlan and Fortin, 1973; Hetrick and Bloom, 1983). Decrease in mean spore density in monsoon season suggests that spore germination takes place during the active growth of host plants. Mason (1964); Ragupathy and Mahadevan (1993) attributed decrease in spore density in monsoon season to increased intra- and extra-metrical mycelium which favours spore germination. AM fungal sporulation is also known to depend on soil moisture and other soil factors (Oliveira and Oliveira, 2005). Associations between AM fungal species and host plant species can vary spatially, even over small distances, which could further explain the high variation in AM fungal community composition.

CHAPTER 5

TAXONOMY OF ARBUSCULAR MYCORRHIZAL FUNGI FROM ORNAMENTAL FLOWERING PLANTS OF GOA AND THEIR MASS MULTIPLICATION

5.1 INTRODUCTION

Arbuscular Mycorrhizal fungi belonging to the phylum *Glomeromycota*, are obligate symbiotic fungi forming mutualistic associations with the roots of most land plants. Approximately 95% of the world's plant species belong to families that are characteristically mycorrhizal (Smith and Read, 1997). Traditionally, glomeromycotan taxonomy has been based on the morphology of the spores. The way the spore is formed on the hypha (*i.e.* mode of spore formation) has been important to describe genera and families and the layer structure of the spore wall to distinguish species (Walker, 1983; Morton, 1988). 200–300 Arbuscular Mycorrhizal species have been described to date (Öpik *et al*., 2010; Schußler and Walker, 2010). The current estimates of AM fungal species are largely based on spore morphology, which does not always separate genetically distinct taxa.

Arbuscular Mycorrhizal fungal identification based purely on morphological characters of field-collected spores is difficult, because sometimes spores are found in low numbers, are parasitized, or are lacking informative taxonomic characteristics, thus impairing an accurate identification. Components of spore walls are susceptible to alteration and deterioration by a wide array of microorganisms in the soil. Establishment of trap cultures using soil or by mixing rhizosphere soil and root pieces with sterilized sand soil mixture and growing with suitable host plants is the most commonly used method to isolate AM fungi (Menge, 1984; Morton *et al*., 1993; Brundrett *et al*., 1999a, b). This method yields a large number of healthy spores which can be readily identifiable and helps the assessment of

local species diversity in different ecosystems (Leal, 2009). This methodology may not allow the identification of all species, because sporulation of the fungal species may be affected by the plant host chosen for trapping (Bever *et al*., 1996) and in some cases it may promote the sporulation of AM fungal species that were not sporulating at the sampling time or field conditions (Stürmer, 2004).

When several spores of AM fungi are inoculated together in a pot culture experiment, some prove to be better competitors than others (Van Nuffelen and Schenck, 1984; Wilson, 1984; Lopez-Aguillon and Mosse, 1987) and the most successful fungi generally are those that colonized roots most rapidly (Abbott and Robson, 1984; Wilson, 1984). The outcome of competition between endophytes is expected to depend on the placement and amount of inoculum, hyphal growth rates in soil and interactions within roots (Hepper *et al*., 1988; Abbott and Robson, 1991a, b). The pot culturing method results in isolation of more species than other methods (Watson and Milner, 1996). It provides additional information on fungal diversity that complements spore occurrence data obtained using the same soil samples and may provide valuable new information about the biology of AM fungi (Brundrett *et al*., 1999a). The objective of the present work was to study the taxonomy of AM fungal spores isolated from the rhizosphere of ornamental flowering plants using trap cultures and to produce pure cultures of dominant AM fungal species and their mass multiplication.

5.2 MATERIALS AND METHODS

5.2.1 Study sites and collection of rhizosphere samples: Was carried out as described under **4.2.1**.

5.2.2 Extraction of AM fungal spores: Was carried out as described under **4.2.2**.

5.2.3 Identification of AM fungi: Identification of spores was carried out using various bibliographies (Schenck and Perez, 1990; Bentivenga and Morton, 1995; Walker and Vestberg, 1998; Redecker *et al*., 2000; Morton and Redecker, 2001, Rodrigues and Muthukumar, 2009) and INVAM (International culture collection of Vesicular Arbuscular Mycorrhizal Fungi) http://invam.caf.wvu.edu.

5.2.4 Establishment of pot cultures: Were carried out as described under **4.2.4**. Trap cultures thus established were allowed to grow for 6 months and the most abundant AM fungal spore morphotypes were recovered and used to establish monospecific cultures.

5.2.5 Mass multiplication: A mixture of sand and soil (2:1) was sieved through a 2 mm mesh and oven sterilised at 180°C (3 h/day) for three consecutive days, to eliminate indigenous AM fungi. Inoculum comprised of sand:soil mixture containing dried roots, hyphae and spores. Six different AM fungal species were mass multiplied separately in pots initially using *S. scutellarioides* and later transferred to trays.

5.3 RESULTS

In all 44 AM fungal species belonging to ten different genera *viz*. *Acaulospora*, *Ambispora, Claroideoglomus*, *Dentiscutata*, *Funneliformis*, *Gigaspora*, *Glomus*, *Racocetra*, *Rhizophagus* and *Simiglomus* were recovered from 43 ornamental flowering plants. *Glomus* was the dominant genus with 13 species followed by *Acaulospora* (12), *Gigaspora* (5), *Rhizophagus* (4), *Dentiscutata* (3), *Funneliformis* (2), *Racocetra* (2), *Ambispora* (1), *Claroideoglomus* (1) and *Simiglomus* (1). Out of total 129 rhizosphere samples collected, 96 trap cultures were established successfully. The AM fungal spores obtained from trap cultures were then isolated and used in the establishment of pure cultures (**Plate 9A**). Of the 10 pure cultures obtained, 6 pure cultures of dominant AM fungal species were used for mass multiplication (**Plate 9B)**. Spore and spore syndrome was also encountered during the study (**Plate 10**). The taxonomic description of various AM fungal species isolated is given below:

***Acaulospora delicata* Walker, Pfeiffer & Bloss.** *Mycotaxon*, 25: 621–628 (1986). **(Plate 11A)**

Spores formed singly in soil, hyaline to pale yellowish-cream, globose to sub-globose, 80–125(–150) x 80–110(–140) μm. Spore wall structure of 4 walls in two groups (A & B). Wall group A consist of a thin, hyaline, outer evanescent wall (wall 1) 1 μm thick, closely attached to wall 2 which is relatively thick (2.5–3.5 μm) laminated wall with up to 6 sub equal laminations. Wall group B with 2 thin, hyaline membranous walls (wall 3 & 4) 0.5 μm and 0.75–1 μm thick respectively. Wall 3 covered by minute granular excrescences.

Plate 9: Culturing and mass multiplication of AM fungi.

A. Pot culture of AM fungi using *Solenostemon scutellarioides* (L.) Codd, as host plant.

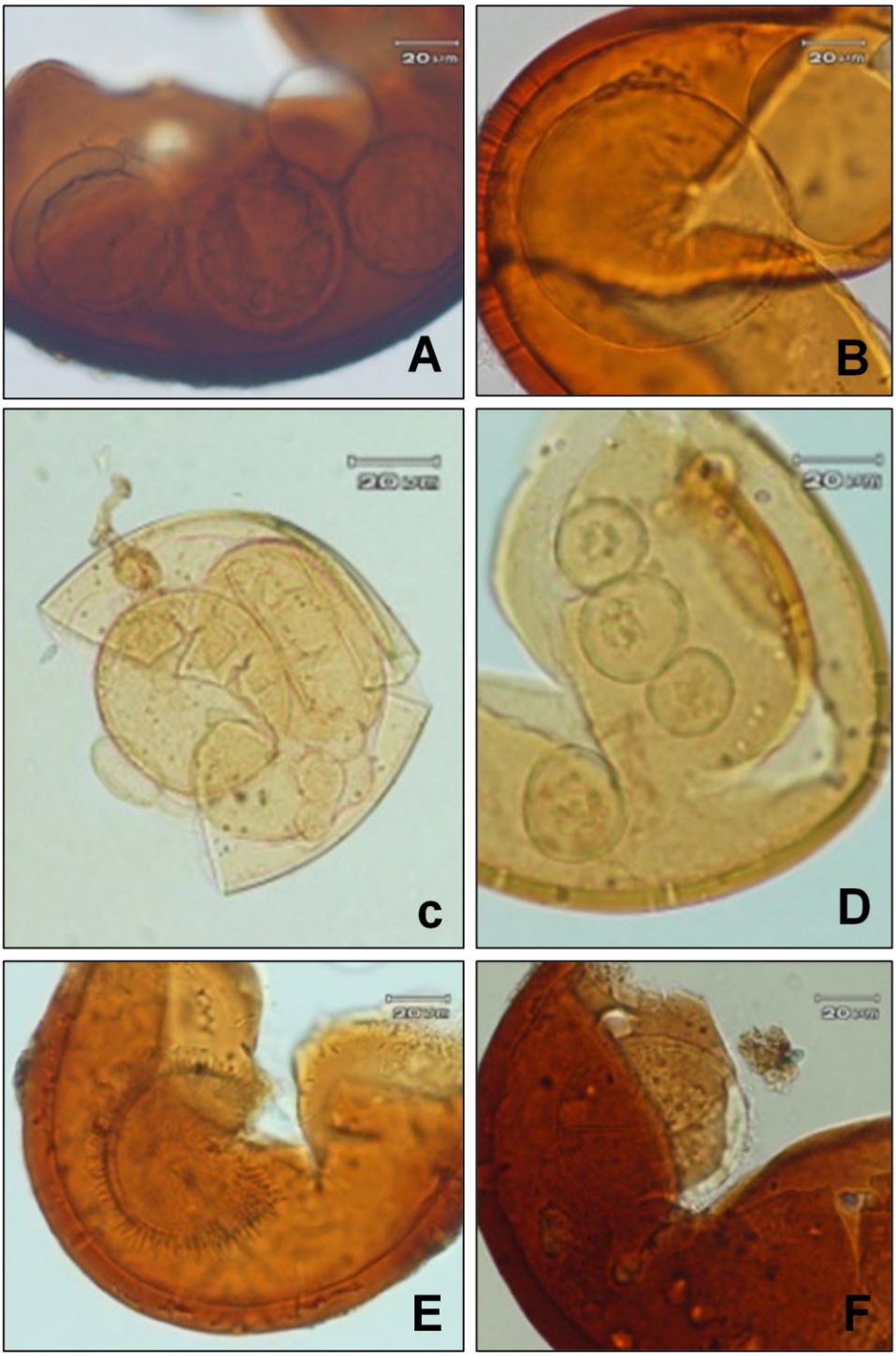
B. Mass multiplication of AM fungi using *Solenostemon scutellarioides* (L.) Codd, as host plant.

Plate 9

Plate 10: Spore in spore syndrome.

A–F. Spore in spore syndrome observed in rhizosphere soils of ornamental flowering plants.

Plate 10



***Acaulospora foveata* Trappe & Janos.** *Mycotaxon*, 15: 515–522 (1982). **(Plate 11C & D)**

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

***Acaulospora laevis* Gerdemann & Trappe.** *Mycologia Memoir*, 5: 76 (1974). **(Plate 11B)**

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

***Acaulospora mellea* Spain & Schenck.** *Mycologia*, 76: 685–699 (1984).

Spores formed singly in soil, honey-coloured to yellow-brown, sub-globose, 96–130 x 78–92 μm. Spore wall 4–8(–11) μm thick, consist of 3 separable walls; the outermost wall (wall 1) yellow–brown to dark brown, 2–6 μm thick, laminate, inseparable from wall 2, 0.5 μm thick; wall 3 hyaline to light yellow, membranous, 0.5–1 μm thick: wall 4 and 5 membranous.

Acaulospora myriocarpa **Spain, Sieverding & Schenck.** *Mycotaxon*, 25: 111–117 (1986).

Spores found singly in soil, hyaline to yellow, sub-globose, (23–)104–114 x (21–)80–96 μm. Spore wall structure of 3 walls (1–3) in one group. Wall 1 rigid, 0.75–2 μm; wall 2 rigid, 0.3–1.5 μm; wall 3 membranous (< 0.3 μm thick) closely appressed to wall 2.

Acaulospora nicolsonii **Walker, Reed & Sanders.** *Transactions of the British Mycological Society*, 83: 360–364 (1984). **(Plate 11E & F)**

Spores formed singly in soil, hyaline to pale yellow brown, globose to sub-globose, 99– 108 x 109–120 (–218) μm. Spore wall an outer, brittle, wall group (Group A) (walls 1–3) enclosing an inner, membranous wall (Group B) (wall 4). Wall group A with an outer thin, hyaline evanescent wall (wall 1), 0.5–1μm thick, tightly adherent to a thick, brittle, hyaline to pale yellow–brown laminated wall (wall 2) 3–10 μm thick, with up to 13 subequal laminae, enclosing a loosely adherent, pale yellow, brittle, unit wall, 0.5–1.5 μm thick (wall 3); wall 1 smooth or roughened as it breaks up and sloughs, leaving granular fragments attached to wall 2 which may crack in an irregular network. Inner wall (Group B, wall 4) very thin, hyaline, membranous (<0.5 μm) and spore contents vacuolate or reticulate.

Acaulospora polonica **Blaszkowski.** *Karstenia*, 27: 37–42 (1988). **(Plate 11G & H)**

Spores produced singly in soil, globose to sub-globose, (80–)90 (–115) μm in diameter, sessile on a hypha tapering to a globose to sub-globose swollen

Plate 11: Arbuscular Mycorrhizal fungal spores.

A. Broken spore of *Acaulospora delicata* (x 400).

B. Broken spore of *Acaulospora laevis* (x 400).

C. Broken spore of *Acaulospora foveata* (x 400).

D. Broken spore of *Acaulospora foveata* showing surface ornamentation (x 1000).

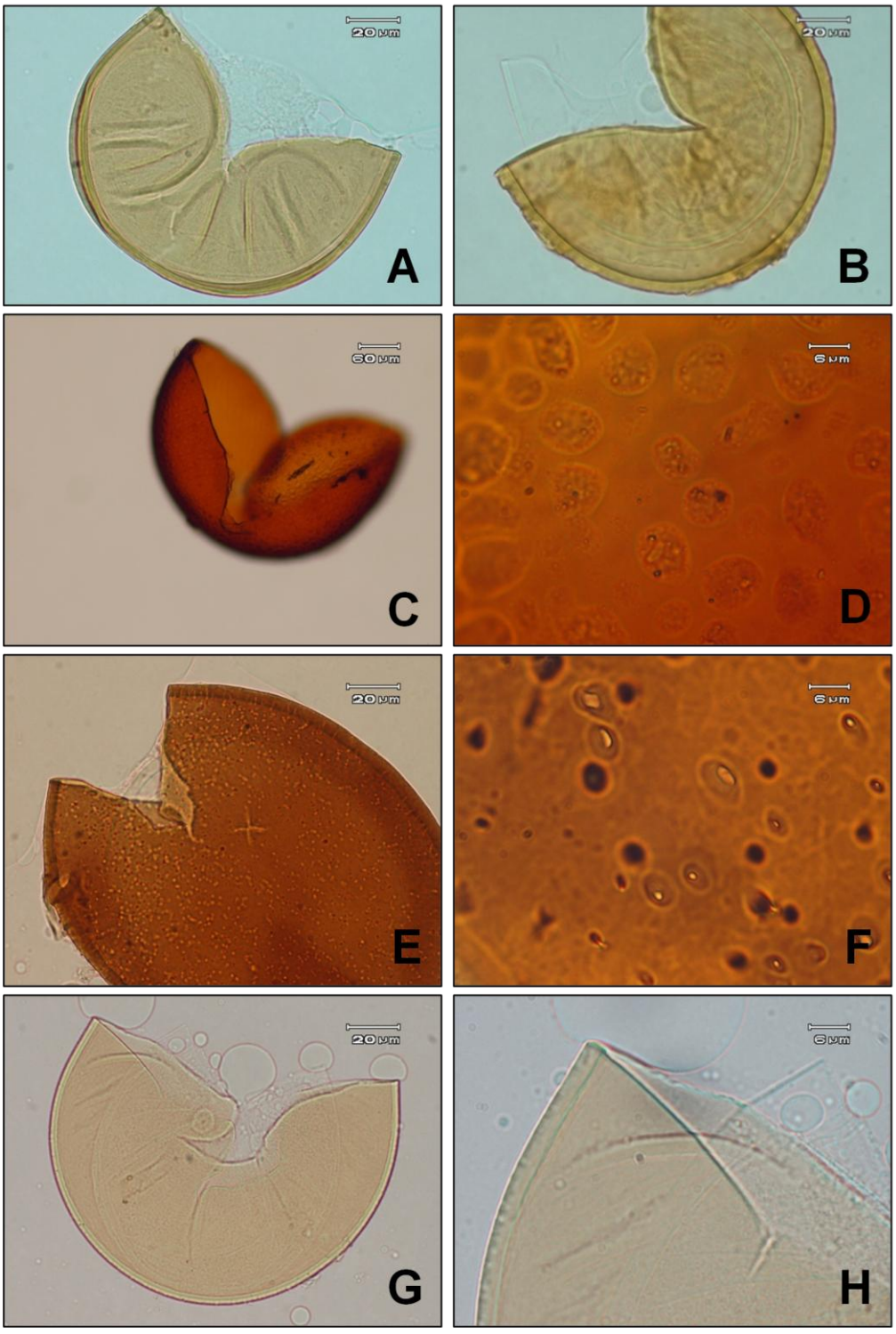
E. Broken spore of *Acaulospora nicolsonii* showing surface ornamentation (x 400).

F. Broken spore of *Acaulospora nicolsonii* showing surface ornamentation (x 1000).

G. Broken spore of *Acaulospora polonica* (x 400).

H. Broken spore of *Acaulospora polonica* showing wall layers (x 1000).

Plate 11



hyphal terminus, 60–90 µm in diameter; hyphal terminus contents hyaline; terminus wall 0.7–1 µm thick. Spore wall 4 layerered (1–4) in three groups (A, B and C). Group A, of a hyaline unit, (1.5–)1.9(–2.3) µm thick outer layer. Group B, of a hyaline membranous, upto 0.5 µm thick layer. Group C, of two hyaline, membranous, (0.5–) 0.8(–1) µm thick, adhering in layers 3 and 4. Spores filled with hyaline droplets, and not reacting in Melzer's reagent.

***Acaulospora rehmii* Sieverding & Toro.** *Angewandte Botanik*, 61: 217–223 (1987). **(Plate 12A–D)**

Spores light yellow in colour, 82–175 µm in diam. Spore wall consists of 4 wall layers. Outer wall, 3–13 µm thick includes the ornamentation of labyrinth form folds with depressions between ridges, 1–4.5 µm thick, middle wall layer hyaline, 0.5–2.0 µm thick, inner wall layer 0.5–1.5 µm attached to innermost wall layer of 0.5 µm thick.

***Acaulospora scrobiculata* Trappe.** *Mycotaxon*, 6: 359–366 (1977). **(Plate 12E–H)**

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

Plate 12: Arbuscular Mycorrhizal fungal spores.

A. Broken spore of *Acaulospora rehmii* (x 400).

B. Broken spore of *Acaulospora rehmii* showing surface ornamentation (x 400).

C. Scanning Electron Microscope image of *Acaulospora rehmii*.

D. Scanning Electron Microscope image of *Acaulospora rehmii*.

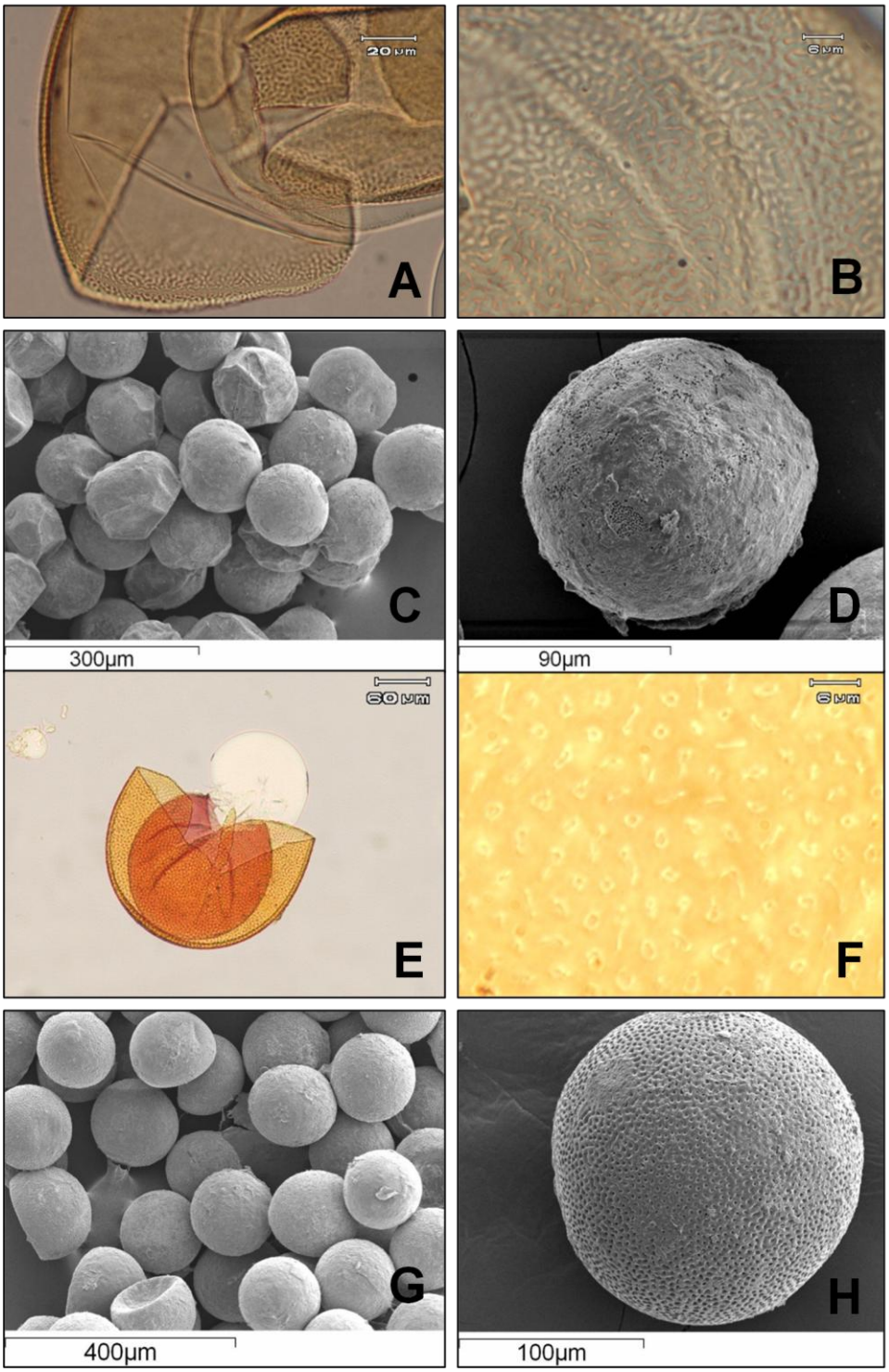
E. Broken spore of *Acaulospora scrobiculata* (x 400).

F. Broken spore of *Acaulospora scrobiculata* showing surface ornamentation (x 400).

G. Scanning Electron Microscope image of *Acaulospora scrobiculata*.

H. Scanning Electron Microscope image of *Acaulospora scrobiculata*.

Plate 12



***Acaulospora soloidea* Vaingankar & Rodrigues sp. nov.** *Mycotaxon*, 115: 323–326 (2011). **(Plate 13A–H)**

Spores formed singly in the soil, sessile, borne laterally on the subtending hypha of the sporiferous saccule. The spores are pale brown to brown, globose to sub-globose (50–)65–84(–90) µm diam. Spore wall structure of 5 walls (1–5) in three groups (A, B, C), exterior, medium and interior, in total 2.0–3.0 µm thick. Group A with a single wall (W1). Wall 1 is hyaline, 0.4–0.6 µm thick, sloughing completely, evanescent and thus present only in young spores. Group B consisting of two walls (W2 and W3). Wall 2 is continuous, brown 1.2–1.6 µm thick, rigid, with numerous acellular, fibrillose, hairy outgrowths forming a pile or thick coat 3–6 µm on the spore surface. The hairs are tightly packed together on the spore surface when dry. The length of each may vary from 10–15 µm, Wall 3 hyaline to light brown, laminated, 0.3–0.4 µm thick tightly adherent to W2. Group C composed of two hyaline flexible walls (W4 and W5), which are formed after the differentiation of spore wall. Wall 4 hyaline, bilayered, very thin 0.1–0.2 µm, does not show any reaction with Melzer's reagent. Wall 5 is hyaline, thin, 0.1–0.2 µm, amorphous and is not beaded. Spore contents of hyaline lipid globules.

***Acaulospora elegans* Trappe & Gerdemann.** *Mycologia Memoir*, 5: 34 (1974).

Spores formed singly in soil, dull, dark brown, globose to sub-globose, 140–156 (–330) µm in diam. Spore surface ornamented with crowed light brown spines 2–0.5 µm, soon developing an alveolate reticulum of hyaline ridges, 5–6 x 1 µm super imposed on the spines, alveoli 4-8 µm long. Spore wall with an

Plate 13: Arbuscular Mycorrhizal fungal spores.

A. Fresh spores of *Acaulospora soloidea* (x 40).

B. Intact spore of *Acaulospora soloidea* (x 100).

C. Broken spore of *Acaulospora soloidea* (x 100).

D. Broken spore of *Acaulospora soloidea* showing surface ornamentation (x 100).

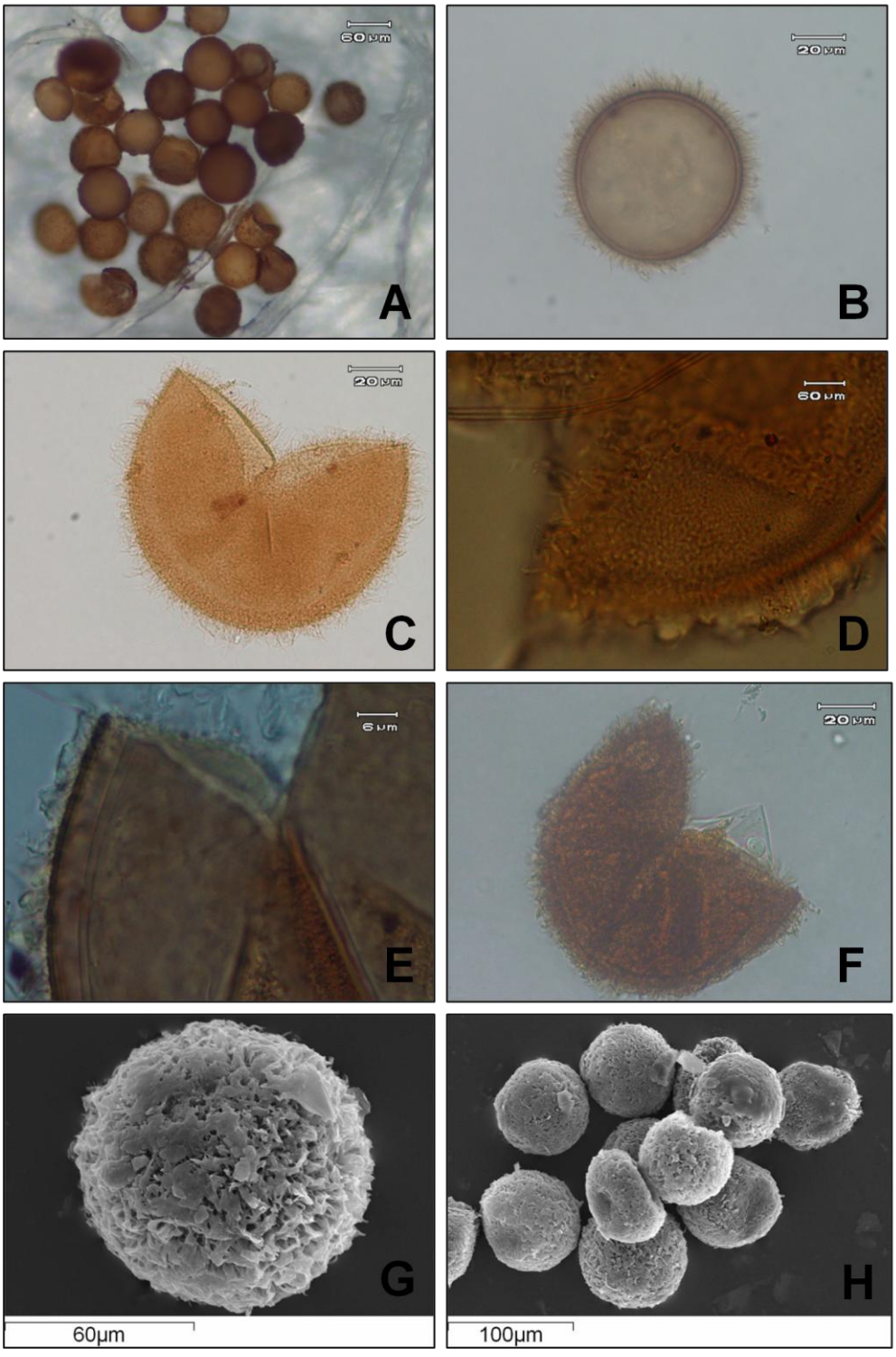
E. Broken spore of *Acaulospora soloidea* showing wall layers (x 100).

F. Broken spore of *Acaulospora soloidea* showing inner wall layer (x 100).

G. Scanning Electron Microscope image of *Acaulospora soloidea* (x 400).

H. Scanning Electron Microscope image of *Acaulospora soloidea* (x 400).

Plate 13



outer layer up to 12 μm thick (including spines and ridges), enclosing 3 hyaline walls which total up to 15 μm thick.

***Acaulospora tuberculata* Janos & Trappe.** *Mycotaxon*, 15: 515–522 (1982).

Spores 255–340 µm, yellowish brown to honey brown. Spore surface uniformly covered with tubercules to 0.7–1.1 µm at the rounded tip. Spore wall consists of three layers, outer layer yellow in colour, 7–12 µm thick, middle layer yellowish brown, 1.5 µm thick, innermost hyaline layer, 1.5–3 µm thick. Spore contents globose to hyaline, guttules of 8–19 µm long.

***Ambispora leptoticha* Walker, Vestberg & Schuβler.** *Mycogical Research*, 13: 111–137 (2007).

Spores 104–262 µm in diameter with single wall, 1.5–10.5 µm thick, spore wall with an indistinct alveolate reticulum of shallow ridges (0.5–1 µm wide). Spore contents globular, hyaline in young spores. Subtending hyphae 9–27 µm diameter at spore attachment, hyphal wall continuous with the spore wall. Spore contents enclosed with a membrane, which occasionally bulges into subtending hyphae.

***Claroideoglomus claroideum* (Schenck & Smith) Walker & Schüβler.** The *Glomeromycota.* A species list with new families and genera: 21 (2010). **(Plate 14A)**

= *Glomus claroideum* Schenck & Smith. *Mycologia*, 74: 77–92 (1982).

= *Glomus maculosum* Miller & Walker. *Mycotaxon*, 25: 217–227 (1986).

= *Glomus fistulosum* Skou & Jakobsen. *Mycotaxon*, 36: 273–282 (1989).

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

***Dentiscutata heterogama* (Nicolson & Gerdemann) Sieverding, Souza & Oehl.** *Mycotaxon*, 106: 342 (2008). **(Plate 14B & C)**

***Scutellospora heterogama* (Nicolson & Gerdemann) Walker & Sanders.** *Mycotaxon*, 27: 169–182 (1986).

Spores borne on a bulbous suspensor cell, 150–230 µm in diam., pale yellow-brown to red-brown. Spore structure of four walls (1–4) into two groups (A and B). Group A ornamented unit wall 1 tightly adherent to an inner laminated wall 2, pale yellow to pale brown, 1–1.5 µm thick. Warts densely crowded 0.5–1 µm diam. Wall 2, 4–7 µm diam., yellow-brown in colour. Group B of two membranous walls (3 and 4) hyaline <1 µm thick. Suspensor cell borne on coenocytic septate subtending hypha, 21–42 µm wide. Wall of suspensor cell

1–2.5 μm thick, distally thickening at the spore base. One or two hyphal peg projections present, 5–9 μm, arising from the suspensor cell and towards the spore. Germination shield 120 μm diameter, pale yellow to brown with smooth margins, with only a few folds. Shape of the shield resembles that of a violin.

***Dentiscutata nigra* (Redhead) Sieverding, Souza & Oehl.** *Mycotaxon*, 106: 342 (2008). **(Plate 14D & E)**

= *Scutellospora nigra* (Redhead) Walker & Sanders. *Mycologia*, 71: 178–198 (1979).

Spores found singly in the soil, laterally on a bulbous sporogenous cell. Spores dark brown to black, spherical, 297–500(–1050) μm diam. with an inner and outer wall. Outer wall black to dark brown, pitted with larger pores, 7–10 mm diameter, overlaying smaller pores consisting of a series of coils; inner wall light brown, transparent of several laminae but continuous 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 subcylindrical 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 0.3–0.7 μm thick. Reticulate ridges on outer wall supporting a detachable alveolate reticulum 0.5–2 μm wide and 2–6 μm high. Inner wall group 3 layered, totaling to 3 μm thickness. Suspensor like cell 45 (–87) x 84 (–140) μm.

***Dentiscutata reticulata* (Koske, Mill. & Walker) Sieverding, Souza & Oehl.** *Mycotaxon*, 106: 342 (2008). **(Plate 14F)**

= *Scutellospora reticulata* (Koske, Miller & Walker) Walker & Sanders. *Mycotaxon*, 16: 429–435 (1983).

Spores found singly in the soil, laterally on a bulbous sporogenous cell. Spores dark red- brown, globose to sub-globose (208–470) x (188–340) μm diam. Spore wall structure complex, consisting of two separate groups of wall layers overlain 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 0.3–0.7 μm thick. Reticulate ridges on outer wall supporting a detachable alveolate reticulum 0.5–2 μm wide and 2–6 μm high. Inner wall group 3 layered, totaling to 3 μm thickness.

***Funneliformis geosporus* (Nicolson & Gerdemann) Walker & Schüβler.** The *Glomeromycota.* A species list with new families and genera: 14 (2010). **(Plate 14G & H)**

= *Glomus geosporum* (Nicolson & Gerdemann) Walker. *Mycotaxon*, 15:49–61 (1982).

= *Glomus macrocarpum* Tulasne & Tulasne. *Canadian Journal of Botany*, 61: 2608–2617 (1983).

Plate 14: Arbuscular Mycorrhizal fungal spores.

A. Intact spores of *Claroideoglomus claroideum* (x 400).

B. Broken spore of *Dentiscutata heterogama* (x 100).

C. Germination shield in *Dentiscutata heterogama* (x 400).

D. Spore of *Dentiscutata nigra* showing sporogenous cell (x100).

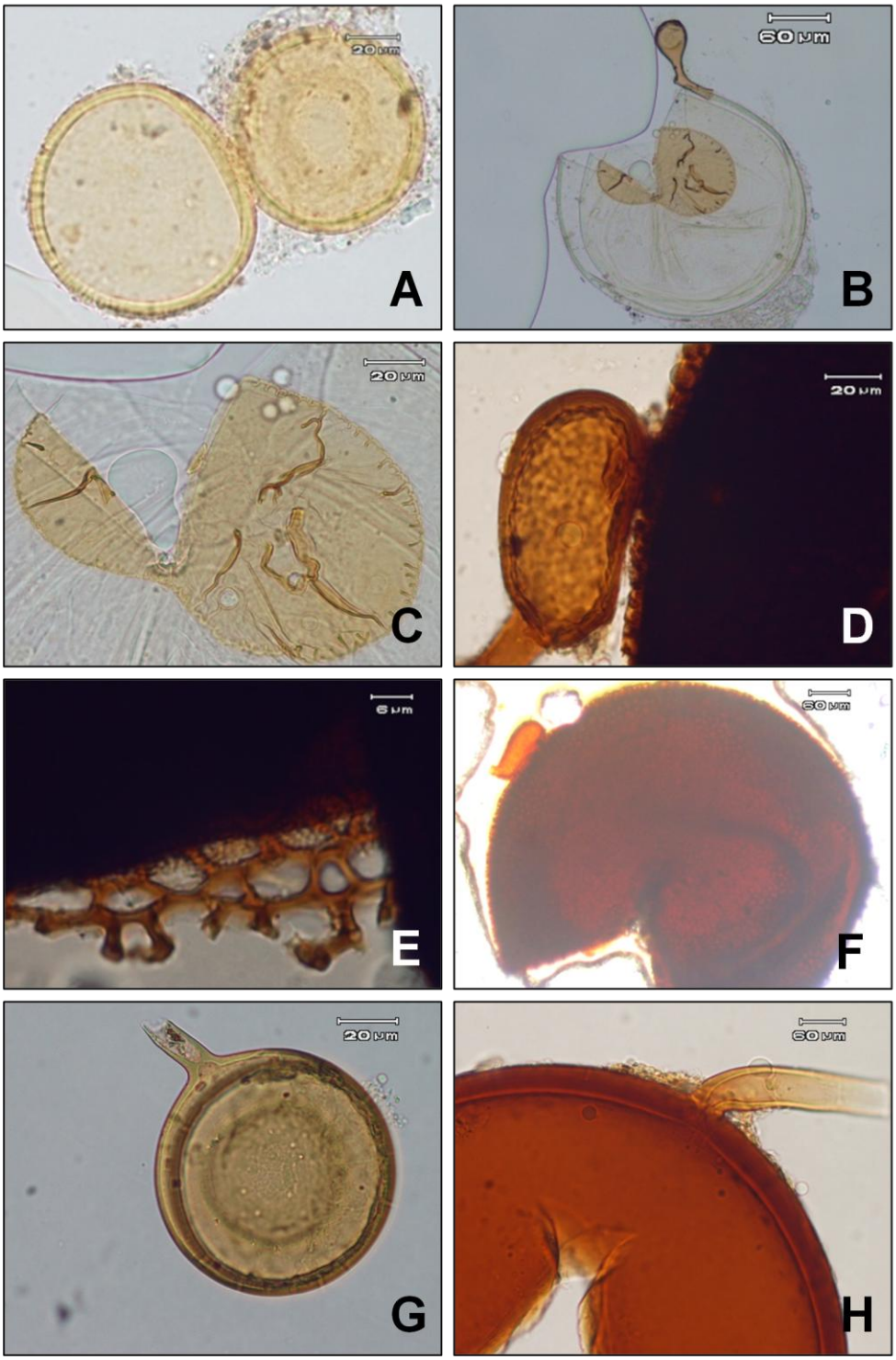
E. Spore of *Dentiscutata nigra* showing surface ornamentation (x 400).

F. Broken spore of *Dentiscutata reticulata* (x 100).

G. Intact spore of *Funneliformis geosporum* (x 100).

H. Broken spore of *Funneliformis geosporum* showing funnel shaped hypha (x 400).

Plate 14



Sporocarps unknown. Spores formed singly in soil, light yellow–brown to dark yellow–brown, globose to sub-globose, 110–225(–290) μm. Spore walls 4–6(–18) μm in thickness, 3 – layered with a thin hyaline, tightly adherent outer wall (<1 μm), a yellow– brown to red–brown laminated middle wall and a yellow to yellow-brown inner wall (<1 μm) that forms a septum. Spore contents granular in appearance, cut off by a thick septum that protrudes slightly into the subtending hypha. Spores with one straight to recurved, simple to slightly funnel shaped subtending hypha up to 200 μm long,10–18(–24) μm diameter, with yellow to dark yellow-brown wall thickening that extends 30–100 μm along the hypha from the spore base.

***Funneliformis mosseae* (Nicolson & Gerdemann) Walker & Schüβler.** The *Glomeromycota.* A species list with new families and genera: 13 (2010).

= *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe. *Mycologia Memoir*, 5: 76 (1974).

Spores yellow to brown, globose to sub-globose, 105–305 μm with one or two funnel shaped bases 20–50 μm diam., divided from subtending hyphae by a curved septum, hyaline 2–7 μm thick irregularly branched septate hyphae of 2–12 μm wide.

***Gigaspora albida* Schenck & Smith.** *Mycologia*, 74(1): 85 (1982). **(Plate 15A & B)**

Spores formed singly in the soil, dull white, mostly spherical, (143–)162–222(–350) μm. Spore wall 4–12 μm thick, with 1–6 walls; outer wall thin, smooth, 1–2 μm thick, readily cracking under light pressure; usually 2 or 3 but

occasionally 4–5 inner walls of varying thickness. Sporogenous cell (24–)38–51 μm broad borne on a septate subtending hypha with fine hyphal branches.

Gigaspora candida **Bhattacharjee, Mukerji, Tewari & Skoropad.** *Transactions of the British Mycological Society*, 78(1): 184 (1982).

Spores found singly in soil, white, globose, 200–240(–300) μm. Spore wall smooth, 2 layered, outer layer 1 μm thick, inner layer up to 6 μm thick. Sporogenous cell, 30–50 μm diameter.

Gigaspora margarita **Becker & Hall.** *Mycotaxon*, 4(1): 155 (1976). **(Plate 15C & D)**

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

Gigaspora ramisporophora **Spain, Sieverding & Schenck.** *Mycotaxon*, 34(2): 668 (1989).

Spores smooth, golden yellow, globose, (96–)200–273(–567) μm in diam. Spore wall structure of 3 walls in a single group with total wall thickness 9–12(–31) μm; wall 1, unit wall, hyaline, brittle, 1.4–4(–5.7) μm thick, continuous with outer wall of sporogenous cell and adherent to wall 2; wall 2 laminate, yellow to yellowish brown, 4–10(–28) μm thick, adherent to wall 3 yellow, 1

μm thick. Sporogenous cell 60–83 μm broad; sporogenous cell develop a branch (sporogenous cell with connecting hypha) which gives rise to another spore; sporogenous cell has thick–walled, 3–4 μm hyphal protrusion, 8–10 μm in diam. Sporophores simple or branched, formed of specialized septate hypha, 9.3–13.9 μm in diam., with 1–3 sporogenous cells.

***Gigaspora rosea* Nicolson & Schenck.** *Mycologia*, 71(1): 190 (1979).

Spores produced singly in soil, predominantly globose, 230–294(–305) μm diam., white to cream colour with a rose–pink tint on the spore wall near the hyphal attachment encompassing up to half the spore. Pink colour variable from distinctly rose pink to barely detectable. Spore wall thickness 2.4–7.5 μm, with 2–5 inseparable layers 1–2 μm thick; outer wall layer smooth. Sporogenous cell 28–40 μm diam. borne on a subtending hypha; subtending hypha 7–14 μm wide, hyphal walls 1–2 μm thick, septate.

***Glomus aggregatum* Schenck & Smith emend. Koske.** *Mycologia*, 77: 619 (1985).

Spores found in loose aggregation, pale yellow, globose to sub-globose, (20–)40–120 μm in diam. Spore wall of 1 or 2 separable, coloured laminated walls, each 1–3(–5) μm thick, thicker near point of attachment; if 2 walls present, the outer is thicker. Subtending hypha straight, curved, swollen or irregular, up to 12 μm wide at the spore base; walls 1–3.5 μm thick. Pore open or closed by a curved septum in the subtending hypha by an inner wall near the spore base.

***Glomus coremioides* (Berk. & Broome) Redecker & Morton.** *Mycologia*, 92: 284 (2000).

Sporocarps dull brown, sub-globose, 340–600 μm broad. Peridium of thickwalled interwoven hyphae. Chlamydospores tightly grouped in a hemisphere around a central plexus of hyphae. Chlamydospores obovoid to ellipsoid, (50–)75–102 x 35–63(–82) μm, septa may or may not be present at the spore base. Spore wall 1.5–2.5 μm and frequently thickened at base up to 6 μm.

***Glomus glomerulatum* Sieverding.** *Mycotaxon*, 29: 73–79 (1987).

Sporocarps dark brown, globose to sub-globose, 290–675 μm diam. Spores yellow to brown, globose to sub-globose, 40–70 μm in diam. Composite spore wall is composed of two walls (walls 1 & 2) in Group A. Wall 1 is yellow to brown laminated, 4–9 μm thick. Wall 2 hyaline, membranous, 0.5 μm thick. Two to three hyphal attachments, straight to recurved, cylindrical to funnel shaped, yellow to brown, 5–7 μm diam. The pore of hyphal attachment is 1–2 μm in diam., closed by the spore wall or by a septum. Spore contents are hyaline and oily.

***Glomus heterosporum* Smith & Schenck.** *Mycologia*, 77: 567 (1985).

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

attachment is 5–15(–31) μm wide. Spores frequently with multiple hyphal attachments.

Glomus microcarpum **Tulasne & Tulasne.** *Mycologia*, 76: 190–193 (1984).

Sporocarps are irregularly ellipsoidal enclosed by a peridium. Peridium 50–100 μm wide, composed of interwoven hyphae. Spores are globose, 30–40 μm in diameter. Spore wall smooth, 4–6 μm thick, hyaline with laminations. Subtending hyphae is 4–8.5 μm thick at the point of attachment.

Glomus multicaule **Gerdemann & Bakshi.** *Transactions of the British Mycological Society*, 66: 340–343 (1976).

Spores dark brown, 149–162 μm, ellipsoidal, occasionally triangular with 1–4 hyphal attachments generally occurring at opposite end of spores. Spore wall 8.6–34 μm, thickest at the point of attachment with rounded projections of 1.2–3.7 μm long distributed all over the spore surface.

Glomus pachycaule **(Wu & Chen) Sieverding & Oehl.** *Mycotaxon*, 116: 75–120 (2011). **(Plate 15E)**

= ***Sclerocystis pachycaulis*** **C.G. Wu & Z.C. Chen.** *Taiwania*, 31: 74–75 (1986).

Sporocarps dark brown, 180–675 μm diam., surrounding a central plexus of hyphae. Peridium absent, individual spores partially enclosed in a thin network of tightly appressed hyphae. Spores dark brown, obovoid to ellipsoid or sub-globose, 37–125 μm with a small pore opening in to thick walled subtending hypha. Spore wall laminate, 3–7.6 μm thick upto 13.5 μm thick at

the spore base, perforated projections appears on the inner surface-walled subtending hyphae.

***Glomus pubescens* (Saccardo & Ellis) Trappe & Gerdemann.** *Mycologia Memoir*, 5: 76 (1974). **(Plate 15F)**

Spores found singly in soil, hyaline, smooth, sub-globose to ellipsoid, 20–48 x 18–45 μm, Spore walls 3–6 μm thick, hyaline to light yellow, the opening may be occluded by wall thickening. Spores filled with oil globules of variable size. Hyphal attachment 2 μm in diam., the attached hypha hyaline, thin walled and inconspicuous.

***Glomus radiatum* (Thaxter) Trappe & Gerdemann.** *Mycologia Memoir*, 5: 76 (1974).

Sporocarps up to 9 x 7 x 3 mm, flattened, off white to grayish-yellow. Peridium absent. Chlamydospores at or near the surface thin-walled, becoming progressively thicker walled in the direction of sporocarp base, 60–110(–120) x 48-65(–85) μm ellipsoid or rarely globose, arranged in a matrix of coarse thin-walled hyphae. Spore wall 4–8 μm wide, light yellow. Subtending hyphae opening into the spore up to 6 μm wide, only partially occluded by spore wall thickening but occluded by a plug below the base.

***Glomus sinuosum* (Gerdemann & Bakshi) Almeida & Schenck.** *Mycologia*, 82: 710 (1990).

Sporocarps reddish brown to dull brown, globose to sub-globose, 220–650 μm in diam. Peridium tightly enclosing a sporocarp, composed of thick walled

Plate 15: Arbuscular Mycorrhizal fungal spores.

A. Broken spores of *Gigaspora albida* (x 100).

B. Broken spore of *Gigaspora albida* showing wall layers (x400).

C. Broken spore of *Gigaspora margarita* (x 100).

D. Spore of *Gigaspora margarita* showing surface ornamentation (x 400).

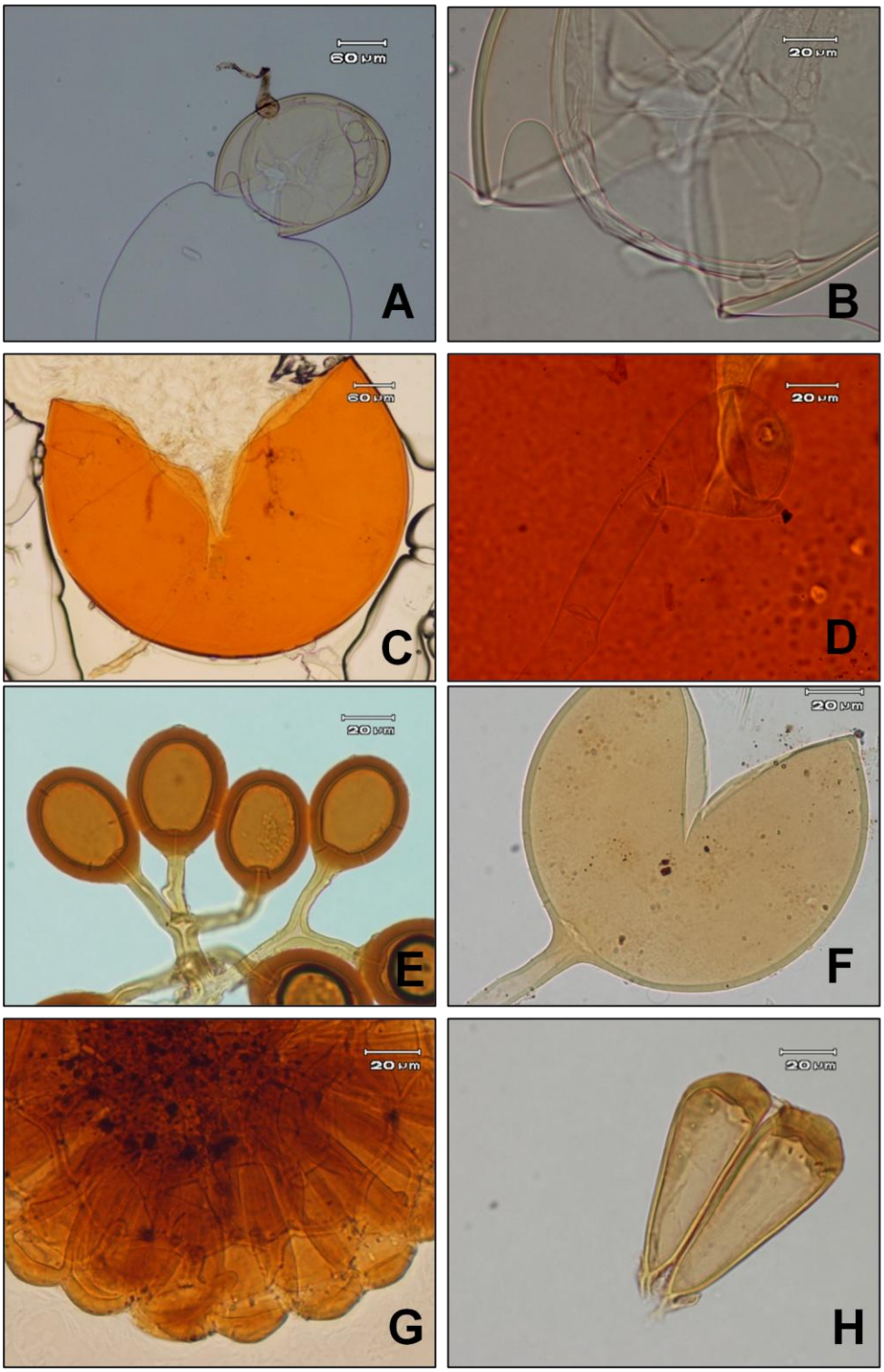
E. Sporocarp of *Glomus pachycaule* (x 400).

F. Intact spore of *Glomus pubescens* (x 400).

G. Sporocarp of *Glomus taiwanense* (x 100).

H. Sporocarp of *Glomus taiwanense* (x 400).

Plate 15



interwoven hyphae. Peridial hyphae sinuous. Sometimes sinuous feature may be indistinct. Chlamydospores globose, sub-globose, obovoid, clavate or irregular, 45–150 x 30–83 μm, walls single with walls evenly thickened or unevenly thickened, usually thickened at the apex or lateral side, (1.5–)5–22.5(–30) μm, with 1–2 attached hyphae.

***Glomus* sp. (Plate 16A–H)**

Spores formed singly in the soil but occasionally adhering in pairs, sessile, pale cream to dull brown, globose, sub-globose to irregular (160–)190–220(–270) µm diameter Spore wall structure of 5 walls (1–5) in two groups (A and B), exterior and interior, in total 3.0–3.5 µm thick, with a mantle of an irregular, reticulate pattern of pale cream to dull brown, fine sinuous hyphae, closely appressed to the spore and flattened, 3–7 µm wide, forming layers on the spore surface up to 20–30 µm thick. Mantle extends above the spore surface forming a funnel shaped, slightly inflated, saucer like structure giving the spore an appearance of pitcher. Saucer like structure constricted at the base with a central pore 2–4 µm wide, open or sealed by the inner wall component. Mantle frequently with adhering debris and soil particles giving the impression of pigmentation. Wall W1 sloughing off completely at maturity. Wall W2 hyaline, flexible and remains inseparable from wall W1, 0.5–0.8 µm in thickness. Group B consisting of three walls (W3, W4 and W5). Wall W3 is hyaline, continuous, flexible, separating the inner spore content from the outer wall, 3–4 µm in thickness. Wall W4 hyaline, rigid, 3–4 µm thick. Wall W5 hyaline, unilayered, tightly adherent to W4, however the staining reaction in

Plate 16: Arbuscular Mycorrhizal fungal spores.

A. Fresh spores of *Glomus* sp. (x 100).

B. Broken spore of *Glomus* sp. showing wall layers (x1000).

C. Intact spore of *Glomus* sp. with outer layer separated (x 100).

D. Spore of *Glomus* sp. showing inner wall layers (x 100).

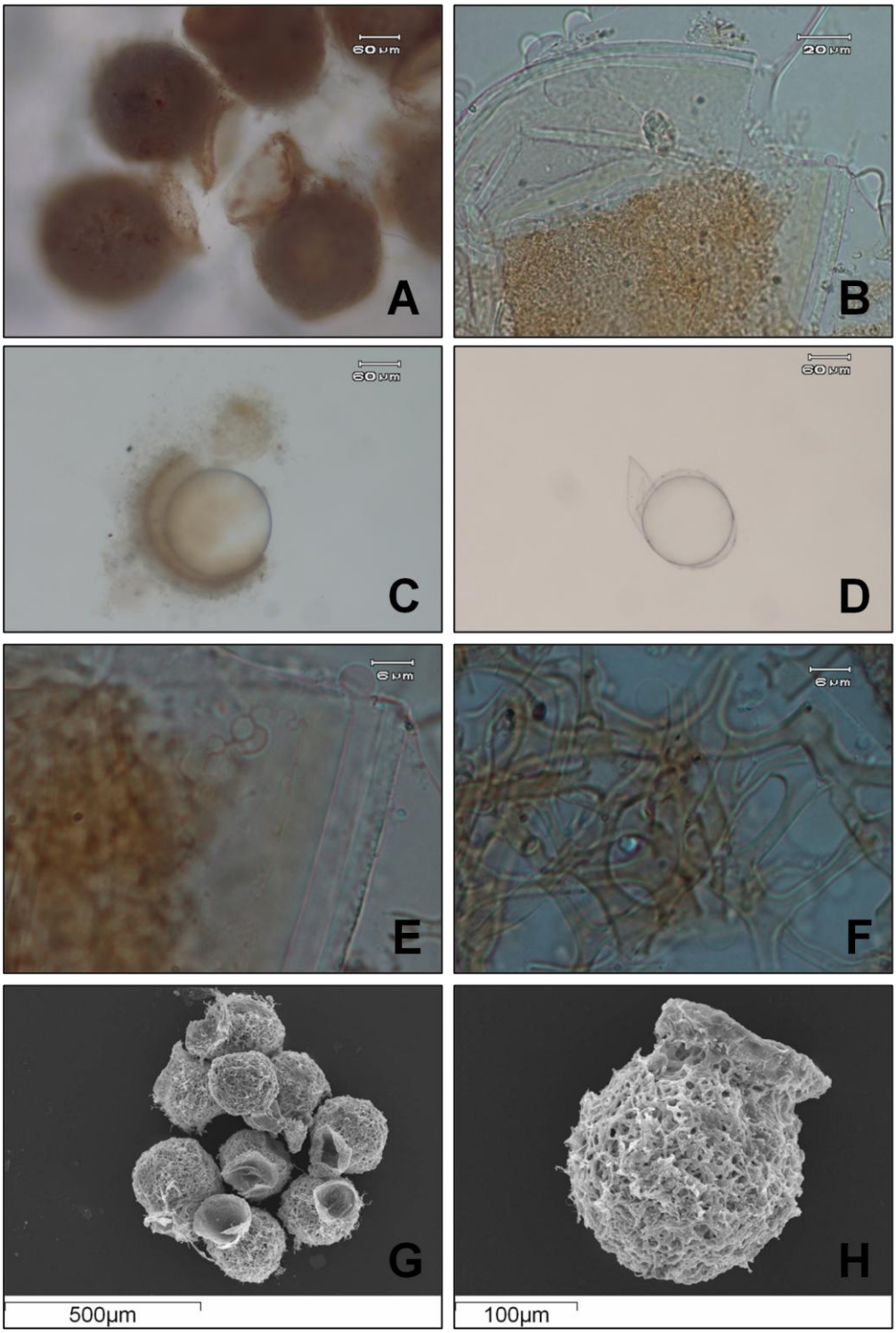
E. Broken spore of *Glomus* sp. showing inner wall layers (x 1000).

F. Spore of *Glomus* sp. showing sinuous hyphal netwok on surface (x 1000)

G. Scanning Electron Microscope image of *Glomus* sp.

H. Scanning Electron Microscope image of *Glomus* sp.

Plate 16



Melzer's reagent helps to reveal its presence, thin, 4–6 μm, amorphous and is not beaded. Spore contents of hyaline lipid globules.

***Glomus taiwanense* (Wu & Chen) Almeida & Schenck.** *Kew Bulletin*, 50(2): 306 (1995). **(Plate 15G & H)**

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

***Glomus tortuosum* Schenck & Smith.** *Mycologia*, 74: 83 (1982).

Spores formed singly in soil but occasionally adhering in pairs, yellow to dull grey-brown with a mantle of sinuous hyphae, closely appressed to the spore and flattened, 4–10 μm wide, forming layers of hyphae on the spore surface up to 25 μm thick. Mantle hyphae hyaline when young, acquiring a brownish pigment with age, and originating from swellings of hyphal attachment 10–20 μm below the spore or arising from other hyphae adjacent to the spore. Mantle frequently with adhering debris and soil particles. Chlamydospores largely globose, (130–)140(–200) μm diameter, some sub-globose 94–180 x 116–220 μm excluding the mantle; spores with a single, laminate, thin wall, 0.5–2 μm diam; spore contents globular but usually obscured by the mantle.

***Racocetra gregaria* (Schenck & Nicolson) Oehl, F.A. Souza & Sieverding.** *Mycotaxon*, 106: 337 (2008). **(Plate 17A & B)**

= *Scutellospora gregaria* (Schenck & Nicol.) Walker & Sanders. *Mycologia*, 77: 702–720 (1985).

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

***Racocetra weresubiae* (Koske & Walker) Oehl, Souza & Sieverding.** *Mycotaxon*, 106: 337 (2008).

= *Scutellospora weresubiae* Koske & Walker. *Mycotaxon*, 27: 219–235 (1986).

Spores found singly in the soil, terminally on a bulbous sporogenous cell. Spores translucent, glistening, pale pink, globose to sub-globose, (125–) 156–265 x 135–294(–414) μm diam. Spore wall structure of six walls (1–6) in three

groups (A, B & C). Group A often with an outer brittle, hyaline, unit wall (wall 1) up to 0.5 μm thick, tightly adherent to an inner brittle, pink, laminated wall (wall 2) (3–)12(–15) μm thick. Group B of two membranous walls (3 & 4), each 1 μm thick. Group C formed of a thin hyaline coriaceous wall (wall 5) (2–8 μm thick), surrounding a hyaline membranous innermost wall (wall 6) 0.5 μm thick. Sporogenous cell, hyaline to pale brownish–yellow, (32–50) μm wide, with 1 or 2 hyphal pegs 27 μm long and 3–8 μm wide, projecting towards the spore base. Sporogenous cell, borne terminally on a sparsely septate or aseptate subtending hyphae.

***Rhizophagus clarus* (Nicolson & Schenck) Walker & Schüβler.** The *Glomeromycota*. A species list with new families and genera: 19 (2010).

= ***Glomus clarum* Nicolson & Schenck.** *Mycologia*, 71: 178–198 (1979).

Spores hyaline 68–290 μm in diam., composed of two wall layers. Spore contents hyaline consisting of globules of variable size, outer wall consists of 5–20 μm thick, inner wall of 2–9 μm thick. Subtending hyphae 15–80 μm wide, becoming thinner with increasing distance from the spore.

***Rhizophagus diaphanus* (Morton & Walker) Walker & Schüβler.** The *Glomeromycota*. A species list with new families and genera: 19 (2010).

= *Glomus diaphanum* Morton & Walker. *Mycotaxon*, 21: 431–440 (1984).

Spore formed singly, or in loose clusters, hyaline, gobose to sub-globose, (39–) 100–121 μm in diam. Spore wall structure consists of two walls (wall 1 and 2) in a single group (A). Wall 1 (2)–4.4(6.5) μm thick, laminate. Wall 2 membranous, (0.2–) 0.8(–1.3) μm, extends 5–12 μm in the subtending hypha

and forms a septum enclosing the spore contents. Spore contents are hyaline and contain oil globules. Subtending hypha 5.4–11 μm in diameter at the spore base; hyphal wall (1.4–) 3(–3.7) μm thick.

***Rhizophagus fasciculatus* (Thaxter) Walker & Schüβler.** The *Glomeromycota*. A species list with new families and genera: 19 (2010).

= *Glomus fasciculatum* (ThaXter) Gerdemann & Trappe emend. Walker & Koske. *Mycotaxon*, 30: 253–262 (1987).

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

***Rhizophagus intraradices* (Schenck & Smith) Walker & Schüβler.** The *Glomeromycota*. A species list with new families and genera: 19 (2010). **(Plate 17C–F)**

= ***Glomus intraradices* Schenck & Smith.** *Mycologia*, 74: 77–92 (1982).

Spores 93–131 μm diameter, brown in colour. Spore walls 3–15 μm thick, yellow to gray brown, with 4 laminated walls, inner walls 1–2 μm thick, darker than outer walls. Spore contents globular, yellow to light brown. Wall of the spore extending into the hyphal attachment forming an apparent tubaeform flare at the juncture with the hyphal attachment. Hyphal attachment 9–33 μm

Plate 17: Arbuscular Mycorrhizal fungal spores.

A. Broken spore of *Racocetra gregaria* (x 40).

B. Spore of *Racocetra gregaria* showing surface ornamentation (x 400).

C. Fresh spores of *Rhizophagus intraradices* (x 100).

D. Intact spore of *Rhizophagus intraradices* (x 400).

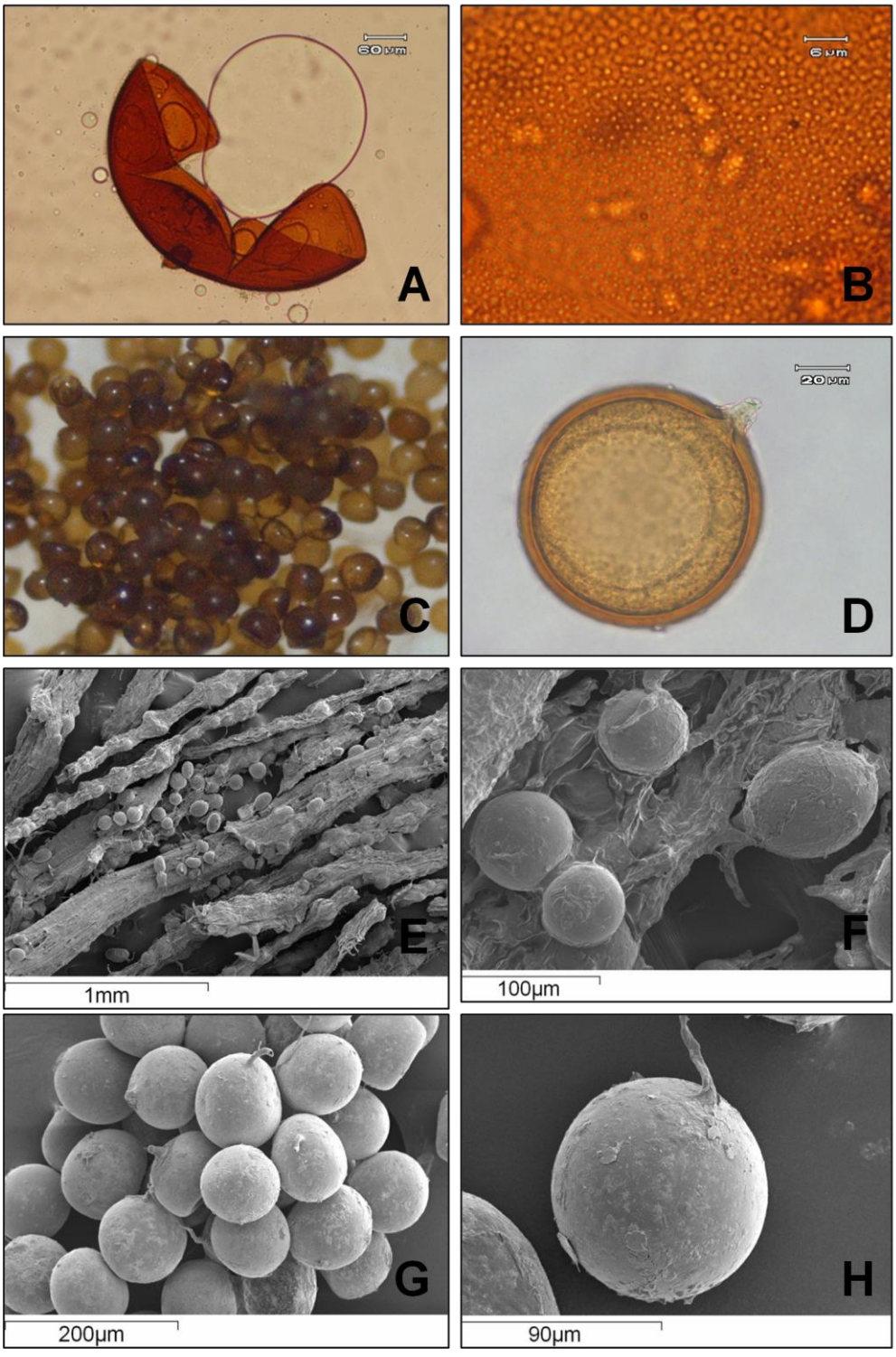
E. Scanning Electron Microscope image of *Rhizophagus intraradices* in roots.

F. Scanning Electron Microscope image of *Rhizophagus intraradices* in roots.

G. Scanning Electron Microscope image of *Simiglomus hoi*.

H. Scanning Electron Microscope image of *Simiglomus hoi*.

Plate 17



wide with wall thickness of 1.5–5.2 µm at the base of the spore. Hyphal attachment constricted 2–3 µm at the base of the spore.

Simiglomus hoi (**Berch & Trappe) Silva, Oehl & Sieverding.** *Mycotaxon*, 116: 75–120 (2011). **(Plate 17G & H)**

= ***Glomus hoi* Berch & Trappe.** *Mycologia*, 77: 654–657 (1985).

Spores borne singly in soil, globose to sub-globose, (50–)80–120(–140) µm, light brown in colour, ellipsoidal, 23–140 µm in diam. Spore wall consists of two distinct separable layers. Outer layer orange–yellow, 2–8 µm diameter. Inner layer 0.5–1 µm thick, hyaline to light yellow. Wall of the subtending hyphae conspicuously thickened over very long distances from the spore base (up to > 1000 µm) continuous and concolorous with the spore wall, or slightly lighter in colour than the spore wall. Subtending hypha cylindrical or slightly flared toward the attachment of spore, 5–13 µm wide, bearing thin walled septate lateral branches. Subtending hypha occluded by a fine curved septum below the attachment of spore.

5.4 DISCUSSION

The study revealed a large number of arbuscular mycorrhizal fungi associated with rhizosphere soils of ornamental flowering plants species. A total 44 AM fungal species was recorded from 43 host plant species the majority of which belonged to the genus *Glomus*. Similar observations on the predominance of *Glomus* species in rhizosphere soils of various plants species from Western Ghats region of Goa have been reported previously (Radhika and Rodrigues, 2010). Raghupathy and Mahadevan (1993); Thaper and Khan (1995) also

reported the predominance of genus *Glomus* in species diversity from tropics. All the 44 species of AM fungi recorded in the present study fit well into the known descriptions. However, two species of AM fungi taxonomically different from the existing world species collection were also recorded. The first species is described as *Acaulospora soloidea* sp. nov. and the other species belongs to the genus *Glomus*. Spore in spore syndrome was also observed during the study. Hall (1977) reported occurrence of *Glomus pallidus* spores within the spores of *Glomus macrocarpus* var. *macrocarpus*. Koske (1975) reported the presence of a yellow punctuate spore of *Acaulospora scrobiculata* inside *Gigaspora* species.

In the present study, no host specificity was observed. Moreira *et al*. (2007) reported the effect of a single AM species differing in two different host plants, each host plant selectively producing a differentiated spore compositition. However, depending on the growth conditions i.e. host plant and environmental conditions, there may be qualitative and quantitative change in the spore composition, which may not reflect the original composition in the field (Carrenho *et al*. 2002). The differences in spore numbers obtained from trap cultures may be due to variations in host plant root type and morphology, carbon biomass or nutrient and endogenous hormonal levels (Cuenca and Meneses, 1996; Stutz and Morton, 1996; Brundrett *et al*., 1999). Host plant and soil factors can influence both diversity and overall levels of P in soil and plant are able to inhibit mycorrhiza formation (Douds and Schenck, 1990) and influence the diversity of AM fungi in field soil (Cuenca and Meneses, 1996). A wide diversity of AM fungi is responsible for

the forms mycorrhizae in ornamental flowering plants and therefore conservation and efficient utilization of arbuscular mycorrhizal diversity is a pre-requisite to sustainable plant productivity.

CHAPTER 6

MYCORRHIZAL STATUS OF SELECTED ORNAMENTAL FLOWERING PLANTS AS INFLUENCED BY ITS PHENOLOGY

6.1 INTRODUCTION

Phenology is the study of periodicity or timing of recurring biological events. Plant phenology is the timing of plant growth and development and events involve flowering, fruiting, leaf flushing and germination (Leith, 1974). Mycorrhizal symbiosis is the most important root-fungus association found in plants. It occurs in nearly all agricultural and natural soils, colonising roots of many plant species (Smith and Read, 1997). The symbiosis is characterized by a bi-directional nutrient transfer where the plant supplies the fungus with carbon and in return receives nutrients, mainly P from the fungal symbiont. Phosphorus is often not freely accessible in the soil and its availability to plants varies between different soils (Javot *et al.*, 2007). One of the main benefits for the plant from the AM symbiosis is improvement of P uptake. Phosphate is a mineral nutrient limiting plant growth at many natural ecosystems due to its poor solubility. Arbuscular Mycorrhizal fungi transport P from distant reservoirs to the plant, extending the reach of plant root system. Even under non-limiting P supply and without apparent growth effects of AM colonization, colonized plant roots are reducing the activity of their own P uptake system and rely mainly on their fungal symbiont for P provision (Smith *et al*., 2003). This mycorrhizal P uptake pathway for P acquisition is reported in several studies (Pearson and Jakobsen, 1993; Smith *et al*., 2003; Poulsen *et al*., 2005), but it is seen that this effective plant P uptake by AM fungi is not directly correlated with the potential P supply capacity *i.e.* a high colonization degree or a high proportion of intraradical structures capable of nutrient exchange (Burleigh *et al*., 2002; Cavagnaro *et al*., 2004; Smith *et al*., 2004; Feddermann *et al*., 2008; Jansa *et al*., 2008). Variation in colonization ability,

P translocation and symbiotic efficiency can occur not only between AM fungal species but also within species (Lerat *et al*., 2003; Munkvold *et al*., 2004; Koch *et al*., 2006). It is therefore practically not possible to generalize functions of a single isolate to the entire species not generalize functions from a single species to a phylum or genus. Under experimental conditions AM colonized plant growth may be improved but in some cases no such improvement or even an inhibition can be detected compared to non-colonized plants (Smith and Read, 1997).

Arbuscular Mycorrhizal fungi can contribute almost 100% of total plant uptake of P without increasing total plant P content over that of a non mycorrhizal control (Smith *et al*., 2003, 2004). The precise physiological mechanisms behind such effects are not known to date. Improvements in P acquisition have a significant impact on plant growth, health, and subsequently on plant biodiversity and ecosystem productivity (van der Heijden *et al*., 1998b). Due to the symbiotic relationship between plant and AM fungi, phenological studies are essential to know the status of AM fungi associated with the rhizosphere soils during the different growth stages of host plants. Arbuscular Mycorrhizal fungal colonization and spore density seems to vary in sensitivity to hosts depending on their stage of growth.

Phenology of host plant species influences mycorrhizal status. A number of studies have shown that the presence of AM fungi influence plant phenology (Allen and Allen, 1986; Garbaye and Churin, 1996; Courty *et al*., 2007). Reciprocally, plants may influence fungal phenology either by

moderating microclimatic conditions or by altering the timing of resource availability such as C flux or *via* the timing of leaf fall. Studies have shown that AM fungal phenology is sensitive to temperature, rainfall and forest stand conditions (Chacón and Guzman, 1995; Luoma et al., 2004) and that this sensitivity can even vary between deciduous and evergreen hosts (Gange *et al*., 2007).

Investigation of AM status during the various growth stages of commercially important ornamental flowering plants offers the potential for future manipulation of AM biofertilizers in floriculture industry establishment with possible favourable consequences for enhanced flower production with minimal inputs. Despite the importance of AM fungi and the importance of these plant species as commercially important floricultural crops there are few studies pertaining to AM status and plant phenology. There are no previous studies on P concentration in ornamental flowering plants in relation to phenology. Studies involving the relationship between AM fungi and ornamental plant species and the influence on AM status may contribute to more sustainable practices in the future. The aim of this study was to determine the mycorrhizal status, percent root colonization and spore density as influenced by phenology of *Chrysanthemum morifolium*, *Crossandra infundibuliformis* and *Tagetes erecta* growing under natural conditions. Total P in these plant species during different growth stages was also determined.

6.2 MATERIALS AND METHODS

6.2.1 Site description: The experimental site (Parsem) is situated in North Goa and lies at latitude 15°71′89″ N and longitude 73°79′68″ E. The soil was lateritic with acidic to near neutral pH ranging from 5.6–6.5.

6.2.2 Plant species selected for the study: Three plant species *viz*., *Chrysanthemum morifolium* Ramat, *Crossandra infundibuliformis* (L.) Nees and *Tagetes erecta* L. were selected for the study.

6.2.3 Sample collection: Plants of uniform age were selected from the study sites. Rhizosphere roots and soil samples were collected from the selected plant species during the three different growth stages *viz*., vegetative, flowering and senescence stage. Samples were collected in triplicate for each plant species during a particular growth stage. Samples were packed in polyethylene bags, labeled and brought to the laboratory. The root samples were processed soon after bringing to the laboratory, whereas soil samples were stored at 4°C until processing.

6.2.4 Processing of root samples and estimation of root colonization by AM fungi: Was carried out as described under **3.2.2 and 3.2.3**.

6.2.5 Extraction of AM fungal spores: Was carried out as described under **4.2.2**.

6.2.6 Taxonomic identification of AM fungal spores: Was carried out as described under **4.2.5**.

6.2.7 Total P estimation by spectrophotometric method: Leaf P concentration during vegetative stage was assessed by leaf analysis after oven drying at 70°C for 72 h (Chapman and Pratt, 1961). Total P was estimated using Ammonium vanadate - ammonium molybdate yellow colour method (Jackson, 1973).

Preparation of reagent: Preparation of 2N Hydrochloric Acid: 165.6 ml concentrated HCl (37%, sp.gr.1.19) was diluted with double distilled water, mixed well, allowed to cool and the final volume was made to 1l with double distilled water.

Preparation of standard Stock Solution: 2.5 g potassium di-hydrogen phosphate (KH_2PO_4) was dried in an oven at 105°C for 1 hour, cooled in a desiccator and stored in tightly stoppered bottle. 0.2197g dried potassium dihydrogen phosphate was then dissolved in double distilled water and the final volume was made to 1l with double distilled water. This solution contains 50 ppm P (Stock Solution). A series of standard solutions from the above stock solution was prepared as follows:

1, 2, 3, 4, and 5 ml stock solution was diluted to 100 ml final volume by using double distilled water. These solutions contained 0.5, 1.0, 1.5, 2.0 and 2.5 ppm P respectively.

Preparation of Ammonium Heptamolybdate-Ammonium Vanadate: 22.5 g ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] was dissolved in 400 ml double distilled water. Then 1.25 g ammonium metavanadate (NH_4VO_3) was dissolved in 300 ml hot double distilled water. Later ammonium metavanadate solution was added to ammonium heptamolybdate solution in 1l volumetric flask and the mixture was allowed to cool at room temperature. After cooling, 250 ml concentrated nitric acid (HNO_3) was slowly added to the mixture, the solution again cooled at room temperature and the final volume made up to 1l with double distilled water.

Dry ash-digestion procedure: Modified method of Chapman and Pratt (1961) was employed. 0.5–1.0 g portions of ground plant material were weighed in 50 ml porcelain crucible. Porcelain crucible was placed into a cool muffle furnace and temperature gradually increased to 550°C. Ashing was continued for 5 hours after attaining 550°C. The cooled ash was dissolved in 5 ml portions 2N HCl and mixed well. After 15–20 minutes, volume was adjusted to 50 ml using double distilled water. The solution was mixed thoroughly and allowed to stand for about 30 minutes, then filtered through Whatman No. 42 filter paper, discarding the first portion of the filtrate. The remaining portion of the filtrate was used for analysis of P (by Ammonium Vanadate-Ammonium Molybdate yellow colour method).

Measurement of P by Ammonium vanadate-ammonium molybdate yellow colour method: 10 ml of the filtrate were pipetted into a 100 ml

volumetric flask, 10 ml ammonium-vanadomolybdate reagent added and the solution diluted to volume with double distilled water.

Preparation of a standard curve: 1, 2, 3, 4 and 5 ml standard stock solutions were pipetted into a 100 ml volumetric flask, 10 ml ammonium-vanadomolybdate reagent added and the solution diluted to volume with double distilled water. A blank was also prepared by adding 10 ml ammonium-vanadomolybdate reagent into a 100 ml volumetric flask and the solution diluted to volume with double distilled water. The absorbance of the blank, standards and samples was read after 30 minutes at 410 nm wavelength. Calibration curve for standards was prepared by plotting absorbance against the respective P concentrations. P concentration in the unknown samples was read from the calibration curve.

Calculations: Percent total Phosphorus in plant,

$$\% P = \text{ppm P (from calibration curve)} \times \frac{R}{Wt} \times \frac{100}{10000}$$

Where R = Ratio between total volume of the filtrate and the filtrate volume used for measurement, and Wt = Weight of dry plant (g).

Relative abundance and Frequency of occurrence: Frequency of occurrence and relative abundance of AM fungi were calculated by using the following formulae by Beena *et al*., (2000).

Frequency of occurrence (%),

F = (Number of soil samples that possessed spores of particular AM species/Total no. of soil samples screened) x 100

Relative abundance (%),

RA = (Number of spores of particular AM species/Total no. of spores of all the AM species) x 100

6.3 RESULTS

Root colonization by AM fungi varied from 35–66% in the three stages of growth in *C. morifolium*. It was maximum (66%) during vegetative stage beyond which the root colonization steadily declined towards reproductive stage and was least during senescence. Hyphal and vesicular colonization was observed from the vegetative stage to the senescent stage. Arbuscules appeared during late flowering stage and were persistent until senescence. In *C. infundibuliformis* root colonization ranged from 39–69% during all three stages of growth. Root colonization was highest (69%) during vegetative stage after which levels slowly declined during reproductive stage and were minimum during senescence. Hyphal and vesicular colonization was observed during all growth stages. Arbuscules appeared only during the flowering stage, later degenerated and were absent during senescence. Root colonization by AM fungi varied from 36–71% in all the three stages of growth in *T. erecta*. It was highest (71%) during vegetative stage after which it steadily declined and was least during senescence. Hyphal and vesicular colonization was observed during vegetative stage and persisted until senescence. The arbuscules appeared late during flowering stage and were persistent till senescence (**Table 11**).

In total 13 AM fungal species belonging to six different genera *viz*., *Acaulospora*, *Claroideoglomus*, *Funneliformis*, *Gigaspora*, *Glomus* and

Rhizophagus were isolated during the various growth stages of the three plant species studied. Only two AM species *viz*., *Gigaspora albida* and *Rhizophagus intraradices* were recorded in all the three plant species during all the stages of growth.

Spore density in the rhizosphere soils of *C. morifolium* ranged from 34–76 spores $100g^{-1}$. Least spore density (34 spores $100g^{-1}$) was recorded during the initial stages of plant growth and steadily increased during flowering, reaching a maximum (76 spores $100g^{-1}$) during senescence, accounting for four AM species (**Table 11**). Frequency of occurrence was similar in all the three growth stages while variation in relative abundance being highest in *R*. *intraradices* in all three stages (**Fig. 17**). In *C. infundibuliformis*, the spore density ranged from 43–95 spores $100g^{-1}$. The least spore density (43 spores $100g^{-1}$) was recorded during the initial stages of plant growth, increased during the flowering stage (83) and was maximum (95 spores $100g^{-1}$) during senescence and accounted for seven AM species (**Table 11**). Frequency of occurrence was similar in all the three growth stages while variation in relative abundance being highest in *R*. *intraradices* during all three stages (**Fig. 18**). Spore density in the rhizosphere of *T. erecta* varied from 38–121 spores. It was lowest (38 spores $100g^{-1}$) during the initial stages of plant growth, steadily increased during flowering stage (73 spores $100g^{-1}$) and was maximum (121 spores $100g^{-1}$) during the senescence stage accounting for a total of seven AM species. Highest spore density was recorded in *T. erecta* (121 spores $100g^{-1}$) during the senescent stage of growth. Frequency of occurrence was similar in all the three growth stages

Table 11: Effect on AM fungal colonization, spore density and AM fungal diversity in stages of host phenology.

Sr. No.	Plant species	Growth stage	AM colonization	AM colonization (%)	Spore density100g^{-1}	AM species
1.	***C. morifolium***	**Vegetative**	H	66.00^{a} ±2.65	34.00^{c} ±2.00	*Acaulospora laevis*, *Gigaspora albida*, *Rizophagus intraradices*, *R. fasciculatus*.
		Flowering	H, V, A	43.00^{b} ±1.53	55.00^{b} ±2.52	*Acaulospora laevis*, *Gigaspora albida*, *Rizophagus intraradices*, *R. fasciculatus*.
		Senescence	H, V, A	35.00^{c} ±1.15	76.00^{a} ±3.61	*Acaulospora laevis*, *Gigaspora albida*, *Rizophagus intraradices*, *R. fasciculatus*.
2.	***C. infundibuliformis***	**Vegetative**	H, V	69.00^{a} ±0.58	43.00^{c} ±3.00	*Acaulospora foveata*, *Claroideoglomus claroideum*, *Gigaspora albida*, *Glomus aggregatum*, *G. multicaule*, *G. pachycaule*, *Rizophagus intraradices*.
		Flowering	H, V, A	56.00^{b} ±2.52	83.00^{b} ±2.65	*Acaulospora foveata*, *Claroideoglomus claroideum*, *Gigaspora albida*, *Glomus aggregatum*, *G. multicaule*, *G. pachycaule*, *Rizophagus intraradices*.
		Senescence	H, V	39.00^{c} ±1.53	95.00^{a} ±4.04	*Acaulospora foveata*, *Claroideoglomus claroideum*, *Gigaspora albida*, *Glomus aggregatum*, *G. multicaule*, *G. pachycaule*, *Rizophagus intraradices*.
3.	***T. erecta***	**Vegetative**	H, V	71.00^{a} ±1.15	38.00^{c}±2.08	*Acaulospora scrobiculata*, *Funneliformis geosporus*, *Gigaspora albida*, *Gi. ramisporophora*, *Glomus heterosporum*, *G. multicaule*, *Rizophagus intraradices*.
		Flowering	H, V, A	57.00^{b} ±2.52	73.00^{b} ±4.58	*Acaulospora scrobiculata*, *Funneliformis geosporus*, *Gigaspora albida*, *Gi. ramisporophora*, *Glomus heterosporum*, *G. multicaule*, *Rizophagus intraradices*.
		Senescence	H, V, A	36.00^{c} ±1.00	121.00^{a} ±2.52	*Acaulospora scrobiculata*, *Funneliformis geosporus*, *Gigaspora albida*, *Gi. ramisporophora*, *Glomus heterosporum*, *G. multicaule*, *Rizophagus intraradices*.

Legend: V=Vesicular colonization, A= Arbuscular colonization, H= Hyphal colonization. Values presented are mean of 3 replicates, ± indicate standard deviation. Data with different letters in the same column are significantly different at $P \leq 0.05$.

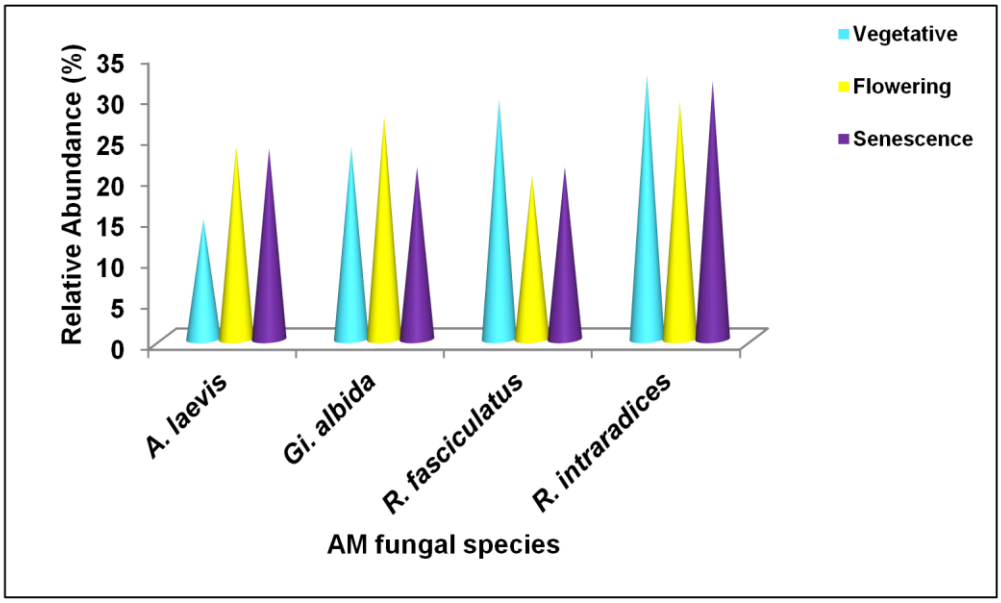


Figure 17: Relative abundance of AM fungi during different stages of growth in *C. morifolium*.

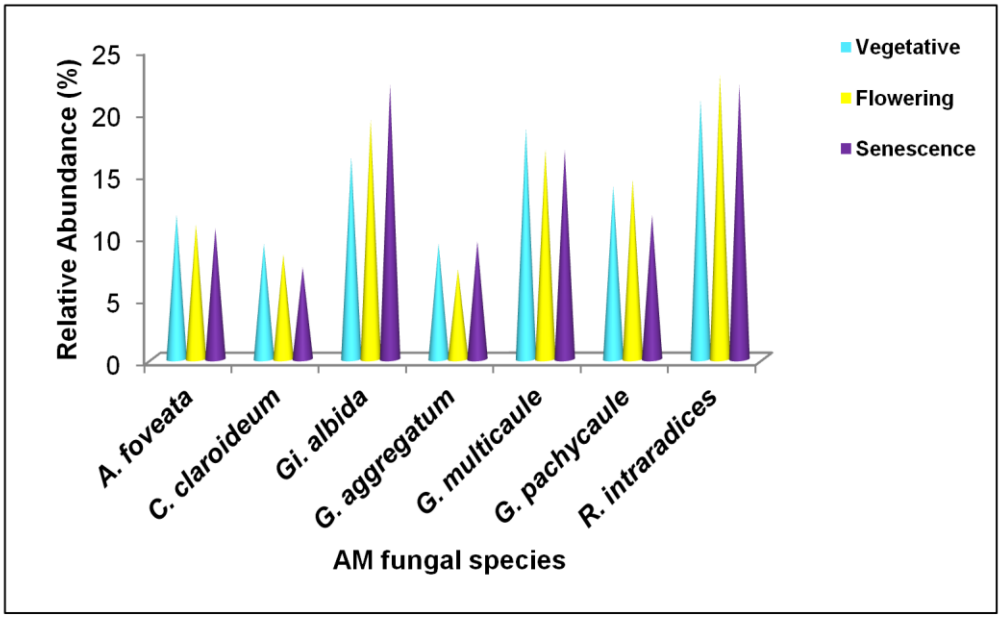


Figure 18: Relative abundance of AM fungi during different stages of growth in *C. infundibuliformis*.

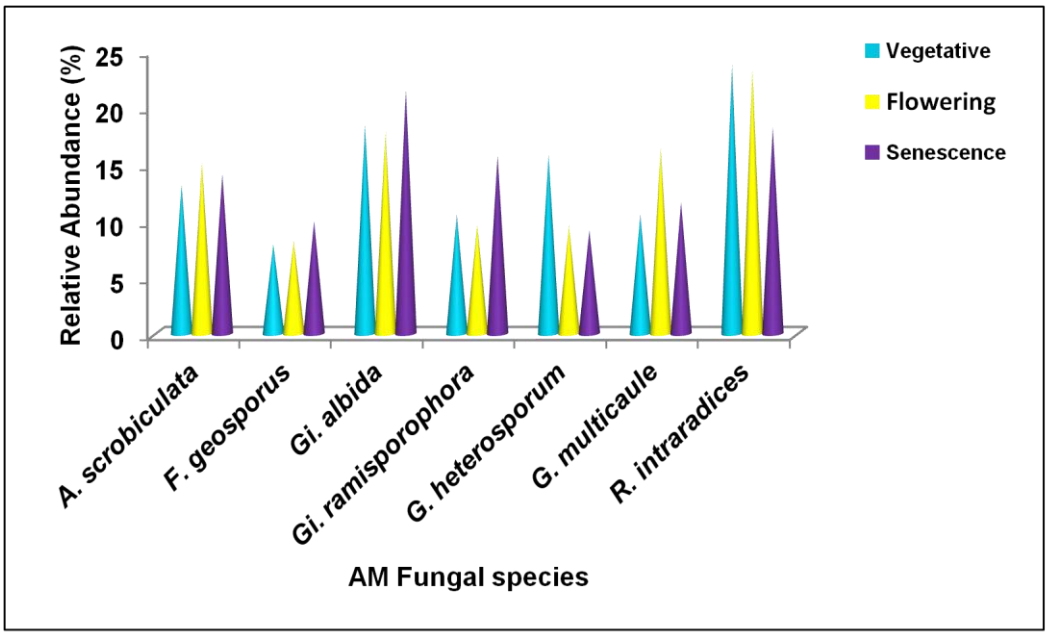


Figure 19: Relative abundance of AM fungi during different stages of growth in *T. erecta*.

Table 12: Total P concentrations at different stages of plant growth in 3 flowering species in Goa.

Sr. No.	Plant species	Growth stage	Total leaf P (%)
1.	*C. morifolium*	Vegetative	0.57^{b} ±0.01
		Flowering	0.59^{a} ±0.01
		Senescence	0.53^{c} ±0.01
2.	*C. infundibuliformis*	Vegetative	0.48^{a} ±0.01
		Flowering	0.49^{a} ±0.01
		Senescence	0.48^{a} ±0.08
3.	*T. erecta*	Vegetative	0.57^{b} ±0.01
		Flowering	0.62^{a} ±0.01
		Senescence	0.56^{c} ±0.01

Legend: Values are mean of 3 replicates, ± indicates standard deviation.
Data with different letters for each plant species are significantly different at $P \leq 0.05$.

while variation in relative abundance being highest in *R*. *intraradices* during all three stages (**Figure 19**).

Phosphorus (P) concentration (g^{-1} dry weight basis) in the leaf gradually increased and reached maximum during flowering stage in all the plant species studied. Total leaf P in *C. morifolium* ranged from 0.53–0.59%. It increased significantly during the initial vegetative stages of plant growth (0.57%) steadily increasing until flowering (0.59%) and then decreased during the senescent stage (0.53%). In case of *C. infundibuliformis* no significant difference was observed in P concentration during the three growth stages. It increased from vegetative (0.48%) stage to flowering (0.49%) and then decreased again at the end of flowering stage (0.48%). The highest leaf P concentration was observed in *T*. *erecta* during the flowering stage. The P concentration during vegetative stage (0.57%) increased significantly in the flowering stage (0.62%) and then decreased (0.56%) during the senescence (**Table 12**).

6.4 DISCUSSION

In the present study the effect of host phenology on AM status and the significance of AM fungi during the various growth stages of three ornamental flowering plants was studied. Experiment was conducted by comparing different growth stages of three different host plants growing under natural conditions. The study revealed that root colonization varied in all the three stages of growth of all the plant species. It was highest during vegetative stage after which it steadily declined and was least during senescence. This

susceptibility of plant roots to AM colonization can be explained by specific compatibility systems, avoidance or failure of AM fungi to elicit host defence mechanisms although there are evidences of host-fungus specificity in AM associations (Harley and Smith, 1983; Duddridge, 1987). Root colonization is influenced by host physiology which includes alterations in nutrient requirements, membrane composition and metabolite levels and occurs even when nutrient input is negligible (Dehne, 1986; Pacovsky, 1986; Escudero and Mendonza, 2005; Brundett, 1991). The extent of root colonization was also dependent on the season together with the phenological stage when the samples were collected.

During the vegetative stage only vesicular and hyphal colonization was observed in all the plant species. However it is difficult to make generalizations about root colonization patterns in AM fungi based on the host phenology (Moreira *et al*., 2006). Variability can be extreme because root colonization is influenced by factors inherent to the host plant, and by climatic and edaphic factors (Giovannetti, 1985). During monsoon season due to the continuous root growth AM fungi are likely to exist in vegetative form (Adiano-Anaya, 2006). Following the onset of flowering arbuscular colonization was observed which was persistent until senescence which shows that phenology and season have an important role in root-AM fungal relationship in the three plant species.

Spore density was lowest during the vegetative stage, increased during flowering stage and reached maximum during senescence. Production

of spores is a means of propagation induced by root senescence during root turnover at plant maturity or triggered by stress as a survival strategy (Smith and Read, 1997). Spore production is an indication of the end of optimum plant growth and coincides with a period of slow growth, absence of root growth or senescence of host roots (Baylis, 1969; Guadarrama and Sanchez, 1999) and is thus related to the phenological stage of the host plant (Hayman, 1970; Giovannetti, 1985; Bononi *et al*., 1988; Gemma and Koske, 1988). Peak period of spore production is generally thought to coincide with the period of fungal resource remobilization from senescing roots (Sutton and Barron, 1972) and is maximum in natural communities when root activity is interrupted by a long dry season (Janos, 1980). Dependence of spore production on moisture and nutrient availability was reported earlier (Augé, 2001; Johnson *et al*., 2003).

Highest spore density was recorded in *T*. *erecta* during the senescent stage. It has been reported that AM fungi have different behavioural patterns in perennial plants, without a condensed sporulation at the end of the life cycle, as in annuals (Gemma and Koske, 1988). Certain stress situations might elicit a stimulus for greater spore production, which could be important for the survival of the endophyte (Moreira *et al*., 2006). Low sporulation numbers with a more or less even distribution throughout the year and high colonization rates were observed in perennials by Baylis (1969). Since perennial plants do not present a definitive final point for root growth there is no special period during which a higher stimulus for sporulation occurs (Baylis, 1969; Sparling and Tinker, 1975). However, perennial plants also

have a physiological cycle with a dynamic root death and root growth sequence, which results in a somewhat continuous development resulting in spore production.

Phosphorus concentration in the leaf increased during plant growth, reached maximum in the flowering stage and decreased during the senescent stage in all of the selected plant species. This reflects on the higher P requirement by plants during flowering stage and this extra requirement of P during early reproductive growth is facilitated by arbuscular colonization that appears during the flowering stage as the arbuscules are considered as sites for P transfer from fungus to plant (Mullen and Schmidt, 1993; Harrison *et al*., 2002; Karandashov *et al*., 2004). The presence of arbuscules in the roots during reproduction suggests a benefit to plant, while persistence of hyphal and vesicular colonization indicates decreased P demand at maturity. Phosphate is taken up by high-affinity phosphate transporters in the extra-radical mycelium (Harrison and van Buuren, 1995) and is transported within the fungus as polyphosphate (poly P), and in the intraradical hyphae the long chains are hydrolysed, facilitating transfer to the host plant (Harrison, 1999; Bago *et al*., 2002; Ohtomo and Saito, 2005). Fungus-to-plant transfer occurs mainly at the arbuscule interface, although expression of P transporters around Paris type hyphal coils has also been demonstrated (Karandashov *et al*., 2004). Plant ATPase activity is strongly expressed at the periarbuscular membrane (Smith *et al*., 2009) and phosphate accumulation as poly-P strongly correlated with AM colonisation (Ohtomo and Saito, 2005). The P supply and demand can vary through time, and this variation occurs in

response to colonization by AM the plant develops. However in case of facultative mycotrophic plant demand for P may be quite low (Fitter, 1991). Some plants may only occasionally require assistance from AM fungi to meet demand for P mainly during the reproductive stage. Therefore these plants need not maintain consistently high levels of colonization by AM fungi. In some plant species, fractional total colonizations (vesicles, arbuscules and hyphae) or fractional arbuscular colonization is highest just before or during reproduction (Dodd and Jeffries, 1986; Boswell *et al*., 1998), and the positive effect of mycorrhizal colonization on P uptake appears to correspond to those times (McGonigle and Fitter, 1988; Dunne and Fitter, 1989). If mycorrhizal colonization increases reproduction of an annual plant, it is essential that the benefit such as nutrient allocation must take place before or during reproduction. Thus it can be concluded that knowledge of AM fungal status and influence of AM association and population during different growth stages of plant species used in floriculture can help to improve flower production in floriculture industry.

CHAPTER 7

EFFECT OF THE DOMINANT ARBUSCULAR MYCORRHIZAL FUNGAL SPECIES ON THE GROWTH AND FLOWER QUALITY IN SELECTED ORNAMENTAL FLOWERING PLANT SPECIES

7.1 INTRODUCTION

Excessive use of chemical fertilizers has resulted in destruction of AM fungal species from agricultural land (Johnson, 1993; Douds and Millner, 1999; Oehl *et al*., 2004). Arbuscular Mycorrhizal fungi are the most commonly forming associations with the majority of plant species (Bever *et al*., 2001). About 95% of the world's plant species belong to families that are characteristically mycorrhizal (Smith and Read, 1997) and depend on AM symbiosis to meet at least some of their primary needs. When a soil is disturbed or is partially removed, due to habitat disturbance, coupled with destructive agricultural practises, a decrease in the number of AM propagules occurs (Miller, 1979). The conservation and efficient utilization of this diversity is of crucial importance for sustainable plant production systems (Giovannetti and Gianinazzi-Pearson, 1994). It is now universally accepted that mycorrhizal symbioses are fundamental for good plant nutrition and health, and soil quality (Smith and Read, 2008). Focus on biofertilizers research has increased all over the world and large amount of evidence has collected to show immense potential of AM fungi used for their abilities as promising biofertilizers. The application of AM fungi as biofertilizers in floriculture sector has thus gained considerable attention in the last few decades. The use of AM fungi is more affordable and accessible to most of the agriculture industry especially in floriculture sector and it is generally believed to be effective. Worldwide, there is an ever-growing demand for AM biofertilizers which can be used in floriculture industry. Species of AM fungi that can be easily cultured, mass multiplied, requiring little maintenance and providing better yield are sought after by mycorrhizologists due to their multiple benefits. The AM fungal

species used for biofertilizer purpose are mostly isolated from the wild and are then applied as biofertilizers either singly or in combination with other useful microorganisms.

A new trend in research concentrating on preparing AM fungal formulations either singly or in combination with other fungi has been established for producing biofertilizers based on scientific criteria. The AM application is a powerful tool for increasing the crop productivity and can be a viable alternative to induce earlier flowering. Rapid mass multiplication of AM fungal species and their long-term germplasm storage are achievable in a small space and short time, with no harm to the nature. Mycorrhizal inocula can be produced under controlled climatic conditions in greenhouse throughout the year without any seasonal limitation (Dalpe and Monreal, 2004). Due to rapid and aseptic nature of mass multiplication techniques, large numbers of uniform mycorrhizal plants can be produced using a limited amount of inocula. The sterile nature of AM inocula facilitates the root colonization even at seedling stage without any pathogenic infection. Inspite of these several advantages, these organisms had received very little attention as far as their potential as biofertilizers. Screening of AM fungi for selecting suitable species is an important preliminary step for the use of AM fungi in floriculture industry. In the past few years extensive efforts have been made by mycorrhizologists to find efficient species of mycorrhizae for the floriculture industry. Therefore, this study was conducted to assess the influence of AM fungal species using active AM fungal bioinoculum on growth and flower quality in four ornamental flowering plants species of commercial

importance *viz*., *Chrysanthemum morifolium* Ramat, *Crossandra infundibuliformis* (L.) Nees, *Jasminum nitidum* Skan. and *Tagetes erecta* L. and to identify the most efficient AM species.

7.2 MATERIALS AND METHODS

7.2.1 AM inoculants: Six AM fungal species *viz*., *Acaulospora laevis*, *A. scrobiculata*, *Gigaspora albida*, *Glomus coremioides*, *Rhizophagus fasciculatus* and *R. intraradices* were used for the study. Spores of these AM fungal species were originally isolated from the rhizosphere soils of ornamental flowering plants growing in various regions of Goa. Identification of spores was done by using AM identification manuals (Schenck and Perez, 1990; Rodrigues and Muthukumar, 2009). A mixture of sand: soil (2:1) was sieved through a 2mm sieve, oven sterilised at 180°C for three consecutive days to eliminate indigenous AM fungi. For this study, pure cultures of six AM fungal species *viz*., *Acaulospora laevis*, *A. scrobiculata, Gigaspora albida*, *Glomus coremioides, Rhizophagus fasciculatus* and *R. intraradices* were used. Pure cultures were prepared in pots using *Solenostemon scutellarioides* (L.) Codd, as host plant. These pure cultures were then mass multiplied in trays for six months using the same host. Root colonization was estimated using slide method (Giovannetti and Mosse, 1980). Spore density was estimated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) followed by sucrose density gradient centrifugation (Daniels and Skipper, 1982). Inoculum was comprised of sand: soil mixture containing live colonized roots, hyphae and spores (350 spores $100g^{-1}$ soil).

7.2.2 Ornamental plant species used for the study:

a. *Chrysanthemum morifolium* Ramat.

Chrysanthemum morifolium is a partly woody erect, branched, perennial herb, up to 1m in height with alternate thick, leaves. The inflorescence consists of many flower heads. Chrysanthemums are widely grown commercially for their showy red, white, or yellow flowers, which are produced after monsoon. The blossoms range from daisy like in appearance to very shaggy. They are used in commercial floriculture as cut flowers, potted plants and garden plants.

b. *Crossandra infundibuliformis* (L.) Nees

Crossandra infundibuliformis is a perennial shrubby plant, up to 1–1.5m in height with alternate, oval, evergreen leaves. It blooms continuously almost throughout the year. Flower colour range from common orange to salmon-orange. Flowers grow from four-sided stalked spikes and are unusually shaped with 5 petals. They have a tube like stalk which makes it easier to string them in a garland. These tiny flowers are often stung together into strands, sometimes along with jasmine flowers and therefore in great demand for making garlands which are offered to temple deities or used to adorn womens' hair.

c. *Jasminum nitidum* Skan.

Jasminum nitidum is a spreading shrub and its habit is mostly climbing. The leaves are opposite pinnate leaflets. Stems are often green and angled. Inflorescence is a cyme which is bi- or trichotomous, simple or flowers rarely solitary. Bracts are linear and simple and their shape is ovate. These flowers

are in great demand for making garlands which are offered to temple deities or used to adorn womens' hair. The main beauty and uniqueness of the flower is its odour which has a unique status in the perfume world.

d. *Tagetes erecta* L.

Tagetes erecta is an annual erect herb, about 60 cm high. Leaves pinnately divided, segments lanceolate-serrate, strongly aromatic. Flowers in terminal heads, colour ranges from a light yellow to deep orange. The plants, with their attractive flower colour bloom for a long period. Marigolds are mostly used as cut flowers for making garlands. They are also planted in beds for mass display, in mixed borders and are also grown as potted plants.

7.2.3 Seedling/sapling treatment: Seeds of *C. infundibuliformis* and *T. erecta* were thoroughly washed with distilled water, treated with fungicide (0.5% Bavistin) for 2 minutes, washed 4-5 times to remove any traces of the fungicide, and finally rinsed in sterile distilled water. These sterilized seeds were then placed in Petri plates lined with a sterile, moist tissue paper and kept for germination at 27°C. Germinated seedlings were transferred to trays containing actively growing AM fungal inoculum and kept in the polyhouse at 27°C. In case of *C. morifolium* and *J. nitidum* healthy cuttings of uniform size were planted in the trays containing mycorrhizal inoculum. Control plants were planted in trays containing sterile sand: soil (2:1) mixture (**Plate 18 A–D**).

7.2.4 Estimation of root colonization by AM fungi: After 60 days, 50 root segments (each measuring one centimeter) per treatment were randomly

selected and stained with trypan blue (Phillips and Hayman, 1970). A segment was considered mycorrhizal when it showed the presence of hypha along with arbuscule and/or vesicle. Degree of colonization was estimated by slide method (Giovannetti and Mosse, 1980). Total root colonization was expressed as percentage.

7.2.5 Transfer of mycorrhizal seedlings to pots: After 60 days, mycorrhizal plantlets were transferred to pots (diameter: 16 cm; height: 15 cm) containing sterile sand: soil (2:1) mixture (one plantlet pot^{-1}) in a shadenet at 30**–**32°C and 45**–**47% RH. The sand-soil mixture had the following properties: pH 7.3; N: 0.57 kg/ha; available P: 2.98 kg/ha; and available K: 336 kg/ha. Each pot was filled with 2 kg of dry sterile soil. The pots were arranged in completely randomized block design with 5 replicates. Hoagland's solution without P was added fortnightly (**Plate 18 E–H**). The three growth stages *i.e.* vegetative, reproductive and senescence were compared among the six AM treatments and un-inoculated control.

7.2.6 Plant growth parameters: Root and shoot length was recorded after 2 months while stem diameter, total leaf P and leaf length after 5 months of plant growth. Number of days required for flowering, flower number, water loss of flowers after every 24h (at 25ºC and 65% RH) and, root and shoot dry weights were recorded. Dry weights of root and shoot samples were calculated after drying at 70°C. Plant growth was assessed in terms of total plant dry weight (d.w.).

Plate 18: Arbuscular Mycorrhizal fungal treatment

A. AM treated *Chrysanthemum morifolium* cuttings.

B. AM treated *Crossandra infundibuliformis* seedlings.

C. AM treated *Jasminum nitidum* cuttings.

D. AM treated *Tagetes erecta* seedlings.

E. Completely randomized block design for *Chrysanthemum morifolium* saplings.

F. Completely randomized block design for *Crossandra infundibuliformis* seedlings.

G. Completely randomized block design *Jasminum nitidum* saplings

H. Completely randomized block design for *Tagetes erecta* seedlings.

Plate 18

Mycorrhizal dependency (MD): Using dry weight data, degree of plant response to AM fungi (Mycorrhizal Dependency) was calculated as the difference between the biomass of the shoot of inoculated and un-inoculated plant and was expressed as percentage of the dry weight biomass of inoculated plants (Plenchette *et al*., 1983).

Mycorrhizal dependency (MD) = (Shoot dry weight of inoculated plant / Shoot dry weight of un-inoculated plant) x 100

Mycorrhizal efficiency index (MEI): Mycorrhizal efficacy in growth enhancement was calculated by taking the average dry weight of the plant. The Mycorrhizal Efficiency Index (MEI) was estimated according to Bagyaraj (1994).

Mycorrhizal efficiency index (MEI) = (Weight of inoculated plant – Weight of un-inoculated plant)/ Weight of inoculated plant x 100

7.2.7 Harvest and determination of fresh weight loss of flower: Flowers of *C. morifolium* were harvested 13 days after calyx opening, flowers of *C. infundibuliformis* flowers were harvested 17 days after the emergence of first bud on the spike and *T. erecta* flowers were harvested 15 days after calyx opening. The percent fresh weight loss was calculated by weighing the flowers kept at 25ºC and 65% relative humidity after every 24 h until constant weight. Percent fresh weight loss per 24 h was calculated using the formula,

Fresh weight loss (%) = (Initial weight – Final weight/ Initial weight) x 100

7.2.8 Nutrient analysis: Mineral status of various AM treated plants during vegetative stage was assessed by tissue elemental analysis of leaves after oven drying (70°C for 72 h). Analysis was performed in triplicates using standard procedure (Chapman and Pratt, 1961). Total P was estimated using Ammonium vanadate - ammonium molybdate yellow colour method (Jackson, 1973). Na and K were estimated using flame photometry (Yoshida *et al*., 1976).

7.2.9 Statistical analysis: Data on selected plant growth variables was subjected to Analysis of Variance using the WASP (Web Agri Stat Package, version 1.0, ICAR, Goa). Means were compared by the LSD test and statistical significance is reported at 5% level.

7.3 RESULTS

Six AM fungal cultures along with control were used to evaluate their effect on growth and flower quality in *C. morifolium, C. infundibuliformis, J. nitidum* and *T. erecta* (**Plate 19A–D**). All AM treatments stimulated flowering with increased size and improved flower quality compared to the un-inoculated control (**Plate 19E–H**).

In *C. morifolium* maximum root colonization was recorded in *R. intraradices* (72%) inoculated plants followed by *A. scrobiculata* (68%) and *R. fasciculatus* (64%) **(Fig. 20)**. Plants inoculated with *R. intraradices* showed increased root length in the early vegetative stages of growth **(Table. 13)**. *Acaulospora scrobiculata* inoculated plants recorded maximum plant height

Plate 19: Effect of Arbuscular Mycorrhizal fungi on plant growth and flowers.

A. **Plant growth and flowering in *Chrysanthemum morifolium* cuttings.**

B. **Plant growth and flowering in *Crossandra infundibuliformis* seedlings.**

C. **Plant growth and flowering in *Jasminum nitidum* cuttings.**

D. **Plant growth and flowering in *Tagetes erecta* seedlings.**

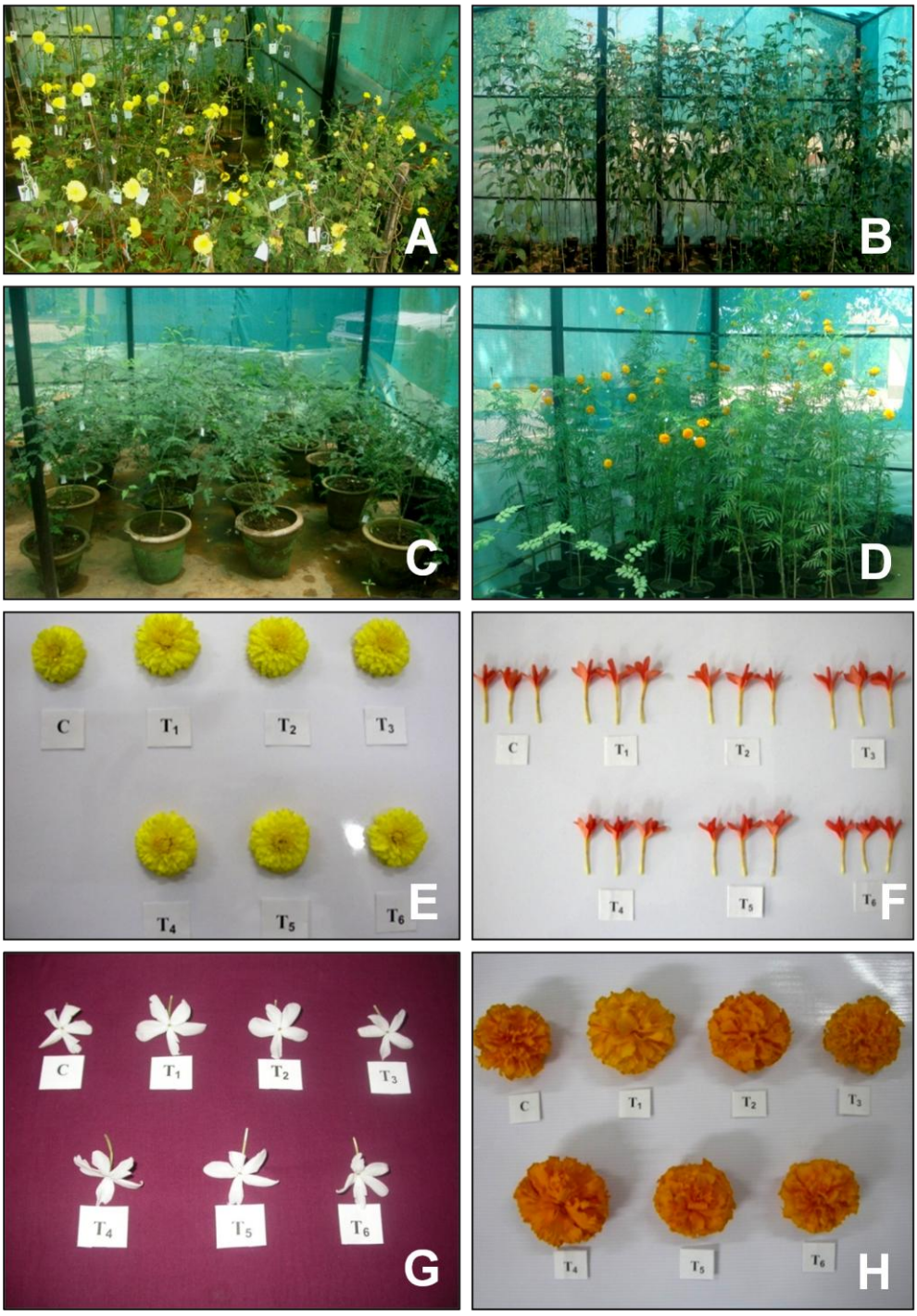
E. **Flower size variation in *Chrysanthemum morifolium.***

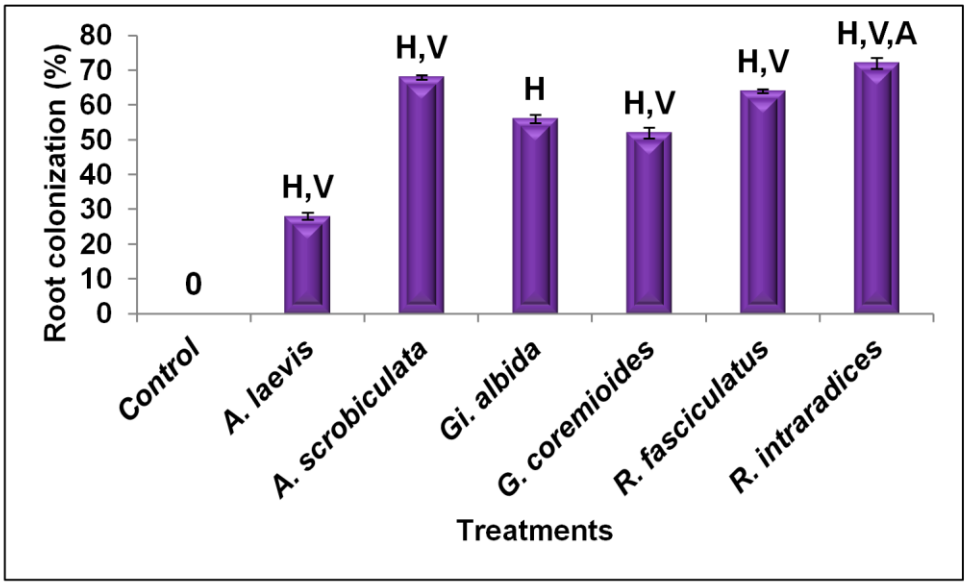
F. **Flower size variation in *Crossandra infundibuliformis.***

G. **Flower size variation in *Jasminum nitidum.***

H. **Flower size variation in *Tagetes erecta.***

Plate 19


A
B
C
D
C
T1
T2
T3
T4
T5
T6
E
C
T1
T2
T3
T4
T5
T6
F
C
T1
T2
T3
T4
T5
T6
G
C
T1
T2
T3
T4
T5
T6
H



Legend: H= Hyphal, V= Vesicular and A= Arbuscular colonization.

Figure 20: Percent root colonization in *C. morifolium* plants inoculated with various AM treatments (after 60 days of growth).

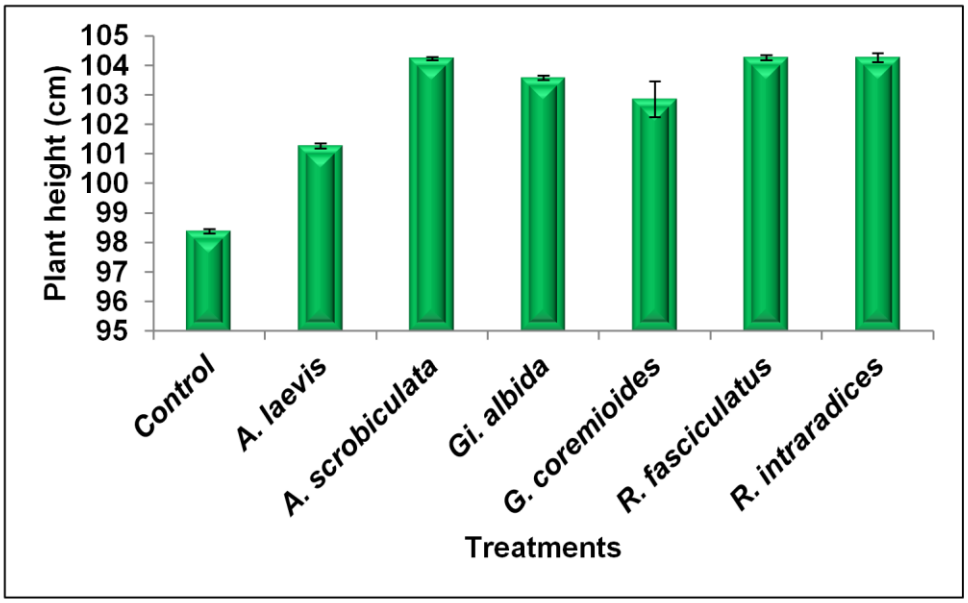


Figure 21: Plant height in *C. morifolium* plants inoculated with various AM treatments (after 150 days of growth).

Table 13: Effect of AM fungi on selected vegetative characteristics in *C. morifolium*.

Sr. No.	Treatments	Root length after 60 days (cm)	Plant height after 60 days (cm)	Stem diameter after 150 days (cm)	No. of lateral branches plant^{-1}
1.	**Control**	09.34^{e} ±0.05	20.28^{e} ±0.08	1.12^{bc} ±0.08	10.8^{b} ±0.84
2.	***A. laevis***	10.28^{d} ±0.04	20.46^{d} ±0.05	1.10^{c} ±0.10	13.4^{a} ±0.45
3.	***A. scrobiculata***	11.46^{b} ±0.09	22.30^{a} ±0.17	1.27a ±0.08	13.6^{a} ±0.55
4.	***Gi. albida***	11.32^{c} ±0.08	21.86^{c} ±0.05	1.20^{ab} ±0.07	13.6^{a} ±0.55
5.	***G. coremioides***	11.32^{c} ±0.11	22.30^{a} ±0.07	1.26^{a} ±0.05	13.4^{a} ±0.55
6.	***R. fasciculatus***	11.52^{b} ±0.11	22.30^{a} ±0.14	1.22^{a} ±0.08	13.6^{a} ±1.00
7.	***R. intraradices***	11.72^{a} ±0.08	22.16^{b} ±0.09	1.26^{a} ±0.05	13.6^{a} ±0.55

Legend: Values presented are mean of 5 replicates, ± indicates standard deviation.
Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = Acaulospora; *Gi. = Gigaspora*; *G = Glomus*; *R = Rhizophagus*.

during the vegetative phase along with plants inoculated with *R. fasciculatus* and *R. intraradices* **(Fig. 21)**. Total Na, P and K content varied significantly within the treatments being relatively higher in *R*. *intraradices* inoculated plants. Total Na concentration was significantly higher (1.39–1.87%) in AM inoculated plants compared to un-inoculated control plants (1.20%). Total P concentration in AM inoculated plants was higher (0.45–0.49%) compared to un-inoculated plants (0.26%). Total K concentration was significantly higher (0.41–0.74%) in AM inoculated plants as compared to un-inoculated plants (0.31%) except in *A. laevis* treated plants (**Table 14**). During the reproductive phase all inoculated plants showed earlier flowering (**Table 15**) with *R. intraradices* showing highest (22%) increase in flower number plant^{-1} followed by *A. scrobiculata*, *Gi. albida*, *R. fasciculatus* and *G. coremioides* with least increase (11%) in *A. laevis* (**Fig. 22**). There was a significant increase in width and fresh weights of flowers except in *A*. *laevis* treated plants **(Table 15)**. Least water loss was recorded in flowers produced by *R. intraradices* inoculated plants **(Fig. 23)**. Total dry weight was maximum in *R*. *intraradices* treated plants (**Table 15**). Root and shoot dry weights varied within the AM treatments, highest in *R*. *intraradices* inoculated plants **(Fig. 24).** Mycorrhizal Dependency and Mycorrhizal Efficiency Index were also maximum in the dry shoots of the plants inoculated with *R. intraradices* **(Fig. 25).**

In *C. infundibuliformis* maximum root colonization was recorded in *R. intraradices* inoculated plants **(Fig. 26)**. *Acaulospora scrobiculata* had a greater effect on plant height during the vegetative stage (**Fig. 27)**, while *R. intraradices* showed greater stem diameter, leaf length and leaf diameter

Table 14: Total Na, P and K concentrations in AM fungi inoculated *C. morifolium* plants against control in the vegetative stage.

Sr. No.	Treatments	Na (%)	P (%)	K (%)
1.	**Control**	1.20^{e} ±0.01	0.26^{d} ±0.01	0.31^{e} ±0.01
2.	***A. laevis***	1.39^{d} ±0.03	0.45^{c} ±0.01	0.34^{e} ±0.01
3.	***A. scrobiculata***	1.83^{a} ±0.01	0.47^{ab} ±0.01	0.51^{b} ±0.03
4.	***Gi. albida***	1.59^{c} ±0.01	0.47^{bc} ±0.01	0.41^{d} ±0.01
5.	***G. coremioides***	1.59^{c} ±0.02	0.46^{bc} ±0.01	0.46^{c} ±0.03
6.	***R. fasciculatus***	1.76^{b} ±0.02	0.47^{ab} ±0.01	0.47^{c} ±0.02
7.	***R. intraradices***	1.87^{a}±0.03	0.49^{a} ±0.01	0.74^{a} ±0.01

Legend: Values presented are mean of 3 replicates, ± indicates standard deviation. Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = Acaulospora; *Gi. = Gigaspora*; *G = Glomus*; *R = Rhizophagus*.

Table 15: Effect of AM fungi on floral characteristics and total dry weight in *C. morifolium* plants.

Sr. No.	Treatment	No. of days for flower initiation (DAT)	Earlier induction of flowering (day)	Flower number plant^{-1}	Flower diameter (cm)	Fresh weight of flower (g)	Total dry weight (g)
1.	Control	219^{a} ±0.84	00^{d} ±0.00	46.4^{e} ±0.89	3.18^{c} ±0.08	0.79^{c} ±0.00	14.68^{d} ±0.45
2.	***A. laevis***	216^{b} ±0.89	04^{c} ±0.89	51.2^{d} ±0.45	3.22^{c} ±0.08	0.79^{c} ±0.01	16.05^{c} ±0.20
3.	***A. scrobiculata***	209^{d} ±0.55	11^{a} ±0.55	54.8^{ab} ±1.10	3.40^{b} ±0.07	0.81^{bc} ±0.01	17.73^{a} ±0.24
4.	***Gi. albida***	209^{d} ±1.00	11^{a} ±1.00	54.6^{b} ±0.89	3.46^{b} ±0.55	0.81^{abc} ±0.02	17.06^{b} ±0.22
5.	***G. coremioides***	209^{cd} ±0.55	11^{ab} ±0.55	53.2^{c} ±0.84	3.56^{a} ±0.55	0.81^{abc} ±0.01	17.28^{b} ±0.12
6.	***R. fasciculatus***	210^{c} ±0.45	10^{b} ±0.45	53.2^{c} ±0.45	3.56^{a} ±0.55	0.82^{ab} ±0.01	17.88^{a} ±0.14
7.	***R. intraradices***	209^{d} ±0.89	11^{a} ±0.89	55.8^{a} ±0.84	3.58^{a} ±0.04	0.83^{a} ±0.00	18.08^{a} ±0.15

Legend: Values presented are mean of 5 replicates, ± indicates standard deviation.
Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = *Acaulospora*; *Gi.* = *Gigaspora*; *G* = *Glomus*; *R* = *Rhizophagus*; DAT = Days after transplant.

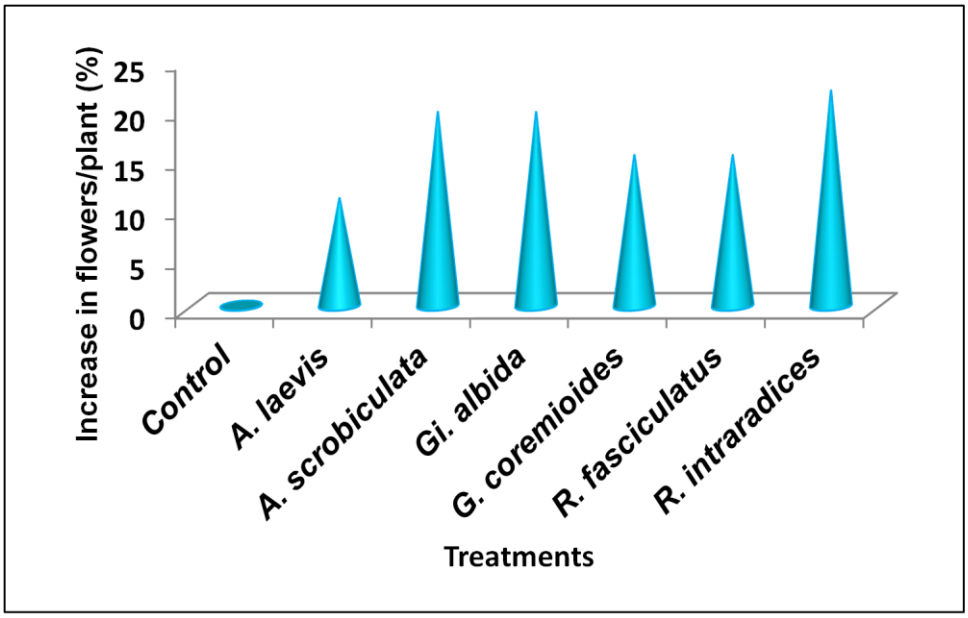


Figure 22: Effect of various AM fungal bioinoculants on percent increase in flower number plant^{-1} in *C. morifolium* plants.

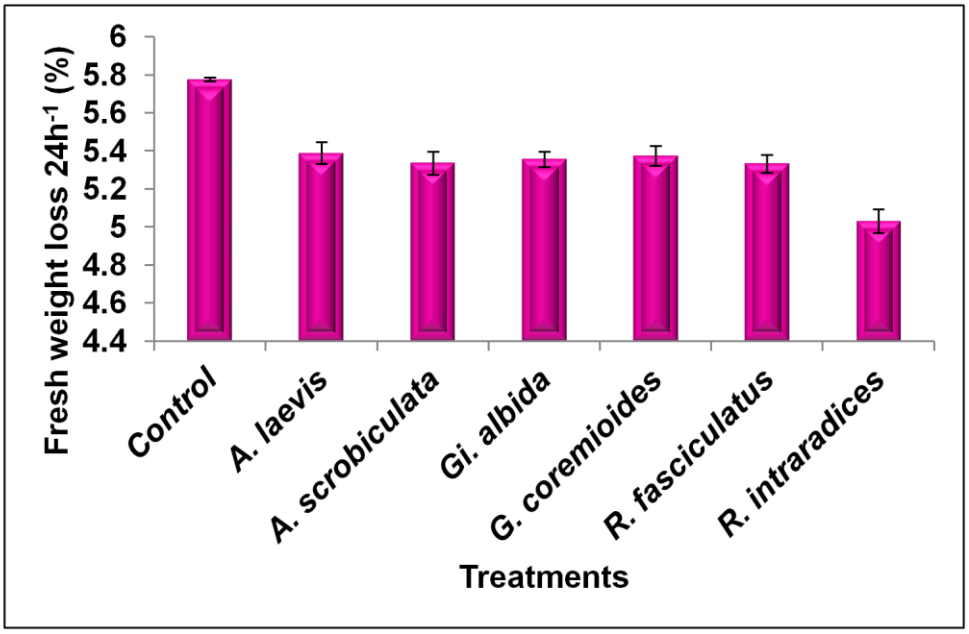


Figure 23: Effect of various AM fungal bioinoculants on percent average fresh weight loss in flowers 24h^{-1} in *C. morifolium* plants.

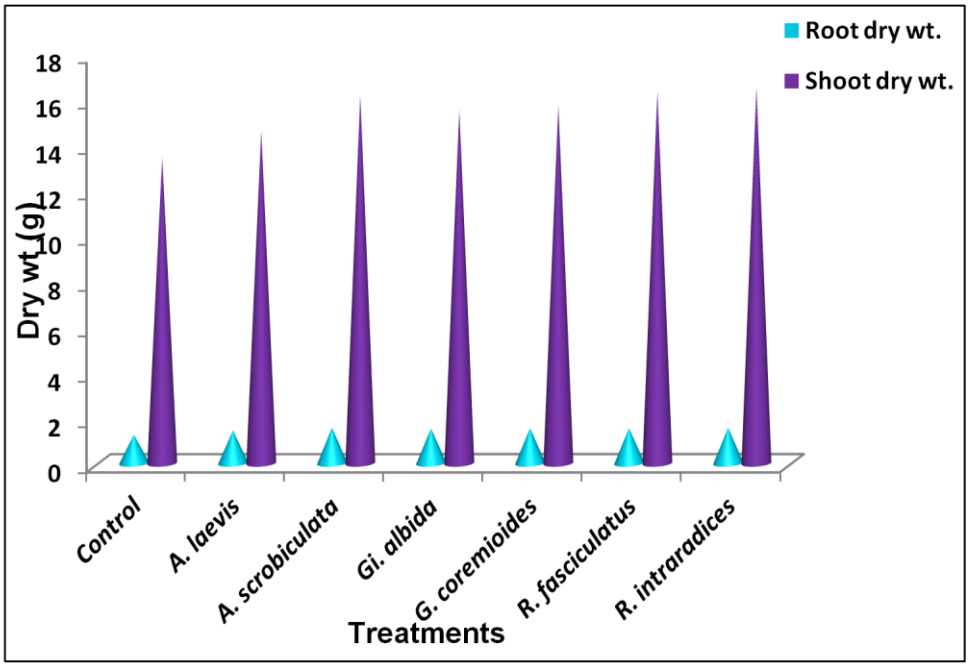


Figure 24: Effect of various AM fungal bioinoculants on root and shoot dry weight in *C. morifolium* plants.

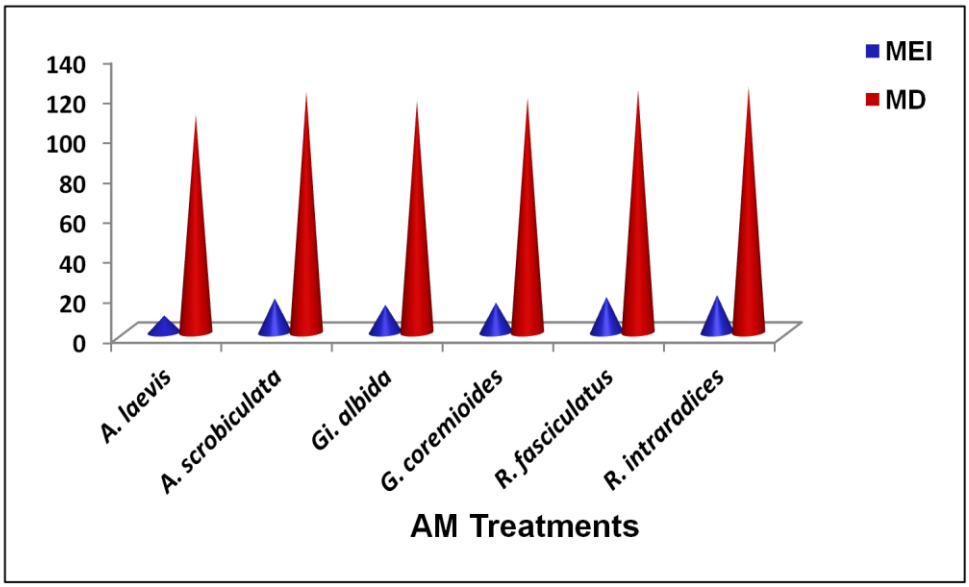
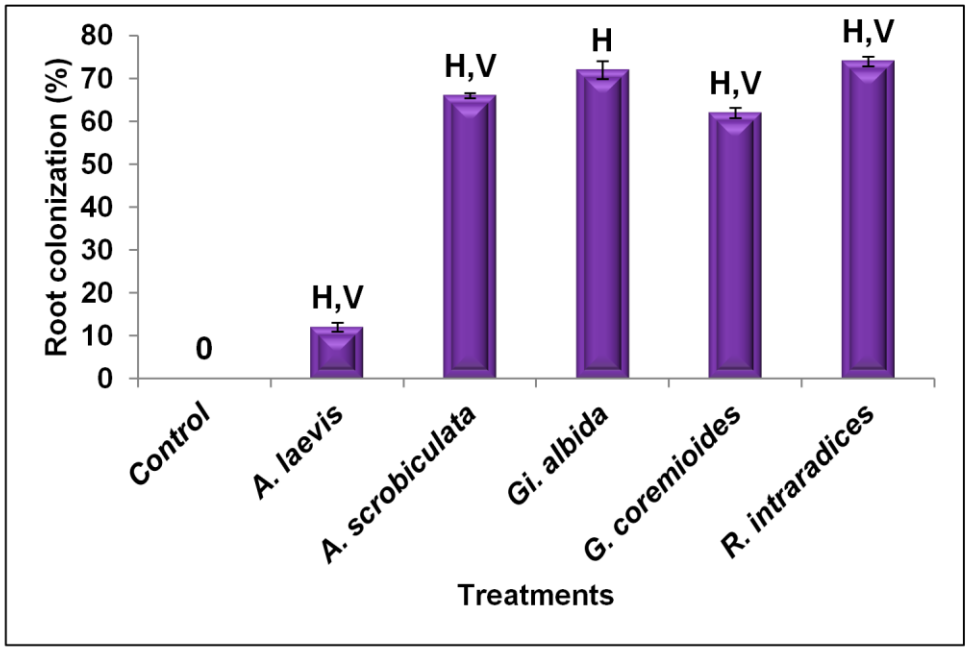


Figure 25: Mycorrhizal Efficiency Index and Mycorrhizal Dependency among various AM fungal bioinoculants in *C. morifolium* plants.



Legend: H= Hyphal, V= Vesicular and A= Arbuscular colonization.

Figure 26: Percent root colonization in *C. infundibuliformis* plants inoculated with various AM treatments (after 60 days of growth).

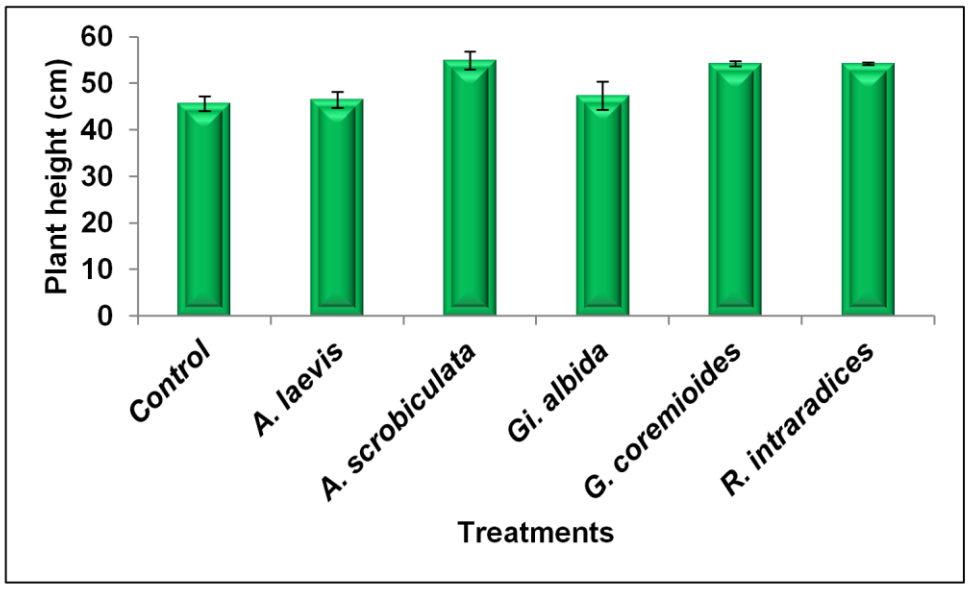


Figure 27: Plant height in *C. infundibuliformis* plants inoculated with various AM treatments (after 150 days of growth).

compared to other AM species **(Table 16)**. Total Na, P and K concentration was relatively higher in *R*. *intraradices* inoculated plants. Total Na concentration was significantly higher (1.98–2.05%) except in *A. laevis* inoculated plants compared to un-inoculated control (1.81%). Total P concentration in AM inoculated plants varied significantly (0.45–0.49%) compared to un-inoculated plants (0.26%). Total K concentration was also significantly higher in AM inoculated plants (**Table 17**). During the reproductive stage *R. intraradices* and *G. coremioides* inoculated plants flowered two weeks earlier than un-inoculated control. Although the number of flowers/inflorescence did not vary significantly, the total number of flowers/plant was more in AM treated plants (**Table 18**) with maximum flowers recorded in *R. intraradices* treated plants (**Fig. 28**). Percent fresh weight loss of flowers/day was least in *G. coremioides* inoculated plants **(Fig. 29).** Total dry weight was maximum in *R*. *intraradices* inoculated plants **(Table 18)**. Root and shoot dry weights were maximum in *R*. *intraradices* inoculated plants **(Fig. 30)**. Mycorrhizal Dependency and Mycorrhizal Efficiency Index were also maximum in the plants inoculated with *R. intraradices* **(Fig. 31)**.

In *J. nitidum* maximum root colonization was recorded in *R. fasciculatus* (67%) inoculated plants followed by plants inoculated with *R. intraradices* (63%), *G. coremioides* (62%), *Gi. albida* (57%), *A. scrobiculata* (56%) and *A. laevis* (38%) **(Fig. 32)**. *Rhizophagus fasciculatus* had greater effect on root and shoot length followed by *R. intraradices* inoculated plants **(Table 19)**. Stem girth was also more in *R. fasciculatus* followed by *G. coremioides* inoculated plants. Plant height after 150 days was maximum in

Table 16: Effect of AM fungi on selected vegetative characteristics in *C. infundibuliformis* plants.

Sr. No.	Treatment	Root length after 60 days (cm)	Plant height after 60 days (cm)	Stem diameter after 150 days (cm)	No. of leaves	Leaf length (cm)	Leaf diameter (cm)
1.	**Control**	3.56^{d} ±0.19	5.70^{d} ±0.34	1.90^{c} ±0.10	28^{cd} ±1.41	18.46^{b} ±0.62	5.48^{c} ±0.24
2.	***A. laevis***	3.50^{d} ±0.12	5.72^{d} ±0.11	1.94^{c} ±0.11	29.2^{bc} ±1.79	19.50^{b} ±0.86	5.58^{bc} ±0.18
3.	***A. scrobiculata***	5.54^{b} ±0.35	8.48^{b} ±0.19	2.18^{ab} ±0.16	29.2^{bc} ±2.28	19.46^{b} ±1.19	5.84^{ab} ±0.25
4.	***Gi. albida***	4.18^{c} ±0.50	7.12^{c} ±0.13	1.96^{c} ±0.18	26.4^{d} ±1.67	18.86^{b} ±0.86	5.72^{abc} ±0.08
5.	***G. coremioides***	6.08^{a} ±0.31	8.82^{ab} ±0.22	2.04^{bc} ±0.15	32.0^{a} ±2.45	18.94^{b} ±0.52	5.52^{bc} ±0.39
6.	***R. intraradices***	5.68^{ab} ±0.13	8.96^{a} ±0.54	2.32^{a} ±0.08	30.8^{ab} ±1.10	20.64^{a} ±0.49	5.98^{a} ±0.27

Legend: Values presented are mean of 5 replicates, ± indicates standard deviation.
Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = Acaulospora; *Gi. = Gigaspora*; *G = Glomus*; *R = Rhizophagus*.

Table 17: Total Na, P and K concentrations in AM fungi inoculated *C. infundibuliformis* plants against control in the vegetative stage.

Sr. No.	Treatments	Na (%)	P (%)	K (%)
1.	**Control**	1.81^{b} ±0.04	0.26^{d} ±0.01	0.76^{e} ±0.01
2.	***A. laevis***	1.95^{b} ±0.02	0.45^{c} ±0.01	0.79^{d} ±0.02
3.	***A. scrobiculata***	2.02^{a} ±0.02	0.47^{ab} ±0.01	0.88^{b} ±0.01
4.	***Gi. albida***	1.98^{a} ±0.52	0.47^{bc} ±0.01	0.83^{c} ±0.01
5.	***G. coremioides***	1.99^{a} ±0.09	0.47^{ab} ±0.01	0.80^{d} ±0.01
6.	***R. intraradices***	2.05^{a} ±0.06	0.49^{a} ±0.01	0.92^{a} ±0.01

Legend: Values presented are mean of 3 replicates ± indicates standard deviation. Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = *Acaulospora*; *Gi.* = *Gigaspora*; *G* = *Glomus*; *R* = *Rhizophagus*.

Table 18: Effect of AM treatments on floral characteristics and total dry weight in *C. infundibuliformis* plants.

Sr. No.	Treatment	No. of days for flower initiation (DAT)	Earlier induction of flowering (day)	No. of flower inflorescence^{-1}	Flower number plant^{-1}	Fresh weight of flower (g)	Total plant dry weight (g)
1.	**Control**	290^{a} ±1.79	00^{d} ±0.00	22.0^{a} ±1.00	132^{a} ±1.53	0.06^{d} ±0.00	41.22^{b} ±1.51
2.	***A. laevis***	288^{b} ±1.52	02^{c} ±1.52	22.4^{a} ±1.14	136^{a} ±1.15	0.06^{c} ±0.00	42.31^{a} ±0.32
3.	***A. scrobiculata***	279^{c} ±1.64	11^{b} ±1.64	21.6^{a} ±1.14	140^{a} ±2.00	0.06^{a} ±0.00	44.56^{a} ±1.23
4.	***Gi. albida***	280^{c} ±1.14	10^{b} ±1.14	21.8^{a} ±0.84	138^{a} ±2.10	0.06^{ab} ±0.00	43.77^{a} ±1.41
5.	***G. coremioides***	277^{d} ±1.48	13^{a} ±1.48	22.6^{a} ±1.14	145^{a} ±1.00	0.06^{b} ±0.00	45.80^{a} ±0.54
6.	***R. intraradices***	276^{d} ±0.55	14^{a} ±0.55	22.8^{a} ±0.84	147^{a} ±2.52	0.07^{a} ±0.00	46.11^{a} ±1.21

Legend: Values presented are mean of 5 replicates, ± indicates standard deviation.
Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = *Acaulospora*; *Gi.* = *Gigaspora*; *G* = *Glomus*; *R* = *Rhizophagus*; DAT = Days after transplant.

Table 19: Effect of AM fungi on selected vegetative characteristics in *J. nitidum*.

Sr. No.	Treatments	Root length after 60 days (cm)	Plant height after 60 days (cm)	Stem diameter after 150 days (cm)
1.	**Control**	10.01^{e} ±0.24	28.96^{d} ±1.95	2.68^{c} ±0.16
2.	***A. laevis***	11.02^{d} ±0.15	29.18^{d} ±1.46	2.68^{c} ±0.18
3.	***A. scrobiculata***	11.62^{c} ±0.23	32.88^{bc} ±1.94	2.08^{bc} ±0.12
4.	***Gi. albida***	12.22^{b} ±0.20	31.00^{cd} ±2.08	2.76^{bc} ±0.15
5.	***G. coremioides***	12.06^{a} ±0.12	32.12^{abc} ±1.77	2.09^{ab} ±0.18
6.	***R. fasciculatus***	12.74^{a} ±0.17	33.84^{ab} ±1.52	3.04^{a} ±0.17
7.	***R. intraradices***	12.56^{a} ±0.28	35.28^{a} ±1.42	2.08^{bc} ±0.12

Legend: Values presented are mean of 5 replicates, $\pm$ indicates standard deviation. Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = Acaulospora; *Gi. = Gigaspora*; *G = Glomus*; *R = Rhizophagus*.

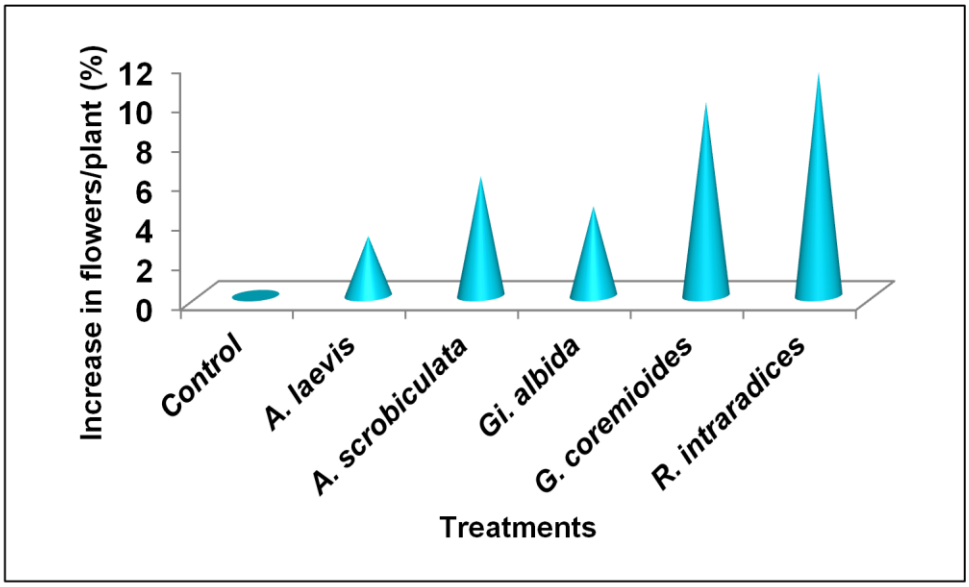


Figure 28: Effect of various AM fungal bioinoculants on percent increase in flower number plant^{-1} in *C. infundibuliformis* plants.

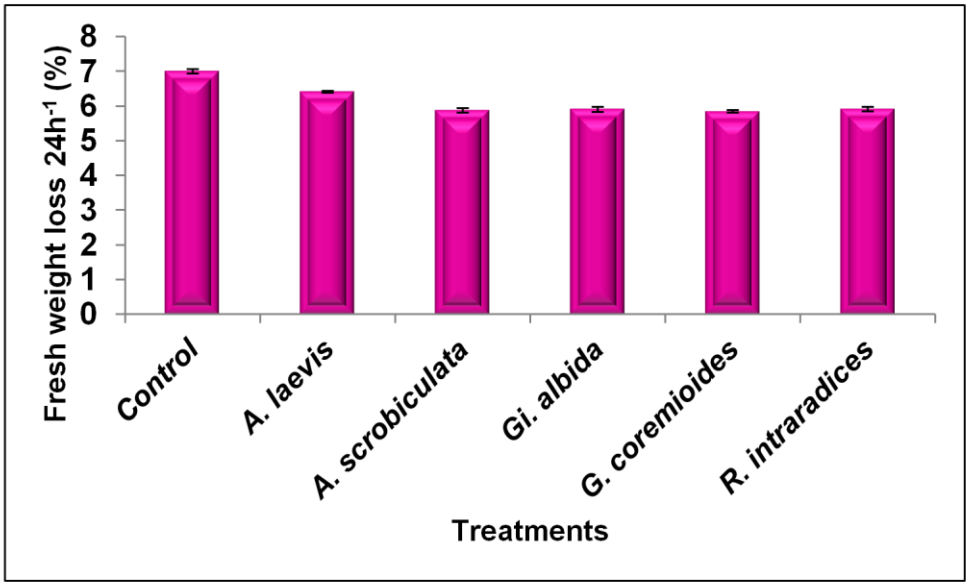


Figure 29: Effect of various AM fungal bioinoculants on percent average fresh weight loss in flowers 24h^{-1} in *C. infundibuliformis* plants.

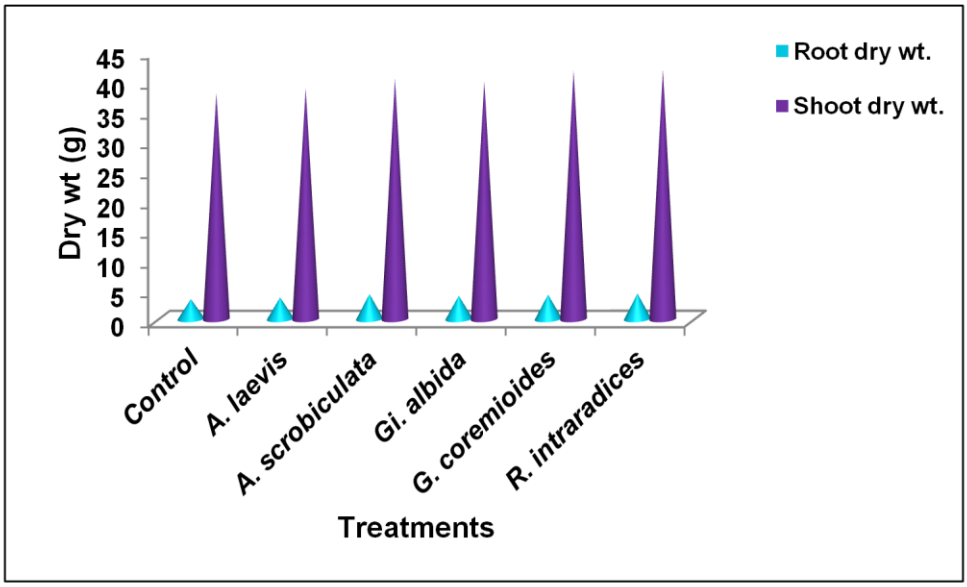


Figure 30: Effect of various AM fungal bioinoculants on root and shoot dry weight in *C. infundibuliformis* plants.

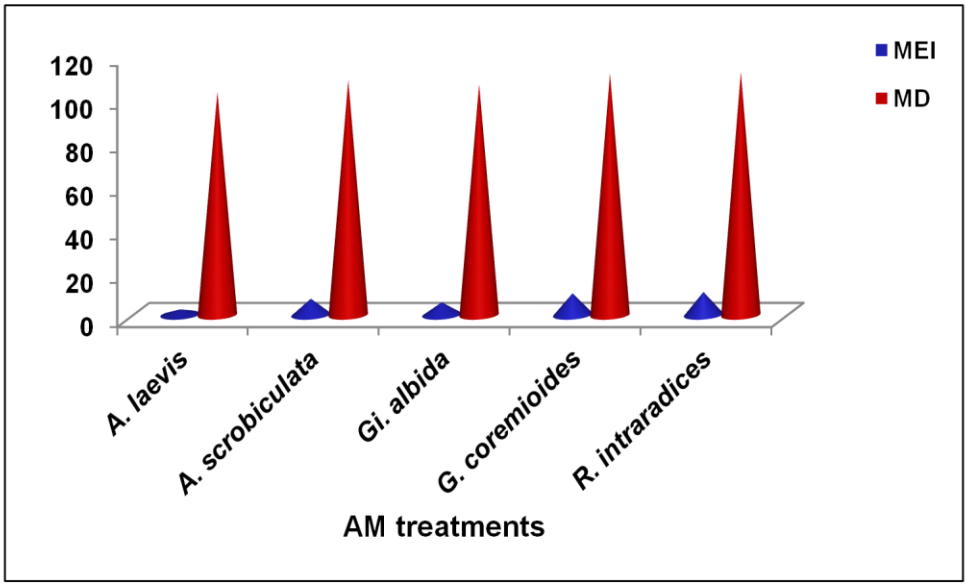


Figure 31: Mycorrhizal Efficiency Index and Mycorrhizal Dependency among various AM fungal bioinoculants in *C. infundibuliformis* plants.

plants inoculated with *R*. *intraradices* (**Fig. 33**). Total Na, P and K concentration was higher in *R*. *intraradices* inoculated plants. Total Na concentration varied significantly (0.75–0.96%) in AM inoculated plants except in *G. coremioides* (0.70%) compared to un-inoculated control (0.68%). Total P concentration in AM inoculated plants varied (0.41–0.44%) compared to un-inoculated control (0.25%). Total K concentration was also higher in AM inoculated plants (0.23–0.43%) than un-inoculated plants (0.14%) (**Table 20**). *Rhizophagus fasciculatus* inoculated plants showed earlier induction of flowering with increased fresh weight (**Table 21**) with increase in number of flowers/plant (**Fig. 34**). Percent fresh weight loss per day was less in flowers of all AM inoculated plants except in *A*. *laevis* as compared to un-inoculated control indicating higher moisture retaining ability of flowers **(Fig. 35)**. Total dry weight was maximum in *R*. *intraradices* inoculated plants **(Table 21)**. Root and shoot dry weights varied within the AM treatments and were recorded highest in *R*. *intraradices* inoculated plants **(Fig. 36)**. Mycorrhizal Dependency and Mycorrhizal Efficiency Index were also maximum in plants inoculated with *R. intraradices* **(Fig. 37).**

In *T. erecta* maximum colonization was recorded in *R. intraradices* (75%) inoculated plants followed by plants inoculated with *Gi. albida* (64%) and *A. scrobiculata* (54%) (**Fig. 38**). *Rhizophagus intraradices* had greater effect on root length *G*. *coremioides* on shoot length during the earlier stages of growth. No significant difference was observed in stem diameter after 150 days among any of the AM treatments. Although *Gi. albida* and *G. coremioides* colonized plants showed greater shoot length than *R.*

Table 20: Total Na, P and K concentrations in AM fungi inoculated *J. nitidum* plants against control in the vegetative stage.

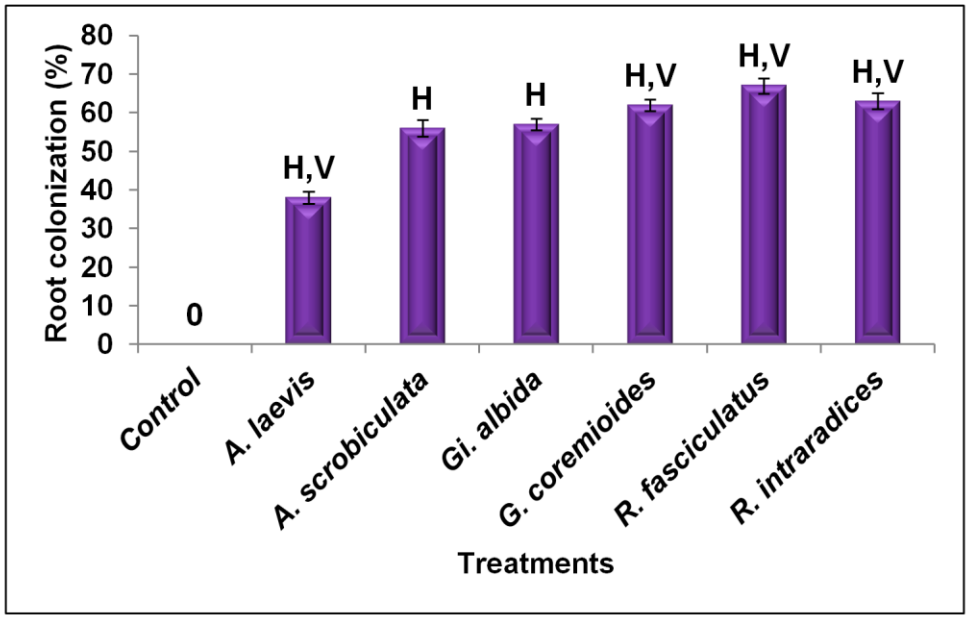
Sr. No.	Treatments	Na (%)	P (%)	K (%)
1.	**Control**	0.68^{f} ±0.02	0.25^{c} ±0.01	0.14^{g} ±0.01
2.	***A. laevis***	0.75^{e} ±0.01	0.41^{b} ±0.01	0.23^{f} ±0.01
3.	***A. scrobiculata***	0.82^{c} ±0.01	0.42^{b} ±0.01	0.30^{c} ±0.01
4.	***Gi. albida***	0.88^{b} ±0.01	0.41^{b} ±0.01	0.32^{b} ±0.00
5.	***G. coremioides***	0.70^{f} ±0.01	0.42^{ab} ±0.01	0.25^{e} ±0.02
6.	***R. fasciculatus***	0.78^{d} ±0.01	0.43^{ab} ±0.01	0.28^{d} ±0.01
7.	***R. intraradices***	0.96^{a} ±0.01	0.44^{a}±0.01	0.43^{a} ±0.01

Legend: Values presented are mean of 5 replicates, ± indicates standard deviation. Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = Acaulospora; *Gi. = Gigaspora*; *G = Glomus*; *R = Rhizophagus*.

Table 21: Effects of AM fungi on floral characteristics and total dry weight in *J. nitidum.*

Sr. No.	Treatment	No. of days for flower initiation (DAT)	Earlier induction of flowering (day)	No. of flower plant^{-1}	Fresh weight of flower (g)	Total plant dry weight (g)
1.	**Control**	357^{a} ±1.87	0^{e} ±0.00	54.3^{f} ±1.52	0.087^{d} ±0.00	52.23^{d} ±1.89
2.	***A. laevis***	355^{ab} ±1.67	2^{de} ±1.51	55.0^{f} ±2.00	0.087^{cd} ±0.00	54.09^{cd} ±2.80
3.	***A. scrobiculata***	351^{c} ±2.36	6^{cd} ±3.36	59.0^{e} ±1.00	0.088^{cd} ±0.00	59.03^{ab} ±2.25
4.	***Gi. albida***	353^{bc} ±2.54	4^{cd} ±2.00	61.6^{d} ±0.57	0.087^{cd} ±0.00	57.00^{bc} ±2.02
5.	***G. coremioides***	351^{c} ±1.58	6^{bc} ±2.91	64.3^{c} ±0.57	0.088^{c} ±0.00	58.83^{ab} ±1.85
6.	***R. fasciculatus***	345^{d} ±2.86	12^{a} ±2.60	70.3^{a} ±1.52	0.094^{a} ±0.00	60.03^{ab} ±0.88
7.	***R. intraradices***	348^{d} ±1.79	9^{ab} ±1.87	66.6^{b} ±1.52	0.912^{b} ±0.00	61.00^{a} ±1.25

Legend: Values presented are mean of 5 replicates, ± indicates standard deviation.
Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = Acaulospora; *Gi. = Gigaspora*; *G = Glomus*; *R = Rhizophagus*; DAT = Days after transplant.



Legend: H= Hyphal and V= Vesicular colonization.

Figure 32: Percent root colonization in *J. nitidum* plants inoculated with various AM treatments (after 60 days of growth).

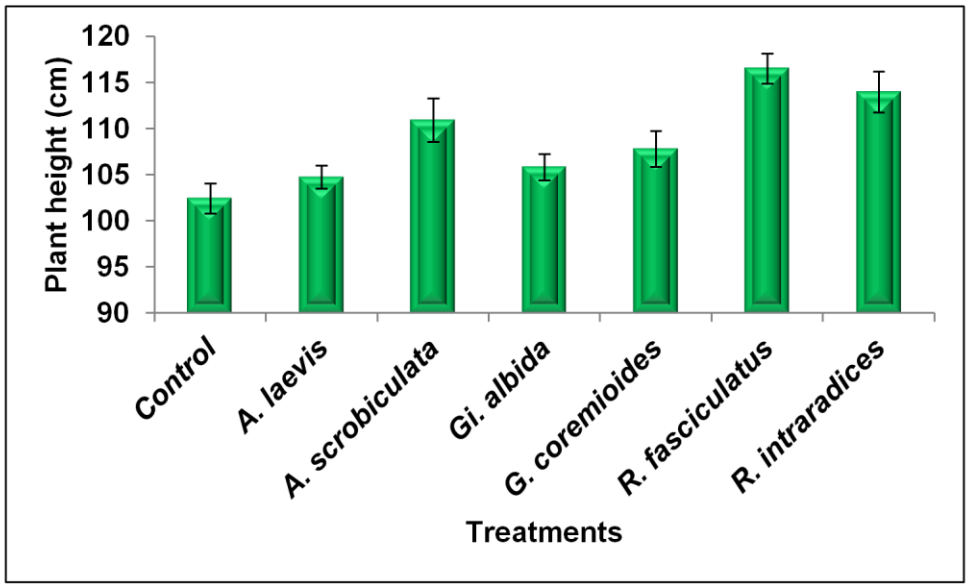


Figure 33: Plant height in *J. nitidum* plants inoculated with various AM treatments (after 150 days of growth).

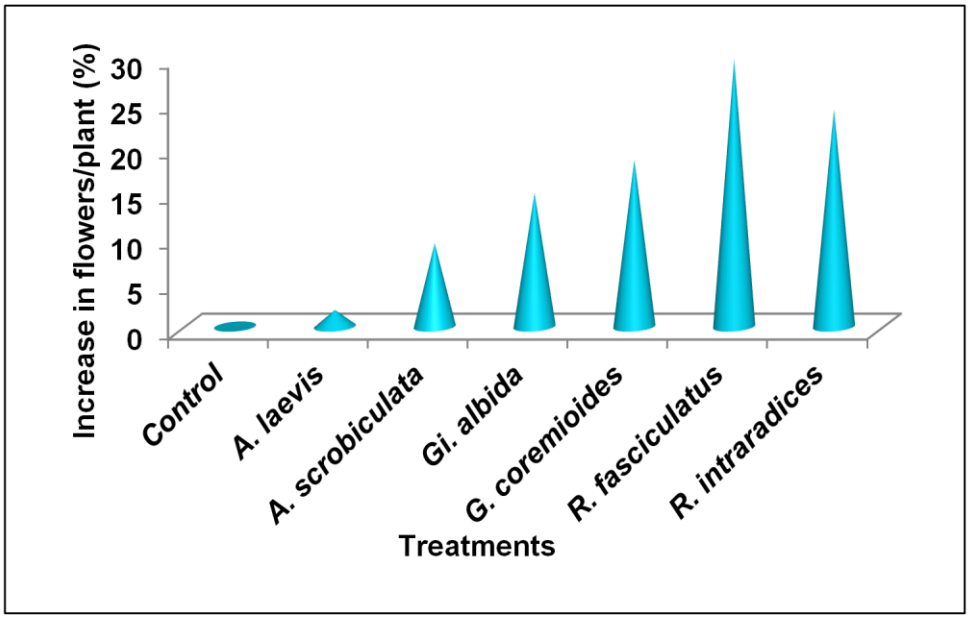


Figure 34: Effect of various AM fungal bioinoculants on percent increase in flower number plant^{-1} in *J. nitidum* plants.

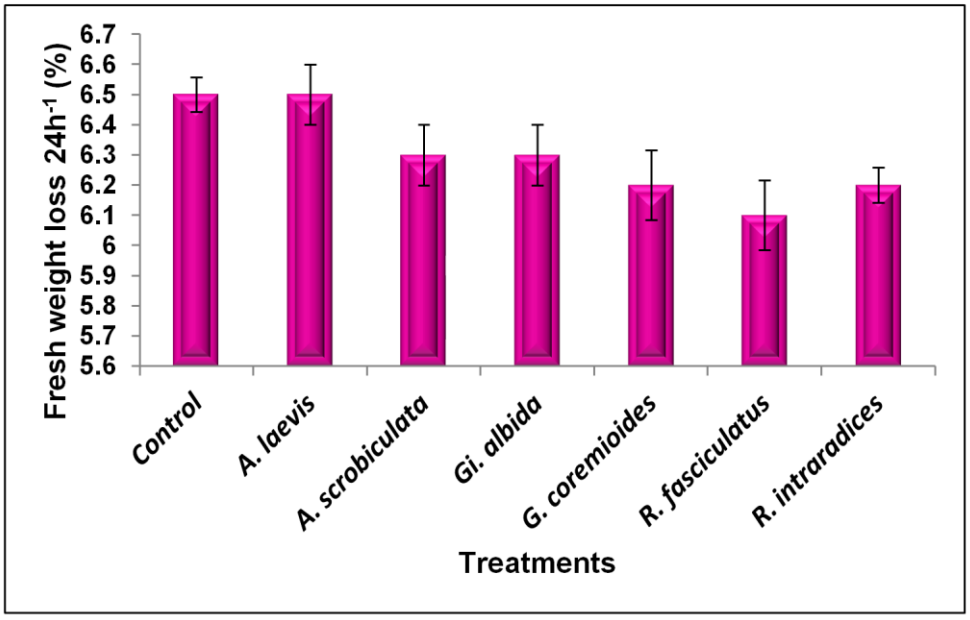


Figure 35: Effect of various AM fungal bioinoculants on percent average fresh weight loss in flowers 24h^{-1} in *J. nitidum* plants.

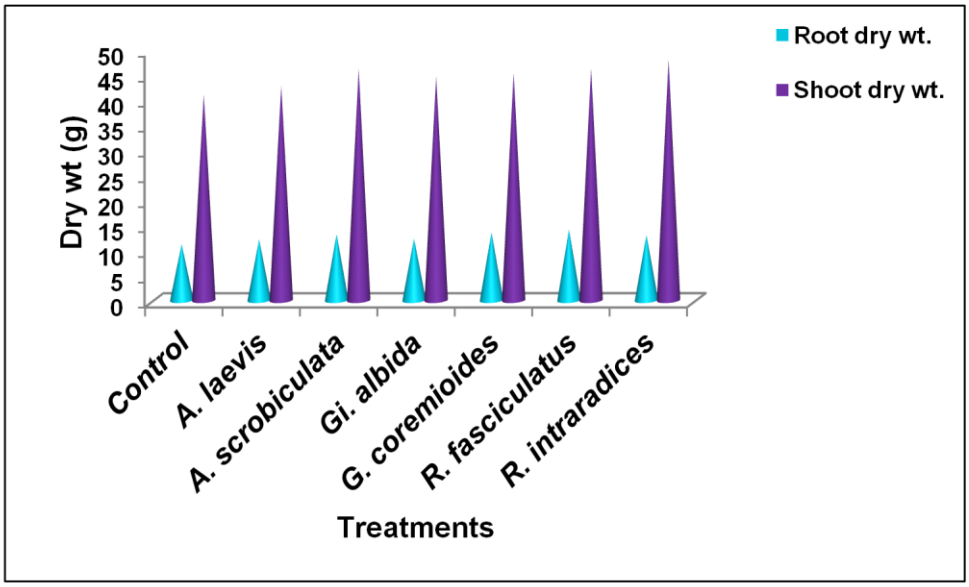


Figure 36: Effect of various AM fungal bioinoculants on root and shoot dry weight in *J. nitidum* plants.

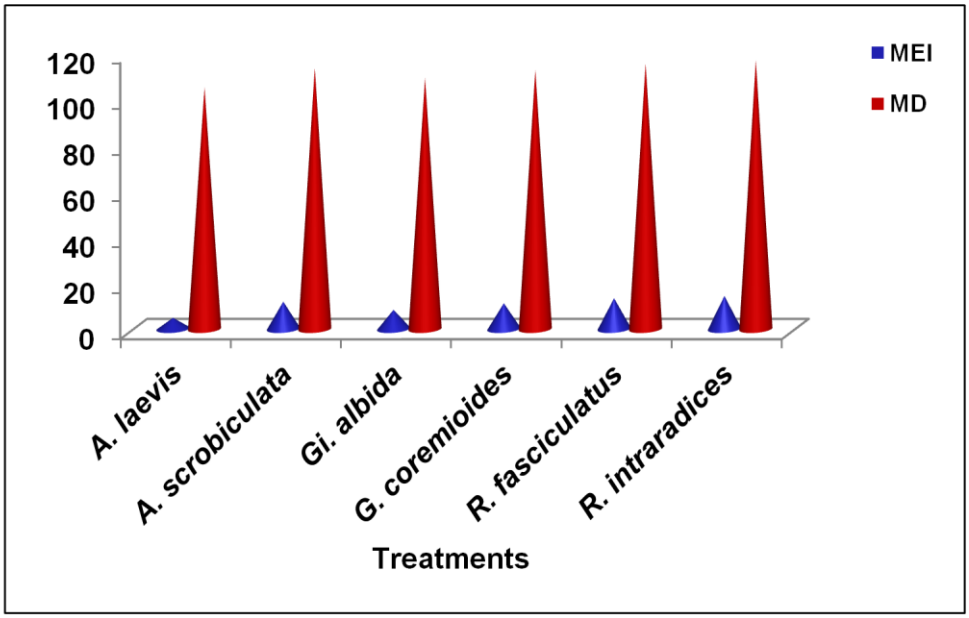
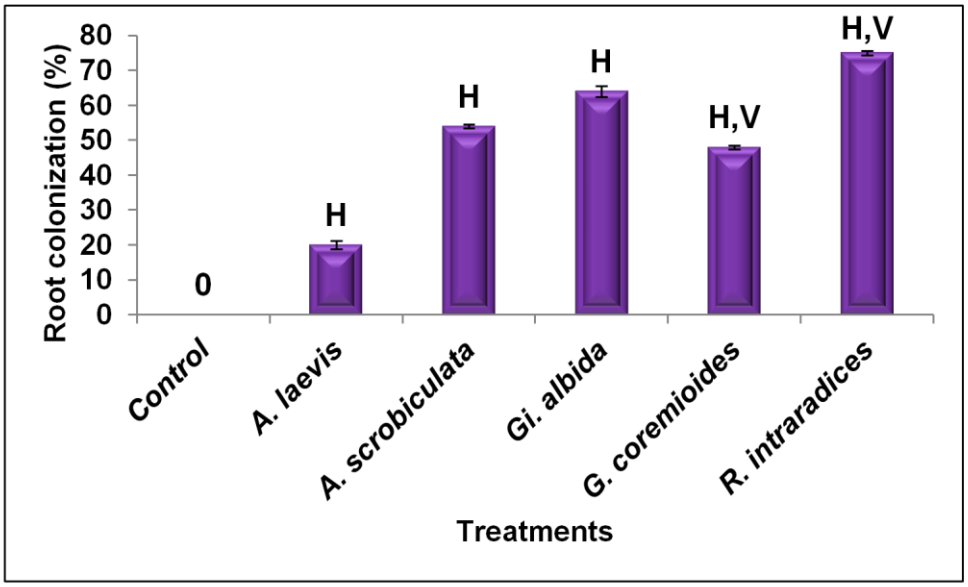


Figure 37: Mycorrhizal Efficiency Index and Mycorrhizal Dependency among various AM fungal bioinoculants in *J. nitidum* plants.



Legend: H= Hyphal and V= Vesicular colonization.

Figure 38: Percent root colonization in *T. erecta* plants inoculated with various AM treatments (after 60 days of growth).

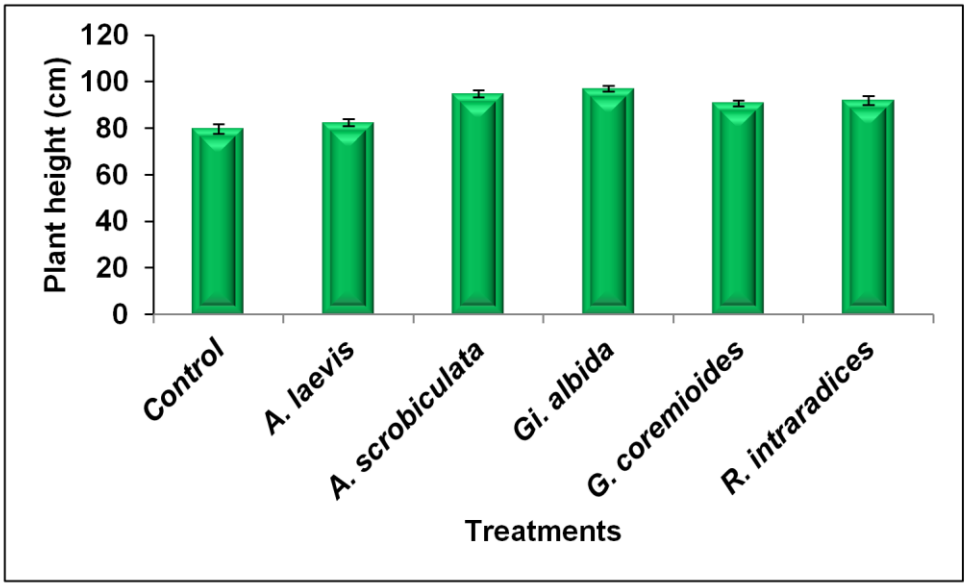


Figure 39: Plant height in *T. erecta* plants inoculated with various AM treatments (after 150 days of growth).

intraradices colonized plants (**Fig. 39)**, number of leaves and leaf length were less when compared to the latter **(Table 22)**. Total Na, P and K concentrations varied significantly within the treatments and were highest in *R*. *intraradices* inoculated plants, while the P concentration in *G*. *coremioides* and *R*. *intraradices* was maximum **(Table 23)**. During the reproductive stage *R. intraradices* and *A. scrobiculata* showed two weeks earlier than un-inoculated plants (**Table 24)**. *Rhizophagus intraradices* and *G. coremioides* showed an increase in number of flowers plant^{-1}, 67% and 53% respectively **(Fig. 40)** and recorded less fresh weight loss (**Fig. 41**). Total dry weight was maximum in *R*. *intraradices* inoculated plants **(Table 24).** Root and shoot dry weights were also recorded highest in *R*. *intraradices* inoculated plants **(Fig. 42)**. Mycorrhizal Dependency and Mycorrhizal Efficiency Index were also maximum in plants inoculated with *R. intraradices* **(Fig. 43)**.

7.4 DISCUSSION

Previous studies have suggested that application of AM fungi either as combination or as a single AM isolate increases the nutrient uptake mainly P by plants (Benthlenfalvay *et al*., 1988) resulting in increased productivity (Al-Karaki *et al*., 2004). The present study was conducted to compare the efficacy of six AM fungal species used singly on growth and flowering parameters in *C. morifolium*, *C. infundibuliformis*, *J. nitidum* and *T. erecta*. The study revealed differential effects on plant growth and floral parameters by different AM treatments. Similar differential effects of AM fungi on a variety of responses in marigold were reported earlier (Linderman and Davis, 2004; Sensoy *et al*., 2007). Initial mycorrhizal colonization depends on germination

Table 22: Effect of AM fungi on selected vegetative characteristics in *T. erecta.*

Sr. No.	Treatment	Root length after 60 days (cm)	Plant height after 60 days (cm)	Stem diameter after 150 days (cm)	No. of leaves after 150 days	Leaf length (cm)	No. of lateral branches plant^{-1}
1.	**Control**	3.14^{c} ±0.11	6.22^{d} ±0.11	2.86^{a} ±0.11	34.0^{d} ±1.58	14.34^{c} ±0.15	8.0^{cd} ±1.00
2.	***A. laevis***	3.30^{c} ±0.10	6.60^{c} ±0.22	2.88^{a}±0.08	34.6^{cd} ±1.52	14.36^{c} ±0.15	9.0^{bc} ±1.00
3.	***A. scrobiculata***	4.96^{b} ±0.15	7.14^{b} ±0.17	2.98^{a} ±0.23	36.8^{abc} ±2.05	15.14^{a} ±0.13	10.2^{b} ±1.48
4.	***Gi. albida***	5.90^{b} ±0.24	9.40^{a} ±0.20	2.96^{a} ±0.21	37.2^{ab} ±1.64	14.80^{b} ±0.12	9.4^{bc} ±1.14
5.	***G. coremioides***	5.38^{a} ±0.08	9.60^{a} ±0.19	2.90^{a} ±0.19	35.4^{bcd} ±1.14	14.70^{b} ±0.16	11.8^{a} ±1.48
6.	***R. intraradices***	5.44_{a} ±0.23	9.50^{a} ±0.16	3.00^{a} ±0.10	39.0^{a} ±2.24	15.22^{a} ±0.18	12.2^{a} ±1.48

Legend: Values presented are mean of 5 replicates, ± indicates standard deviation.
Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = Acaulospora; *Gi. = Gigaspora*; *G = Glomus*; *R = Rhizophagus*.

Table 23: Total Na, P and K concentrations in AM fungi inoculated *T. erecta* plants against control in the vegetative stage.

Sr. No.	Treatments	Na (%)	P (%)	K (%)
1.	**Control**	1.14^{e} ±0.01	0.25^{f} ±0.03	0.33^{f} ±0.01
2.	***A. laevis***	1.16^{d} ±0.01	0.46^{e} ±0.01	0.45^{e} ±0.01
3.	***A. scrobiculata***	1.21^{c} ±0.01	0.50^{bc} ±0.01	0.70^{b} ±0.01
4.	***Gi. albida***	1.27^{b} ±0.01	0.49^{cd} ±0.01	0.58^{c} ±0.01
5.	***G. coremioides***	1.29^{b} ±0.01	0.53^{ab} ±0.01	0.53^{d} ±0.01
6.	***R. intraradices***	1.36^{a} ±0.03	0.53^{a} ±0.02	0.75^{a} ±0.01

Legend: Values presented are mean of 3 replicates, ± indicates standard deviation.
Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = *Acaulospora*; *Gi.* = *Gigaspora*; *G* = *Glomus*; *R* = *Rhizophagus*.

Table 24: Effect of AM fungi on floral characteristics and total dry weight in *T. erecta* plants.

Sr. No.	Treatment	No. of days for flower initiation (DAT)	Earlier induction of flowering (day)	Flower number plant^{-1}	Flower width (cm)	Fresh weight of flower (g)	Toal plant dry weight (g)
1.	**Control**	128^{a} ±1.87	00^{e} ±0.00	14.8^{d} ±3.27	5.14^{d} ±0.19	5.61^{d} ±0.19	11.00^{b} ±0.33
2.	***A. laevis***	125^{b} ±0.71	03^{d} ±0.71	16.8^{cd} ±3.56	5.16^{d} ±0.58	5.80^{c} ±0.015	11.16^{b} ±0.26
3.	***A. scrobiculata***	114^{e} ±2.00	14^{a} ±2.00	21.8^{ab} ±2.28	5.60^{bc} ±0.16	6.03^{b} ±0.07	13.72^{a} ±0.13
4.	***Gi. albida***	117^{d}±2.00	11^{b} ±2.00	18.4^{bc} ±3.44	5.78^{b} ±0.11	6.60^{a} ±0.13	13.94^{a} ±0.13
5.	***G. coremioides***	121^{c} ±0.84	07^{c} ±0.84	23.0^{a} ±1.87	6.34^{a} ±0.11	6.59^{a} ±0.09	13.78^{a} ±0.32
6.	***R. intraradices***	113^{e} ±1.52	15^{a} ±1.52	25.0^{a} ±1.58	6.44^{a} ±0.11	6.73^{a} ±0.03	13.92^{a} ±0.19

Legend: Values presented are mean of 5 replicates, ± indicates standard deviation.
Data with different letters in the same column are significantly different at $P \leq 0.05$.
A = *Acaulospora*; *Gi.* = *Gigaspora*; *G* = *Glomus*; *R* = *Rhizophagus*; DAT = Days after transplant.

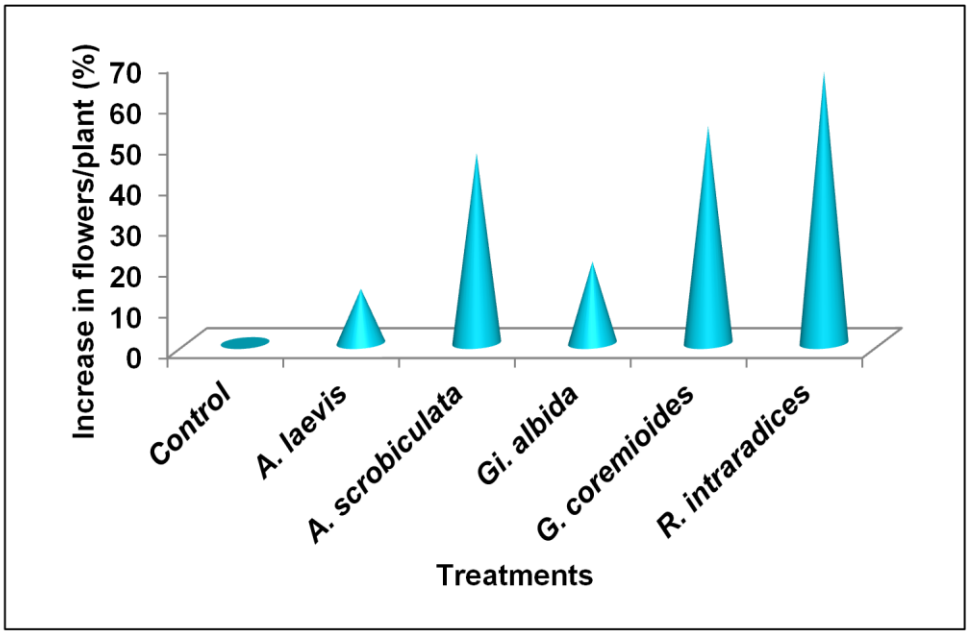


Figure 40: Effect of various AM fungal bioinoculants on percent increase in flower number plant^{-1} in *T. erecta* plants.

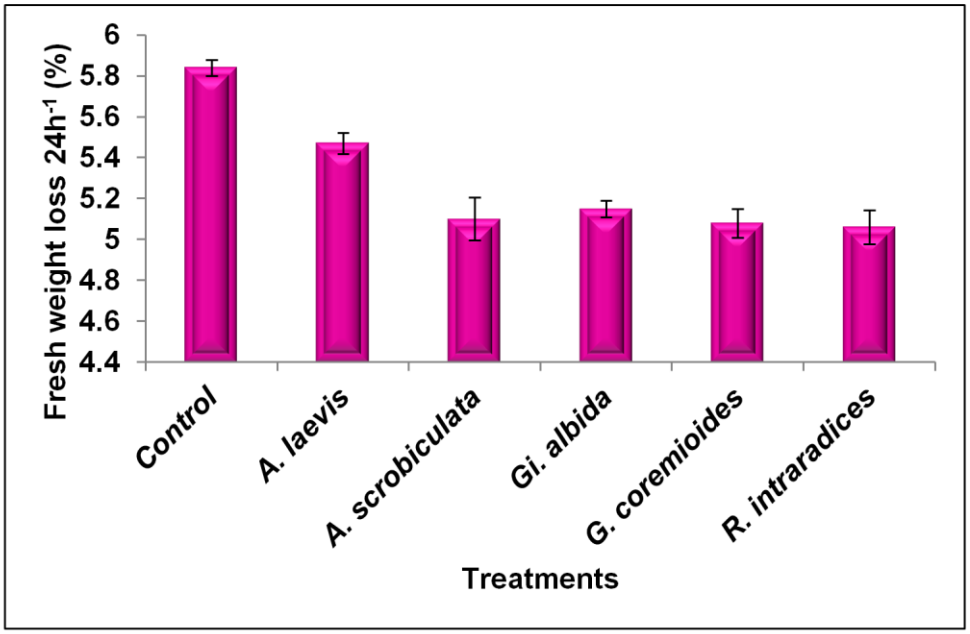


Figure 41: Effect of various AM fungal bioinoculants on percent average fresh weight loss in flowers 24h^{-1} in *T. erecta* plants.

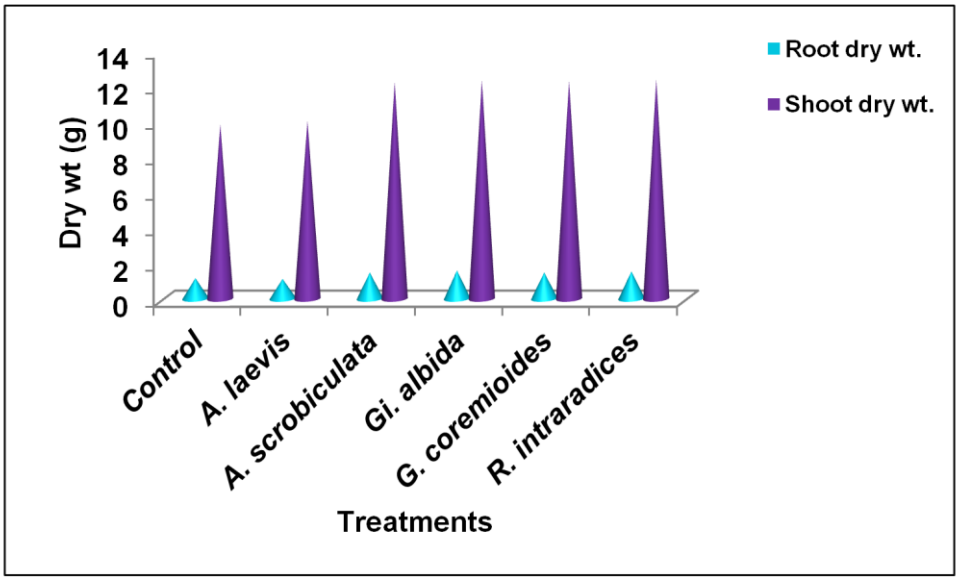


Figure 42: Effect of various AM fungal bioinoculants on root and shoot dry weight in *T. erecta* plants.

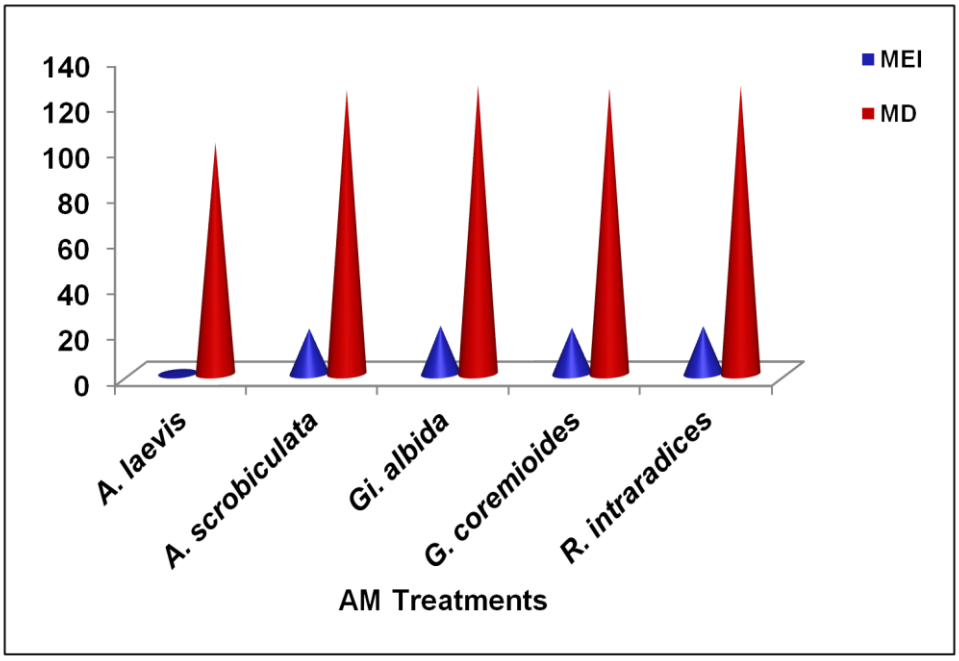


Figure 43: Mycorrhizal Efficiency Index and Mycorrhizal Dependency among various AM fungal bioinoculants in *T. erecta* plants.

of spores or other fungal propagules in the soil, growth of hyphae through the soil and finally hyphal entry into the roots (Bowen, 1987). Plant growth responses to AM fungi varies with the host plant, endophyte and soil (Entry *et al*., 2002; Hart and Reader, 2002) and is related to the timing and extent of AM fungal colonization (Graham *et al*., 1991; Abbott and Gazey, 1994; Wilson and Hartnett, 1998). Significant differences in root colonization between different AM fungal species and a distinct relationship between root colonization and the effect on plant growth and flower yield was observed. It is well known that colonizing ability and growth enhancing effect of different AM species or even strains for a given plant in terms of plant growth (Linderman and Davis, 2004; Sensoy *et al*., 2007) and P uptake (Graham *et al*., 1982) are variable, indicating that not all AM fungi are functionally equivalent (Trent *et al*., 1993; Clark and Zeto, 1996; van der Heijden *et al.*, 1998a, b). Mycorrhizal inoculation had a positive effect on root length and also on plant height similar to results in earlier studies (Aboul-Nasr, 1996; Long *et al*., 2010). The total P concentration in leaf was significantly higher in AM inoculated plants compared to un-inoculated plants which is in conformity with earlier studies (George, 2000; Matysiak and Falkowski, 2010; Asar and Elhindi, 2011).

The effects of AM colonization on vegetative growth may differ quantitatively from their effects on reproduction (Koide *et al*., 1988; Bryla and Koide, 1990; Stanley *et al*., 1993; Nakatsubo, 1997; Karagiannidis and Hadjisavva-Zinoviadi, 1998), because the extent of P deficiency for vegetative growth and reproduction may differ (Koide, 1991) and because some of the nutrient requirement for reproduction may be met by reallocation from

vegetative structures. Arbuscular Mycorrhizal inoculation resulted in early induction of flowering. Kandasamy *et al*. (1986) recorded 7–10 days earlier flowering in AM inoculated *Pyrethrum* over non-mycorrhizal plants. Increase in flower number was reported earlier by Aboul-Nasr (1996); Ranganayaki and Manoharachary (2001); Scagel (2003); Gaur and Adholeya (2005).

Arbuscular Mycorrhizal inoculated plants also showed a significant increase in diameter and fresh weight of flowers. Gange and Smith (2005) made similar observations wherein it was found that AM fungi increased a number of plant traits which include total plant size, flower number and flower size. Further, fresh weight loss per day was less in flowers of AM inoculated plants compared to un-inoculated plants reflecting on the quality of flowers. This indicates that AM plants produce flowers that have better ability to retain moisture and hence can remain fresh for a longer period than non-mycorrhizal plants.

Arbuscular Mycorrhizal inoculated plants also exhibited marked difference in root and shoot dry weights compared to un-inoculated control. Similar results were reported earlier by Lee and George (2005). The mycorrhizal effect on plant growth is quantified by measuring host's growth response, mycorrhizal dependency, and was identified by Gerdemann (1975) as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility. Menge *et al*. (1978) defined mycorrhizal dependency by expressing the dry mass of a mycorrhizal plant as a percentage of the dry mass of a non-mycorrhizal plant

at a given level of soil fertility. Mycorrhizal dependency and mycorrhizal efficiency index varied among all the AM fungal species and *R*. *intraradices* showed highest mycorrhizal dependency in all four plant species studied. This variation is mainly due to differential growth response by plant species to specific AM fungi as observed earlier (van der Heijden *et al*., 1998a). Enhanced nutrient uptake by AM fungi helps in increased plant growth. Baylis (1975) hypothesized that mycorrhizal dependency is largely controlled by root system architecture. Plants with coarsely branched roots and with few or no root hairs are expected to be more dependent on mycorrhiza than are plants with finely branched or fibrous roots.

After comparing the overall effect of all AM species used in the experiment, *R. intraradices* was found to be the most efficient AM fungal bioinoculant for all the ornamental flowering plant species studied as it had enhanced effect on plant growth and flower yield. *Rhizophagus intraradices* inoculated plants showed maximum increase in fresh weight of flowers compared to other AM species. This effect is mainly due to its more efficient colonization and rapid multiplication in the roots. The effectiveness of each AM fungal species varied depending upon the extent of colonization by the fungus. The present study suggests that the beneficial effects on plants were greater by those AM fungal species wherein percent colonization was higher during the treatment period. Root colonization by AM fungi causes increase in plant growth which is the most common response (Mosse, 1973; Tinker, 1975; Wu and Xia, 2006; Wu *et al*., 2008), demonstrated in many different plants and environments, indicating the mutual beneficial relationship between

the fungus and the plant involved in the association. This interaction between AM fungi and the plant can be a beneficial relation, wherein AM colonization in plants improve their growth and reproduction, or it may be a parasitic relationship when colonized plants show a reduction in growth and flower number (Gaur and Adholeya, 2005) due to the carbon drain caused by the fungus (Fitter, 2006). The physiological reasons behind a high or low extent of functional compatibility between plants and AM fungi are not yet understood. It is possible that in some plant/AM fungal combinations, establishment of a symbiotic interface allowing for nutrient exchange at high rates is not possible due to physiological incompatibility between the two organisms at the cellular level (Neumann and George, 2010). Differences in functional compatibility between a given host plant and various AM fungal strains are due to differences in AM life-cycle strategies or mycelia development (Burkert and Robson, 1994; Graham and Abbott, 2000; Smith *et al*., 2000, 2004). When plants are grown in the presence of a single AM fungal species the effect of plant nutrient uptake is dependent on environmental conditions such as soil properties, genotypes of the plant and AM fungal strain involved (Hamel *et al*., 1997; Jakobsen *et al*., 2001).

The functional compatibility of two symbiotic partners to develop association resulting in improved plant performance compared with a non-mycorrhizal control under given environmental conditions is well demonstrated (Pearson and Jakobsen, 1993; Ravnskov and Jakobsen, 1995). Therefore it is necessary to study the host and fungal compatibility before using them as biofertilizers. Incompatibility effects can be overcome by using

the native AM fungal strains which are isolated from natural habitats that are adapted to local environment. In order to get more promising results it is necessary to provide the AM bioinoculants at earlier stages of plant growth. Increased effects of AM species in pot experimental conditions indicate that the AM species used perform much better in natural environmental conditions due to more availability of space and resources. Therefore selective application and proper management of AM symbiosis to floricultural plants is essential to allow reduction of chemical fertilizers, pesticides input and high economic output. Further studies on promising AM fungi should be conducted to develop a commercial bioinoculant which can be used in floriculture industry instead of chemical fertilizers.

CHAPTER 8

SUMMARY

A survey of arbuscular mycorrhizal fungal (AMF) diversity from ornamental flowering plant species from Goa state was undertaken with the need to establish AM fungal diversity and distribution data and also to examine the occurrence of mycorrhizal symbiosis and to search for structures related to possible functional status of this symbiosis. Randomly sampled rhizosphere soils of ornamental flowering plants were assessed for the species richness and diversity of AM fungi. A total of 43 host plant species were examined. The presented data show that although there was less mycorrhizal colonization, roots of all the plants investigated showed the presence of mycorrhizal structures. It can be suggested that fluctuating and low intensity of mycorrhizal symbiosis is caused by unfavourable environmental conditions within the vegetation with seasonal variation. The average percent root colonization ranged from 4 and 99%. Maximum percentage of root colonization was observed by *Canna indica* (99%) and least in *Murraya paniculata* (4%). Only 20 plant species showed vesicular, arbuscular and hyphal colonization. Average root colonization was higher in garden plants (52.63%) than in wild plants (44.47%). Seasonal studies showed that the degree of root colonization was least during pre-monsoon season, increased during monsoon and reached maximum during post-monsoon season. Plants species growing in Western Ghats region showed maximum root colonization followed by those growing in coastal and plateau regions. Altogether 44 AM fungal species belonging to ten genera *viz*., *Acaulospora*, *Ambispora*, *Claroideoglomus*, *Dentiscutata*, *Funneliformis*, *Gigaspora*, *Gigaspora*, *Glomus*, *Racocetra and Rhizophagus* were recovered. *Glomus* was the most dominant genus, with 13 species followed by *Acaulospora* (12), *Gigaspora*

(5), *Rhizophagus* (4), *Dentiscutata* (3), *Funneliformis* (2), *Racocetra* (2), *Ambispora* (1), *Claroideoglomus* (1) and *Simiglomus* (1). The spore population varied from 23 to 350 spores $100g^{-1}$ of soil. Weak negative non-significant correlation was found between percent colonization and spore density (r = –0.1, $P \leq 0.05$). Highest spore density was recorded in *Ixora duffii* (350) and lowest spore density was found in *M*. *paniculata* (23). Average spore density $100g^{-1}$ rhizosphere soil was higher in garden plants (84.68%) than in wild plants (52.09%). *Glomus intraradices* exhibited highest relative abundance (14.51%) and the lowest was shown by *Racocetra gregaria*, *R. weresubiae* and *Rhizophagus diaphanus* (0.17%). Maximum species richness was found in *I. duffii* (8) and least in *Caesalpinia pulcherrima* and *M. paniculata* (2). The species richness was greater in wild plants (37) compared to garden plants (33). Also the species evenness was greater in wild (0.1381) than in garden (0.0899) plants. Mycorrhizal status in *C. morifolium*, *C. infundibuliformis* and *T. erecta* during the various growth stages differed. During vegetative stage only vesicular and hyphal colonization was observed and no arbuscular colonization was recorded in all the selected plant species. The total leaf P (%) was higher in the the plant species studied during flowering stage. Phosphorus levels in the plants increased immediately after arbuscules peaked indicating P uptake is related to arbuscules that participate directly in nutrient exchange. This reflects on the higher P requirement by plants during flowering stage. Out of total of 43 sets of different rhizosphere soil samples collected, 38 sets of trap cultures were established successfully. The AM fungal spores obtained from trap cultures were then isolated and used in the establishment of pure cultures. Of the 10 pure cultures obtained,

cultures of 6 dominant AM fungal cultures were used for mass multiplication. Based on the AM fungal diversity and distribution data in ornamental plant species, the dominant AM fungal species were screened to evaluate effects of their inoculation on growth, yield and flower quality in four ornamental plant species *i.e. Chrysanthemum morifolium* Ramat.*, Crossandra infundibuliformis* (L.) Nees*, Jasminum nitidum* Skan. and *Tagetes erecta* L. Pure culture inocula of six AM fungal species *viz., Acaulospora laevis*, *A. scrobiculata*, *Gigaspora albida*, *Glomus coremioides*, *Rhizophagus fasciculatus* and *R. intraradices* were used separately as treatments against an un-inoculated control. The AM fungal species used in the study were mass multiplied in trays using sterilized sand: soil (2:1) mixture. Healthy and uniform seedlings of *C. infundibuliformis,* and *T. erecta* and, cuttings of *C. morifolium* and *J. nitidum* were transferred to trays containing active and viable AM inoculum to achieve mycorrhizal plants. Mycorrhizal seedlings (one/pot) were transferred into 15 cm diameter pots containing sand: soil (2:1) mix. A completely randomized block design in factorial arrangement with 5 replicates per treatment was employed in the study. The three growth stages *i.e.* vegetative, reproductive and senescence were compared among the AM treatments and the control. Various parameters *viz*., root length, plant height, stem diameter, leaf number, leaf length, number of lateral branches, number of days required for flowering, flower width, flower number, flower fresh weight, fresh weight loss of flowers after every 24h period (at 25ºC and 65% RH) and, root and shoot dry weights were recorded. Data over one blooming season showed that AM fungal inoculation had a significant effect on plant growth and flower quantity as well as quality. Shoot analysis revealed that the total Na, P and K

content varied significantly within the treatments. It was higher in inoculated plants compared to control plants. AM inoculated plants also showed increased root and shoot dry weights compared to un-inoculated control. Increase in flower diameter and the moisture retaining ability of flowers in AM inoculated plants was also recorded. Percent fresh weight loss of flowers per day was less in AM inoculated plants compared to un-inoculated control indicating the better quality of flowers produced by AM inoculated plants. AM inoculated plants also exhibited a marked difference in root and shoot dry weights compared to the un-inoculated control. Mycorrhizal Dependency (MD) and Mycorrhizal Efficiency Index (MEI) was higher of *R. intraradices* treated plants compared to other treatments. The results indicate that *R. intraradices* is the most efficient AM fungal bioinoculant which gave highest yield among all the AM species used. Besides its ability to colonize and multiply was maximum as compared to other AM fungal species used in the study.

It is concluded that it will be advisable for ornamental plant growers to inoculate their horticultural crops with selected mycorrhizal inoculants during the nursery stage, as it cannot be predicted from the soil conditions whether the native AM fungal community is sufficient to sustain a stable horticultural production in the region.

CHAPTER 9

BIBLIOGRAPHY

Abbott LK, Gazey C. 1994. An ecological view of the formation of VA mycorrhizas. *Plant and Soil*, 159: 69–78.

Abbott LK, Robson AD. 1984. The effect of VA mycorrhizae on plant growth. In: VA mycorrhiza (eds). Powell CL, Bagyaraj DJ. CRC Press, Boca Raton, FL. pp. 113–130.

Abbott LK, Robson AD. 1991a. Factors influencing the occurrence of vesicular arbuscular mycorrhizas. *Agriculture*, *Ecosystems and Environment*, 35: 121–150.

Abbott LK, Robson AD. 1991b. Field management of VA mycorrhizal fungi. In: The rhizosphere and plant growth (eds). Keister DL, Cregan PB. Kluwer, Dordrecht. pp. 355–362.

Abbott LK. 1982. Comparative anatomy of vesicular-arbuscular mycorrhizas formed on subterranean clover. *Australian Journal of Botany*, 30: 485–489.

Abdalla ME, Abdel-Fattah GM. 2000. Influence of the endomycorrhizal fungus *Glomus mosseae* on the development of peanut pod rot disease in Egypt. *Mycorrhiza*, 10: 29–35.

Abdel-Fattah GM, Mankarios AT. 1995. Functional activity of *Glomus mosseae* in the protection of soybean from infection by the pathogenic fungus *Chalara elegans*. *Egyptian Journal of Microbiology*, 30: 207–305.

Aboul-Nasr A. 1996. Effects of vesicular-arbuscular mycorrhiza on *Tagetes erecta* and *Zinnia elegans*. *Mycorrhiza*, 6: 61–64. doi: 10.1007/s005720050107.

Adriano-Anaya, ML, Solis-Dominguez F, Gavito-Pardo ME, Salvador-Figueroa M. 2006. Agronomical and environmental factors influence root colonization, sporulation and diversity of arbuscular mycorrhizal fungi at specific phenological stage of Banana trees. *European Journal of Agronomy*, 5(1): 11–55.

Akiyama K, Matsuzaki K-I, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*, 435: 824–827.

Al-Karaki GN, Clark RB. 1999. Effect of varied rates of mycorrhizal inoculum on growth and nutrient acquisition by barley grown with drought stress. *Journal of Plant Nutrition*, 22: 1775–1784.

Al-Karaki GN, Hammad R, Rusan M. 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. *Mycorrhiza*, 11: 41–47.

Al-Karaki GN, MC Michael B, Zak J. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza*, 14: 263–269.

Allen EB, Allen MF, Helm DJ, Trappe JM, Molina R, Rincon E. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. *Plant and Soil*, 170: 47–62.

Allen EB, Allen MF. 1986. Water relations of xeric grasses in the field: interactions of mycorrhizas and competition. *New Phytologist*, 104: 559–571.

Allen MF, Smith WK, Moore Jr TS, Christensen M. 1981. Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis* H.B.K. *New Phytologist*, 88: 683–693.

Allen MF. 1996*.* The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peek into the 21st. *Mycological Research*, 100*:* 769–*782.*

Almeida, RT, Schenck NC, 1990. A revision of the genus *Sclerocystis* (*Glomaceae*: *Glomales*). *Mycologia*, 82: 703–714.

Al-Whaibi MH, Khaliel AS. 1994. The effect of Mg and Ca, K and P content of date palm seedlings under mycorrhizal and nonmycorrhizal conditions. *Mycoscience*, 35: 213-217.

Ames RN, Schneider RW. 1979. *Entrophospora*, a new genus in the Endogonaceae. *Mycotaxon*, 8: 347–352.

Anderson RC, Liberia AE, Dickman LA. 1984. Interaction of vascular plants and vesicular-arbuscular mycorrhizal fungi across a soil moisture- nutrient gradient. *Oecologia*, 64: 111–117.

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant and Soil*, 192: 71–79.

Arora JS. 1993. Introductory Ornamental Horticulture. Kalyani Publishers, Ludhiana, India.

Asrar AWA, Elhindi KM. 2011. Alleviation of drought stress of marigold (*Tagetes erecta*) plants by using arbuscular mycorrhizal fungi. *Saudi Journal of Biological Sciences*, 18: 93–98.

Augé RM, Duan X, Ebel RC, Stodola AJ. 1994. Nonhydraulic signaling of soil drying in mycorrhizal maize. *Planta*, 193: 74–82.

Augé RM. 2001. Water relations, drought and VA mycorrhizal symbiosis. *Mycorrhiza*, 11: 3–42.

Augé RM. 2004. Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science*, 84: 373–381.

Avrahami S, Bohannan BJA. 2007. Response of *Nitrosospira* sp strain AF-Like ammonia oxidizers to changes in temperature, soil moisture content and

fertilizer concentration. *Applied and Environmental Microbiology*, 73: 1166–1173.

Azaizeh HA, Marschner H, Römheld V, Wittenmayer L. 1995. Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil grown maize plants. *Mycorrhiza*, 5: 321–327.

Azcón-Aguilar C, Barea JM. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens- An overview of the mechanisms involved. *Mycorrhiza*, 6: 457–464.

Azcón-Aguilar C, Barea JM.1997. Applying mycorrhiza biotechnology to horticulture: Significance and potentials. *Scientia Horticulturae*, 68: 1–24.

Bago B, Pfeffer PE, Abubaker J, Jun J, Allan JW, Brouillette J, Douds DD, Lammers PJ, Shachar-Hill Y. 2003. Carbon export from arbuscular mycorrhizal roots involves the translocation of glycogen as well as lipid. *Plant Physiology*, 131: 1496–1507.

Bago B, Vierheilig H, Piche Y, Azcón-Aguilar C. 1996. Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. *New Phytologist*, 133: 273–280.

Bago B, Zipfel W, Williams RM, Jun J, Arreola R, Lammers PJ, Pfeffer PE, Shachar-Hill Y. 2002. Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. *Plant Physiology*, 128: 108–124.

Bagyaraj DJ, Brya Reddy MS, Nalini PA. 1989. Selection of an efficient inoculant VA mycorrhizal fungus for *Leucaena*. *Forest Ecology and Management*, 27: 81–85.

Bagyaraj DJ. 1994. Vesicular arbuscular mycorrhiza: Application in Agriculture. In: Techniques for the study of mycorrhiza (eds). Norris JR, Read DJ, Varma AK. Academic Press, London. pp. 819–833.

Balestrini R, Bonfante P. 2005. The interface compartment in arbuscular mycorrhizae: A special type of plant cell wall? *Plant Biosystems*, 139: 8–15.

Balestrini R, Romera C, Puigdomenech P, Bonfante P. 1994. Location of a cell wall hydroxyproline-rich glycoprotein, cellulose and -1,3-glucans in apical and differentiated regions of maize mycorrhizal roots. *Planta*, 195: 201–209.

Bansal M, Kukreja K, Dudeja SS. 2012. Diversity of Arbuscular mycorrhizal fungi, prevalent in rhizosphere of different crops grown in the university farm. *African Journal of Microbiology*, 6(21): 4557–4566. doi: 10.5897/AJMR12.222.

Barea J, Pozo MJ, Azcon R, Azcón-Aguilar C. 2005. Microbial co-operation in the rhizosphere. *Journal of Experimental Botany*, 56: 1761–1778.

Bashan Y, Khaosaad T, Salazar BG, Ocampo JA, Wiemken A. 2007. Mycorrhizal characterization of the boojum tree, *Fouquieria columnaris*, an endemic ancient tree from Baja California Peninsula, Mexico. *Trees*, 21: 329–335.

Baylis GTS. 1969. Mycorrhizal nodules and growth of *Podocarpus* in nitrogen-poor soil. *Nature*, 223: 1385–1386.

Baylis GTS. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Endomycorrhizas (eds.) Sanders FE, Mosse B, Tinker PB. Academic Press, New York, London. pp. 391–407.

Bécard G, Douds DD Jr, Pfeffer PE. 1992. Extensive *in vitro* hyphal growth of vesicular-arbuscular mycorrhizal fungi in the presence of CO_2 and flavonols. *Applied and Environmental Microbiology*, 58: 821–825.

Bécard G, Piché Y. 1989. Fungal growth stimulation by CO_2 and root exudates in vesicular arbuscular mycorrhizal symbiosis. *Applied and Environmental Microbiology*, 55: 2320–2325.

Becker WN, Hall IR. 1976. *Gigaspora margarita*, a new species in the Endogonaceae. *Mycotaxon*, 4(1): 155.

Beena KR, Raviraja NS, Arun AB, Sridhar KR. 2000. Diversity of arbuscular mycorrhizal fungi on the coastal sand dunes of the West Coast of India. *Current Science*, 79(10): 1459–1465.

Bending G. 2007. What are the mechanisms and specificity of mycorrhization helper bacteria? *New Phytologist*, 174: 707–710.

Bending GD, Turner MK, Rayns FR, Marx MC, Wood M. 2004. Microbial and biochemical indicators of soil quality and their potential for differentiating areas under contrasting agricultural management regimes. *Soil Biology and Biochemistry*, 36: 1785–1792.

Bentivenga SP, Morton JB. 1995. A monograph of the genus *Gigaspora*, incorporating developmental patterns of morphological characters. *Mycologia*, 87: 719–731.

Bentivenga SP, Morton JB. 2005. A monograph of the genus *Gigaspora*, incorporating developmental patterns of morphological characters. *Mycologia*, 87: 720–732.

Berch SM, Fortin JA. 1984. A lectotype for *Glomus microcarpum* (Endogonaceae, Zygomycetes). *Mycologia*, 76: 190–193.

Besserer A, Puech-PagésV KP, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biology*, 4(7): 1239–1247.

Bethenfalvay GJ, Brown MS, Ames RN, Thomas RS. 1988. Effects of drought on host and endophyte development in mycorrhizal soyabeans in relation to water use and phosphate uptake. *Plant Physiology*, 72: 565–571.

Bethlenfalvay GJ, Cantrell IC, Mihara KL, Schreiner RP. 1999. Relationships between soil aggregation and mycorrhizae as influenced by soil biota and nitrogen nutrition. *Biology and Fertility of Soils*, 28: 356–363.

Bever JD, Schultz PA, Pringle A, Morton JB. 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. *Bioscience*, 51: 923–931.

Bever JD. 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant and Soil*, 244: 281–290.

Bharathiraja S, Tholkappian P. 2011. AM fungal interaction and its beneficial effects on *Crossandra infundibuliformis*. *International Journal of Current Research*, 33(6): 100–102.

Bhaskaran C, Selvaraj T. 1997. seasonal incidence and distribution of VAM fungi in native saline soils. *Journal of Environmental Biology*, 18: 209–212.

Bhattacharjee M, Mukerji KG, Tewari JP, Skoropad WP. 1982. Structure and hyperparasitism of a new species of *Gigaspora*. *Transactions of the British Mycological Society*, 78(1): 184.

Bhattacharjee S, Sharma GD. 2011. The Vesicular arbuscular mycorrhiza associated with three cultivars of rice (*Oryza sativa* L.). *Indian Journal of Microbiology,* 51(3): 377–383.

Bhattacharya S, Bagyaraj DJ. 2002. Arbuscular mycorrhizal fungi associated with *Arabica coffea*. *Geobios*, 29: 93–96.

Biermann B, Linderman RG. 1983. Use of vesicular arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. *New Phytologist*, 95: 97–105.

Binet MN, Tuinen D van, Deprêtre N, Koszela N, Chambon C, Gianinazzi S. 2011. Arbuscular mycorrhizal fungi associated with *Artemisia umbelliformis* Lam, an endangered aromatic species in Southern French Alps, influence plant P and essential oil contents. *Mycorrhiza,* 21: 523–535.

Biró B, Köves-Péchy K, Vörös I, Takács T, Eggenberger P, Strasser RJ. 2000. Interrelations between *Azospirillum* and *Rhizobium* nitrogen-fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile, AMF-free or normal soil conditions. *Applied Soil Ecology*, 15: 159–168.

Blaszkowski J. 1988. Four new species of the Endogonaceae (Zygomycotina) from Poland. *Karstenia*, 27: 37–42.

Blee KA, Anderson AJ.1998. Regulation of arbuscule formation by carbon in the plant. *The Plant Journal*, 16: 523–*530.*

Boglárka Oláh, C. B., Guillaume Bécard, Jean Dénarié and Clare Gough (2005). "Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway." *The Plant Journal*, 44(2): 195–207. doi: 10.1111/j.1365-313X.2005.02522.x.

Bohrer KE, Friese CF, Amon JP. 2004. Seasonal dynamics of arbuscular mycorrhizal fungi in differing wetland habitats. *Mycorrhiza*, 14: 329–337.

Bolan NS. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil*, 134: 189–207.

Bonfante P, Perotto S. 1995. Strategies of arbuscular mycorrhizal fungi when infecting host plants. *New Phytologist*, 130: 3–21.

*Bononi VLR, Barbosa LM, Viriato A. 1988. Micorrizas vesículo-arbusculares em amendoim e em plantas invasoras da cultura. *Hoehnea*, 15: 1–9.

Borowicz VA. 2001. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology*, 82: 3057–3068.

Boswell EP, Koide RT, Shumway DL, Addy HD. 1998. Winter wheat cover cropping, VA mycorrhizal fungi and maize growth and yield. *Agriculture*, *Ecosystems and Environment*, 67: 55–65.

Bouamri R, Dalpé Y, Serrhini MN, Bennani A. 2006. Arbuscular mycorrhizal fungi species associated with rhizosphere of *Phoenix dactylifera* L. in Morocco. *African Journal of Biotechnology*, 5: 510–516.

Bouyoucos GJ. 1939. Effect of organic matter on the water-holding capacity and the wilting point of mineral soils. *Soil Science*, 47: 377–384.

Bowen GD. 1987. The biology and physiology of infection and its development. In: Ecophysiology of VA Mycorrhizal Plants. (ed). Safir GR. CRC Press, Boca Raton, FL, U.S.A. pp. 27.

Braunberger PG, Abbott LK, Robson AD. 1996. Infectivity of arbuscular mycorrhizal fungi after wetting and drying. *New Phytologist*, 134: 673–684.

Bray RH, Kurtz LT. 1945. Determination of total, organic and available forms of phosphorus in soils. *Soil Science*, 59: 39–45.

Brundett M, Melvelle L, Peterson L. 1994. Practical methods in mycorrhizal research. Mycologue Publications, University of Guelph, Ontario, Canada.

Brundett M. 1991. Mycorrhizae in natural ecosystems. *Advances in Ecological Research*, 21: 171–313.

Brundrett M. 2004. Diversity and classification of mycorrhizal associations. *Biological Reviews*, 79(3): 473–495. doi: 10.1017/S1464793103006316.

Brundrett MC, Abbott LK, Jasper DA. 1999. Glomalean mycorrhizal fungi from tropical Australia I: Comparison of the effectiveness and specificity of different isolation procedures. *Mycorrhiza*, 8: 305: 314.

Brundrett MC, Abbott LK, Jasper DA. 1999a. Glomalean mycorrhizal fungi from tropical Australia I. Comparison of the effectiveness and specificity of different isolation procedures. *Mycorrhiza*, 8: 305–314.

Brundrett MC, Jasper DA, Ashwath N. 1999b. Glomalean mycorrhizal fungi from tropical Australia II. The effect of nutrient levels and host species on the isolation of fungi. *Mycorrhiza*, 8: 315–321.

Brundrett MC. 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist*, 154: 275–304.

Bryla DR, Duniway JM. 1997. Effects of mycorrhizal infection on drought tolerance and recovery in safflower and wheat. *Plant and Soil*, 197: 95–103.

Bryla DR, Koide RT. 1990. Regulation of reproduction in wild and cultivated *Lycopersicon esculentum* Mill. by vesicular-arbuscular mycorrhizal infection. *Oecologia*, 84: 74–81.

Bryla DR, Koide RT. 1998. Mycorrhizal response of two tomato genotypes relates to their ability to acquire and utilize phosphorus. *Annals of Botany*, 82: 849–857.

Buée M, Rossignol M, Jauneau A, Ranjeva R, Bécard G. 2000. The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. *Molecular Plant Microbe Interactions*, 13: 693–698.

Bürkert B, Robson AD. 1994. 65Zn uptake in subterraneam clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root-free sandy soil. *Soil Biology and Biochemistry*, 26: 1117–1124.

Burleigh SH, Cavagnaro TR, Jakobsen I. 2002. Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *Journal of Experimental Botany*, 53: 1–9.

Burrows R, Pfleger FL. 2002. Host plant responses to arbuscular-mychorrhizal fungal inoculum from plots of increasing plant diversity. *Plant and Soil* 240: 169–179.

Butler SJ, Vickery JA, Norris K. 2007. Farmland biodiversity and the footprint of agriculture. *Science*, 315: 381–384.

Callaway RM, Thelen GC, Barth S, Ramsey PW, Gannon JE. 2004. Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. *Ecology*, 85: 1062–1071.

Carrenho R, Trufem SFB, Bononi VLR. 2002. Effects of using different host plants on the detected biodiversity of arbuscular mycorrhizal fungi from an agro ecosystem. *Revista Brasileira de Botanica*, 25: 93–101.

Carvalho LM, Caçador I, Martins-Louçao MA. 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza*, 11: 303–309.

Castillo P, Nico AL, Azcón-Aguilar C, Del Río Rincón C, Calvet C, Jiménez-Díaz RM. 2006. Protection of olive planting stocks against parasitism of root-knot nematodes by arbuscular mycorrhizal fungi. *Plant Pathology*, 55: 705–713.

Cavagnaro TR, Gao LL, Smith FA, Smith SE. 2001. Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytologist*, 151(2): 469–475. doi: 10.1046/j.0028-646x.2001.00191.x.

Chabaud M, Venard C, Defaux PA, Bécard G, Barker DG. 2002. Targeted inoculation of *Medicago truncatula in vitro* root cultures reveals *Mt*ENOD11 expression during early stages of infection by arbuscular mycorrhizal fungi. *New Phytologist*, 156: 265–273.

Chacón S, Guzmán G. 1995. Observations on the phenology of ten fungal species in the subtropical forests at Xalapa, Mexico. *Mycological Research*, 99: 54–56.

Chang DC. 1994. What is the potential for management of vesicular-arbuscular mycorrhizae in horticulture? In: Management of mycorrhizas in agriculture, horticulture and forestry (eds). Robson AD, Abbott LK, Malaczuk N. The Netherlands, Kluwer Academic Publishers. pp. 187–190.

Chapin III FS, Mooney HA, Chapin MC, Matson P. 2004. Principles of Terrestrial Ecosystem Ecology. Springer-Verlag, New York Berlin Heidelberg.

Chapmann HD, Pratt PF. 1961. Methods of Analysis for Soils, Plants and Waters. University of California, Division of Agricultural Sciences. Berkeley.

Chaurasia B, Pandey A, Palni LMS. 2005. Distribution, colonization and diversity of arbuscular mycorrhizal fungi associated with central Himalayan rhododendrons. *Forest Ecology Management*, 207: 315–324.

Chen BD, Li XL, Tao HQ, Christie P, Wong MH. 2003. The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. *Chemosphere*, 50: 839–846.

Chen TT, Chang DCN. 1996. Effect of vesicular-arbuscular mycorrhiza on growth and flowering of two cultivars of cineraria. Memoirs of the College of Agriculture, National Taiwan University, 36: 99–111.

Chen X, Wu C, Tang J, Hu S. 2005. Arbuscular mycorrhizae enhance metal lead uptake and growth of host plants under a sand culture experiment. *Chemosphere*, 60: 665–671.

Clark RB, Zeto SK. 1996. Mineral acquisition by mycorrhizal maize grown on acid and alkaline soil. *Soil Biology and Biochemistry*, 28: 1495–1503.

Clark RB, Zeto SK. 2000. Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition*, 23: 867–902.

Cooper KM. 1984. Physiology of VA mycorrhizal associations. In: VA mycorrhiza (eds). Powell CL, Bagyaraj DJ. CRC Press, Boca Raton, FL. pp. 155–186.

Cordier C, Gianinazzi S, Gianinazzi-Pearson V. 1996. Colonization patterns of root tissues by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato. *Plant and soil*, 185: 223–232.

Corkidi L, Rincön E. 1997. Arbuscular mycorrhizae in a tropical sand dune ecosystem on the Gulf of Mexico. I: Mycorrhizal status and inoculum potential along a successional gradient. *Mycorrhiza*, 7: 9–15.

Courty PE, Bréda N, Garbaye J. 2007. Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. *Soil Biology and Biochemistry*, 39: 1655–1663.

Croll D, Giovannetti M, Koch AM, Sbrana C, Ehinger M, Lammers PJ, Sanders IR. 2009. Nonself vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist*, 181: 924–937.

Cuenca G, Meneses E. 1996. Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant and Soil*, 183: 315–322.

Currie WS, Aber JD. 1997. Modeling leaching as a decomposition process in humid Montane forests. *Ecology*, 78: 1844–1860.

Daft MJ, Hogarth BG. 1983. Competitive interactions amongst four species of *Glomus* on maize and onion. *Transactions of the British Mycological Society*, 80: 339–345.

Dalpe Y, Monreal M. 2004. Arbuscular mycorrhiza inoculum to support sustainable cropping systems. *Crop Management*, doi: 10.1094/CM–2004–0301–09–RV.

Dalpe Y, Plenchette C, Gueye M. 2000. Glomales species associated with surface and deep rhizosphere of Faidherbia albida in Senegal. *Mycorrhiza*, 10: 125–129.

D'Amelio R, Massa N, Gamalero E, D'Agostino G, Sampò S, Berta G, Faoro F, Iriti M, Bosco D, Marzachì C. 2007. Preliminary results on the evaluation of the effects of elicitors of plant resistance on chrysanthemum yellows phytoplasma infection. *Bulletin of Insectology*, 60(2): 317–318.

Daniels BA, Skipper HD. 1982. Methods for recovery and quantitative estimation of propagules from soil. In: Methods and Principles of Mycological Research (ed). Schenck NC. The American Phytological Society, St Paul, MN. pp. 29–35.

Dar GH, Zargar MY, Beigh GM. 1997. Biocontrol of *Fusarium* root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. *Microbial Ecology*, 34: 74–80.

Das P, Kayang H. 2010. Mycorrhizal colonization and distribution of arbuscular mycorrhizal fungi associated with *Michelia champaca* L. under plantation system in northeast India. *Journal of Forestry Research*, 21(2): 137−142. doi: 10.1007/s11676–010–0022–2.

Davidson EA, Matson PA, Vitousek PM, Riley R, Dunkin K, Garciamendez G, Maass JM. 1993. Processes regulating soil emissions of NO and N_2O in a seasonally dry tropical forest. *Ecology*, 74: 130–139.

Davies FJ, Potter JR, Linderman RG. 1992. Mycorrhiza and repeated drought exposure affects drought resistance and extra radical hyphae development of pepper plants. *Journal of Plant Physiology*, 139: 289–294.

Davies FT, Olalde-Portugal V, Aguilera-Gomez L, Alvarado MJ, Ferrera-Cerrato RC, Boutton TW. 2002. Alleviation of drought stress of Chile ancho pepper (*Capsicum annuum* L. CV. San Luis) with arbuscular mycorrhiza indigenous to Mexico. *Scientia Horticulturae*, 92: 347–359.

De Boer W, Kowalchuk GA. 2001. Nitrification in acid soils, microorganisms and mechanisms. *Soil Biology and Biochemistry*, 33: 853–866.

Declerck S, Plenchette C, Strullu DG. 1995. Mycorrhizal dependency of banana (*Musa acuminata*, AAA group) cultivar. *Plant and Soil*, 176: 183–187.

Declerck S, Risede JM, Rufyikiri G, Delvaux B. 2002. Effects of arbuscular mycorrhizal fungi on severity of root rot of bananas caused by *Cylindrocladium spathiphylli*. *Plant Pathology*, 51(1): 109–115.

Degens BP, Sparling GP, Abbott LK. 1996. Increasing the length of hyphae in a sandy soil increases the amount of water-stable aggregates. *Applied Soil Ecology*, 3: 149–159.

Dehn B, Schűepp H. 1989. Influence of VA mycorrhizae on the uptake and distribution of heavy metals in plants. *Agriculture*, *Ecosystems and Environment*, 29: 79–83.

Dehne HW. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology*, 72: 1115–1119.

Del Val. C, Barea JM, Azcon-Aguilar C. 1999. Assessing the tolerance of heavy metals of arbuscular mycorrhizal fungi isolated from sewage-sludge contaminated soils. *Applied Soil Ecology*, 11: 261–269.

DeMars BG, Boerner REJ. 1995. Mycorrhizal dynamics of three woodland herbs of contrasting phenology along topographic gradients. *American Journal of Botany*, 82: 1426–1431.

Dhar PP, Mridha MAU. 2006. Biodiversity of arbuscular mycorrhizal fungi in different trees of Madhupur forest, Bangladesh. *Journal of Forestry Research*, 17(3): 201−205. doi: 10.1007/s11676–006–0047–8.

Diaz G, Azcón-Aguilar C, Honrubia M. 1996. Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. *Plant and Soil*, 180: 241–249.

Dickson S, Smith FA, Smith SE. 2007. Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza*, 17: 375–393.

Dickson S, Smith SE. 2001. Cross walls in arbuscular trunk hyphae form after loss of metabolic activity. *New Phytologist*, 151: 735–742.

Dickson S. 2004. The *Arum-Paris* continuum of mycorrhizal symbioses. *New Phytologist*, 163: 187–200.

Dodd JC, Jeffries P. 1986. Early development of vesicular-arbuscular mycorrhizas in autumn-sown cereals. *Soil Biology and Biochemistry*, 18: 149–154.

Douds DD, Pfeffer PE, Shachar-Hill Y. 2000. Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. In: Kapulnik Y, Douds DD (eds) Arbuscular mycorrhizas: physiology and function. Kluwer, Dordrecht. pp. 107–129.

Douds Jr HDD, Millner PD. 1999. Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agriculture*, *Ecosystems and Environment*, 74: 77–93.

Douds Jr. DD, Schenck NC. 1990. Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrient and carbohydrate contents. *New Phytologist*, 116: 621–627.

Drigo B, Pijl AS, Duyts H, Kielak AM, Gamper HA, Houtekamer MJ, Boschker HTS, Bodelier PLE, Whiteley AS, Veen JAV, Kowalchuk GA. 2010. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. Proceedings of the National Academy of Sciences of the United States of America. 107: 10938–10942.

Duan T, Shen Y, Facelli E, Smith SE, Nan Z. 2010. New agricultural practices in the Loess Plateau of China do not reduce colonisation by arbuscular mycorrhizal or root invading fungi and do not carry a yield penalty. *Plant and Soil,* 331: 265–275. doi: 10.1007/s11104–009–0251–3.

Dubey A, Mishra MK, Singh PK, Vyas D. 2008. Occurrence of AM fungi at varying stages of growth of rice plants. Proceedings *National Academy of Sciences* India, 78(1): 51–55.

Duddridge JA. 1987. Specificity and recognition in ectomycorrhizal associations. In: Fungal Infection of Plants (ed). Pegg GF, Abres PG. Cambridge University Press, Cambridge. pp. 25–44.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. ISME (International Society for Microbial Ecology) Journal, 4: 337–345.

Dunne MJ, Fitter AH. 1989. The phosphorus budget of a field-grown strawberry (*Ffragaria* x *ananassa* cv. Hapil) crop: evidence for a mycorrhizal contribution. *Association of Applied Biologist*, 114: 185–193.

Dwivedi D, Johri BN, Ineichen K, Wray V, Wiemken A. 2009. Impact of antifungals producing rhizobacteria on the performance of *Vigna radiata* in the presence of arbuscular mycorrhizal fungi. *Mycorrhiza*, 19: 559–570.

Eason WR, Scullion J, Scott EP. 1999. Soil parameters and plant responses associated with arbuscular mycorrhizas from contrasting grassland management regimes. *Agriculture*, *Ecosystems and Environment*, 73: 245–255.

Edathil TT, Manian S, Udaiyan K. 1996. Interaction of multiple VAM fungal species on root colonization, plant growth and nutrient status of tomato seedlings (*Lycopersicon esculentum* Mill.). *Agriculture*, *Ecosystems and Environment*, 59: 63–68. doi: 10.1016/0167–8809(96)01040–7.

Elsen A, Baimey H, Swennen R, De Waele D. 2003. Relative mycorrhizal dependency and mycorrhiza-nematode interaction in banana cultivars (*Musa* spp.) differing in nematode susceptibility. *Plant and Soil*, 256: 303–313.

Entry JA, Rygiewicz PT, Watrud LS, Donnelly PK. 2002. Influence of adverse soil conditions on the formation and function of arbuscular mycorrhizas. *Advances in Environmental Research*, 7(1): 123–138.

Entz MH, Penner KR, Vessey JK, Zelmer CD, Thiessen Martens JR. 2004. Mycorrhizal colonization of flax under long-term organic and conventional management. *Canadian Journal of Plant Science*, 84: 1097–1099.

Eom AH, Hartnett DC, Wilson GWT. 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia*, 122: 435–444.

Escudero V, Mendoza R. 2005. Seasonal variation of arbuscular mycorrhizal fungi in temperature grassland along a wide hydrologic gradient. *Mycorrhiza*, 15(4): 291–299.

Faber BA, Zasoski RJ, Burau RG, Uriu K. 1990. Zinc uptake by corn as affected by vesicular-arbuscular mycorrhizae. *Plant and Soil*, 129: 121–130.

Facelli E, Smith SE, Smith FA. 2009. Mycorrhizal symbiosis- overview and new insights into roles of arbuscular mycorrhizas in agro- and natural ecosystems. *Australian Plant Pathology*, 38(4): 338–344.

Feddermann N, Boller T, Salzer P, Elfstrand S, Wiemken A, Elfstrand M. 2008. *Medicago truncatula* shows distinct patterns of mycorrhiza related gene expression after inoculation with three different arbuscular mycorrhizal fungi. *Planta,* 227: 671–680.

Feddermann N, Finlay R, Boller T, Elfstrand M. 2010. Functional diversity in arbuscular mycorrhiza - the role of gene expression, phosphorus nutrition and symbiotic efficiency. *Fungal Ecology*, 3: 1–8.

Feldmann F, Boyle C. 1998. Concurrent development of arbuscular mycorrhizal colonization and powdery mildew infection on three *Begonia hiemalis* cultivars. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 105(2): 121–129.

Fenchel T, Finlay BJ. 2005. The ubiquity of small species: patterns of local and global diversity. *Bio-Science*, 54: 777–784.

Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z. 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza*,12: 185–190.

Ferrer RL, Prikryl Z, Gryndler M, Vancura V. 1987. Natural occurrence of vesicular-arbuscular fungi in grape vine and apple trees. In: Inter-relationships between Micro-organisms and Plants in Soil (eds). Vancura V, Kunc F. Amsterdam, Netherlands. pp. 141–147.

Fester T, Strack D, Hause B. 2001. Reorganization of tobacco root plastids during arbuscule development. *Planta*, 213*:* 864–868.

Filion M, St-Arnaud M, Fortin JA. 1999. Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. *New Phytologist*, 141: 525–533.

Finlay BJ. 2002. Global dispersal of free-living microbial eukaryote species.*Science*, 296: 1061–1063.

Fitter AH. 1991. Costs and benefits of mycorrhizas: Implications for functioning under natural conditions. *Experientia*, 47: 350–355.

Fitter AH. 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist*, 172: 3–6.

Flores AC, Luna AAE, Portugal VO. 2007. Yield and quality enhancement of marigold flowers by inoculation with *Bacillus subtilis* and *Glomus fasciculatum*. *Journal of Sustainable Agriculture*, 31(1): 21–31. doi: 10.1300/J064v31–04.

Francis P. 1984. Plants as Human Adornment in India. *Economic Botany*, 38(2): 194–209. doi: 0.1007/BF02858832.

Francis R, Read DJ. 1995. Mutualism and antagonism in the mycorrhizal symbiosis with special reference to impacts on plant community structure. *Canadian Journal of Botany*, 73(1): 1301–1309.

Frank AB. 1885. "Über die auf Würzelsymbiose beruhende Ehrnährung gewisser Bäum durch unterirdische Pilze". *Berichte der Deutschen Botanischen Gesellschaft*, 3: 128–145.

Franzluebbers AJ, Wright SF, Stuedemann JA. 2000. Soil aggregation and glomalin under pastures in the southern Piedmont, USA. *Soil Science Society of America*, 64: 1018–1026.

Furlan V, Bernier-Cardou M. 1989. Effect of N, P and K on formation of endomycorrhizae, growth and mineral content of onion. *Plant and Soil*, 113: 167-174.

Furlan V, Fortin JA. 1973. Formation of vesicular-arbuscular endomycorrhizas by *Endogone calospora* on *Allium cepa* under three temperature regimes. *Naturaliste Canadian*, 100: 467–477.

Galleguillos C, Aguirre C, Barea JM, Azcon R. 2000. Growth promoting effect of two *Sinorhizobium meliloti* strains (a wild type and its genetically modified derivative) on a non-legume plant species in specific interaction with two arbuscular mycorrhizal fungi. *Plant Science*, 159: 57–63.

Galván GA, Parádi I, Burger K, Baar J, Kuyper TW, Scholten OE, Kik C. 2009. Molecular diversity of arbuscular mycorrhizal fungi in onion roots from organic and conventional farming systems in the Netherlands. *Mycorrhiza*, 19(5): 317–328. doi: 10. 1007/s00572-009-0237–2.

Galvez L, Douds Jr. DD, Drinkwater LE, Wagoner P. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant and Soil*, 228: 299–308.

Gamage HK, Singhakumara BMP, Ashton M. 2004. Effects of light and fertilization on arbuscular mycorrhizal colonization and growth of tropical rain-forest *Syzygium* tree seedlings. *Journal of Tropical Ecology*, 20: 525–534.

Gange AC, Bower E, Brown VK. 2002. Differential effects of insects herbivory on arbuscular mycorrhizal colonization. *Oecologia*, 131: 103–112.

Gange AC, Smith AK. 2005. Arbuscular mycorrhizal fungi influence visitation rates of pollinating insects. *Ecological Entomology*, 30: 600–606.

Gange AC, West HM. 1994. Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytologist*, 128: 79–87.

Garbaye J, Churin JL. 1996. Effect of ectomycorrhizal inoculation at planting on growth and foliage quality of *Tilia tomentosa*. *Journal of Arboriculture*, 22: 29–34.

Gardes M, Dahlberg A. 1996. Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytologist*, 133: 147–157.

Gaur A, Adholeya A. 1994. Estimation of VAM spores in soil - A modified method. *Mycorrhiza News*, 6: 10–11.

Gaur A, Adholeya A. 2002. Arbuscular–mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biology and Fertility of Soils*, 35: 214–218.

Gaur A, Adholeya A. 2005. Diverse response of five ornamental plant species to mixed indigenous and single isolate arbuscular-mycorrhizal inocula in marginal soil amended with organic matter. *Journal of Plant Nutrition*, 28: 707–723.

Gaur A, Gaur A, Adholeya A. 2000. Growth and flowering in *Petunia hybrida*, *Callistephus chinensis* and *Impatiens balsamina* inoculated with mixed AM inocula or chemical fertilizers in a soil of low P fertility. *Scientia Horticulturae*, 84: 151–162.

Gemma JK, Koske RE. 1988. Seasonal variation in spore abundance and dormancy of *Gigaspora gigantea* and in mycorrhizal inoculum potential of a dune soil. *Mycologia*, 80: 211–216.

Gemma JN, Koske RE, Carreiro M (1989). Seasonal dynamics of selected species of VA mycorrhizal fungi in a sand dune. *Mycological Research*, 92: 317–321.

Genre A, Chabaud M, Faccio A, Barker DG, Bonfante P. 2008. Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both Medicago truncatula and Daucus carota. *Plant Cell*, 20: 1407–1420.

Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell*, 17: 3489–3499.

George E. 2000. Nutrient uptake - Contributions of arbuscular mycorrhizal fungi to plant mineral nutrition. In: Arbuscular Mycorrhizas: Physiology and Function (eds). Kapulnik Y, Douds Jr. DD. Kluwer Academic Publishers, Dordrecht. pp. 307–343.

Gerdemann JW, Bakshi BK. 1976. Endogonaceae of India: two new species. *Transactions of the British Mycological Society*, 66: 340–343.

Gerdemann JW, Nicolson TH. 1963. Spore density of *Endogone* species extracted from soil wet sieving and decanting. *Transactions of the British Mycological Society*, 46: 235–244.

Gerdemann JW, Trappe JM. 1974. The Endogonaceae in the Pacific Northwest. *Mycologia Memoir*, 5: 1–76.

Gerdemann JW. 1968. Vesicular-arbuscular mycorrhiza and plant growth. *Annual Review of Phytopathology*, 6: 397–418.

Gerdemann JW. 1975. Vesicular-arbuscular mycorrhizae. In: The development and function of root (eds). Torrey JG, Clarkson DT. Academic Press, New York. pp. 575–591.

Gernns H, Alten H, Poehling HM. 2001. Arbuscular mycorrhiza increased the activity of a biotrophic leaf pathogen-is a compensation possible? *Mycorrhiza*, 11: 237–243.

Giananazzi S, Schűepp H, Barea JM, Haselwandter K. 2001. Mycorrhizal technology in agriculture: from genes to bioproducts. Birkhauser, Basel, Switzerland.

Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D. 2010. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza*, 20: 519–530.

Gianinazzi S, Trouvelot A, Lovato P, van Tuinen D, Franken P, Gianinazzi-Pearson V. 1995. Arbuscular mycorrhizal fungi in plant production of temperate agroecosystems. *Critical Reviews in Biotechnology*, 15: 305–311.

Gianinazzi-Pearson V, Smith SE, Gianinazzi S, Smith FA. 1991. Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas VIs H+-ATPase a component of ATP-hydrolysing enzyme activities in plant-fungus interfaces? *New Phytologist*, 117: 61–76.

Gianinazzi-Pearson V. 1996. Plant cell responses to arbuscular mycorrhizal fungi: Getting to the roots of the symbiosis. *Plant Cell*, 8: 1871–1883.

Gildon A, Tinker PB. 1981. A heavy metal-tolerant strain of mycorrhizal fungus. *Transactions of the British Mycological Society*, 77: 648–649.

Gilmore AE. 1968. Phycomycetous mycorrhizal organisms collected by open pot cultures. *Hilgardia*, 39: 87–107.

Giovanetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 84: 489–500.

Giovanetti M. 1985. Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. *Transactions of the British Mycological Society*, 84: 679-684.

Giovannetti M, Avio L, Sbrana C. 2010. Fungal spore germination and pre-symbiotic mycelial growth- Physiological and genetic aspects. In: Arbuscular Mycorrhizas: Physiology and Function (eds). Koltai H, Kapulnik Y. Springer Dordrecht Heidelberg London, New York. pp. 3–32. doi: 10.1007/978–90–481–9489–6–1.

Giovannetti M, Gianinazzi-Pearson V. 1994. Biodiversity in arbuscular mycorrhizal fungi. *Mycological Research*, 98: 705–715.

Giovannetti M, Sbrana C, Avio L, Citernesi AS, Logi C. 1993. Differential hyphal morphogenesis in arbuscular-mycorrhizal fungi during pre-infection stages. *New Phytologist*, 125: 587–593.

Giovannetti M, Sbrana C, Logi C. 2000. Microchambers and video-enhanced light microscopy for monitoring cellular events in living hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, 226: 153–159.

Giovannetti M, Sbrana C. 1998. Meeting a non-host: the behavior of AM fungi. *Mycorrhiza*, 8: 123–130.

González-Chávez MC, Carrillo-Gonzalez R, Wright SF, Nichols K. 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Environmental Pollution*, 130(3): 317–323. doi: 10.1016/j.envpol.2004.01.004.

González-Guerrero M, Azcón-Aguilar C, Mooney M, Valderas A, Mac Diarmid CW, Eide DJ, Ferrol N. 2005. Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. *Fungal Genetics and Biology*, 42: 130–140.

Gosling P, Hodge A, Goodlass G, Bending GD. 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture*, *Ecosystems and Environment*, 113: 17–35.

Gosling P, Shepherd M. 2005. Long term changes in soil fertility in organic farming systems in England, with particular reference to phosphorus and potassium. *Agriculture*, *Ecosystems and Environment*, 105: 425–432.

Graham JH, Abbot LK. 2000. Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. *Plant and Soil*, 220: 207–218.

Graham JH, Eissenstat DM, Drouillard DL. 1991. On the relationship between a plant's mycorrhizal dependency and rate of vesicular-arbuscular mycorrhizal colonization. *Functional Ecology*, 5: 773–779.

Graham JH, Linderman RG, Menge JA. 1982. Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of *Troyer citrange*. *New Phytologist*, 91: 183–189.

Grime JP, Mackey JML, Hillier SH, Read DJ. 1987. Floristic diversity in a model system using experimental microcosms. *Nature*, 328: 420–422.

Guadarrama P, Álvarez-Sánchez FJ. 1999. Abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest, Veracruz, Mexico. *Mycorrhiza*, 8: 267–270.

Guillemen JP, Gianinazzi S, Gianinazzi-Pearson V, Marchall J. 1995. Effect of arbuscular endomycorrhizae on growth and mineral nutrition of pineapple vitro-plants in a soil with salinity. *Fruits-Paris*, 50: 333–341.

Gutjahr C, Casieri L, Paszkowski U. 2009. *Glomus intraradices* induces changes in root system architecture of rice independently of common symbiosis signaling. *New Phytologist*, 182: 829–837.

Hall IR. 1977. Species and mycorrhizal infections of New Zealand Endogonaceae. *Transactions of the British mycological Society*, 68: 341–356.

Hall JL, Williams LE. 2003. Transition metal detoxification and tolerance. *Journal of Experimental Botany*, 53: 1–11.

Hamel C, Dalpe Y, Furlan V, Parent S, 1997. Indigenous populations of arbuscular mycorrhizal fungi and soil aggregate stability are major determinants of leek (*Allium porrum* L.) response to inoculation with *Glomus intraradices* Schenck & Smith or *Glomus versiforme* (Karsten) Berch. *Mycorrhiza*, 7: 187–196.

Hanway JJ, Heidel H. 1952. Soil analysis method as used in Iowa State College soil testing laboratory. Iowa State College of Agriculture. 57: 1–31.

Harley JL, Smith SE. 1983. Mycorrhizal Symbiosis. Academic Press, London.

Harrier LA, Watson CA. 2004. The potential role of Arbuscular Mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. *Pest Management Science*, 60: 149–157.

Harrison MJ, Dewbre GR, Liu J. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell*, 14: 2413–2429.

Harrison MJ, Pumplin N, Breuillin JF, Noar RD, Park HJ. 2010. Phosphate Transporters in Arbuscular Mycorrhizal Symbiosis. In: Arbuscular Mycorrhizas: Physiology and Function (eds). Koltai H, Kapulnik Y. Springer Dordrecht Heidelberg London, New York. pp.117–135. doi: 10.1007/978–90-481–9489–6–6.

Harrison MJ, Van Buuren ML.1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme. Nature*, 378: 626–629.

Harrison MJ. 2005. Signaling in the Arbuscular Mycorrhizal Symbiosis. *Annual Review of Microbiology*, 59: 19–42.

Harrison MJ.1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50: 361–389.

Hart MM, Reader RJ. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist*, 153: 335–344.

Hartnett DC, Wilson GWT. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. *Plant and Soil*, 244: 319–331.

Hause B, Fester T. 2005. Molecular and cell biology of arbuscular mycorrhizal symbiosis. *Planta*, 221: 184–196. doi: 10.1007/s00425–004–1436–x.

Hause B, Fester T. 2005. Molecular and cell biology of arbuscular mycorrhizal symbiosis. *Planta*, 221: 184–196. doi: 10.1007/s00425–004–1436–x.

Hawkes VH, Belnap J, D'Antonio C, Firestone MK. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. *Plant and Soil*, 281: 369–380.

Hayman DS. 1970. Endogone spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Transactions of the British Mycological Society*, 54: 53–63.

Hayman DS. 1975. The occurrence of mycorrhiza in crops as affected by soil fertility. In: Endomycorrhizas (eds). Sanders FE, Mosse B, Tinker PB. Academic Press, London. pp. 495–509.

Hayman, DS. 1982. Influence of soils and fertility on activity and survival of VAM fungi. *Phytopathology*, 72: 1119–1125.

He X, Mouratov S, Steinberger Y. 2002. Temporal and spatial dynamics of vesicular-arbuscular mycorrhizal fungi under the canopy of *Zygophyllum dumosum* Boiss. in the Negev Desert. *Journal of Arid Environment*, 52: 379–387.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. 1998. Ploughing up the wood-wide web. *Nature*, 394: 431–431.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *Journal of Ecology*, 90: 371–384.

Hepper CM, Azon-Ahuilar C, Rosenclahl S, Sen R. 1988. Competition between three species of *Glomus* used as spatially introduced and indigenous mycorrhizal inocula for leek *(Allium porrum* L.). *New Phytologist*, 110: 207–215.

Hepper CM, Smith GA. 1976. Observation on the germination of *Endogone* spores. *Transactions of the British Mycological Society*, 66: 189–194.

Hepper CM. 1984. Isolation and culture of VA mycorrhizal (VAM) fungi. In: VA Mycorrhizae (eds). Powell CL, Bagyaraj DJ. CRC Press, Florida, USA. pp. 95–112.

Hetrick BAD, Bloom J. 1983. Vesicular-arbuscular mycorrhizal fungi and associated with native tallgrass prairie and cultivated winter wheat. *Canadian Journal of Botany*, 61: 2140–2146.

Hetrick BAD, Wilson GWT, Todd TC. 1996. Mycorrhizal response in wheat cultivars: Relationship to phosphorus. *Canadian Journal of Botany*, 74: 19–25.

Hijri I, Sýkorová Z, Oehl F, Ineichen K, Mäder P, Wiemken A, Redecker D. 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Molecular Ecology*, 15:2277–2289. doi:10.1111/j.1365–294X.2006.02921.x.

Hildebrandt U, Kaldorf M, Bothe H. 1999. The zinc violet and its colonisation by arbuscular mycorrhizal fungi. *Journal of Plant Physiology*, 154: 709–717.

Hildebrandt U, Regvar M, Bothe H. 2007. Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry*, 68: 139–146.

Hindumathi A, Reddy BN. 2011. Occurrence and distribution of arbuscular mycorrhizal fungi and microbial flora in the rhizosphere soils of mungbean [*Vigna radiata* (L.) wilczek] and soybean [G*lycine max* (L.) Merr.] from Adilabad, Nizamabad and Karimnagar districts of Andhra Pradesh state, India. *Advances in Bioscience and Biotechnology*, 2: 275–286.

Hoagland DR, Arnon DI. 1939. The water culture methos for growing plants without soil. *California Agricultural Experimental Station Circular*, 347: 35.

Hodge A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, 413: 297–299.

Holford ICR. 1997. Soil phosphorus: its measurement, and its uptake by plants. *Australian Journal of Soil Research*, 35: 227–239.

Horner-Devine MC, Lage M, Hughes JB, Bohannan BJM. 2004. A taxa area relationship for bacteria. *Nature*, 432: 750–753.

Horner-Devine MC, Silver JM, Leibold MA, Bohanna BJM, Colwell RK, Fuhrman JA, Green JL, Kuske CR, Martiny JBH, Muyzer G, Overeas L, Reysenbach A, Smith VH. 2007. A comparison of taxon co-occurrence patterns for macro- and micro-organisms. *Ecology*, 88(6): 1345–1353.

Husband R, Herre EA, Young JP. 2002. Temporal variation in the AM communities colonising seedlings in a tropic forest. *FEMS Microbiology Ecology*. 42: 131–136.

Hussain AI, Anwar F, Sherazi STH, Przybylski R. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, 108: 986–995.

Isobe K, Tsuboki Y. 1998. The relationship between growth promotion by arbuscular mycorrhizal fungi and root morphology and phosphorus absorption in gramineous and leguminous crops. *Crop Science*, 67: 347–352.

Jackson MI. 1973. Soil Chemical Analysis, Prentice Hall of Inc. Engle Wood Cliffs, New Jersey, U.S.A. pp. 183–203.

Jacquelinet-Jeanmougin S, Gianinazzi-Pearson V, Gianinazzi S. 1987. Endomycorrhizas in the Gentianaceae. II. Ultrastructural aspects of symbiont relationships in *Gentiana lutea* L. *Symbiosis*, 3: 269–286.

Jacquot-Plumey E, van Tuinen D, Chatagnier O, Gianinazzi S, Gianinazzi-Pearson V. 2001. 25S rDNA-based molecular monitoring of glomalean fungi in sewage sludge-treated field plots. *Environmental Microbiology*, 3: 525–531.

Jaiti F, Meddich A, Hadrami I. 2007.Effectiveness of arbuscular mycorrhizal fungi in the protection of date palm (*Phoenix dactylifera* L.) against bayoud disease. *Physiological and Molecular Plant Pathology*, 71: 166–173.

Jakobsen I, Abbott LK, Robson AD. 1992. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. I: spread of hyphae and phosphorus in flow into roots. *New Phytologist*, 120: 371– 380.

Jakobsen I, Gazey C, Abbott LK. 2001. Phosphate transport by communities of arbuscular mycorrhizal fungi in intact soil cores. *New Phytologist*, 149: 95–103.

Jakobsen I, Rosendahl L. 1990. Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist*, 115: 77–83.

Jalali BL, Chand H. 1988. Role of VAM in Biological Control of Plant Diseases. In: Mycorrhizae for Green Asia (eds). Mohadevan A, Raman N, Natarajan K. Madras Express Service, India. pp: 209–215.

Janos DP, Trappe JM. 1982. Two new *Acaulospora* species from Tropical America. *Mycotaxon*, 15: 515–522.

Janos DP. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology*, 61: 151–162.

Jansa J, Mozafar A, Frossard E. 2005. Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site. *Plant and Soil*, 276: 163–176.

Jansa J, Mozafar A, Khun G, Anken T, Ruh R, Sanders IR, Frossard E. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Application*, 13: 1164–1176.

Jansa J, Smith FA, Sally ES. 2008. Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytologist*, 177: 779–789.

Jastrow JD, Miller RM, Lussenhop J. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. *Soil Biology and Biochemistry*, 30: 905–916.

Javot H, Pumplin N, Harrison MJ. 2007. Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. *Plant*, *Cell and Environment*, 30: 310–322.

Jensen A, Jacobsen P. 1980. The occurrence of vesicular-arbuscular mycorrhiza in barley and wheat grown in some Danish soil with different fertiliser treatments. *Plant and Soil*, 55: 403–414.

Johansson J, Paul L, Finlay RD. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiology Ecology*, 48:1–12.

Johnson D, Leake JR, Ostle N, Ineson P, Read DJ. 2002. In situ $13CO_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist*, 153: 327–334.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, 135(4): 575–585.

Johnson NC, Pfleger FL, Crookston RK, Simmons SR, Copeland BJ. 1991. Vesicular-arbuscular mycorrhizas respond to corn and soybean cropping history. *New Phytologist*, 117: 657–663.

Johnson NC, Rowland DL, Corkidi L, Eggerton-Warburton LM, Allen EB. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology*, 84: 1895–1905.

Johnson NC. 1993. Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications*, 3: 749–757.

Joner EJ, Briones R, Leyval C. 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and Soil*, 226: 227–234.

Jumpponen A, Trowbridge J, Mandyam K, Johnson L. 2005. Nitrogen enrichment causes minimal changes in arbuscular mycorrhizal colonization but shifts community composition-evidence from rDNA data. *Biology and Fertility of Soils*. 41:217–224.

Kabir Z, O'Halloran IP, Fyles JW, Hamel C. 1998. Dynamics of the mycorrhizal symbiosis of corn: effects of host physiology, tillage practice and fertilization on spatial distribution of extraradical mycorrhizal hyphae in the field. *Agriculture*, *Ecosystems* and *Environment*, 68: 151–163.

Kahiluoto H, Ketoja E, Vestberg M, Saarela I. 2001. Promotion of AM utilization through reduced P fertilization. 2. Field studies. *Plant and Soil*, 231: 65–79.

Kalita RK, Bora DP, Dutta D. 2002. Vesicular arbuscular mycorrhizal associations with some native plants. *Indian Journal of Forestry*, 25: 143–146.

Kandasamy DG, Oblisami S, Santhanakrishnan P. 1986. Influence of VA-mycorrhizal inoculation on the growth of *Pyrethrum* in the nursery. *Pyrethrum Post*, 16: 81–83.

Karaarslan E, Uyanöz R. 2011. Occurrence of arbuscular mycorrhizal fungi in some native plants grown on saline soils around the lake Tuz in Turkey and

its relations with some physical and chemical properties of soil. *Scientific Research and Essays*, 6(20): 4238–4245. doi: 10.5897/SRE11.331.

Karagiannidis N, Blestos F, Stavropoulos N. 2002. Effect of Verticillium wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. *Scientia Horticulturae*, 94: 145–156.

Karagiannidis N, Hadjisavva-Zinoviadi S. 1998. The mycorrhizal fungus *Glomus mosseae* enhances growth, yield and chemical composition of a durum wheat variety in 10 different soils. *Nutrient Cycling in Agroecosystems*, 52: 1–7.

Karandashov V, Nagy R, Wegmüller S, Amrhein N, Bucher M. 2004. Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences*, USA, 101: 6285–6290.

Karishma, Aggarwal A, Tanwar A, Neetu. 2011. Efficacy of bioinoculants, plant growth regulators and nutrients in prolonging vase life of *Chrysanthemum indicum* L. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 11(4): 593–599.

Kaushal S. 2000. Influence of edaphic factors on VAMF spore population and root colonization in *Acacia nilotica* in Rajasthan. *Journal of Mycology and Plant Pathology*, 30: 386–388.

Khaliel AS, Abou-Heilah AN. 1985. Formation of vesicular-arbuscular mycorrhizae in *Phoenix dactylifera* L., cultivated in Qassim region, Saudi Arabia. *Pakistan Journal of Botany*, 17: 267–270.

Khalil S, Loynachan TE, McNabb HS. 1992. Colonization of soybean by mycorrhizal fungi and spore populations in Iowa soils. *Agronomy Journal*, 84: 832–836.

Khan AG, Kuck C, Chandhry TM. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, 41(1): 197–207.

Khan AG. 1993. Occurrence and importance of mycorrhizae in aquatic trees of New South Wales, Australia. *Mycorrhiza*, 3: 31–38.

Khare V, Narayan RP, Kehri HK. 2011. Population dynamics of arbuscular mycorrhizal fungi in saline-alkaline soils of Gangetic planes of India. *Scholarly Journal of Agricultural Science*, 1(3): 41–46.

Kim TT, Kim CK. 1992. Vesicular- arbuscular mycorrhizal fungi found in the soils around the roots of the leguminous plants. *Korea Journal of Mycology*, 20: 171–182.

Kirk PM, Cannon PF, David JC, Stalpers J. 2001. Ainsworth and Bisby's Dictionary of the Fungi (9th ed.), CAB International, Wallingford, UK.

Kivlin SN, Hawkes CV, Treseder KK. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 43: 2294–2303.

Klironomos JN. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, 84: 2292–2301.

Koch AM, Croll D, Sanders IR. 2006. Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. *Ecology Letters*, 9: 103–110.

Koide R, Huenneke L, Hamburg S, Mooney H. 1988. Effects of applications of fungicide, phosphorus and nitrogen on the structure and productivity of an annual serpentine plant community. *Functional Ecology*, 2: 335–344.

Koide RT. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist*, 117: 365–386.

Koide RT. 2010. Mycorrhizal Symbiosis and Plant Reproduction. In: Arbuscular Mycorrhizas: Physiology and Function (eds). Koltai H, Kapulnik Y. Springer Dordrecht Heidelberg, London, New York. pp. 297-320. doi: 10.1007/978–90–481–9489–6–14.

Koske RE, Gemma JN, Olexia PD. 1986. *Glomus microaggregatum*, a new species in the Endogonaceae. *Mycotaxon*, 26: 125–132.

Koske RE, Tessier B. 1983. A convenient permanent slide mounting medium. *Mycological Society of America Newsletter*, 34: 59.

Koske RE. 1975. Endogone spores in Australian sand dunes. *Canadian Journal of Botany*, 53: 668–672.

Koske RE. 1982. Evidence for a volatile attractant from plant roots affecting germ tubes of a VA mycorrhizal fungus. *Transactions of the British Mycological Society*, 79: 305–310.

Koske RE. 1985. *Glomus aggregatum* emended: A distinct taxon in the *Glomus fasciculatum* complex. *Mycologia*, 77: 619.

Koske RE. 1987. Distribution of VA mycorrhizal fungi along latitudinal temperature gradient. *Mycologia*, 79: 55–68.

Kosuta S, Chabaud M, Lougnon G, Gough C, Denarie J, Barker DG, Bécard G. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *Mt*ENOD11 expression in roots of *Medicago truncatula*. *Plant Physiologist*, 131: 952–962.

Kothari SK, Marschner H, Romheld V. 1991. Effect of a vesicular-arbuscular mycorrhizal fungus and rhizospheric microorganism on manganese reduction in the rhizosphere and manganese concentration in maize (*Zea mays* L.) *New Phytologist*, 117 : 649–655.

Kothari SKF Marschner H, George E. 1990. Effect of VA-mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. *New Phytologist*, 116: 303–311.

Kruckelmann HE. 1975. Effects of fertilizers, soils, soil tillage and plant species on the frequency of *Endogone* chlamydospores and mycorrhizal infection in arable soils. In: Endomycorrhiza (eds). Sanders FE, Mosse B, Tinker PB. Academic Press, New York. pp. 511–525.

Kurle JE, Píleger FL. 1994. The effects of cultural practices and pesticides on VAM fungi. In: *Mycorrhizae and Plant Health* (eds). Pfleger FL, Lindennan RG. APS Press, St. Paul. pp. 101–131.

Landis FC, Gargas A, Givnish TJ. 2004. Relationship among arbuscular mcorrhizal fungi, vascular plants and environmental conditions in oak savannas. *New Phytologist*, 164: 493–504.

Landwehr M, Hilderbrandt U, Wilde P, Nawarth K, Tóth T, Biró B. 2002. The arbuscular mycorrhizal fungus *Glomus geosporum* in Europaen saline, sodic and gypsum soils. *Mycorrhiza*, 12: 199–211.

Leal PL, Stürmer SL, Siqueira JO. 2009. Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from soils under different land use systems in the Amazon, Brazil. *Brazilian Journal of Microbiology*, 40: 111–121.

Lee YJ, George E. 2005. Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply. *Plant and Soil*, 278: 361–370.

Leith H. 1974. Phenology and seasonality modeling. Springer, Berlin.

Lekberg Y, Koide RT, Rohr JR, Aldrich-Wolfe L, Morton JB. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *Journal of Ecology*, 95: 95–105.

Lekberg Y, Koide RT. 2005. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytologist*, 168: 189–204.

Lerat S, Lapointe L, Gutjahr S, Piché Y, Vierheilig H. 2003. Carbon partitioning in a split-root system of arbuscular mycorrhizal plants is fungal and plant species dependent. *New Phytologist*, 157: 589–595.

Leyval C, Turnau K, Haselwandter K. 1997. Interactions between heavy metals and mycorrhizal fungi in polluted soils: physiological, ecological and applied aspects. *Mycorrhiza*, 7: 139–153.

Li XL, George E, Marschner H. 1991. Phosphorus depletion and pH decrease at the root soil and hyphae soil interfaces of VA mycorrhizal white clover fertilized with ammonium. *New Phytologist*, 119: 379–404.

Linderman RG, Davis EA. 2004. Varied response of marigold (*Tagetes* spp.) genotypes to inoculation with different arbuscular mycorrhizal fungi. *Scientia Horticulturae*, 99: 67–78. doi: 10.1016/S0304–4238(03)00081–5.

Linderman RG. 1994. Role of VAM Fungi in Biocontrol. In: Mycorrhizae and Plant Health, Pfleger, FL, R.G. Linderman (Eds.). The American Phytopathological Society, St. Paul, MN., USA. pp. 1–27.

Lingfei L, Anna Y, Zhiwei Z. 2005. Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. *FEMS Microbiology Ecology*, 54: 367–373.

Lingua G, D'Agostino G, Antosiano NMM, Berta G. 2002. Mycorrhiza induced differential response to a yellows disease in tomato. *Mycorrhiza*, 12: 191–198.

Liu A, Hamel C, Hamilton RI, Ma BL Smith DL. 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza*, 9: 331–336.

Lodge DJ, McDowell WH, Swiney CP. 1997. The importance of nutrient pulses in tropical forests. *Trends in Ecology and Evolution*, 9: 384–387.

Loes AK, Ogaard AF. 2001. Long term changes in extractable soil phosphorus (P) in organic dairy farming systems. *Plant and Soil*, 237(2): 321–332.

Long LK, Huang YH, Yang RH, Guo J, Zhu H. 2010. Effects of arbuscular mycorrhizal fungi on zinnia and the different colonization between *Gigaspora* and *Glomus*. *World Journal of Microbiology and Biotechnology*, 26(8): 1527–1531. doi: 10.1007/s11274–010–0313–y.

Lopez-Aguillon R, Mosse B. 1987. Experiments on competitiveness of three endomycorrhizal fungi. *Plant and Soil*, 97: 155–170.

López-Ráez JA, Charnikhova T, Gómez-Roldán V, Matusova R, Kohlen W, De Vos R. 2008. Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. *New Phytologist*, 178(4): 863–874.

Lotter DW. 2003. Organic agriculture. *Journal of Sustainable Agriculture*, 21: 59–128.

Lovato PE, Schuepp H, Trouvelot A, Gianinazzi S. 1995. Application of arbuscular mycorrhiza fungi (AMF) in orchard and ornamental plants. In: Mycorrhiza structure, function, molecular biology and biotechnology (eds). Varma A, Hock B. Springer Verlag, Heidelberg. pp. 521–559.

Lugo M, González Maza ME, Cabello MN. 2003. Arbuscular mycorrhizal fungi in a mountain grassland II: seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia*, 95: 407–415.

Luoma DL, Eberhart JL, Molina R, Amaranthus MP. 2004. Response of ectomycorrhizal fungus sporocarp production to varying levels and patterns of green-tree retention. *Forest Ecology and Management*, 202: 337–354.

Mader P, Edenhofer S, Boller T, Wiemken A, Niggli U. 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biology and Fertility of Soils*, 31: 150–156.

Malcova R, Vosátka M, Gryndler M. 2003. Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L., and *Agrostis capillaris* L. *Applied Soil Ecology*, 23: 55–67.

Malloch DW, Pirozynsky KA, Raven PH. 1980. Ecological and evolutionary significance of mycorrhizal symbiosis in vascular plants. *Proceedings of the National Academy of Sciences USA*, 77: 2113–2118.

Mangan SA, Adler GH. 2000. Consumption of arbuscular mycorrhizal fungi by terrestrial and arboreal small mammals in a Panamanian cloud forest. *Journal of Mammalogy*, 81: 563–570.

Marschner H. 1995. The Mineral Nutrition of Higher Plants. Academic Press, London. pp. 674.

Marschner H. 2002. Mineral nutrition of higher plants. Academic Press, London. pp. 889.

Marschner P, Baumann K. 2003. Changes in bacterial community structure, induces by mycorrhizal colonization in split-root maize. *Plant and Soil*, 251: 279–289.

Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem, S, Ovreas L, Reysenbach, AL, Smith VH, Staley JT. 2006. Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology*, 4: 102–112.

Mason DT. 1964. A survey of numbers of *Endogone* spores in soil cropped with barley, raspberry and strawberry. *Horticultural Research*, 4: 98–103.

Matysiak B, Falkowski G. 2010. response of three ornamental plant species to inoculation with arbuscular mycorrhizal fungi depending on compost addition to peat substrate and the rate of controlled release fertilizer. *Journal of Fruit and Ornamental Plant Research*, 18(2): 321–333.

Mc Gonigle TP, Fitter AH. 1988. Growth and phosphorus inflows of *Trifolium repens* L. with a range of indigenous vesicular-arbuscular mycorrhizal infection levels under field conditions. *New Phytologist*, 108: 59–65.

Mehrotra VS. 1997. Problems associated with morphological taxonomy of AM fungi. *Mycorrhiza News*, 9: 1–10.

Menge JA, Johnson ELV, Platt RG. 1978. Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. *New Phytologist*, 81: 553–559.

Menge JA. 1984. Inoculum production. In: VA mycorrhiza (eds). Powell CL, Bagyaraj DJ. CRC Press, Boca Raton, FL. pp. 187–203.

Miller RL, Jackson LE. 1998. Survey of vesicular-arbuscular mycorrhizae in lettuce production in relation to management and soil factors. *Journal of Agricultural Science Cambridge*, 130: 173–182.

Miller RM. 1979. Some occurrences of vesicular-arbuscular mycorrhiza in natural and disturbed ecosystems of the Red Desert. *Canadian Journal of Botany*, 57: 619–623.

Miller RM. 1987. Mycorrhizae and succession. In: Restoration Ecology: A Synthetic Approach to Ecological Research (eds). Jordan WR, Gilpin ME, Aber JD. Cambridge University Press, Cambridge, UK.

Miransari M, Smith D. 2009. Alleviating salt stress on soybean (*Glycine max* (L.) Merr.) - *Bradyrhizobium japonicum* symbiosis, using signal molecule genistein. *European Journal of Soil Biology*, 45: 146–152.

Miransari M, Smith DL. 2007. Overcoming the stressful effects of salinity and acidity on soybean [Glycine max (L.) Merr.] nodulation and yields using signal molecule genistein under field conditions. *Journal of Plant Nutrition*, 30: 1967–1992.

Miransari M, Smith DL. 2008. Using signal molecule genistein to alleviate the stress of suboptimal root zone temperature on soybean-*Bradyrhizobium* symbiosis under different soil textures. *Journal of Plant Interactions*, 3: 287–295.

Mohammad M, Pan WL, Kennedy AC. 1998. Seasonal mycorrhizal colonization of winter wheat and its effect on wheat growth under dry land field conditions. *Mycorrhiza*, 8: 139–144.

Mohammad MJ, Malkawi HI, Shibli R. 2003. Effects of mycorrhizal fungi and phosphorus fertilzation on growth and nutrient uptake of barley grown on soils with different levels of salts. *Journal of Plant Nutrition*, 26: 125–137.

Mohan V, Bappammal M, Malathy N, Manokaran P (2005). Distribution of arbuscular mycorrhizal (AM) fungi in association with some important medicinal plants of Tamil Nadu. *Indian Forestry*, 131(6): 797–804.

Moreira M, Baretta D, Tsai SM, Cardoso EJBN. 2006. Spore density and root colonization by arbuscular mycorrhizal fungi in preserved or disturbed *Araucaria angustifolia* (Bert.) O. Ktze. ecosystems. *Scientia Agricola*, 63: 380–385.

Moreira M, Nogueira MA, Tsai SM, Gomes-da-cost SM, Cardoso EJBN. 2007. Sporulation and diversity of arbuscular mycorrhizal fungi in Brazil Pine in the field and in greenhouse. *Mycorrhiza*, 17: 519–526.

Morton JB, Bentivenga SP, Bever JD. 1995. Discovery, measurement, and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales. Zygomycetes). *Canadian Journal of Botany*, 73: 25–32.

Morton JB, Bentivenga SP, Wheeler WW. 1993. Germplasm in the international collection of vesicular-arbuscular mycorrhizal fungi (INVAM) and procedures for culture development, documentation and storage. *Mycotaxon*, 48: 491–528.

Morton JB, Redecker D. 2001. Concordant morphological and molecular characters reclassify five arbuscular mycorrhizal fungal species into new genera *Archaeospora* and *Paraglomus* of new families Archaeosporaceae and Paraglomaceae respectively. *Mycologia*, 93(1): 181–195.

Morton JB, Redecker D. 2001. Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. *Mycologia*, 93(1): 181–195.

Morton JB. 1988. Taxonomy of VA mycorrhizal fungi: Classification, nomenclature and identification. *Mycotaxon*, 32: 267–324.

Morton JB. 1995. Taxonomy and phylogenetic divergence among five Scutellospora spp. based on comparative developmental sequences. *Mycologia*, 87: 122–137.

Morton JB. 2000. Evolution of endophytism in arbuscular mycorrhizal fungi in Glomales. In: Microbial Endophytes (eds). Bacon CW, White JH. Dekker M. New York. pp. 121–140.

Morton JB. 2002. International Culture Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi. West Virginia University. http://invam.caf.wvu.edu.

Morton JB. Benny GL. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): A new order Glomales, two new suborders Glomineae and Gigasporineae and two new families Acaulosporaceae and Gigasporaceae with an emendation of Glomaceae. *Mycotaxon*, 37: 471–491.

Mosse B. 1959. The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular mycorrhiza*. Transactions of the British Mycological Society*, 42: 273–286.

Mosse B. 1973. Advances in the study of VA-mycorrhiza. *Annual Review of Phytopathology*, 11: 1711–194.

Mullen RB, Schmidt SK. 1993. Mycorrhizal infection, phosphorus uptake, and phenology in *Ranunculus adoneus*: implications for the functioning of mycorrhizae in alpine systems. *Oecologia*, 94: 229–234.

Munkvold L, Kjøller R, Vestberg M, Rosendahl S, Jakobsen I. 2004. High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist*, 164: 357–364.

Muthukumar T, Senthilkumar M, Rajangam M, Udaiyan K. 2006. Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. *Mycorrhiza*, 17: 11–24. doi: 10.1007/s00572–006-0077–2.

Muthukumar T, Udaiyan K. 1999. Spore-in-spore syndrome in vesicular-arbuscular mycorrhizal fungi and its seasonality in a tropical grassland. *Nova Hedwigia*, 68: 339–349.

Muthukumar T, Udaiyan K. 2000. Arbuscular mycorrhiza of plants growing in the Western Ghats region, Southern India. *Mycorrhiza*, 9: 297–313.

Muthukumar T, Udaiyan K. 2002. Seasonality of vesicular-arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta Oecologica*, 23: 337–347.

Nagahashi G, Douds Jr. DD. 2000. Partial separation of root exudate components and their effects upon the growth of germinated spores of AM fungi. *Mycological Research*, 104: 1453–1464.

Nagahashi G, Douds Jr. DD. 2007. Separated components of root exudate and cytosol stimulate different morphologically identifiable types of branching responses by arbuscular mycorrhizal fungi. *Mycological Research*, 111: 487–492.

Naik HB, Nalawadi UG, Sreenivasa MN, Patil AA. 1995. Field responses of China aster (*Callistephus chinensis* (L) Nees.) cv. ‘Ostrich plume’ to the inoculation of vesicular arbuscular mycorrhizal fungi at different phosphorus levels. *Scientia Horticulturae*, 62: 129–133.

Nair MG, Safir GR, Siqueira JO. 1991. Isolation and identification of vesicular-arbuscular mycorrhiza-stimulatory compounds from clover (*Trifolium repens*) roots. *Applied and Environmental Microbiology*, 57: 434–439.

Nakatsubo T. 1997. Effects of arbuscular mycorrhizal infection on the growth and reproduction of the annual legume *Kummerowia striata* growing in a nutrient-poor alluvial soil. *Ecological Research*, 12: 231–237.

Neumann E, George E. 2010. Nutrient Uptake: The Arbuscular mycorrhizal fungal symbiosis as a plant nutrient acquisition strategy. In: Arbuscular Mycorrhizas: Physiology and Function (eds). Koltai H, Kapulnik Y. Springer Dordrecht Heidelberg, London, New York. pp. 137–167. doi: 10.1007/978–90–481–9489–6–7.

Newsham KK, Fitter AH, Watkinson AR. 1995a. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology*, 83: 991–1000.

Newsham KK, Fitter AH, Watkinson AR. 1995b. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution*, 10: 407–411.

Nicolson TH, Schenck NC. 1979. Endogonaceous mycorrhizal endophytes in Florida. *Mycologia*, 71(1): 190.

Norman JR, Hooker JE. 2000. Sporulation of *Phytophthora fragariae* shows greater stimulation by exudates of nonmycorrhizal than by mycorrhizal strawberry roots. *Mycological Research*, 104: 1069–1073.

Nowak J. 2004. Effects of arbuscular mycorrhizal fungi and organic fertilization on growth, flowering, nutrient uptake, photosynthesis and transpiration of geranium (*Pelargonium hortorum* L.H. Bailey ‘Tango Orange’). *Symbiosis*, 37: 259–266.

Oehl F, Laczko E, Bogenrieder A, Stahr K, Bösch R, van der Heijden M, Sieverding E. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry*, 42: 724–738.

Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology*, 69: 2816–2824. doi: 10.1128/AEM.69.5.2816-2824.2003.

Oehl F, Sieverding E, Mäder P, Dubois D, Ineichen K, Boller T, Wiemken A. 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia*, 138: 574–83.

Oehl F, Sieverding E. 2004. *Pacispora*, a new vesicular-arbuscular mycorrhizal fungal genus in the Glomeromycetes. *Journal of Applied Botany and Food Quality*, 78(1): 72–82.

Oehl F, Sieverding E, Palenzuela J, Ineichen K, Silva GA. 2011a. Advances in *Glomeromycota* taxonomy and classification. *IMA Fungus*, 2(2): 191–199. doi: 10.5598/imafungus.2011.02.02.10.

Oehl F, Silva DKA, Maia LC, Sousa NMF, Vieira HEE, Silva GA. 2011b. *Orbispora* gen. nov., ancestral in the Scutellosporaceae (Glomeromycetes). *Mycotaxon*, 116: 161–169.

Oehl F, Silva GA, Goto BT, Maia LC, Sieverding E. 2011c. Glomeromycota: two new classes and a new order. *Mycotaxon*, 116: 365–379.

Oehl F, Silva GA, Goto BT, Sieverding E. 2011d. Glomeromyceta: three new genera and glomoid species reorganized. *Mycotaxon*, 116: 75–120. doi: 10.5248/116.75.

Oehl F, Silva GA, Sánchez-Castro I, Goto BT, Maia LC, Vieira Hee, Barea JM, Sieverding E, Palenzuela J. 2011e. Revision of Glomeromycetes with entrophosporoid and glomoid spore formation with three new genera. *Mycotaxon*, 117: 297–316.

Oehl F, Souza FA, Sieverding E. 2008. Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming Glomeromycetes. *Mycotaxon*, 106: 311–360.

Ohtomo R, Saito M. 2005. Polyphosphate dynamics in mycorrhizal roots during colonization of an arbuscular mycorrhizal fungus. *New Phytologist*, 167: 571–578.

Olah B, Briere C, Bécard G, Denarie J, Gough C. 2005. Nodfactors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. *Plant Journal*, 44: 195–207.

Oliveira AN, Oliveira LA. 2005. Seasonal dynamics of arbuscular mycorrhizal fungi in plants of *Theobroma grandiflorum* Schum and *Paullinia cupana* Mart. of an agroforestry system in Central Amazonia, Amazonas state, Brazil. *Brazilian Journal of Microbiology*, 36: 262–270.

Oliveira AN, Oliveira LA. 2010. Influence of edapho-climatic factors on the sporulation and colonization of arbuscular mycorrhizal fungi in two Amazonian native fruit species. *Brazilian archives Biology and Technology*, 53(3): 653–661.

Oliveira RS, Dodd JC, Castro PML. 2001. The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialized region of Northern Portugal. *Mycorrhiza*, 10: 241–247.

Opik M, Metsis M, Daniell TJ, Zobel M, Moora M. 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist*, 184: 424–437.

Öpik M, Moora M, Liira J, Zobel M. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology*, 94: 778–790.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (*Glomeromycota*). *New Phytologist*, 188: 223–241.

Ortas I. 2003. Effect of selected mycorrhizal inoculation on phosphorus sustainability in sterile and no-sterile soils in the Harran Plain in south Anatolia. *Journal of Plant Nutrition*, 26(1): 1–17.

Ortas I. 2008. Field trials on mycorrhizal inoculation in the eastern mediterranean horticultural region. In: Mycorrhiza works (ed). Feldmann F, Kapulnık Y, Baar J. Hannover, Germany. pp. 56–77.

Ouimet R, Camiré C, Furlan V. 1996. Effect of soil K, Ca and Mg saturation and endomycorrhization on growth and nutrient uptake of sugar maple seedlings. *Plant and Soil*, 179: 207–216.

Pacovsky RS. 1986. Micronutrient uptake and distribution in mycorrhizal or phosphorus-fertilized soybeans. *Plant and Soil*, 95: 379–388.

Palenzuela J, Ferrol N, Boller T, Azcón-Aguilar C, Oehl F. 2008. *Otospora bareai*, a new fungal species in the Glomeromycetes from a dolomitic shrub-land in the National Park of Sierra de Baza (Granada, Spain). *Mycologia*, 100(2): 282–291.

Panna D, Highland K. 2010. Mycorrhizal colonization and distribution of arbuscular mycorrhizal fungi associated with *Michelia champaca* L. under plantation system in northeast India. *Journal of Forestry Research*, 21(2): 137−142.

Parniske M. 2006. Molecular genetics of the arbuscular mycorrhizal symbiosis. *Current Opinion in Plant Biology*, 7: 414–421.
Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology*, 6: 763–775.

Paszkowski U, Kroken S, Roux C, Briggs SP. 2002. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences* U.S.A. 99: 13324–13329.

Paulitz TC, Linderman RG. 1991. Mycorrhizal interactions with soil organisms. In: Handbook of Applied Mycology. Soil and Plants (ed). Arora DK. Marcel Dekker, New York. pp. 77–129.

Pearson JN, Jakobsen I. 1993. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants measured by dual labelling with ^{32}P and ^{33}P. *New Phytologist*, 124: 489–494.

Peay KG, Bruns TD, Kennedy PG, Bergemann SE, Garbelloto M, 2007. A strong species-area relationship for eukaryotic soil microbes: island size maters for ectomycorrhizal fungi. *Ecology Letters*, 10: 470–480.

Perner H, Schwarz D, Bruns C, Mäder P, George E. 2007. Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of *Pelargonium* plants. *Mycorrhiza*, 17: 469–474. doi:10.1007/s00572–007-0116–7.

Pezzani F, Montaña C, Guevara R. 2006. Associations between arbuscular mycorrhizal fungi and grasses in the successional context of a two-phase mosaic in the Chihuahua desert. *Mycorrhiza*, 16: 285–295.

Pfeffer PE, Douds DD, Bécard G, Shachar-Hill Y. 1999. Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. *Plant Physiology*, 120: 587–598.

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining of mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55: 158–161.

Piotrowski JS, Denich T, Klironomos JN, Graham JM, Rillig MC. 2004. The effects of arbuscular mycorrhizae on soil aggregation depend on the interaction between plant and fungal species. *New Phytologist*, 164: 365–373.

Pirozynski KA, Malloch DW. 1975. The origin of land plants: a matter of mycotrophism. *Biosystems*, 6: 153–64.

Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA. 2005. Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian Journal of Plant Science*, 85: 31–40.

Plenchette C, Furlan V, Fortin JA. 1983. Response of endomycorrhizal plants grown in calcined montmorillonite clay to different levels of soluble phosphorus. I. Effect on growth and mycorrhizal development. *Canadian Journal of Botany*, 61: 1377–1383.

Poulsen KH, Nagy R, Gao L-L, Smith SE, Bucher M, Smith FA, Jakobsen I. 2005. Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. *New Phytologist*, 168: 445–454. doi: 10.1111/j.1469–8137.2005.01523.x.

Powell CL, Bagyaraj DJ. 1984. VA mycorrhiza, CRC press, USA.

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H. 2009. Phylogenetic trait conservatism and the evolution of functional tradeoffs in arbuscular mycorrhizal fungi. *Proceedings of the Royal Society B*, 276: 4237–4245.

Pozo MJ , Cordier C , Dumas-Gaudot E , Gianinazzi S , Barea JM , Azcón-Aguilar C. 2002. Localized *vs* systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. *Journal of Experimental Botany*, 53: 525–534.

Price N, Roncadori R, Hussey R. 1989. Turgor, solute import and growth in maize roots treated with galactose. *New Phytologist*, 31: 1095–1103.

Pringle A, Bever JD. 2002. Divergent phenologies may facilitate the coexistence of arbuscular mycorrhizal fungi in a North Carolina grassland. *American Journal of Botany*, 89: 1439–1446.

Rabie G. 1998. Induction of fungal disease resistance in *Vicia faba* by dual inoculation with *Rhizobium leguminosarum* and vesicular-arbuscular mycorrhizal fungi. *Mycopathologia*, 141: 159–166.

Radhika KP, Rodrigues BF. 2007. Arbuscular mycorrhizae in association with aquatic and marshy plant species of Goa, India. *Aquatic Botany*, 86: 291–294.

Radhika KP, Rodrigues BF. 2010. Arbuscular mycorrhizal fungal diversity in some commonly occurring medicinal plants of Western Ghats, Goa region. *Journal of Forestry Research*, 21: 45–52.

Ragupathy S, Mahadevan A. 1993. Distribution of vesicular- arbuscular mycorrhizae in the plants and rhizosphere soils of the tropical plains, Tamil Nadu, India. *Mycorrhiza*, 3: 123–136.

Rajan SK, Reddy BJD, Bagyaraj DJ. 2000. Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. *Forest Ecology Management*, 126: 91–95.

Ranganayaki N, Manoharachary C. 2001. AM fungal fssociation with *Tagetes erecta* L. and their impact on plant growth. *Philipine Journal of Science*, 130(1): 21–31.

Rausch C, Daram P, Brunner S, Jansa J, Laloi M, Leggewie G, Amrhein N, Bucher M. 2001. A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature*, 414: 462–466.

Ravnskov S, Jakobsen I. 1995. Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. *New Phytologist*, 129: 611–618.

Read DJ, Duckett JG, Francis R, Ligrone R, Russell A. 2000. Symbiotic fungal associations in “lower’ land plants. *Philosophical Transactions of the Royal Society* B, 355: 815–831.

Read DJ, Perez M. 2003. Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? *New Phytologist*, 157: 475–492.

Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia*, 47: 376–390.

Read DJ. 1998. Plants on the web. *Nature,* 396: 22–23.

Redecker D, Kodner R, Graham LE. 2000. Glomalean fungi from the Ordovician. *Science*, 289: 1920–1921.

Redecker D, Morton JB, Bruns TD. 2000. Molecular phylogeny of the arbuscular mycorrhizal fungi *Glomus sinuosum* and *Sclerocystis coremioides*. *Mycologia*, 92(2): 282–285.

Remy W, Taylor TN, Hass H, Kerp H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences* USA, 91: 11841–11843.

Repetto O, Bestel-Corre G, Dumas-Gaudot E, Berta G, Gianinazzi-Pearson V, Gianinazzi S. 2003. Targeted proteomics to identify cadmium-induced protein modifications in *Glomus mosseae* inoculated pea roots. *New Phytologist*, 157: 555–567.

Requena N, Jimenez I, Toro M, Barea JM. 1997. Interactions between plant-growth- promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of Anthyllis cytisoides, a model legume for revegetation in Mediterranean semi-arid ecosystems. *New Phytologist*, 136: 667–677.

Requena N, Perez-Solis E, Azcon-Aguilar C, Jeffries P, Barea JM. 2001. Management of indigenous plant–microbe symbioses aids restoration of desertified ecosystems. *Applied and Environmental Microbiologyy*, 67: 495–498.

Reynolds HL, Packer A, Bever JD, Clay K. 2003. Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology*, 84: 2281–2291.

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganism. *Plant and Soil*, 321: 305–339.

Rillig MC, Maestre FT, Lamit LJ. 2003. Micro-site differences in fungal hyphal length, glomalin, and soil aggregate stability in semiarid Mediterranean steppes. *Soil Biology and Biochemistry*, 35: 1257–1260.

Rillig MC, Wright SF, Eviner V. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant and Soil*, 238: 325–333.

Rillig MC, Wright SF, Kimball BA, Leavitt SW. 2001. Elevated carbon dioxide and irrigation effects on water stable aggregates in a *Sorghum* field: a possible role for arbuscular mycorrhizal fungi. *Global Change Biology*, 7: 333–337.

Rillig MC. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science*, 84: 355–363.

Rivera-Becerril F, van Tuinen D, Martin-Laurent F, Metwally A, Dietz K-J, Gianinazzi S, Gianinazzi- Pearson V. 2005. Molecular changes in *Pisum sativum* L. roots during arbuscular mycorrhiza buffering of cadmium stress. *Mycorrhiza*, 16: 51–60.

Rodrigues BF, Muthukumar T. 2009. Arbuscular Mycorrhizae of Goa- A manual for identification protocols. Goa University, India. pp. 1–135.

Rodriguez-Echeverria, S, Hol WHG, Freitas H, Eason WR, Cook R. 2008. Arbuscular mycorrhizal fungi of *Ammophila arenaria* (L.) Link: spore abundance and root colonisation in six locations of the European coast. *European Journal of Soil Biology*, 44: 30–36.

Ryan M, Ash J. 1999. Effects of phosphorus and nitrogen on growth of pasture plants and VAM fungi in SE Australian soils with contrasting fertiliser histories (conventional and biodynamic). *Agriculture, Ecosystems and Environment*, 73: 51–62.

Ryan MH, Angus JF. 2003. Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P uptake or yield. *Plant and Soil*, 250: 225–239.

Ryan MH, Chilvers GA, Dumaresq DC. 1994. Colonisation of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour. *Plant and Soil*, 160: 33–40.

Ryan MH, Graham JH. 2002. Is there a role for arbuscular mycorrhizal fungi in production agriculture. *Plant and Soil*, 244: 263–271.

Sainz MJ, Taboada-Castro MT, Vilarino A. 1998. Growth, mineral nutrition and mycorrhizal colonization of red clover and cucumber plants grown in a soil amended with composted urban wastes. *Plant and Soil*, 205: 85–92.

Saito M, Kato T. 1994. Effects of low temperature and shading on relationships between nodulation, vesicular-arbuscular mycorrhizal infection and shoot growth of soybean. *Biology and Fertility of Soils*, 17: 206–211.

Sanders FE, Tinker BP, Black RLB, Palmerly SM. 1977. The development of endomycorrhizal root systems. I. Speed of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. *New Phytologist*, 78: 257–268.

Sanders IR, Clapp JP, Wiemken A. 1996. The genetic diversity of arbuscular mycorrhzal fungi in natural ecosystem: a key to understanding the ecology and functioning of the mycorrhizal symbiosis. *New Phytologist*, 133: 123–134.

Sanders IR, Fitter AH. 1992. Evidence for differential responses between host-fungus combinations of vesicular-arbuscular mycorrhizas from a grassland. *Mycological Research*, 96: 415–419.

Sannazzaro AI, Ruiz OA, Alberto E, Menendez AB. 2004. Presence of different arbuscular mycorrhizal infection patterns in roots of *Lotus glaber* plants growing in the Salado river basin. *Mycorrhiza*, 14: 139–142.

Santos-Gonza´lez JC, Finlay RD, Tehler A. 2007. Seasonal dynamics of arbuscular mycorrhizal fungal communities in roots in a seminatural grassland. *Applied Environmental Microbiology*, 73: 5613–5623.

Sawaki H, Saito M. 2001. Expressed genes in the extraradical hyphae of an arbuscular mycorrhizal fungus, *Glomus intraradices*, in the symbiotic phase. *FEMS Microbiology Letters*, 195: 109–113.

Scagel CF, Schreiner RP, 2006. Phosphorus supply alters tuber composition, flower production, and mycorrhizal responsiveness of container-grown hybrid *Zantedeschia*. *Plant and Soil*, 283: 323–337. doi: 10.1007/s11104–006–0022–3.

Scagel CF. 2003a. Inoculation with Arbuscular mycorrhizal Fungi alters nutrient and flowering of *Freesia* x *hybrid*. *Journal of Environmental Horticulture*, 21(4): 196–205.

Scagel CF. 2003b. Soil pasteurization and inoculation with mycorrhizal fungi alters flower production and corm composition of *Zephyranthes* spp. *Journal of Horticulture Science and Biotechnology*, 78(6): 798–812.

Scagel CF. 2004a. Soil pasteurization and mycorrhizal inoculation alter flower production and corm composition of *Brodiaea laxa* ‘Queen Fabiola’. *HortScience*, 39(6): 1432–1437.

Scagel CF. 2004b.Inoculation with VA mycorrhizal fungi and rhizobacteria alters nutrient allocation and flowering of Harlequin flower. *Horticulture Technology*, 14(1): 39–48.

Scervino JM, Ponce MA, Erra-Bassells R, Vierheilig H, Ocampo JA, Godeas A. 2005. Flavonoids exhibit fungal species and genus specific effects on the presymbiotic growth of *Gigaspora* and *Glomus*. *Mycological Research*, 109: 789–794.

Schachtman DP, Reid RJ, Ayling SM. 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiology*, 116: 447–453.

Schenck NC, Perez Y. 1990. Manual for identification of VA Mycorrhizal Fungi. INVAM, University of Florida, Gainesville, U.S.A. pp. 1–283.

Schenck NC, Smith GS. 1982. Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. *Mycologia*, 74: 77–92.

Schenck NC, Spain JL, Howeler RH. 1984. Several new and unreported vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Colombia. *Mycologia*, 76: 685–699.

Schenck NC, Spain JL, Sieverding E. 1986. A new sporocarpic species of *Acaulospora* (*Endogonaceae*). *Mycotaxon*, 25: 111–117.

Scheublin TR, Ridgway KP, Young JPW, van der Heijden MGA. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Applied and Environmental Microbiology*, 70: 6240–6246.

Schmidt B, Şumălan R. 2011. The influence of arbuscular mycorrhizal fungi on ornamental characters of *Tagetes patula* L. *Journal of Horticulture, Forestry and Biotechnology*, 15(1): 170–174.

Schmidt SK, Sobieniak-Wiseman LC, Kageyama SA, Halloy SRP, Schadt CW. 2008. Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the andes and rocky mountains. *Arctic and Alpine Research*, 40: 576–583.

Schram JE. 1966. Plant colonization studies on black wastes from anthracite mining in Pennsylvania. *American Philosophical Society*, 56: 1–194.

Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research*, 105: 1413–1421.

Schüßler A, Walker C. 2010. The *Glomeromycota.* A species list with new families and new genera. Gloucester, UK. pp. 56.

Schüßler A. 2000. *Glomus claroideum* forms an arbuscular mycorrhiza-like symbiosis with the hornwort *Anthoceros punctatus*. *Mycorrhiza,* 10: 15–21.

Schwab SM, Menge JA, Leonard RT. 1983. Comparison of stages of vesicular-arbuscular mycorrhiza formation in sudangrass grown at two levels of phosphorus nutrition. *American Journal of Botany*, 70: 1225–1232.

Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A. 2006. The promise and the potential consequences of

the global transport of mycorrhizal fungal inoculum. *Ecology Letters*, 9: 501–515.

Sengupta A, Chaudhuri S. 2002. Arbuscular mycorrhizal relations of mangrove plant community at the Ganges river estuary in India. *Mycorrhiza*, 12: 169–174.

Sensoy S, Demir S, Turkmen O, Erdinc C, Savur OB. 2007. Responses of some different pepper (*Capsicum annum* L.) genotypes to inoculation with two different arbuscular mycorrhizal fungi. *Scientia Horticulturae*, 113: 92–95. doi: 10.1016/j.scienta.2007.01.023.

Sequeira JO, Saggin-Júnior OJ. 2001. Dependency on arbuscular mycorrrhizal fungi and responsiveness of some Brazilian native woody species. *Mycorrhiza*, 11: 245–255.

Sequeira JO, Sylvia DM, Gibson J, Hubbell DH. 1985. Spores, germination, and germ tubes of vesicular-arbuscular mycorrhizal fungi. *Canadian Journal of Microbiology*, 31: 965–972.

Shannon CE, Weaver W. 1949. The mathematical Theory of communication. University of Illinois, Urbana, Illinois.

Sharma AK, Srivastava PC, Johri BN, Rathore VS. 1992. Kinetics of zinc uptake by mycorrhizal (Vam) and non-mycorrhizal corn (*Zea mays* L.) Roots. *Biology and Fertility of Soils*, 13: 206–210.

Shetty KG, Hetrick BAD, Schwab AP. 1995. Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. *Environmental Pollution*, 88: 307–314.

Shi ZY, Feng G, Christie P, Li XL. 2006. Arbuscular mycorrhizal status of spring ephemerals in the desert ecosystem of Junggar Basin, China. *Mycorrhiza*, 16: 269–275.

Sieverding E, Oehl F. 2006. Revision of *Entrophospora* and description of *Kuklospora* and *Intraspora*, two new genera in the arbuscular mycorrhizal Glomeromycetes. *Journal of Applied Botany and Food Quality*, 80: 69–81.

Sieverding E, Toro ST. 1987. *Acaulospora denticulata* sp. nov. and *Acaulospora rehmii* sp. nov. (*Endogonaceae*) with ornamented spore walls. *Angewandte Botanik*, 61: 217–223.

Sieverding E. 1987. A VA-mycorrhizal fungus, *Glomus glomerulatum* sp. nov., with two hyphal attachments and spores formed only in sporocarps. *Mycotaxon*, 29: 73–79.

Sieverding E. 1991. Vesicular-arbuscular mycorrhiza management in tropical ecosystems. GTZ Escborn Germany.

Siguenza C, Especiel I, Allen EB. 1996. Seasonality of mycorrhizae in Coastal sand dunes of Basa California. *Mycorrhiza*, 6: 151–157.

Sikes BA, Cottenie K, Klironomos JN. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology*, 97: 1274–1280.

Simon L, Bousquet J, Lévesque RC, Lalonde M. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature*, 363: 67–69.

Simpson EH. 1949. Measurement of diversity. *Nature*, 163: 688.

Siqueira JO, Saggin-Júnior OJ. 2001. Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species. *Mycorrhiza*, 11: 245–255.

Sivakumar N. 2012. Effect of edaphic factors and seasonal variation on spore density and root colonization of arbuscular mycorrhizal fungi in sugarcane fields. *Annals of Microbiology*, doi: 10.1007/s13213–012–0455–2.

Smith FA, Jakobsen I, Smith SE. 2000. Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. *New Phytologist*, 147: 357–366.

Smith FA, Smith SE. 1996. Mutualism and parasitism: Diversity in function and structure in the “arbuscular” (VA) mycorrhizal symbiosis. *Advances in Botanical Research*, 22*:* 1–43.

Smith FW, Mudge SR, Rae AL, Glassop D. 2003. Phosphate transport in plants. *Plant and Soil*, 248: 71–83.

Smith GS, Schenck NC. 1985. Two new dimorphic species in the Endogonaceae: *Glomus ambisporum* and *Glomus heterosporum*. *Mycologia*, 77: 567.

Smith SE, Gianinazzi-Pearson V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 39: 221–244.

Smith SE, Read DJ. 1997. Mycorrhizal Symbiosis. 2nd edition. Academic Press, London. pp. 605.

Smith SE, Read DJ. 2008. Mineral Nutrition, Toxic Element Accumulation and Water Relations of Arbuscular Mycorrhizal Plants. In: Mycorrhizal Symbiosis. 3rd Ed., Academic Press, London. pp. 145–148.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology*, 133: 16–20.

Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth and total P uptake. *New Phytologist*, 162: 511–524.

Sohn BK, Kim KY, Chung SJ, Kim WS, Park SM, Kang JG, Cho JS, Kim T, Lee JH. 2003. Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum. *Scientia Horticulturae*, 98(2): 173–183.

Sorensen JN, Larsen J, Jakobsen I. 2005. Mycorrhiza formation and nutrient concentration in leeks (*Allium porrum*) in relation to previous crop and cover crop management on high P soils. *Plant and Soil*, 273: 101–114.

Spain JL, Miranda JC. 1996. *Glomus brasilianum*: an ornamented species in the *Glomaceae*. *Mycotaxon*, 60: 137–142.

Spain JL, Sieverding E, Oehl F. 2006. *Appendicispora*, a new genus in the arbuscular mycorrhizal-forming Glomeromycetes, with a discussion of the genus *Archaeospora*. *Mycotaxon*, 97: 163–182.

Spain JL, Sieverding E, Schenck NC. 1989. *Gigaspora ramisporophora*: a new species with novel sporophores from Brazil. *Mycotaxon*, 34(2): 668.

Sparling GP, Tinker PB. 1975. Mycorrhizas in Pennine grassland. In: *Endomycorrhizas* (eds). Sanders FE, Mosse B, Tinker PB. Academic Press, London. pp. 545–560.

Šrámek F, Dubsky M, Vosatka M., 2000. Effect of arbuscular mycorrhizal fungi and *Trichoderma harzianum* on three species of balcony plants. *Rostlinna Vyroba*, 46: 127–131.

Srivastava PS, Bharti N, Pande D, Srivastava S. 2002. Role of mycorrhiza in *in vitro* micropropagation of plants In: Techniques in Mycorrhizal Studies (eds). Mukerji KG, Manoharachari C, Chamola BP. Kluwer Academic Publishers, Netherland. pp. 443–460.

St. John TV, Coleman DC. 1983. The role of mycorrhizae in plant ecology. *Canadian Journal of Botany*, 61: 1005–1014.

Staddon PL, Ramsey CB, Ostle N, Ineson P, Fitter AH. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of 14C. *Science,* 300: 1138–1140.

Stanley MR, Koide RT, Shumway DL. 1993. Mycorrhizal symbiosis increases growth, reproduction and recruitment *of Abutilon theophrasti Medic*. in the field. *Oecologia*, 94: 30–35.

Strack D, Fester T, Hause B, Schliemann W, Walter MH. 2003. Arbuscular mycorrhiza: biological, chemical and molecular aspects. *Journal of Chemical Ecology*, 29: 1955–1979.

Stürmer SL, Bellei MM. 1994. Compositional and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. *Canadian Jounal of Botany*, 72: 359–363.

Stürmer SL. 2004. Effect of different fungal isolates from the same mycorrhizal community on plant growth and phosphorus uptake in soybean and red clover. *Revista Brasileira Ciencia Do Solo*, 28: 611–622.

Stutz JC, Morton JB. 1996. Successive pot cultures reveal high species richness of vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. *Oecologia*, 64: 111–117.

Subramanian KS, Charest C. 1997. Nutritional, growth, and reproductive responses of maize (Zea mays L.) to arbuscular mycorrhizal inoculation during and after drought stress at tasselling. *Mycorrhiza*, 7: 25–32.

Subramanian KS, Charest C. 1999. Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions. *Mycorrhiza*, 9: 69–75.

Sundar SK, Palavesam A, Parthipan B (2011). AM fungal diversity in selected medicinal plants of Kanyakumari district, Tamil Nadu, India. *Indian Journal of Microbiology*, 51(3): 259–265.

Sunilkumar CP, Garampalli RH. 2010. Diversity of Arbuscular Mycorrhizal fungi in agricultural fields of Hassan district. *World Journal of Agricultural Sciences*, 6(6): 728–734.

Sutton JC, Barren GL. 1972. Population dynamics of *Endogone* spores in soil. *Canadian Journal of Botany*, 50: 1909–1914.

Tabin TA, Arunachalam A, Shrivastava K, Arunachalam K. 2009. Effect of arbuscular mycorrhizal fungi on damping off-disease in *Aquilaria agallocha* Roxb. seedlings. *Tropical Ecology*, 50: 243–248.

Tamasloukht M, Sejalon-Delmas N, Kluever A, Jauneau A, Roux C, Bécard G, Franken P. 2003. Root factors induce mitochondrial-related gene expression and fungal respiration during the developmental switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea*. *Plant Physiology*, 131: 1468–1478.

Tawaraya K, Hashimoto K, Wagatsuma T. 1998. Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonization by the arbuscular mycorrhizal fungus *Gigspora margarita*. *Mycorrhiza*, 8: 67–70.

Taylor TN, Remy W, Hass H, Kerp H. 1995. Fossil arbuscular mycorrhizae from early devonian. *Mycologia*, 87: 560–573.

Thaper HS, Khan SN. 1985. Distribution of VA mycorrhizal fungi in forest soils of India. *Indian Journal of Forestry*, 8: 5–7.

Thingstrup I, Rubaek G, Sibbesen E, Jakobsen I. 1998. Flax (*Linum usitatissimum* L.) depends on arbuscular mycorrhizal fungi for growth and P uptake at intermediate but not high soil P levels in the field. *Plant and Soil*, 203: 37–46.

Thompson JP. 1987. Decline of vesicular-arbuscular mycorrhizae in long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. *Australian Journal of Agricultural Research*, 38: 847–867.

Thompson JP. 1991. Improving the mycorrhizal condition of the soil through cultural practices and effects on growth and phosphorus uptake in plants. In: Phosphorus Nutrition of Grain Legumes in the Semi Arid Tropics(eds). Johansen C, Lee KK, Sahrawat KL. ICRISAT Publishing, India. pp. 117–137.

Thompson JP. 1994. Inoculation with vesicular-arbuscular mycorrhizal fungi from cropped soil overcomes long-fallow disorder of linseed (*Linum usitatissimum* L.) by improving P and Zn uptake. *Soil Biology and Biochemistry*, 26: 1133–1143.

Tinker PB. 1975. Effect of vesicular-arbuscular mycorrhizas on higher plants. *Symposium of the Society of Experimental Biology*, 29: 325–349.

Tisdall JM, Oades JM. 1979. Stabilization of soil aggregates by the root system of ryegrass. *Australian Journal of Soil Research*, 17: 429–441.

Tisdall JM, Smith SE, Rengasamtn P. 1997. Aggregation of soil by fungal hypahe. *Australian Journal of Soil Research*, 35: 55–60.

Titus JH, Titus PJ, Nowak RS, Smith RD. 2002. Arbuscular mycorrhizae of Mojave Desert plants. *West North American Naturalist*, 62: 327–334.

Tommerup IC. 1983. Temperature relations of spore germination and hyphal growth of vesicular-arbuscular mycorrhizal fungi in soil. *Transactions of the British Mycological Society*, 81: 381–387.

Trappe JM, Luoma DL. 1992. The ties that bind: fungi in ecosystems. In: The Fungal Community: Its Organization and Role in the Ecosystem (eds). Carroll GC, Wicklow DT. 2nd ed. Marcel Dekker, New York. pp. 17–27.

Trappe JW. 1977. Three new Endogonaceae: *Glomus constrictus, Sclerocystis clavispora* and *Acaulospora scrobiculata. Mycotaxon*, 6: 359–366.

Trent JD, Svejcar AJ, Bethlenfalvay GJ. 1993. Growth and nutrition of combinations of native and introduced plants and mycorrhizal fungi in a semiarid range. *Agriculture, Ecosystems and Environment*, 45: 13–23.

Treseder KK, Allen MF. 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. *New Phytologist*, 147: 189–200.

Treseder KK, Cross A. 2006. Global distributions of arbuscular mycorrhizal fungi. *Ecosystems*, 9: 305–316.

Trotta A, Varese GC, Gnavi E, Fusconi A, Sampo S, Berta G. 1996. Interactions between the soil borne root pathogen *Phytophthora nicotianae* var. *parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. *Plant and Soil*, 185: 199–209.

Tsimilli-Michael M, Eggenberg P, Biro B, Köves-Péchy K, Vörös I and Strasser RJ. 2000. Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and *Azospirillum* and *Rhizobium* nitrogen-fixers on the photosynthetic activity of alfalfa, probed by the polyphasic chlorophyll a fluorescence transient O-J-I-P. *Applied Soil Ecology*, 15: 169–182.

Turnau K, Mesjasz-Przybylowicz J. 2003. Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza*, 13: 185–190.

Udaiyan K, Karthikeyan A, Muthukumar T. 1996. Influence of edaphic and climatic factors on dynamics of root colonization and spore density of vesicular-arbuscular fungi in *Acacia farnesiana* Willd. and *A. planifrons* W. et A. *Tree*, 11: 65–71.

Uhlmann E, Görke C, Petersen A, Oberwinkler F. 2004. Arbuscular mycorrhizae from semiarid regions of Namibia. *Canadian Journal of Botany*, 82: 645–653.

Vaingankar JD, Rodrigues BF. 2011. *Acaulospora soloidea*, a new arbuscular mycorrhizal fungus from rhizosphere soils of *Murraya paniculata*. *Mycotaxon*, 115: 323–326.

Valentine AJ, Osborne BA, Mitchell DT. 2001. Interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber. *Scientia Horticulturae,* 88: 177–189.

van Aarle IM, Olsson PA, Soderstrom B. 2002. Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. *New Phytologist*, 155: 173–182.

van der Heijden MGA, Boller T, Wiemken A, Sanders IR, 1998a. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, 79: 2082–2091.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998b. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396: 69–72.

van der Heijden MGA, Scheublin TR. 2007. Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytologist*, 174: 244–250.

van der Heijden MGA, Wiemken A, Sanders IR. 2003. Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. *New Phytologist*, 157: 569–578.

van der Putten WH. 2003. Plant defense below ground and spatio temporal processes in natural vegetation. *Ecology*, 84: 2269–2280.

Van Nuffelen M, Schenck NC. 1984. Spore germination penetration and root colonization of six species of vesicular-arbuscular mycorrhizal fungi on soybean. *Canadian Journal of Botany*, 62: 624–628.

Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW. 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology*, 12: 3085–3095.

Vázquez MM, Barea JM, Azcón R. 2002. Influence of arbuscular mycorrhizae and a genetically modified strain of *Sinorhizobium* on growth, nitrate reductase activity and protein content in shoots and roots of *Medicago sativa* as affected by nitrogen concentrations. *Soil Biology and Biochemistry*, 34: 899–905.

Vellinga EC, Wolfe BE, Pringle A. 2009. Global patterns of ectomycorrhizal introductions. *New Phytologist*, 181: 960–973.

Veresoglou SD, Chen B, Rillig MC. 2012. Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biology and Biochemistry*, 46: 53–62.

Vierheilig H, Alt M, Mohr U, Boller T, Wiemken A. 1994. Ethylene biosynthesis and activities of chitinase and beta-1,3-glucanase in the roots of host and nonhost plants of vesicular–arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae*. *Journal of Plant Physiology*, 143: 337–343.

Vierheilig H, Iseli B, Alt M, Raikhel N, Wiemken A, Boller T. 1996. Resistance of *Urtica dioica* to mycorrhizal colonization: A possible involvement of *Urtica dioica* agglutinin. *Plant and Soil*, 183: 131–136.

Vigo C, Norman JR, Hooker JE. 2000. Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. *Plant Pathology*, 49: 509–514.

Vogelsang KM, Reynolds HL, Bever J D. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist*, 172: 554–562.

Vosatka M. 1995. Influence of inoculation with arbuscular mycorrhizal fungi on the growth and mycorrhizal infection of transplanted onion. *Agriculture, Ecosystems and Environment*, 53: 151–159.

Walker C, Pfeiffer CM, Bloss HE. 1986. *Acaulospora delicata* sp. nov. an endomycorrhizal fungus from Arizona. *Mycotaxon*, 25: 621–628.

Walker C, Reed LE, Sanders FE. 1984. *Acaulospora nicolsonii*, a new endogonaceous species from Great Britain. *Transactions of the British Mycological Society*, 83: 360–364.

Walker C, Sanders FE. 1986. Taxonomic concepts in the Endogonaceae: III. The separation of *Scutellospora* gen. nov. from *Gigaspora* Gerdemann & Trappe. *Mycotaxon*, 27: 169–182.

Walker C, Schüßler A. 2004. Nomenclatural clarifications and new taxa in the Glomeromycota. *Mycological Research*, 108: 979–982.

Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H, Nishmura I, Schüßler A. 2007a. Molecular phylogeny and new taxa in the Archaeosporales (Glomeromycota): *Ambispora fennica* gen. sp. nov., Ambisporaceae fam. nov., and emendation of *Archaeospora* and Archaeosporaceae. *Mycological Research*, 111: 137–153. doi: 10.1016/j.mycres.2006.11.008.

Walker C, Vestberg M, Schüßler A. 2007b. Nomenclatural clarifications in Glomeromycota. *Mycological Research*, 111: 253–255.

Walker C, Vestberg M. 1998. Synonymy amongst the arbuscular mycorrhizal fungi: *Glomus claroideum*, *G*. *maculosum*, *G*. *multisubstensum* and *G*. *fistulosum*. *Annals of Botany*, 82: 601–624. doi: 10.1006/anbo.198.0714.

Walker C. 1983. Taxonomic concepts in the Endogonaceae. Spore wall characteristics in species descriptions. *Mycotaxon*, 18: 443–455.

Walker C. 1992. Systematics and taxonomy of the arbuscular endomycorrhiza fungi (Glomales) a possible way forward. *Agronomie*, 12: 887–897.

Walkley A, Black JA. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic titration method. *Soil Science*, 37: 29–38.

Wang C-L, Tschen JSM, Wang CL. 1997. Factors on the spore germination of arbuscular mycorrhizal fungi, *Glomus* spp. *Archives of International Medicine*, 157: 109–116.

Warner NJ, Allen MF, MacMahon JA. 1987. Dispersal agents of vesicular-arbuscular mycorrhizal fungi in a disturbed arid ecosystem. *Mycologia*, 79: 721–730.

Watson CA, Atkinson D, Gosling P, Jackson LR, Rayns FW. 2002. Managing soil fertility in organic farming systems. *Soil Use Management*, 18: 239–247.

Watson DMH, Millner PD. 1996. Assessment of Glomalean species biodiversity as influenced by trapping methods. In 'Programs and *abstracts of the First International Conference on mycorrhizae.' (eds). Szaro TM, Bruns TD. University of California, Berkeley. pp. 125.

*Wen CL. 1991. Effects of temperature and *Glomus* sp. on the growth and cut flower quality of micropropagated *Gerbera jamesonii*. M.S. thesis, Department of Horticulture, National Taiwan University, Republic of China. pp.158.

West HM. 1995. Soil phosphate status modifies response of mycorrhizal and nonmycorrhizal *Senecio vulgaris* L. to infection by the rust, *Puccinia lagenophorae* Cooke. *New Phytologist*, 129: 107–116.

Whipps JM. 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany*, 82: 1198–1227.

Wilson GWT, Hartnett DC. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *American Journal of Botany*, 85: 1732–1738.

Wilson JM. 1984. Comparative development of infection by three vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, 97: 413–426.

Wiseman PE, Wells CE. 2005. Soil inoculums potential and arbuscular mycorrhizal colonization of *Acer rubrum* in forested and developed landscapes. *Journal of Arboriculture*, 31: 296–302.

Wright DP, Read DJ, Scholes JD. 1998. Mycorrhizal sink strength influences wholeplant carbon balance of *Trifolium repens* L. *Plant Cell and Environment*, 21: 881–891.

Wright SF, Anderson RL. 2000. Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. *Biology and Fertility of Soils*, 31: 249–253.

Wright SF, Upadhyaya A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, 198: 97–107.

Wright SF, Upadhyaya A.1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science*, 161(9): 1–12.

Wu QS, Xia RX, Zou YN. 2008. Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *European Journal of Soil Biology*, 44: 122–128.

Wu QS, Xia RX. 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology*, 163: 417–425.

Yang AN, Lu L, Wu CX, Xia MM. 2011. Arbuscular mycorrhizal fungi associated with Huangshan Magnolia (*Magnolia cylindrica*). *Journal of Medicinal Plants Research* , 5(18): 4542–4548.

Yano K, Yamauchi A, Kono Y. 1996. Localized alteration in lateral root development in roots colonized by an arbuscular mycorrhizal fungus. *Mycorrhiza*, 6: 409–415.

Yaseen T, Burni T, Jabeen M, Hussain F. 2006. Mycorrhizal status of *in vitro* raised *Chrysanthemum cinerariifolium*. *Pakistan Journal of Plant Sciences*, 12(2): 131–135.

Yasuda N, Sugimoto Y, Kato IS, Yoneyama K. 2003. (+)-Strigol, a witchweed seed germination stimulant, from *Menispermum dauricum* root culture. *Phytochemistry*, 62: 1115–1119.

Yoshida S, Forno DA, Cock JH, Gomez KA. 1976. Laboratory Manual for Physiological Studies of Rice. The International Rice research Institute, Los Banos, Laguna, Philippines.

Zhang T, Sun Y, Shi Z, Feng G. 2012. Arbuscular mycorrhizal fungi can accelerate the restoration of degraded spring grassland in central asia. *Rangeland Ecology and Management*, 65(4): 426–432.

Zhao ZW, Xia YM, Qin XZ, Li XW, Cheng LZ, Sha T, Wang GH. 2001. Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China. *Mycorrhiza*, 11: 159–162.

***Literature not seen.**

Webliography:

http://icargoa.res.in/wasp/rbd2.php

http://invam.caf.wvu.edu.

http://www.iiem.com/em/floriculture/chapter2.html