SUMMARY

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.

Flowers symbolize purity, beauty, peace, love, and passion. 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. 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. 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.

Haryana 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, Kamal, 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 fungi. 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. AM fungi are environment compatible and economically feasible alternatives for improving crop production. Inoculation of AM fungi 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.

AM fungi interact with microorganisms, which develop activities that involved in plant growth and plant protection. Mycorrhizal mycelium releases energy-rich organic compounds responsible for an increased growth and activity of rhizosphere microorganisms. Mycorrhizal inoculation improved the establishment of both inoculated and indigenous phosphate-solubilizing rhizobacteria. On the other hand many microorganisms can also benefit mycorrhiza formation and functioning. Some biologically active substances such as amino acids, plant hormones and vitamins are produced by soil microorganisms which can stimulate the growth rates of mycorrhizal fungi. One important example among the beneficial effects is that exerted by the so-called mycorrhiza helper bacteria known to stimulate mycelial growth of AM fungi and enhance mycorrhizal formation. These non-symbiotic beneficial rhizobacteria, that colonize the root system and affect the plant growth favourably, are called as Plant Growth Promoting Rhizobacteria (PGPR). These bacteria solubilize organic and inorganic phosphate from soil particles by releasing organic acids and make P as well as micronutrients more readily available for plant growth. Similarly, Pseudomonas fluorescens improves the plant growth parameters and hence called plant growth promoting rhizobacteria. Fungus, Trichoderma has been reported to have both antagonistic and stimulatory effects on AM fungi. It is considered as wonder organism because different species act as biological control agents of plant diseases; produce cellulolytic, hemicellulolytic, chitinolytic and proteolytic enzymes; capable of biodegrading chlorophenolic compounds helpful in soil bioremediation and also produce secondary metabolites with antibiotic activity.

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*.

**Objective-1**

**Survey and enumeration of ornamental flowering plants of Haryana**

Although several studies have been done for the enumeration and distribution of flowering plants in India but only a few have highlighted the enumeration and distribution of ornamental flowering plants in Haryana. Due to large demands of ornamental flowering plants, it is very important to know about presence and distribution of ornamental flowering plants in different parts of the Haryana and to provide knowledge to flower growers that which area is suitable for particular flowering plant. Therefore, the purpose of the present study was to visit different areas of Haryana and relocate maximum number of ornamental flowering plants with emphasis on documenting their local name, family and economic importance.

A total of 13 districts of Haryana were surveyed for collecting samples of ornamental flowering plants. Out of these 13 districts, 11 districts showed maximum production of ornamental flowering plants in Haryana. Main flower production in these 11 districts were *Gladiolus huttoni*, *Tagetes erecta*, *Chrysanthemum indicum*, *Polianthes tuberosa*, *Dianthus caryophyllus*, *Lilium longiflorum* and *Rosa indica*. A total of 52 plants were collected which were identified and their economic importance was studied. The collected plants belong to thirty one families, of which twenty seven were grouped under dicot group and four were under monocot group. Asteraceae was found to be the dominating dicot family having 12 genera followed by Rubiaceae (4 genera), Apocynaceae (4 genera), Amaranthaceae (2 genera) and Malvaceae (2 genera) while in monocot group Liliaceae and Amaryllidaceae was dominant families having two genera each.
The findings of this study enhanced the knowledge on various ornamental flowering plants growing in Haryana. This study revealed that Haryana is rich in ornamental flowering plants and it is necessary for further studies to validate their uses in cosmetics, vegetables as well as in potential medicine.

Objective-2

Biodiversity of Arbuscular Mycorrhizal (AM) fungi associated with some ornamental flowering plants of Haryana

Plant diversity and plant community structure are influenced by arbuscular mycorrhizal (AM) fungal communities. AM fungi play a significant role in ecosystem processes, such as soil aggregation and nutrient cycling. Once the roots are colonized by AM fungi, plants appear to be able to regulate further colonization through the exudates released. After some time these fungi form structures i.e. mycelium, vesicles and arbuscules. Arbuscules are tree like structures formed by the repeated branching of the hyphae when it enters in a cell. Vesicles are like an oval bag and used as storage organ.

Arbuscular mycorrhizal diversity and richness is measured in terms of extent of root colonization and spore number. AM fungal composition is governed by a number of interacting factors like soil type and texture, soil nutrient, moisture content, temperature and host plant genotype. The quantitative as well as qualitative composition of AM fungi results from the complex fungus, host plant and habitat interaction. Agroecosystem plant species, soil type, weeds and previous crop also have an effect on AM fungal population. Species composition and richness is also influenced by the depth of the sampling plant.

Therefore, different districts of Haryana (Faridabad, Kurukshetra, Yamunanagar, Panchkula, Ambala, Karnal, Kaithal, Panipat, Sonipat, Jind, Rohtak, Gurgaon and Mewat) were surveyed for the collection of soil samples and roots of ornamental flowering plants.

The present objective reveals the status of root colonization as well as sporulation of AM fungi associated with selected 52 ornamental flowering plants. While assessing root colonization, presence of mycelium, arbuscules and vesicles were observed. Mycelium of various kinds (H-shaped, Y-shaped, coiled and parallel) was seen in the studied root samples. Root colonization ranged from 0 to 100 per cent. Plants showing very high root colonization were *Lilium longiflorum*, *Narcissus jonquilla*, *Gladiolus huttoni*, *Heliconia pendula*, *Lagerstroemia indica*, *Tagetes erecta*, *Aloe vera*, *Arctotis stoechadifolia*, *Aster amellus*, *Chrysanthemum indicum* and *Dahlia rosea*. Least mycorrhizal root colonization was recorded in *Dianthus caryophyllus*, *Gaillardia aristata*, *Iberis amara* and *Plumeria obtusa*, while in
Mesembryanthemum crystallinum, AM root colonization was absent. Different shapes of vesicles like elliptical, round, globose, oval, beaked and elongated were reported. Arbuscules were also found.

Rhizospheric soil sample of each plant were processed and number of AM spores present in 50 gm of soil sample were counted. The spore number ranged from 9.00±1.00 to 470.00±5.50. Narcissus jonquilla showed highest spore number while lowest spore number was recorded in Mesembryanthemum crystallinum. Among fifty two plant species investigated, nineteen plants species showed spore number below 150, eighteen plant showed between 150-200, seven species showed between 200-250, six species between 250-300, and two plant species showed above 300 spores per 50 gm of soil sample.

A total of eighty five species of six genera i.e. Glomus, Acaulospora, Gigaspora, Entrophospora, Scutellospora and Sclerocystis were reported. Dominance of genus Glomus was evident but the genus Acaulospora was also present in abundance. The genera Gigaspora and Sclerocystis were next in abundance. Three species of Entrophospora and two species of Scutellospora were present.

Objective-3

Mass multiplication of dominant AM fungi associated with some ornamental flowering plants of Haryana using different hosts and substrates.

AM fungi are known as obligate symbions and are difficult to multiply under laboratory condition. The reason for the obligate biotrophy is that AM fungi lost some of its carbon fixing abilities or the genetic machinery that supports them during the long evolution of symbiotic relationship with the host. AM fungi are known to completely dependent upon host plant for fixing carbon supply. Significant development of AM fungi to complete life cycle is only achieved in the presence of compatible host plant.

Several culture techniques based on this constraint are applicable for commercial scale production of the inoculum. The most widely used technique is pot culture, where the fungi are usually maintained and multiplied in conjunction with suitable host plant roots and substrate. Pot culture is the classical, cheapest and most preferred method of multiplication for large scale production of AM fungal propagules. Addition of any substrate to the soil mixture should also promote the growth of host plant and avoid harmful or detrimental effect on the inoculum.

Therefore in this objective, three different host plants namely Maize (Zea mays),
Lemon grass (Cymbopogon nardus) and Barley (Hordeum vulgare) were used as trap plant for multiplication of Glomus mosseae and Acaulospora laevis in the presence of different concentrations of two substrates i.e., textile effluent (textile waste) and sunflower seed waste with six concentration (Without substrate, 25, 50, 75, 100 and 200 gm/pot).

Regarding A. laevis, when Barley and Maize were used as host and sunflower seed waste as substrate, maximum AM root colonization, AM spore number, plant height, shoot weight and root weight were found maximum at 100 gm of substrate while for Lemon grass 75 gm of concentration was most effective. When textile waste was used as substrate for mass production of A. laevis with Barley, Maize and Lemon grass as host plants in different concentrations, it was observed that all treated plants showed positive response with respect to different parameters. While using Barley and Lemon grass as host, maximum AM spores and root colonization were recorded in plants treated with 75 gm of textile waste followed by 50 gm. Plant height, shoot weight and root weight were also maximum in plants amendment with 75 gm followed by 50 gm and 25 gm of textile waste. When Maize was used as host plant for AM fungal propagation, 50 gm concentration of textile waste resulted in maximum spore count as well as highest root colonization.

For mass multiplication of A. laevis, while comparing all the three hosts, Maize proved to be better host than Barley and Lemon grass as it enhanced maximum AM root colonization and spore production. Among the two substrates used, sunflower seed waste was found to be most suitable substrate for maximum AM root colonization and sporulation.

Inoculum production of G. mosseae also varied considerably with different hosts and substrates. When Barley was used as host and sunflower seed waste as substrate, maximum root colonization as well as spore number was recorded at 200 gm of substrate concentration. Likewise, plant height, shoot biomass and root biomass was also maximum at 200 gm followed by 100 and 75 gm of sunflower seed waste. While using Maize and Lemon grass as host plants, maximum root colonization, spore number, plant height, shoot biomass and root biomass (fresh and dry) were maximum at 100 gm concentration of sunflower seed waste. Textile waste was also tried for mass culturing of G. mosseae. When Barley was used as host, maximum root colonization as well as spore number was recorded highest at 100 gm concentration of substrate. While using Maize and Lemon grass as host plants, AM root colonization as well as spore number was maximum at 75 gm concentration of substrate. Plant height, shoot biomass and root biomass also showed an enhancement at this concentration.
Among both the substrates tested for mass multiplication of \textit{G. mosseae}, sunflower seed waste proved to be the best for AM root colonization and spore population. Similarly, out of the three hosts, Barley resulted in maximum mycorrhization and spore production.

**Objective-4**

Effect of AM fungi alone and in combinations with \textit{Trichoderma viride} and \textit{Pseudomonas fluorescens} on growth parameters of \textit{Chrysanthemum indicum}, \textit{Gerbera jamesonii}, \textit{Gladiolus huttoni} and \textit{Tagetes erecta}.

\begin{itemize}
  \item[a)] In pots under polyhouse conditions
  \item[b)] On \textit{Chrysanthemum indicum} under field conditions
\end{itemize}

Cultivation of non-conventional crops has great prospects to meet financial needs of farming community. In this regard ornamental flowering plants have attracted farmers globally. \textit{Chrysanthemum}, which occupies a prominent place in ornamental horticulture, is one of the most commercially exploited flower crop. Extract of \textit{Chrysanthemum} plants (stem and flower) have been shown to have a wide variety of potential medicinal properties. \textit{Gerbera} is one of the ten most popular cut flowers in the world and according to global trends in floriculture; it occupies the fourth place in cut flowers. In India, it is fast catching up among the general circles of Indian public and fetches a good market price. \textit{Gladiolus} also occupies a prominent position among the cut flowers owing to the elegant appearance of its spike and most valuable flowering bulbs. It is commercially important flower both in domestic and international market. \textit{Tagetes} too as one of the most popular flowering plant is suitable for potted plant, bedding, edging, garland making, religious offering and for making different products. Aerial parts of plant contain high quality of essential oil that are used in soaps, perfumery, cosmetic and pharmaceutical industries.

Hence, this investigation was carried out to check the effect of two AM fungi i.e. \textit{Glomus mosseae} and \textit{Acaulospora laevis}, phosphate solubilizing bacteria (\textit{Pseudomonas fluorescens}) and \textit{Trichoderma viride} alone and in different combinations on different growth parameters and flowering of \textit{Chrysanthemum indicum}, \textit{Gerbera jamesonii}, \textit{Gladiolus huttoni} and \textit{Tagetes erecta} under polyhouse conditions. Effect of these bioinoculants was also seen on \textit{Chrysanthemum indicum} under field conditions.

In case of \textit{C. indicum}, among all the growth parameters, shoot height, number of flowers, phosphorus uptake both in shoot and root and total chlorophyll content were recorded highest in triple inoculation of \textit{G. mosseae} + \textit{A. laevis} + \textit{T. viride} while fresh and
dry shoot weight and stomatal conductance was found highest in plants inoculated with dual combination of *G. mosseae* + *T. viride*. *G. mosseae* along and in combination with *T. viride* enhanced some growth parameter but *G. mosseae* along with *A. laevis* and *T. viride* proved to be best combination for growth and flowering of *Chrysanthemum*.

In case of *G. jamesonii*, inoculation of *G. mosseae* + *T. viride* increased root length, fresh and dry root weight, mycorrhizal root colonization, number of flowers, chlorophyll content and phosphorus uptake while maximum number of leaves, fresh and dry shoot weight, AM spore count and stomatal conductance were observed maximum in the combination of *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens*. Maximum leaf area was present in *G. mosseae* + *A. laevis* + *P. fluorescens*.

In case of *G. huttonii* inoculation of *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* produced more number of flowers and daughter cormels, increased root length, fresh and dry root weight, mycorrhizal root colonization, AM spore count, phosphorus uptake and chlorophyll content. Shoot weight (fresh and dry) and stomatal conductance was maximum in the triple combination of *G. mosseae* + *A. laevis* + *T. viride*. Maximum height was observed in dual combination of *G. mosseae* + *T. viride*.

In case of *T. erecta* combination of *A. laevis* + *T. viride* + *P. fluorescens* was best as it increased root length, fresh and dry root weight, mycorrhizal root colonization, number of flowers, phosphorus uptake and chlorophyll content. Inoculation of *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* produced increased fresh and dry shoot weight, AM spore count while dual inoculation of *G. mosseae* + *A. laevis* increased maximum height. In case of field condition of *C. indicum*, most of morphological as well as physiological parameters were found maximum in triple combination of *G. mosseae* + *A. laevis* + *T. viride*.

The results of the present investigation showed an increase in different growth parameters and flowering of studied ornamental plant on inoculation of AM fungi and other bioinoculants. So this study provides a great potential for utilizing the efficient strains of mycorrhizal fungi to exploit them for the beneficial effects in establishment of seedlings, increase in productivity and reduce the fertilizer application required for obtaining economic production of these plants under poly-house and field conditions.

**Objective 5**

**Influence of bioinoculants treated plants, growth regulators and nutrients on vase life regulation of *Chrysanthemum indicum*, *Gerbera jamesonii* and *Tagetes erecta*.**
Vase life of cut flower is most attractive and economic component of cut flower. Consumers of cut flowers demand high quality flowers at the time of purchase and a guaranteed long vase life so that aesthetic value of the flowers will last. Commercial floriculture is one of the most profitable agro-industry in the world and in the floriculture industry. The quality of flowering crops is limited by its longevity, which is influenced by senescence. Wilting and senescence of the petals determine the longevity of the flower. The techniques of prolonging the vase life of cut flowers are a great asset to the growers and users. Beauty and fresh look of the flower can be retained only for a few days even when some chemical preservatives are used to prolong their vase life. Growth regulators [Kinetin (KN) and Salicylic acid (SA)], sugar, macronutrients, micronutrients etc. play an important in increasing vase life of cut flower. Similarly, beneficial microbes like arbuscular mycorrhizal fungi (AMF), Phosphate solubilizing bacteria (PSB) and Trichoderma viride also play an important role in increasing flower quality as well as vase life.

Therefore, present investigation was planned to compare the efficiency of two AM fungi (Glomus mosseae and Acaulospora laevis) alone and in combination with Pseudomonas fluorescense, Trichoderma viride, growth regulators and nutrients on the vase life of C. indicum, G. jamesonii and shelf life of T. erecta flowers.

Different parameters like visible effect, flower diameter, volume of holding solution, vase life and peroxidase activity, protein content, total and reducing sugar was recorded at 0, 8 and 16 days of holding cut flowers in 100 ml different solutions.

For C. indicum, triple combinations of G. mosseae + A. laevis + T. viride, was found to be most effective in increasing vase life, flower diameter and protein content etc. followed by G. mosseae + T. viride, G. mosseae (G), A. laevis (A) and kinetin. So, it is evident from our result that the vase life of Chrysanthemum flowers increased by inoculation of different bioinoculants as compared to the treated with different growth regulators and nutrients.

While for G. jamesonii, combination of G. mosseae + T. viride treated flowers showed increased flower diameter, vase life, protein content, slow down peroxidase activity, reduce the breakdown of total soluble sugar and reducing sugar. Sucrose was also effective in increasing vase life of cut gerbera flowers.

In case of T. erecta, A. laevis + T. viride + P. fluorescens bioinoculants treated flowers showed increased flower diameter, vase life, increased protein content, decreased peroxidase activity, reduced breakdown of sugar.
Cut flowers deteriorate very quickly and hence, to maintain freshness of flowers, they have to be treated with different plant growth regulators or nutrient solutions and even some biostimulants like arbuscular mycorrhizal fungi alone and along with some other beneficiary microorganisms like *T. viride* and *P. fluorescens*. It was found that pretreatment of plants in soil with AM fungi alone or in different combination with other microbes gave better results as compared to control and post harvest treatments of cut flower with different growth regulators and nutrients.

**Objective-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.*

Phosphorus is known as ‘the key of life’ because it is directly involved in most essential life processes. It is an integral component of several important compounds in the plant cells, including the sugar-phosphate intermediates of respiration and photosynthesis and the phospholipids that make up plant membranes. Phosphorus is essential for cell division, development of meristematic tissue and causing a stimulating effect on the number of floral buds and flowers. It influences many plant functions including flowering and fruiting, root development, disease resistant and maturation. Due to this P have a particular importance to plants grown for their flowers or to newly transplanted plants undergoing establishment.

Phosphate fertilizers in appropriate doses are important for getting better yield and growth of plants. But most of the grower seems to believe that high fertilizer supply can result in high crop yield. The repeated and injudicious applications of chemical P fertilizers, leads to the loss of soil fertility by reducing and disturbing microbial diversity, activity and consequently reducing yield of crops.

Therefore, AM fungal species used in the earlier objective along with *Pseudomonas fluorescens* were tested to find a fertilizers treatment adequate for supporting the presence and development of both partners of the symbiosis, resulting in improving the growth enhancement and flowering response of selected ornamental flowering plants i.e. *C. indicum, G. jamesonii, G. huttoni* and *Tagetes erecta*. The present investigation was therefore designed to evaluate the effect of various bioinoculants at different levels of superphosphate (low, medium and high) on various growth parameters, P acquisition and phosphatase activity of selected ornamental plants.
In case of *C. indicum* it was found that mycorrhizal inoculation (*G. mosseae* and *A. laevis*) with superphosphate and *P. fluorescens* increased the growth and physiological parameters of the plant in comparison to the control. The most effective results were observed in medium concentration where various bioinoculants were applied to the plant. Plant height, root biomass (fresh and dry), flower number, P content in shoots and roots, percent mycorrhizal root colonization and AM spore number was maximum in the dual combination of *A. laevis* + *P. fluorescens* followed by *G. mosseae* + *P. fluorescens*. Both the enzymes (acid phosphatase and alkaline phosphatase) were found most active in the dual combination of *A. laevis* + *P. fluorescens*. Maximum root length and leaf area enhancement was observed in combination of *G. fluorescens* + *A. laevis* + *P. fluorescens*.

In case of *G. jamesonii*, low concentration of superphosphate showed best result with combination of *G. mosseae* + *A. laevis* + *P. fluorescens*. Increased root length, fresh and dry root weight, number of flowers, AM root colonization, AM spore count, phosphorus uptake and phosphatase activity was maximum in this combination.

*G. huttoni* also showed best results in low concentration of superphosphate. Growth parameters i.e. height, root length, fresh and dry shoot and root weight, AM root colonization and spore number were maximum in dual combinations of *G. mosseae* + *P. fluorescens*.

In case of *T. erecta*, low concentration with *G. mosseae* + *A. laevis* + *P. fluorescens* was best. It increased root length, number of flowers, fresh and dry root weight, AM spore count, AM root colonization, phosphorus uptake and phosphatase activity.

Chemical fertilizers are expensive, pose a risk for pollution, produce short term benefits and additional threat of chemical fertilizers may reduce plant beneficial microorganisms. Besides the need of synthetic chemicals, there is also need for farmers to achieve better production in ornamental plants. Hence, it is recommended that application of AM fungi with PSB and *Trichoderma viride* as a biofertilizer for *C. indicum*, *G. jamesonii*, *G. huttoni* and *T. erecta* with aim of increasing productivity, vase life and reducing fertilizer use.