CHAPTER – I
GENERAL INTRODUCTION

Man’s life has always been intensively connected with plants and nature surrounding him and nature plays a dynamic role in human life. Plants have been used by human beings for nourishment, defence, protection, food, fibre, medicine and decoration. Ornamental flowering plants are grown for display of their flowers as flowers are one of the nature’s most beautiful gift to man. It is well known that flowers are associated with mankind from the dawn of civilization. The Aryans of the Vedic period were great lovers of flowers. The Lotus has been mentioned frequently in the Sanskrit scriptures of the Vedic times. The epic of the Aryans, the Ramayana and the Mahabharta, believed to have been compiled during 500 B.C. mention about gardens and flowers. Huen Tsang, the famous Chinese pilgrim, travelled widely in India and he wrote that the Hindus needed flowers every morning for worshipping and they used flowers to adorn their hair. The different frescos at Ajanta (100-600 A.D.) also are indicative of the importance of flowers and gardening in ancient India.

Flowers symbolize purity, beauty, peace, love, and passion. To a Japanese flower-arranger, each flower express one or more meaning. To an Indian, flowers have a much greater significance. It is said that in India man is born with flowers, lives with flowers and finally dies with flowers. A Hindu needs flowers every morning for religious offering to the family deity. It is estimated that about 30 per cent of the total cut flower in the Calcutta are used for worship (Anonymous, 1976). In our society no social function is complete without the use of flowers. Floral garland and gajras are needed for marriage ceremonies. Floral ornaments, bouquets, or flower arrangements also find a pride of place in social gatherings, birthday parties, welcoming a home-coming friend or relative and honouring dignitaries. So, ornamental flowering plants play an important role in modern society as flowers are an integral part of human life due to their diversity in beauty, texture, colour, shape and fragrance and also constitute one of its main fresh export products. Flowers of shrubs and trees beautifully decorate our yards and parks, while household plants add a pleasant living touch to our indoor environment. Extracts from many fragrant flowers such as rose (Rosa spp.), lavender (Lavandula angustifolia) and champak (Canaga odorata) are generally used in perfume industry. The essential oils from jasmine, rose, and tuberose form the base for many of the internationally renowned perfumes. One ton of jasmine is said to yield about 3 kg of concrete which sells at Rs. 8,000 to Rs. 10,000 per kg. Roses are also grown for
preparing gulkand, a product mainly used in sweets, and for chewing in combination with pan (betel leaf). The dried fruit of *Vanilla planifolia* yields the vanilla of commerce which is now produced synthetically.

Flower cultivation has been practiced in India since time immemorial but floriculture has blossomed into a profitable business only in recent time. It has emerged as most lucrative business due to much higher return than other crops (Pandey *et al*., 2010). Indian floriculture industry has been shifting from traditional flowers to cut flowers mainly for export purposes. Area under cultivation in floriculture was about 253.65 thousand hectares and production of flowers was estimated to be 1.652 million tonnes (MT) for loose flowers and 750.66 MT for cut flowers in 2011-12 in India. The global trade of cut flowers and potted plants is worth US $40 billion per annum (Ezhilmathi *et al*., 2007). Cut flowers make up about one-third of the value of the global ornamental horticulture market (Gupta *et al*., 2006). The quality of flowering plants is limited by its longevity, which is influenced by senescence (Wani *et al*., 2012). The market loss of cut flowers due to inefficient postharvest management in India is estimated to be around 20-40% (Waheeduzzama *et al*., 2006). The techniques of prolonging the vase life of cut flowers are a great asset to the growers and users (Nair *et al*., 2003).

Ornamental plant research in India is of recent origin. Floriculture research was strengthened with the establishment of Division of Floriculture and Landscape Gardening at the IIHR, Bangalore, in 1969 and Division of Vegetable Crops and Floriculture at the IARI, New Delhi in 1971. In India the research on floricultural crops is still infacy in comparison to western countries. The major flower growing states in India are Karnataka, Tamil Nadu, Andhra Pradesh, West Bengal, Maharashtra, Rajasthan, Delhi and Haryana.

Haryana belongs to the Kuru region of North India was formed on 1st November 1966. 'Abode of God' is the Sanskrit meaning of the word Haryana. The state is referred as the cradle of Indus Valley and Vedic civilization that flourished at the banks of the extinct Saraswati River. This state has a rich diversity of horticultural crops due to presence of diverse agro climatic conditions ranging from subtropical and semi-arid to sub-humid. There was less flower cultivation in the Haryana up to mid nineties but covered 4,810 hectare (ha) during 2004-05 and 6.3 thousand ha during 2010-11. The main flower growing areas are Faridabad, Karnal, Panchkula, Panipat, Sonipat, Gurgaon, Kaithal, Jind and Jhajjar, mainly growing Marigold, Rose, Tuberose and *Gladiolus*.

Ornamental plant cultivation has received an impetus in the recent years due to large demands of flowers for various occasions. So the enhancement of the growth and bioactive agents of these plants is desirable. One of the methods by which this may be achieved is by
inoculation of the roots of plants with microorganisms like arbuscular mycorrhizal (AM) fungi. AM fungi are environment compatible and economically feasible alternatives for improving crop production. AM fungi inoculation offers noble additive effects to crops-owing to: increased productivity, increased crop uniformity, reduced transplant losses, reduced fertilizer and fungicide applications, increased disease resistance, improved crop marketability, accelerated growth rates, accelerated budding and flowering.

**MYCORRHIZA**

The term mycorrhiza is coined by Albert Bernard Frank in 1885 who was a German Forest Plant Pathologist. Mycorrhiza is the combination of two words, one Greek Mykes (Mushroom/Fungus) and other Latin Rhiza (roots), it literally means fungus root. It is defined as an association or symbiosis between plants and non-pathogenic fungi that colonize the cortical tissue of roots during periods of active plant growth (Sharma et al., 2014). It is characterized by bidirectional movement of nutrients where carbon flows to fungus and inorganic nutrients move to the plant. About 95% plant species harbour mycorrhizal association.

Plants having branched, fine, long roots with numerous root hairs are less dependent on mycorrhiza for nutrient acquisition (Manjunath and Habte, 1992; Hetrick et al., 1992) than plant with coarse roots (Graham et al., 1991). More than 6000 fungal species are capable of establishing mycorrhizal association of approximately with 2,40,000 plant species (Sharma, 2001). On the basis of morphological and anatomical features, mycorrhiza is divided into three broad groups: ectomycorrhizae, ectendomycorrhiza and endomycorrhiza. However, on the basis of morphology, anatomy, host plant taxonomy and fungal taxonomy, Marks (1991) divided mycorrhizal fungi into seven basic types- Ectomycorrhiza, Ectendomycorrhiza, Arbutoid, Monotropoid, Ericoid, Orchidoid and Arbuscular mycorrhiza.

1. **Ectomycorrhizae (ECM)**

The ectomycorrhizae means mycorrhizae which occur outside the cell. The fungal member of ectomycorrhizae are mostly Basidiomycota (4000 spp.) although few may be Ascomycota (Morchella) and are often with those species which have quite large and conspicuous fruiting bodies e.g. *Amanita* spp., *Boletus edulis*, *B. betulicola*, *Pisolithus arhizus*, *Lactarius deliciosus*, *Rhizopogon roseolus* and *Paxillus luteolus*, *Suillus luteus*, *Thelephora terrestris* etc.
Ectomycorrhiza are very common on forest trees and shrubs particularly in sub-arctic and temperate regions. Most of the host plants belong to families Pinaceae, Fagaceae, Betulaceae, Myrtaceae, Juglandaceae, Tiliaceae etc. have ectomycorrhizal association.

Ectomycorrhiza are characterized by presence of fungal hyphae between cortical cells of root producing net like structure called Hartig net (Robert Hartig Father of Forest Biology) and a covering sheath called mantle that covers the root surface externally. In this infection modification of infected roots and loss of absorbent hair take place.

Mantle may vary in thickness, colour, and texture that depending on particular combination. This mantle increases the surface area of absorbing roots and affects root morphology resulting in root bifurcation and clustering.

2. Ectendomycorrhizae (ECEM)
Ectendomycorrhiza term was first coined by Melin in 1923. It is also known as mixed infection as it is intermediate type and showing features of both ectomycorrhizae and endomycorrhizae (Harley, 1989). Ectendomycorrhizal formation induces the growth of short roots, similar to ECM association in apple seedlings (Thakur and Sharma, 2013). It is having both intracellular as well as intercellular hyphae in cortical region of roots. Emergent roots become covered in a matrix of highly branched hyphae, a coarse sheath develop behind the apex, between root hairs, and eventually cover the entire root, except when lateral root grow very rapidly (Sharma et al., 2014). They persist in moist and neutral soil. Fungi involved in this infection belong to Ascomycota and placed in genus Wilcoxina and the plants like Alnus spp., Salix spp. and Populus spp. show this type of association.

3. Ericoid mycorrhizae (EM)
These mycorrhizae occur in cold and wet soils in which mineralization and decomposition processes are inhibited (Bajwa et al., 1985). Inner cortical cells of the host root become packed with fungal hyphae and upto 80 per cent of the root portion can be fungal hyphae. It may form a loose weft of hyphae that grows over the root surface, but a true mantle is lacking. The extraradical phase of ericoid mycorrhizae consists of sparse hyphae that do not extend very far into the soil. Arbuscules are not formed in this association. The ericoid mycorrhizae are associated with Ascomycetous fungi of the genus Hymenoscyphus, and plants in the Ericaceae family such as Calluna, Rhododendron and Vaccinium that have very fine root system and typically grow in acid and peaty soils.

4. Arbutoid Mycorrhizae (ARM)
It occurs in plant species of Arbutus, Arctostaphyles, Arctotis, Gutheria and Leucothoe. Fungal partners of Arbutoid mycorrhiza includes Amanita, Cortinarius, Lactarius and
Boletus (Largent et al., 1980). In this a fungal sheath surrounds the infected roots as a result of intercellular fungal penetration and in certain cases due to development, it forms intracellular network of hyphae.

5. Monotropoid Mycorrhizae (MM)
It is found in achlorophyllous plants in family Monotropaceae (e.g. Indian pipe) and produce hyphal mantle and Hartig net which is one cell deep in the root cortex and thus limited intracellular penetration.

Monotropoid mycorrhizae also form ectomycorrhizal relationship with trees and thereby form a link through which carbon and other nutrients can flow from the autotrophic host plant to the heterotrophic, parasitic plant. The roots are enclosed in a fungal sheath. In this relationship species of Monotropa, Pterospora obtain some carbon and energy via the shared mycorrhizal fungus.

6. Orchidaceous Mycorrhizae (OM)
Orchid mycorrhiza form association with the plants of the family Orchidaceae. Orchids typically possess very small seeds with little nutrient reserve. The fungi colonize either the embryo of the minute orchid seeds or the roots of chlorophyllous and achlorophyllous orchid species as well as protocorm. Fungus enters the plant, shortly after the germination and forms hyphal coils called pelotons (Hadley, 1975) within the cell. The pelotons are active for only few days, after which they degenerate and growing orchids absorb the nutrients.

Orchidaceous mycorrhizal infection in orchids differs from all other mycorrhizal infection is that the main role of the fungus is to supply carbohydrates to the achlorophyllous plants. Besides carbon they also supply other nutrients like nitrogen and other inorganic nutrients. Evidence of this was provided by Alexander (1987), Alexander and Hadley (1983) and Alexander et al. (1984). Orchid mycorrhiza forming fungi are Ceratobasidium, Tulasnella, Armillaria, Rhizoctonia, Fomes, Marasmius and Coriolus.

7. Arbuscular Mycorrhizae (AM)
Arbuscular mycorrhiza is endomycorrhiza and has got its name from the fungal tree shaped, short lived structure that develops in plant root cells (arbus tree). Earlier, the name Vesicular Arbuscular Mycorrhizae (VAM) fungus was used, but since not all the groups produce vesicles, the term AM fungi is preferred (Smith, 1995; Walker, 1995; Friberg, 2001).

AM fungi are ubiquitous, important for terrestrial ecosystem and have potential applications. AM fungi are found under all climates and in all ecosystems regardless of the type of soil, vegetation or growing conditions. It is found in all angiospermic families, except some families such as Betulaceae, Commelinaceae, Urticaceae etc. (Gerdemann, 1975).
Families that rarely form arbuscular mycorrhiza are Brassicaceae, Chenopodiaceae, Cyperaceae and Polygonaceae (Gerdemann, 1975; Tester et al., 1987; Meney et al., 1993).

AM fungi are characterized by the presence of intracellular hyphae in the primary cortex which form specific structures called vesicles and arbuscules later on. As the fungus grows, the host cell membrane invaginates and develops the vesicles and arbuscules, creating a new compartment where material is deposited. This apoplastic space prevents direct contact between the plant and fungus cytoplasm and allows for efficient transfer of nutrients between the symbionts. Arbuscules are small tree like, hyphae filled invagination of the cortical cells, which provide intimate contact between plasmalemmae of the two symbiotic partners and are presumably the point of material exchange between host plant and fungus. Arbuscules are named by Gallaud (1905) because they looked like trees. Vesicles are thin or thick-walled, globose to subglobose, irregular shaped structures, terminally or intercalary having inter or intracellular hyphae within the roots. Some of genera of AM are Glomus, Acaulospora, Gigaspora, Scutellospora, Entrophospora and Sclerocystis. Glomus is very common AM genus with common species like fasciculatum, mosseae, constrictum, monosporum and macrosporum.

Gallaud (1905) divided arbuscular mycorrhiza into two structural classes, Paris and Arum type after the plants in which they were first described. These are known as coiling and linear association respectively. In both the types, the initial epidermal penetration results in intracellular hyphae, often forming coils (pelotons) in hypodermis and the outer cortex. Paris type occurs more frequently in plant kingdom than Arum type. It is reported that Paris type of arbuscular mycorrhiza is found in 41 Angiosperms families, while Arum type is found in 30 families.

**Paris type**

It is named after Paris quadrifolia and characterized by the presence of extensive intracellular hyphal coils and absence of intercellular phase. Hyphae spread by intracellular growth following a twisted path through cortical cells because there are no continuous longitudinal spaces. The resultant colonies generally possess a convoluted appearance (Gallaud, 1905). Vesicles are intracellular. Arbuscules are small, complex and may be restricted to a single layer of cells in the inner cortex.

**Arum type**

Arum type is named on the host plant Arum maculatum and is characterized by the presence of an extensive intercellular phase of hyphal growth in the root cortex and the development of
terminal arbuscules on intracellular hyphal branches. Hyphae proliferate in the cortex by growing longitudinal between host cells and resulting colonies of AM fungi have a linear appearance. Arbuscules are relatively simple and scattered in the infected zone.

**STRUCTURAL DIVERSITY IN AM FUNGI**

AM fungi form a variety of structures in the host roots such as hyphae, vesicles and arbuscules. AM symbionts also form certain structures such as appressorium, auxillary cell, extra-radical hyphae in the soil.

1) **Appresoria**

AM fungal hyphae produce swelling called appresoria on the epidermal cell wall and the formation of appresoria is the sign of fungal recognition of a potential host plant (Staples and Macko, 1980). Thus appressorium can be defined as fungal structure formed after the first contact of the AM hyphae with the host root (Tawaraya et al., 2007).

2) **Arbuscules**

Dichotomously branched, haustoria like intracellular fungal structures appearing as little tree are called arbuscules (Gallaud, 1905). These are considered as the sole structure defining features of an AM fungus (Gianinazzi et al., 1979). Phosphates and fixed carbon molecules are exchanged at this interface. Arbuscules are short lived structures and believed to have a turnover period of 4-15 days.

3) **Auxillary cell**

These are also called as accessory bodies. Auxillary cells are clusters of thin-walled cells, often ornamented by spines or knobs and formed on extraradical hyphae of Gigaspora and Scutellospora. They have a different role than propagules and act as the structure for temporary storage of carbon compounds.

4) **Colony**

It is referred as infection units formed by hyphal colonization of the root resulting from the external hyphae.

5) **Intra-radical hyphae**

These can be either intercellular, when it grows between the walls of adjacent root cells or intracellular, when it grows within the root cells. Intraradical hyphae can be parallel or with H-shaped or Y-shaped branches. The main function of intraradical hyphae is the transport of substances absorbed by extraradical hyphae from the soil.
6) **Extraradical mycelium**

The extraradical mycelium also known as soil hyphae or external hyphae plays a critical role in the absorption and rapid translocation of the absorbed nutrients to the intraradical mycelium. Extraradical mycelium encounters excessive environmental variations, acquire nutrients from the soil, propagate the association and produce spores and other structures.

7) **Spores**

Spores are formed as swellings on one or more subtending hyphae in the soil. Sometimes, these are also formed inside the roots, on root surface, on plants or on their decaying fragment. The AM fungal spores are relatively large (40-800 µm) with different wall layers (Becard and Pfeffer, 1993), and many nuclei (1,500-2,000). Colour of spores varies from hyaline to black and texture from smooth to highly ornamented. Spores function as storage structures, resting stages and propagules.

8) **Sporocarps**

These are compound, unorganized structures containing many spores. Sporocarp may accommodate specialized hyphae and can be sometime encased in an outer layer known as peridium.

9) **Vesicles**

Vesicles are sac like hyphal swellings of varying shapes that are believed to act as the storage organ for food as they are rich in lipids and glycolipids (Mosse, 1973). They are formed terminally, intercalary or laterally on an undifferentiated non-gametangial hyphae. Initially they are thin- walled, but later develop smooth to ornamented thick layers (Wu et al., 1995). Vesicles remain viable for long periods (Diop et al., 1994) and can act as infective propagules.

**HOST SPECIFICITY**

AM fungi are strictly obligate biotrophs feeding on the products of photosynthesis of their host. AM symbiosis is the widest spread symbiosis in natural ecosystems (Smith and Smith, 2011). As AM fungi are cosmopolitan in distribution, not only bound to particular group of plants but can be found extensively associated with Pteridophytes, Gymnosperms and Angiosperms. It is also found associated with gametophytes of some mosses, Psilotales and Lycopods, which do not have true roots (Pocock and Duckett, 1985) and even in aquatic plants (Beck-Nielsen and Madsen, 2001). AM fungi colonize most of the plants except some members of the families Brassicaceae, Chenopodiaceae, Cyperaceae, Amaranthaceae, Pinaceae, Betulaceae, Juncaceae, Proteaceae and Polygonaceae. These members oppose
formation of AM fungi association due to the presence of some fungitoxic substances in the root cortical tissues or through their secretion in the rhizosphere. AM fungi growth response can be influenced by genotype and developmental stages of the partners, the environmental conditions and community interactions (Facelli et al., 2010) and growth response can be positive, negative or even neutral. The host specificity of AM fungi could contribute to the maintenance of diversity within fungal community (Bever et al., 2001).

**BIOLOGY OF AM FUNGI**

Life cycle of AM fungi involves: A) Establishment of the symbiosis (propagule activation, host search, appressorium formation, root penetration and arbuscule formation), B) Vegetative growing phase (intra and extraradical mycelium growth, and an overall increase of fungal biomass, formation of mycelial structures and expansion of the AM fungi colonization within and between plants) and C) Reproductive phase (formation of reproductive structures- Resting spores). Symbiotic association establishment between AM fungi and host root involves exchange of signals between the symbionts, leading to the expression of certain genes and programmed cellular events (Harrison, 2005; Bonfante and Genre, 2008). The fungal hyphae after entering in the cortical cells with subsequent branching resulting in highly branched hyphal structures called as arbuscules. Vesicles are initiated soon after the initiation of arbuscular colonization, but continue to develop when the arbuscules senesce. At the same time root colonization is accompanied by the development of an extraradical mycelium that includes characteristic branched structure (Mosse and Hepper, 1975). The external spores develop on some of these branched structures completing the fungal life cycle. Spores may occur inside roots as well as in soil and can survive in adverse conditions. The timing of the onset of sporulation varies with species and in most of the cases it occurs within 3 to 4 weeks after the onset of mycorrhizal colonization.

**ECOLOGY OF AM FUNGI**

AM fungi occur in most ecosystems including dense rain forests, scrub, savanna, open woodlands, grasslands, heaths, sand dunes, deserts and more commonly in agricultural lands. They are virtually ubiquitous and so have a broad ecological range (Mosse and Thompson 1981), being present in temperate, tropical, subtropical, arid, semi-arid and arctic regions of the earth. There is considerable evidence that AM fungi have been reported to occur in most ecosystem including tropical forests (Bagyaraj et al., 2002) and subtropical forests (Sharma et al., 1987). Various factors like change in pH, temperature, soil moisture content and soil
depth etc., influence the distribution of AM fungi. Soil pH from acidic to neutral, high temperature and light intensity are known to increase AM fungus sporulation (Cardoso et al., 2003; Koide and Mosse, 2004). Spore of *Acaulospora laevis* showed optimum germination at pH 4.5, and this germination capacity was reduced by 10% in neutral or alkaline conditions (Hepper, 1984). In contrast, some *Glomus* spp. showed maximum germination at neutral to alkaline pH (Siquera et al., 1984). It is believed that most of the AM fungi are present at the depth of 0-30 cm of soil and their population decreases as soil depth increases (Higo et al., 2013). Extra matrical mycelium of AM fungi has been shown to be important in the binding sand and thus in the stabilization of dunes and sandy soil. Development of soil aggregates is also dependent upon binding by mycorrhizal hyphae. AM species richness also decreases with increase in the altitude (Gaur and Kaushik, 2011a). Chen et al. (2012) showed that agriculture practices and cropping time also play a major role in regulating mycorrhizal communities. Adverse conditions like water logging, water stress, salinity, presence of heavy metals in the soil and soil erosion also affects the presence of AM fungi.

**TAXONOMY OF AM FUNGI**

The original taxonomy of the AM fungi was based on morphology of the large soil borne spores which were found near colonized plant host's roots. Distinguishing AM fungi spore characteristics used in classification are cell wall morphology, size, shape, colour, hyphal attachment and reaction to staining compound (Wright, 2005).

AM fungi of the phylum Zygomycota were placed within the order Glomales, divided in two suborders (Glomineae and Gigasporineae) by Morton and Benny (1990) on the basis of spore morphology and spore formation characteristics. Suborder, Glomineae consisted of the type family Glomaceae with two genera *Glomus* and *Sclerocystis* and the Acaulosporaceae family with *Acaulospora* and *Entrophospora*. Suborder, Gigasporineae was proposed to include type family Gigasporaceae with the genera *Gigaspora* and *Scutelllospora*. Redecker et al. (2000) by utilizing morphological and molecular data, transferred *Sclerocystis* to the genus *Glomus* thereby eliminating the genus *Sclerocystis* from AM fungi. Schüßler et al. (2001) by using molecular data established the relationship among AM fungi and between other fungi. He divided Glomeromycota phylum into 4 orders (Archaeosporales, Paraglomerales, Diversisporales and Glomerales) and 12 genera. In 2001, Morton and Redecker erected two new families in the order Glomales (now Glomerales): Archaeosporaceae and Paraglomaceae based on data from molecular, morphological and biochemical investigation with one genus in each family i.e., *Archaeospora* and *Paraglomus*. 
Now, the classification system substantially changed in recent years (Schüßler et al., 2001; Sharma et al., 2008; Schüßler and Walker, 2010; Oehl et al., 2011a). According to Oehl et al. (2011a, b) and Goto et al. (2012), there are currently three classes, 15 families and 31 genera in the phylum Glomeromycota. Several genera have increased in the number of known species over the past few years, such as *Ambispora*, *Diversispora*, *Racocetra*, and *Septoglomus* (Gamper et al., 2009; Oehl et al., 2011a; Palenzuela et al., 2011) that include approximately 10-40 species each.

**Table-1: Classification of AM fungi based on Redecker et al., (2013)**

**Phylum: Glomeromycota, Class: Glomeromycetes**

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<tr>
<th>Orders</th>
<th>Families</th>
<th>Genera</th>
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<td>Glomerales</td>
<td>Glomeraceae</td>
<td><em>Glomus</em> &lt;br&gt; <em>Funnelliformis</em> &lt;br&gt; <em>Rhizophagus</em> &lt;br&gt; <em>Sclerocystis</em> &lt;br&gt; <em>Septoglomus</em></td>
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<td>Claroideoglomeraceae</td>
<td><em>Claroideoglomus</em></td>
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<td>Gigasporaceae</td>
<td><em>Cetraspora</em> &lt;br&gt; <em>Denticutata</em> &lt;br&gt; <em>Gigaspora</em> &lt;br&gt; <em>Racocetra</em> &lt;br&gt; <em>Scutellospora</em></td>
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<td>Acaulosporaceae</td>
<td><em>Acaulospora</em></td>
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<td>Diversisporales</td>
<td>Pacisporaceae</td>
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<td></td>
<td>Diversisporaceae</td>
<td><em>Corymbiglomus</em> &lt;br&gt; <em>Diversispora</em> &lt;br&gt; <em>Otospora</em> &lt;br&gt; <em>Redeckera</em> &lt;br&gt; <em>Tricispora</em></td>
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<td>Sacculosporaceae</td>
<td><em>Sacculospora</em></td>
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<td>Paraglomerales</td>
<td>Paraglomeraceae</td>
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<td>Geosiphonaceae</td>
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<td>Archaeosporales</td>
<td>Ambisporaceae</td>
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<td>Archaeosporaceae</td>
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The brief description of some AM genera is given below:

1. *Glomus*

The genus includes both sporocarpic and non-sporocarpic species. Those with chlamydospores develop the spores terminally on a single undifferentiated non-gametangial hyphae. In sporocarpic species, the spores are arranged not in orderly manner except a few species. The hypha subtending the spore differentiates at the same rate and synthesizes the same component layers as that found in the spore wall. Spores developed by blastic expansion of a hyphal tip and the outer layers of spores slough as the spores ages.

2. *Sclerocystis*

The species of *Sclerocystis* AM fungi are only sporocarpic with spores arranged in an orderly manner around a central plexus of sterile hyphae.

3. *Gigaspora*

*Gigaspora* means giant spore and all known species produce spores without ornamentation. These spores consist of a bilayered spore wall and germination takes place directly through the spore wall. Germ tube arises from a thin papillate layer arising from inner surface of the laminate layer. Auxillary cells are thin walled and echinuate. Spores development proceeds blastically from a hyphal tip and become the sporogenous cell.

4. *Scutellospora*

*Scutellospora* means shield spore. This genus includes those species which produce spores that germinate by means of a ‘germination shield’. Spores may be with or without ornamentation. Spore is mostly bilayered or one to three walls layered. These wall layers are flexible. Thin walled auxillary cells with smooth to knobby surface are produced on hyphae. *Scutellospora* species produce azygospores terminally on a large swollen suspensor like cell.

5. *Acaulospora*

*Acaulospora* means sessile spores. *Acaulospora* is characterized by production of azygospores. The spore is developed from the side of the saccule as a small swelling and as the swelling enlarges, the contents of the saccule are poured into the spore and the saccule disappears. In some species saccule may get completely detached and produce sessile spore.

6. *Entrophospora*

*Entrophospora* means spore nourished from within. These species develop azygospores but in different manner as compared to *Acaulospora*. Azygospores are produced from a sporiferous saccule but inside its neck, rather than from its side. Later on contents of the saccule are poured into the spore and saccule get collapsed.
7. *Archaeospora*

*Archaeospora* spores are either monomorphic or dimorphic. Only acaulosporoid spores are formed when the spores are monomorphic, both acaulosporoid and glomoid spores are formed when the spores are dimorphic. In *Archaeospora* spores, the spore wall is multilayered and includes a thick flexible innermost layer.

8. *Paraglomus*

This genus is strictly monomorphic and is glomoid in formation and structure. They differ from *Glomus* spores, as colonization occurs in patches.

**FUNCTIONS OF ARBUSCULAR MYCORRHIZAL FUNGI**

Arbuscular mycorrhizal fungi are one of the most important beneficial microorganisms in the rhizosphere. AM fungi infect almost all the plants. The AM fungi penetrate the living cells of plants without harming them and form the typical organs such as vesicles and arbuscules in the root. By doing so, the fungi link plant and soil, transporting mineral nutrients, especially phosphorus, to plant and carbon compounds to the soil and its biota. This symbiotic association offers several manifold benefits to ecosystem, some of which are explained below:

1. **AM in nutrient uptake**

The primary role of arbuscular mycorrhiza is to increase the absorption and translocation of essential nutrient. Plant species are benefited from mycorrhizal association because of greater efficiency in nutrient uptake (Harley, 1989; Thakur and Sharma, 2013). Arbuscular mycorrhizal roots have enhanced nutrient absorption ability due to morphological and physiological changes in the root system (Clarke and Zeto, 2000). This includes effective absorption surface area because of proliferated hyphae, exploration of greater soil volume, longevity of absorbing roots, enhanced utilization of available nutrients and better retention of soluble nutrients (Sharma *et al*., 2014). Benefits of AM association includes improved uptake of Phosphorus (P) (Koide, 1991; Chandreshkara *et al*., 1995; Fattah, 2013), Zinc (Zn) (Kothari *et al*., 1991; Jamal *et al*., 2002), Nitrogen (N) (Ames *et al*., 1983), Copper (Cu), (Faber *et al*., 1990), Potassium (K) (Clarke and Zeto, 2000) and Iron (Caris *et al*., 1998). AM plants accumulate large quantities of some micronutrients under conditions of low soil nutrient availability (Faber *et al*., 1990)

**Phosphorus uptake**

One of the most dramatic effects of mycorrhizal infection on host plant is increase in the phosphorous (P) uptake (Bolan, 1991). Phosphorus is a major plant nutrient required in
relatively very small amount and plays a vital role in all biological functions. The mycorrhizal roots invade the nutrients depletion zone surrounding roots and capture the available phosphorous even in phosphorous deficient soil (Koch et al., 1997; Kapoor and Mukerji, 2000; Xavier and Germida, 2003; Bagheri et al., 2012; Fattah, 2013). This is because large soil volume becomes available to phosphorous and then to plants through the extended hyphae of AM fungi. Mycorrhizal fungi produce phosphatase and organic acids that allows utilization of organic phosphorous under the humid tropical conditions.

**Nitrogen uptake**

Nitrogen is required for the synthesis of amino acids, purines, pyrimidines and thus indirectly involved in protein and nucleic acid synthesis. AM fungi enable the host plant to access nitrogen in an organic form that would be unavailable (Mukopadhyay and Maiti, 2009). The hyphae of AM fungi have potential to extract nitrogen and transport it from the soil to the plant. AM fungi improve growth, nodulation and nitrogen fixation in legume-Rhizobium symbiosis. Mycorrhizal association has tendency to supply more than 50 per cent of nitrogen required by plant (McFarland et al., 2010).

2. **AM in Water Uptake**

AM fungi exhibit an important role in water uptake. Mycorrhizal plants show better adaptations to adverse conditions as compared to non-mycorrhizal plants (Rani et al., 1998; Takacs and Voros, 2003). AM fungi bind soil into semi stable aggregates. Increased aggregation of silt, clay and loamy soil by AM fungi makes the soil more porous with greater water permeability than uninoculated soil. AM fungi promote root growth, more extensive exploration of soil volume resulting in more flow of water (Mukopadhyay and Maiti, 2009). AM fungi also reduce the water stress by increasing stomatal conductance and photosynthetic activity (Dell’ Amico et al., 2002) and by more effective scavenging of soil water.

3. **AM in biochemical and physiological properties**

AM fungi affect the plant hormone level by exerting the control over the root morphology (Selvaraj, 1998). Manoharachary et al. (2009) observed an increase in growth hormones such as Indole Acetic acid (IAA), Gibberellins, Cytokinins, Auxin and growth regulators such as Vitamin B in AM inoculated plants as compared to non-mycorrhizal ones. Increase in phytohormone activity could elevate photosynthetic rates by stomatal opening influencing ion transport and regulating chlorophyll levels (Allen et al., 1982). Selvaraj (1998) noticed an increase in photosynthetic activity in leaves of AM inoculated plants. It was also reported that AM fungi improve the enzymatic machinery of the enzyme involved in absorption, translocation and assimilation of major mineral ions. Increased activities of anti-oxidant
enzymes like superoxidizedimutase, polyphenoloxidase and phosphatase enzyme in arbuscular
mycorrhizal plants was observed by Aziz et al. (2011).

4. AM as biocontrol agent
Biocontrol is defined as the reduction in the inoculum density or disease producing activity of
pathogens, accomplished naturally or through addition of one or more organisms other than
man himself (Baker and Cook, 1974). Plant roots colonized by AM fungi remain functional
longer than the non-mycorrhizal roots and are less susceptible to the attack by certain types of
pathogens. American et al. (2001) observed that AM fungi are able to postpone the onset of
wilting in maize plant. Nehra et al. (2003) studied the role of AM fungi in controlling root
knot nematode on ginger. Aggarwal et al. (2006) and Sharma et al. (2007) also found VAM
as biocontrol against soil borne plant pathogens and root rot of Acacia nilotica respectively.

5. AM in plant drought resistance
A drought resistant plant would be one that exhibits behaviour closer to that of amply water
controls. AM colonization can modify a species capacity for drought resistance, avoidance
and tolerance. Arbuscular mycorrhizal colonization can help plants to cope with drought
stress by maintaining detrimental effect caused by water stress (Medina and Azcon, 2010).
They increase drought resistance by enhancing plant nutrition and water uptake (Levy and
Krikom, 1980; Safir and Nelson, 1985) or increased stomatal conductance through regulation
of absissic acid or cytokinin level or osmoregulation (Allen and Boosalis, 1983).

6. Salinity stress tolerance
Salinity in the soil affects the establishment, growth and development of plants leading to
huge losses in crop productivity (Evelin et al., 2009). AM fungi can reduce the impact of
salinity due to improved mineral nutrition and physiological processes like photosynthesis or
water use efficiency, and a better osmotic adjustment by accumulation of compatible solutes
such as proline, glycine, or soluble sugars, higher K⁺/Na⁺ ratios and compartmentalization of
sodium within some plant tissues (Ruiz-Lozano et al., 1996; Giri et al., 2003; Al-Karaki,
2006; Porcel and Aroca, 2012; Kadian et al., 2013). Pandey and Tarafdar (2002) reported
improvement in survival of AM fungi treated Azadirecta indica under high salinity level.
Wild plants have enormous ability to establish mycorrhizal association under saline
environment as AM fungi are efficient saline tolerant (Selvaraj and Chellapan, 2006).

7. Reclamation of wastelands and adverse site
The use of arbuscular mycorrhizal fungi in reclamation of wasteland has been shown. The
AM fungi have dramatic role in faster forestation in barren lands, degraded and arid lands
(Srivastava et al., 1996; Dixon et al., 1997). AM fungi helps the plant in increasing the
fertility and high productivity of soil (Mukerji and Dixon, 1992; Rani et al., 1998). Role of AM fungi in afforestation is reported by Barna (2002).

8. Soil conservation

Soil-aggregate stability can be efficiently enhanced and maintained by AM fungi (Dalpe and Monreal, 2004). AM fungi produce immuno-reactive glycoprotein, Glomalin, extracellularly on the mycelia in the bulk soil (Wright and Upadhyaya, 1996) thus improving aeration and water percolation and stabilize soil particles into aggregates (Miller and Jastrow 1990; Borie et al., 2006) and reduce erosion potential (Sharma and Adholeya, 2010).

9. Phytoremediation

Heavy metals in the soil have detrimental effects on ecosystem when present in high concentration (Göhre and Paszkowski, 2006). The AM fungi play an important role in phytoremediation directly or indirectly by different processes including phytostabilization, phytoextraction or phytodegradation (Vosatka et al., 2006; Muthukumar and Bagyaraj, 2010). The significance of AM fungi in soil remediation has been recognized by several workers (Srinath et al., 2003; Al-Garni, 2006; Wang et al., 2006; Kapoor et al., 2007). AM plants can reduce translocation of metals from roots to shoots by binding heavy metals on the cell wall of the hyphae in the root (Mukopadhyay and Maiti, 2009). Joner et al. (2000) showed the metal binding capacity of AM mycelium.

10. AM as biohardening tool

Micropropagation has become an important method of propagating plants (Chu and Kurtz, 1990). Transferring micropropagated plants from tubes to in vivo conditions is normally difficult, because they have a poorly developed cuticle (Wetzstein and Sommer, 1982), functionally impaired stomata (Lee and Wetzstein, 1988), and a poorly developed root system (Pierik, 1987). AM fungi modify root architecture for better nutrient uptake thereby envisaging physical, chemical and environmental conditioning of the micro-propagated plantlets (Berta et al., 1995).

11. AM in petal senescence and vase life

Vase life of cut flower is most attractive and economic components of cut flower (Kazami and Ameri, 2012). AM fungi play an important role in increasing vase-life of cut flowers and shelf life of loose flower by reducing ethylene production (Besmer and Koide, 1999) and increasing water absorption. Under low availability of water, O2 can produce reactive oxygen species (ROS) such as superoxide (O2•−), H2O2 and highly toxic hydroxyl radicals (OH•) resulting in petal senescence. AM fungi check the formation of free radicals by increasing antioxidants enzymes. Bhalla et al. (2006) observed improved vase life with the addition of
AM fungi. Scagel (2004) found that flowers on AM fungi inoculated plants generally lasted longer than non-mycorrhizal plants. Meir et al. (2010) observed an increase in the vase life of *Eustoma grandiflorum* due to AM fungi.

### 12. AM in ecosystem

AM fungal diversity is the major factor in maintenance of plant diversity and ecosystem stability. AM fungi alter the plant community structure by affecting the relative abundance of plant species (Sander and Koide, 1994). Another mechanism by which AM fungi may affect plant-species diversity is the differential growth response of plant species to colonization by AM fungi, the so-called ‘mycorrhizal dependence’ (Habte and Manjunath, 1991).

AM fungal hyphae are conduits that play an important role in nutrient cycling by transporting carbon from plant roots to other soil organisms, thus promoting soil C sequestration in agricultural systems (Dalpe and Monreal, 2004).

### 13. Interaction of AM with beneficial micro-organisms

A change in the amount or composition of root exudates and fungal exudates due to existing Arbuscular mycorrhizal colonization determines the diversity and abundance of the bacterial community in the rhizosphere (Maschener and Timonen, 2004; Sharma and Adholeya, 2010). Mycorrhizal colonization can also increase the nodulation and symbiotic nitrogen fixation in mycorrhizal legumes (Hamel, 2004). There is also evidence that, soil micro-flora such as plant growth promoting rhizobacteria, soil pseudomonads, phosphate solubilizing bacteria and acid producing bacteria affect mycorrhizal development on plant roots and the survival of arbuscular mycorrhizal fungi in one way or other (Singh and Kapoor, 1998).

Keeping all these facts in view, the present investigation was therefore, undertaken in order to find out the mycorrhizal association of ornamental flowering plants of Haryana. The present work entitled, **“BIODIVERSITY AND RESPONSE OF ARBUSCULAR MYCORRHIZAL FUNGI ON SOME ORNAMENTAL FLOWERING PLANTS OF HARYANA”**, includes the following objectives:

1. Survey and enumeration of ornamental flowering plants of Haryana.
2. Biodiversity of Arbuscular Mycorrhizal Fungi (AMF) associated with some ornamental flowering plants of Haryana.
3. Mass multiplication of dominant AM fungi associated with some ornamental flowering plants of Haryana using different hosts and substrates.
4. Effect of AM fungi alone and in combinations with *Trichoderma viride* and *Pseudomonas fluorescens* on growth parameters of *Chrysanthemum indicum*, *Gerbera jamesonii*, *Gladiolus huttoni* and *Tagetes erecta*.

   a) In pots under polyhouse conditions

   b) On *Chrysanthemum indicum* under field conditions

5. Influence of bioinoculants treated plants, growth regulators and nutrients on vase life regulation of *Chrysanthemum indicum*, *Gerbera jamesonii* and *Tagetes erecta*.

6. Effectiveness of AM fungi alone or with *Pseudomonas fluorescens* fortified with different concentrations of superphosphate on growth parameters of *Chrysanthemum indicum*, *Gerbera jamesonii*, *Gladiolus huttoni* and *Tagetes erecta*. 
DESCRIPTION OF SELECTED ORNAMENTAL FLOWERING PLANTS

1. *Chrysanthemum indicum* Linn.

   **Family** : Asteraceae

   **Common name** : Mums, Guldaudi

*Chrysanthemum* is one of the leading commercial floriculture crops in India as well in north eastern states (Verma et al., 2012). *Chrysanthemum* plant is not very attractive but it produces most showy flowers. It is grown in pots to decorate verandahs, window gardens and to create colourful islands in the lawn. It is a short- day plant and grown for cut flower production and as well as potted flowering plant for exhibition and decoration. Flower occurs in various colours. Flowers are used in hanging baskets. It can also be used to create many amazing plant forms, large disbudded blooms, spray forms as well as many artistically trained forms.

   Medicinally, stem, leaves and flowers of *Chrysanthemum* are used to treat various infectious diseases such as pneumonia, colitis, stomatitis and fever. Leaves prescribed for migraine. It’s flowers are boiled to make a sweet drink known as *Chrysanthemum* tea which has many medicinal uses, including an aid in recovery from influenza.

2. *Gerbera jamesonii* Linn.

   **Family** : Asteraceae

   **Common name** : African daisy

*Gerbera jamesonii* known as Transvall Daisy or Barberton Daisy is a tender perennial having brilliantly- coloured disc-shaped flowers. It is of commercial significance and fifth most used cut flower in the world (Parthasarathy and Nagaraju, 1999; Anisha, 2011). The plants are
stemless, dwarf, 30-45 cm tall, and are hairy throughout. The leaves are 12-20 cm long, 5-7 cm broad, deeply lobed, with a fairy long (15 cm) petiole. Flowers are daisy-like, 7-10 cm across. It’s flowers are hard and stand the rigours of transportation and have a comparatively long vase life and hence the flowers are very popular for garden decoration as well as vase. Gerbera jamesoni makes ideal ornament ground covers in garden beds and borders. It is used in floral arrangements, indoor decoration, as gifts for special occasions and as wedding bouquets. In India, it is fast catching up among the general circles of Indian public (Thomas et al., 2004) and fetches a good market price.

Plant extract of Gerbera is used in cosmetic and bath preparation showing skin moisturizing and conditioning effects. It is also used to purify or clean air. In, The National Aeronautics and Space Administration (NASA), Gerbera was proven effective in removing benzene, formaldehyde and dichloroethylene.

3. *Gladiolus huttoni* Linn.

**Family** : Iridaceae

**Common name** : Sword lily

*Gladiolus* is popularly known as queen of the bulbous flowers and occupies a prominent position among the cut flowers owing to the elegant appearance of its spike (Bose et al., 2003) and most valuable flowering bulbs (Khattab et al., 2000). Leaves are sword-like. The spike is slender and the florets have a hooded upper petal. It is commercially important flower both in domestic and international market (Chanda et al., 2000). *Gladiolus* is ideal as cut flower. It is also very good for beds, herbaceous borders, and does quite well in pots. It also has multiutility in bouquet and flower arrangements.

Medically, *Gladiolus* is used in treating a variety of ailments including diarrhoea and cold. It is common component of African herbalists medicine, the 'Lenaka'. It is also used in treating dysentery, constipation and conjunction. Patient feel well after taking *Gladiolus* and is often prescribed as a booster for patients.

**Family** : Asteraceae

**Common name** : Marigold, Gainda

Marigold is one of the easiest annual flowers to cultivate and have wide adaptability to different soil and climatic conditions. These are tall-growing plants having large flowers measuring about up to 15 cm. The plants, with their attractive flower colours, bloom for a considerably long period and flowers keep remarkably well when cut.

Marigold as one of the most popular flowering plants is suitable for potted plant, bedding, edging, garland making, religious offering and for making different products (Swaroop et al., 2007). It is ideal for cut flowers. Sometime, the whole plant is cut and used for decoration. Due to its huge potential in value addition, it is gaining industrial importance (Swaroop et al., 2007). Aerial parts of plant contain high quality of essential oil that are used in soaps, perfumery, cosmetic and pharmaceutical industries (Abdul-Wasaea et al., 2011).