

Impact of *Arbuscular Mycorrhizal* Fungi and *Pseudomonas fluorescens* with Various Levels of Superphosphate on Growth Enhancement and Flowering Response of *Gerbera*

Karishma, Kuldeep Yadav, Anju Tanwar and Ashok Aggarwal*

Department of Botany, Kurukshetra University, Haryana 136119, India.

Received: 30 May 2013

Accepted: 20 July 2013

*Corresponding author's email: aggarwal_vibha@rediffmail.com; karishmabotany@rediffmail.com

Gerbera jamesonii is of commercial significance and fifth most used cut flower in the world today. A pot experiment was performed to see the effect of co-inoculation of *arbuscular mycorrhizal* fungi (AMF) i.e. (*Glomus mosseae* and *Acaulospora laevis*) with phosphate solubilizing bacteria *Pseudomonas fluorescens* in the presence of different doses of superphosphate (low, medium, high) on growth establishment and flowering response of *Gerbera*. Among all treatments, plants inoculated with mix culture of *G. mosseae* + *A. laevis* + *P. fluorescens* showed best response in terms of greater root length, root biomass, percent root colonization, AM spore number, number of flowers, phosphorus content and phosphatase activity at lower concentration of superphosphate. Moreover, maximum increase in leaf area and shoot biomass was found in plants treated with dual combination of *G. mosseae* + *P. fluorescens* at lower concentration of superphosphate. This study provides a good scope for commercially utilizing the efficient strains of AM fungi with *P. fluorescens* in the establishment and growth improvement of *Gerbera*.

Abstract

Keywords: *Gerbera jamesonii*, *Glomus mosseae*, *Acaulospora laevis*, *Pseudomonas fluorescens*, Growth response, Phosphatase activity.

INTRODUCTION

Gerbera jamesonii Bolus ex. Hook. (family Asteraceae) commonly known as Transvall Daisy or Barberton Daisy is a tender perennial having brilliantly- coloured disc-shaped flowers and leafless stems. Gerbera is very popular and widely used as a decorative garden plant. It is of commercial significance and fifth most used cut flower in the world (Parthasarathy and Nagaraju, 1999, Anisha, 2009). The flowers are hard and stand the rigorous of transportation and a long vase life fetches a good market price. In India, it is fast catching up among the general circles of Indian public (Thomas *et al.*, 2004). Thus, the improvement for quality attributes such as number of flowers, longevity and flower size are important economic goals.

Phosphorus (P) 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 (Taiz and Zeiger, 2003). Phosphorus is essential for cell division, development of meristematic tissue and causing a stimulating effect on the number of floral buds and balls per plant (Katkar *et al.*, 2002). Photosynthesis activity and stomatal conductance were reduced due to P deficiency (Vieira *et al.*, 1998).

Because of side effect of different agrochemicals, there is an increasing interest in understanding the co-operative activities of soil microbial population and their application in the field of agriculture (Lucy *et al.*, 2004). Two major groups of microbial inoculants that act as biofertilizers are *arbuscular mycorrhizal* fungi and phosphate solubilizing bacteria. AMF are very important symbiotic organisms of most terrestrial plants (Parniske, 2008). Beneficial interactions between AM fungi and horticultural crops have been well documented (Menge, 1983, Wang *et al.*, 2006). Mycorrhizal association increase plant growth (Wang *et al.*, 2006, Yadav *et al.*, 2012, Yadav *et al.*, 2013a) and productivity by increasing nutrient uptake (Pedraza *et al.*, 2001, Al- Karaki, 2002, Prasad *et al.*, 2012, Tanwar *et al.*, 2013), reducing injury of transplants (Menge *et al.*, 1978) and by improving resistance to biotic and abiotic stress factors (Chen *et al.*, 2006, Yadav *et al.*, 2013b). Furthermore, the symbiosis favours the development of more robust plants due to increased water absorption, hormone production, tolerance to adverse environmental conditions and pathogens (Lovato *et al.*, 1996).

Preferential application of AMF to horticulture crop is significant and realistic for their low cost and high economic output (Azcon-Aguilar and Barea, 1997). Sohan *et al.*, (2003) indicated that inoculation of AMF induced early flowering in *Chrysanthemum*. Liang *et al.*, (2010) observed increased number and size of *Zinnia* flower when inoculated with efficient strain of AMF.

AMF interaction with certain plant growth promoting rhizobacteria has been reported to enhance the activity of AMF and consequently plant growth (Sumana *et al.*, 2003). One of the most beneficial members of plant growth promoting rhizobacteria is phosphate solubilizing bacteria which increases the P availability in the soil (Barea *et al.*, 2005). Phosphate released by phosphate solubilizing bacteria (PSB) is taken up by mycorrhizal mycelium and this co-operative synergistic microbial interaction improves P acquisition by the plant (Barea *et al.*, 2005).

The objective of this study was 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 *Gerbera*. The present investigation was therefore designed to evaluate the effect of various bioinoculants at different levels of superphosphate on various growth parameters, P acquisition and phosphatase activity of *Gerbera*.

MATERIALS AND METHODS

Collection of soil sample

For isolation of dominant AMF, composite soil sample from rhizospheric soil of *Gerbera* was collected. It was done by digging out a small amount of soil close to the plant roots up to the depth of 15-30 cm and kept in sterilized polythene bags at 10°C for further processing.

Isolation and identification of dominant AMF spores

Isolation of AMF spores was done by using 'Wet Sieving and Decanting Technique' of

Gerdemann and Nicolson (1963). The quantification of dominant AMF spores was done by 'Grid line intersects method' (Adholeya and Gaur, 1994). These spores were picked up by hypodermic needle under stereobinocular microscope and identified with the help of identification manual of Walker (1983), Scheneck and Perez (1990) and Aggarwal *et al.*, (2012) on the basis of conventional morphological characters. *Glomus mosseae* and *Acaulospora laevis* were found to be the two dominating AMF strains.

Mass production of AMF spores

The dominant AMF obtained were purified by following the funnel technique (Menge and Timmer, 1982). The pure culture of *G. mosseae* and *A. laevis* were further mass multiplied in pots using sand: soil (1:3) as substrate and maize as host plant. During multiplication, host plants were nourished by Hoagland's nutrient solution (without P source) at the interval of 15 days up to three months.

Mass culture of *Pseudomonas fluorescens*

P. fluorescens (MTCC No. 103) was procured from Institute of Microbial Technology, Chandigarh, India and multiplied in nutrient broth medium (1.25 g peptone, 0.75 g beef extract, 1.25g NaCl, 250 mL distilled water) for 24 hrs for proper growth of bacteria.

Different concentrations of superphosphate

Granules of superphosphate were grounded using pestle and mortar to make it a fine powder. Then, different concentrations of superphosphate i.e., low, medium and high were used. Medium concentration is the recommended one (40 kg/ha), lower concentration is half of the recommended (20 kg/ha) and higher is the double dose of recommended one (80 kg/ha).

Experimental set up

The experiment was laid out in a randomized complete block design, with five replicates for each treatment. Soil was collected from botanical garden of Kurukshetra University, India having 20.8% silt, 3.78 clay, 8.05 pH, 0.0485 total N and 0.015 available P. Initially, sand: soil (1:3) mixture was passed through 2 mm sieve and then sterilized in autoclave for 20 minutes at 121°C and 15 psi. Earthen pots (25 × 25 cm) were taken and amended with air-dried sterilized soil: sand mixture (3:1). To this different levels of superphosphate were applied i.e. low (20 kg/h), medium (40 kg/h) and high (80 kg/h) concentrations. In each pot 10% of inoculum of each AMF (*G. mosseae*, *A. laevis*), and *P. fluorescens* alone and in combination were added. For single inoculation of AM fungi, 180 g of soil containing around 900 spores and colonized root fragments of maize plant with an infection level of 90-95% were added, while this quantity was reduced to half (90 g) for treatment with combined inoculation of both the AMF together. Afterwards, 10 ml of culture suspension of *Pseudomonas fluorescens* was mixed in each pot having cfu 1×10^9 /ml. Two seedlings of *Gerbera* were planted in each pot. The experiment was carried out in a polyhouse, where humidity was approximately 55-70% with temperature 25–30°C. Light was provided by cool white fluorescent lamps (8000 lux) under a 16-h photoperiod. The polyhouse also received natural sunlight. The effects of these bioinoculants alone or in combination at different levels of superphosphate were recorded on different growth parameters of *Gerbera* at the flowering stage.

Different treatments were used during the present investigation:

- 1- Control
 - 2- *Glomus mosseae*
 - 3- *Acaulospora laevis*
 - 4- *G. mosseae* + *P. fluorescens*
 - 5- *A. laevis* + *P. fluorescens*
 - 6- *G. mosseae* + *A. laevis* + *P. fluorescens*
- Five replicates of each treatment were taken.

Analysis of growth parameters

Plants were harvested at the flowering stage and then plant height as well as root length was measured with the help of a scale. For root and shoot fresh and dry weight, roots and shoots were harvested after 120 days, weighed for their fresh weight and then, oven dried at 70°C for dry weight. Leaf area was measured by using Leaf Area Meter 211 (Systronics Ltd., Ahmadabad, India). Estimation of phosphorus was done by 'vanadomolybdo phosphoric yellow colour method' (Jackson, 1973). Phosphatase activity was assayed by using p-nitrophenyl phosphate (PNPP) as substrate which is hydrolyzed by the enzyme to p-nitrophenol. For this ice cold sodium acetate buffer (0.05 M with pH 4.8) for acid phosphatase and sodium carbonate-bicarbonate buffer (0.05 M with pH 10) for alkaline phosphatase activity was used and was measured in terms of IU/g FW (Tabatabai and Bremner, 1969).

Quantification of AMF spores

It was done by Adholeya and Gaur 'Grid Line Intersect Method' (1994). Spores were counted under stereo binocular microscope by using a counter.

Mycorrhizal root colonization

Roots were washed from the soil, blotted dry for determination of root fresh and dry weight, P content and mycorrhizal root colonization. Mycorrhizal root colonization was done by 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970). Percent AM root colonization was determined as mentioned under:

Percentage root colonization=(Number of root segments colonized)/(total number of root segments studied)×100

Statistical analysis

All results were analyzed using Analysis of Variance (ANOVA), followed by post hoc test through computer software SPSS 11.5 version. Means were ranked at $p \leq 0.05$ level of significance using Duncan's Multiple Range Test for comparison.

RESULTS

Number of leaves

Inoculation of *Gerbera* with significant bioinoculants (AMF, *P. fluorescens*) at different levels of superphosphate significantly increased the leaves number over control. It is evident from Table 1 that at flowering stage, leaves were maximum in the dual combination of *A. laevis* + *P. fluorescens* followed by *G. mosseae* + *P. fluorescens* at lower concentration of superphosphate. Generally, a decline in the leaves number was noticed with an increment in the superphosphate level.

Leaf area

It was found that at flowering stage, maximum leaf area was found in the lower concentration of superphosphate with *G. mosseae* + *P. fluorescens* treatment followed by *G. mosseae* + *A. laevis* + *P. fluorescens* (Table 1).

Root length

The lower concentration of superphosphate was found to be more effective for root length increment. The uttermost increase in root length was observed in low concentration with *G. mosseae* + *A. laevis* + *P. fluorescens* treatment followed by dual combination of *G. mosseae* + *P. fluorescens*.

Shoot and root fresh and dry weight

Biomass of all the inoculated plants of *Gerbera* increased significantly in terms of fresh and dry weight with all the levels of superphosphate at the flowering stage. Maximum increase in

shoot biomass (fresh & dry) was recorded in the dual combination of *G. mosseae* + *P. fluorescens* at lower concentration of superphosphate followed by *G. mosseae* + *A. laevis* + *P. fluorescens* at medium concentration of superphosphate. While, the consortium treatment (*G. mosseae* + *A. laevis* + *P. fluorescens*) showed maximum increase in root biomass followed by *G. mosseae* + *P. fluorescens* at lower concentration of superphosphate.

Root colonization and AMF spore number

It is evident from Table 1, that maximal mycorrhizal population as well as root colonization was recorded in plants inoculated with mix culture of *G. mosseae* + *A. laevis* + *P. fluorescens* followed by dual combination of *G. mosseae* + *P. fluorescens* at lower concentration of superphosphate. The vesicles, arbuscules and hyphal infection were present in all the treatments least in control.

Number of flowers

Plants inoculated with *G. mosseae* + *A. laevis* + *P. fluorescens* treatment at half of recommended dose i.e. lower of superphosphate showed higher number of flowers followed by *G. mosseae* + *P. fluorescens*. Similarly, maximum increment in number of flowers at medium and higher concentration of superphosphate was also observed at *G. mosseae* + *A. laevis* + *P. fluorescens* treatment in comparison to control.

Phosphorus content and phosphatase activity

The P content in shoots and roots significantly increased in all the treated plants as compared to control at the flowering stage of *Gerbera* (Table 2). The low concentration of superphosphate with *G. mosseae* + *A. laevis* + *P. fluorescens* showed maximum P content in shoots. Second most effective results were observed in the combination of *G. mosseae* + *P. fluorescens* at low superphosphate concentration. Similarly, in roots, maximum P uptake was observed in *G. mosseae* + *A. laevis* + *P. fluorescens*. This combination was also effective in increasing shoot and root P content in medium and higher concentration of superphosphate. Plants inoculated with either of the AMF i.e. *G. mosseae* or *A. laevis* along with *P. fluorescens* at half of the recommended dose of superphosphate significantly increased both root acid phosphatase and alkaline phosphatase activity. Acid phosphatases were found to be more active than alkaline phosphatases. Alkaline and acid phosphatase activity was observed maximal in the consortium of *G. mosseae* + *A. laevis* + *P. fluorescens* followed by *G. mosseae* + *P. fluorescens* at all the levels of superphosphate with maximal at low concentration.

DISCUSSION

In the current study, mix inoculation of AMF and PSB phosphate at low concentration of superphosphate proved to be most effective than single (solo) inoculation in increasing growth, flowering and other physiological parameters of *Gerbera*. The phosphate solubilizing bacteria (PSB) behave as mycorrhiza helper bacteria by promoting the root colonization percent (Azcon-Aguilar and Barea, 1992). *Pseudomonas* could soften the cell walls and middle lamella between the cells of the root cortex by producing specific enzymes and thus making fungal penetration easier (Duponnois, 1992). Padamadevi *et al.*, (2004) also reported higher growth in *Anthurium* by application of PSB and AMF along with inorganic nutrients (N, P, K). Gaur *et al.*, (2000) also noted an increment in vegetative growth of *Petunia hybrida*, *Callistephus chinensis* and *Impatiens balsamina* inoculated with AMF and chemical fertilizers at low P level. AMF with PSB stimulate the nutrient uptake resulting in biosynthesis of various plant growth regulators (Azcon-Aguilar and Barea, 1992). An increased growth of AMF inoculated plants might be due to enhancement in the anabolic processes (especially photosynthesis) due to better uptake and mobilization of various essential nutrients and water (Sbrana *et al.*, 1994, Schmedit *et al.*, 2010). The increment of nutrient absorption in AMF inoculated plants may be due to increase root surface area, the physical exten-

sion of the mycorrhizal hyphae system, hyphae absorptive power and exploration of sites rich in nutrients (Bolan, 1991).

The mycorrhized plants have a higher number of flowers, a characteristic which is highly important in ornamental plant production. The positive increment in flower production by the application of bio-fertilizers may be due to the increase in availability of micro and macro nutrients to the plants resulting in enhancement of hormonal activities within the plant.

The effectiveness of lower concentration of superphosphate in increasing the growth parameters may be due to the direct effect of superphosphate fertilizers or indirectly through the microbial propagation activation i.e. AMF, PSB. Inoculation of plants with both the AMF and *P. fluorescens* resulted in the highest mycorrhizal colonization might be due to synergistic interaction between the AM fungi and *P. fluorescens*. Higher sporulation and root colonization increased fungal host contact and the exchange of nutrients. Das *et al.*, (2007) reported the positive influence of AMF along with rhizobacteria on AMF root colonization. However root colonization and AMF population decreased with increase in concentration of phosphate fertilizer. Excess concentration of phosphate reduces the carbohydrate supply to the endo-mycorrhizal fungi resulting in reduction in its beneficial activity (Koide, 1991). High P levels inhibit AM fungi more directly by reducing spore germination and hyphal growth from the germinated spores (Zubek *et al.*, 2012). A wide range of microorganisms including mycorrhizas are known to have the ability to solubilize inorganic P from insoluble sources. In the current study, acid and alkaline activity was higher at low concentration of superphosphate. The reason could be that phosphatase induced in the presence of AM fungi, especially *Glomus* spore are sensitive to the higher level of phosphate in the environment (Pacovsky *et al.*, 1991).

The acid phosphatase activity was much greater than alkaline phosphatase activity. It is believed that acidic phosphatase was involved in the increased uptake of phosphorus from the soil, while alkaline phosphatase may be linked to active phosphate assimilation or transport in mycorrhizal roots. According to Kumar *et al.*, (2008) the acid phosphatase activity actually increases with increased root colonization by AMF. Supatra and Mukherji (2004) reported that enhanced phosphatase activities resulted in an increment in P availability in P-deficient soil. With low phosphorus availability, P demand increases, resulting in an increase in the phosphatase activity. Acid phosphatase may be associated with the growth and development of the fungus within the host tissue (Gianinazzi *et al.*, 1979) as well as with phosphorus acquisition in the rhizosphere.

CONCLUSION

Inoculation of AM fungi with *P. fluorescens* helps in effective utilization of superphosphate by changing it into available forms, later taken up by the plants for their better growth and development. On the basis of results, it can be concluded that inoculation of *Gerbera* with bioinoculants (*G. mosseae* + *A. laevis* + *P. fluorescens*) at lower concentration of superphosphate provides a great future for utilizing the efficient AMF fungi and *P. fluorescens* for the beneficial effect in the growth establishment and flowering response of *Gerbera*.

ACKNOWLEDGEMENTS

The authors are thankful to Kurukshetra University, Kurukshetra, India for providing basic infrastructure and laboratory facilities to carry out the research work.

Litrature Cited

- Adholeya, A. and Gaur, A., 1994. Estimation of VAM fungal spores in soil. Mycorrhiza News. 6, 10-11.
- Aggarwal, A., Tanwar, A. and Neetu Mehrotra, R.S. 2012. Taxonomy of *Arbuscular mycorrhizal* fungi with special refernce to *Glomus* species: A review. Microbial Diversity and Functions, Edited by D.J. Bagyaraj. K. V. B. R. Tilak and H. K. Kehri, NIPH, Delhi.
- Al- Karaki, G.N. 2002. Growth, sodium and potassium uptake and translocation in salt stressed tomato. J. Plant Nut. 23, 369-379.

- Anisha, P.N. 2009. Studies on inducing variability *In vitro* and use of mycorrhizae in hardening of gerbera. M.Sc. thesis. University of Agricultural Sciences, Dharwad, India.
- Azcon-Aguilar, C. and Barea, J.M. 1992. Interactions between mycorrhizal fungi and other rhizosphere microorganism. In: Mycorrhizal functioning: an integrative plant-fungal process. Allen, M.J. (ed.), Capman and Hall, New York, N.Y., pp. 163-198.
- Azcon-Aguilar, C. and Barea, J.M. 1997. Applying mycorrhiza biotechnology to horticulture: significance and potentials. *Sci. Hortic.* 68, 1-24.
- Barea, J.M., Pozo, M.J., Azcon, R. and Azcon-Aguilar, C. 2005. Microbial co-operation in the rhizosphere. *J. Exp. Bot.* 56, 1761-1778.
- Chen, B.D., Zhu, Y.G. and Smith, F.A. 2006. Effect of *arbuscular mycorrhizal* inoculation on uranium and arsenic accumulation by Chinese brake fern (*Pteris vittata* L.) from uranium mining impacted soil. *Chemosphere* 62, 1464-1473.
- Duponnois, R. 1992. Les bacteries auxiliaires de la mycorrhization on douglas (*Pseudotsuga menziesii* (Mirb.) France par *Laccaria laccata* souche S 238. There del' Universite' de Nancy I. France.
- Gaur, A., Gaur, A. and 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 Hort.* 84, 151-162.
- Gerdemann, J.W. and Nicolson, Y.H. 1963. Spores of mycorrhizae *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.* 46, 235-244.
- Gianiazzi, S., Gianiazzi-Pearson, V. and Dexheimer, J. 1979. Enzymatic studies on the metabolism of vesicular- *arbuscular mycorrhizal*. III. Ultrastructural localization of acid and alkaline phosphatase in onion roots infected by *Glomus mosseae* (Nicol. and Gerd.). *New Phytol.* 82, 127-132.
- Jackson, M.L. 1973. Soil chemical analysis. Published by Printice Hall, New Delhi, p 485.
- Katkar, R.N., Turkhade, A.B., Solanke, U.M., Wankhade, S.T. and Sakhare, B.A. 2002. Effect of foliar sprays of nutrients and chemicals on yield and quality of cotton under rainfed condition. *Res. on Crops.* 3(1), 27-29.
- Koide, R.T. 1991. Nutrient supply, nutriend demand and plant response to mycorrhizal infection. *New Phytol.* 117, 365-386.
- Kumar, K.V.C., Chandrashekhar, K.R. and Lakshmipathy R. 2008. Variation in *arbuscular mycorrhizal* fungi and phosphatase activity associated with *Sida cardifolia* in Karnataka. *World J. Agric. Sci.* 4, 770-774.
- Liang-Kun Long., Qing Yao., Yong-Heng Huang., Rui-Heng Yang., Jun Guo. and Hong-Hui Zhu. 2010. Effect of *arbuscular mycorrhizal* fungi on *Zinnia* and the different colonization between *Gigaspora* and *Glomus*. *World J. Microbiol. Biotechnol.* 26, 1527-1531.
- Lovato, P.E., Gianiazzi-Pearson, V., Trouvelot, A. and Gianiazzi, S. 1996. The state of art of mycorrhizas and micropropagation. *Adv. Hort. Sci.* 10, 46-52.
- Lucy, M., Reed, E. and Glick, B.R. 2004. Application of free living plant growth- promoting rhizobacteria. *Anton. Leeuw. Int. J. Gen. Mol. Microbiol.* 86, 1-25.
- Menge, J.A., 1983. Utilization of vesicular-*arbuscular mycorrhizal* fungi in agriculture. *Can. J. Bot.* 61, 1015-1024.
- Menge, J. A., Davis, R.E., Johanson, E.L.V. and Zentmeyer, G.A. 1978. Mycorrhizal fungi increases growth and reduce transplant injury in avocado. *Calif. Agr.* 32, 6-10.
- Mengue, J.A. and Timmer, L.M. 1982. Procedure for inoculation of plants with VAM in the laboratory, greenhouse and field. In: *Methods and principles of mycorrhizal research*, (Ed.). Schenck, N.C., American Phytopath. Soc., St. Paul, pp. 59-68.
- Pacovsky, R.S., De Silva, P., Carvalho, M.T.V. and Tsai, S.M. 1991. Growth and nutrient allocation in *Phaseolus vulgaris* L. colonized with endomycorrhizae or Rhizobium. *Plant Soil.* 132, 127-137.
- Padmadevi, K., Jawaharlal, M. and Vijayakumar, M. 2004. Effect of biofertilizers on floral characters and vase life of *Anthurium andreaeanum* Lind.) cv. Temptation. *South Indian Hort.* 52, 228-231.

- Parniske M., 2008. *Arbuscular mycorrhiza: the mother of plant root endosymbiosis*. Nat Rev Microbiol. 6, 763-775.
- Parthasarthy, V.A. and Nagaraju, V. 1999. *In vitro* propagation in *Gerbera jamesonii* Bolus. Indian Journal of Horticulture. 56, 82–85.
- Pedraza-Santos, M., Jaen-Contreras, D., Guitierrez-Espinosa, A., Colinas-Leon, T. and Lopez- Peralta, C. 2001. Growth and nutrition of gerbera microplants inoculated with *Arbuscular mycorrhizal* fungi. Agrociencia. 35, 151-158
- Philips, J.M., Hayman D.S., 1970. Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55, 158-161.
- Prasad, K., Aggarwal, A., Yadav, K. and Tanwar, A. 2012. Impact of different levels of superphosphate using *arbuscular mycorrhizal* fungi and *Pseudomonas fluorescens* on *Chrysanthemum indicum* L. J. Soil Sci. Plant Nut., 12 (3), 451-462
- Sbrana, C., Giovannetti, M. and Vitagliano, C. 1994. The effect of mycorrhizal infection on survival and growth renewal of micropropagated fruit rootstocks. Mycorrhiza. 5, 153-156.
- Schemidt, B., Domonkos, M., Sumalan, R. and Biro, B. 2010. Suppression of *arbuscular mycorrhiza*'s development by high concentration of phosphorus at *Tagetes patula* L. Res. J. Agr. Sci. 42, 156-162.
- Schenck, N.C. and Perez, Y. 1990. Manual for the identification of VA mycorrhizal (VAM) fungi. University of Florida, Synergistic Pub Florida, USA, 241 pp.
- Sohan, B.K., Kim, K.Y., Chung, S.J., Kimc, W.S., Park, S.M., Kang, J.G., Rim, Y.S., Cho, J.S., Kim, T.H. and Lee, J.H. 2003. Effect of the different timing of AMF inoculation on plant growth and flower quality of *Chrysanthemum*. Sci. Hortic. 98,173-183.
- Sumana, D.A., Bagyaraj, D.J. and Arpana, J. 2003. Interaction between *Glomus mosseae*, *Azotobacter chroococcum* and *Bacillus coagulans* and their influence on growth and nutrition of neem. J. Soil Biol. Ecol. 23, 80-86.
- Supatra, S. and Mukherji, S. 2004. Alterations in activities of acid phosphatase, alkaline phosphatase, ATPase and ATP content in response to seasonally varying Pi status in okra (*Abelmoschus esculentus*). Journal of Environmental Biology. 25, 181-185.
- Tabatabai, M.A. and Bremner, J.M. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Bioch, 301-307.
- Taiz, L. and Zeiger, E. 2003. Plant Physiology 3rd edn, pp750-751.
- Thomas, C.D., Cameron, A. and Green, R.E. 2004. Extinction risk from climate change. Nature. 427, 145-148.
- Walker, C. 1983. Taxonomic concepts in the Endogonaceae spore wall characteristics in species description. Mycotaxon. 18, 443-445
- Wang, F.Y., Lin, X.G., Yin, R. and Wu, L.H. 2006. Effect of arbuscular mycorrhizal inoculation on the growth of *Elsholtzia splendens* and *Zea mays* and the activities of phosphatase and urease in a multi-metal- contaminated soil under unsterilized conditions. App. Soil Ecol. 31, 110-119.
- Yadav, K., Aggarwal, A. and Singh, N. 2013a. *Arbuscular mycorrhizal* fungi (AMF) induced acclimatization, growth enhancement and colchicine content of micropropagated *Gloriosa superba* L. plantlets. Ind. Crop Prod. 45, 88-93.
- Yadav, K., Aggarwal, A. and Singh, N. 2013b. *Arbuscular mycorrhizal* fungi (AMF) induced acclimatization and growth enhancement of *Glycyrrhiza glabra* L. - a potential medicinal plant. Agric. Res. 2, 43-47
- Yadav, K., Singh, N. and Aggarwal, A. 2012. *Arbuscular mycorrhizal* (AM) technology for the growth enhancement of micropropagated *Spilanthes acmella* Murr. Plant Prot. Sci. 48, 31-36.
- Zubek, S., Mjelcarek, S. and Tarnau, K. 2012. Hypericin and pseudohypericin concentrations of valuable medicinal plant *Hypericum perforatum* L. are enhanced by *arbuscular mycorrhizal* fungi. Mycorrhiza. 22, 149-156.

Table 1. Inoculative effect of AM fungi, *P. fluorescens* and superphosphate on growth parameters of *Gerbera jamesoni* at flowering stage.

Concentration	Treatments	No. of leaves/plant	Leaf area (sq.cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	AM Spore number/10 gm of soil	AM Root colonization (%)	No. of flowers
Low	Control	9.8±1.92 ^{hi}	40.86±1.68 ⁱ	20.06±1.86 ^{hi}	12.36±0.52 ^{hi}	3.48±0.31 ⁱ	6.12±0.48 ^{hi}	3.54±0.29 ^{hi}	20.4±1.94 ^m	19.00±1.61 ^k	1.40±0.54 ^e
	<i>G. mosseae</i>	14.8±1.92 ^d	55.58±1.26 ^{ef}	25.00±1.40 ^{ef}	18.24±1.16 ^{cd}	5.82±0.23 ^d	9.18±1.15 ^e	5.14±0.29 ^d	124.4±5.31 ^e	77.84±3.01 ^d	2.8±0.44 ^e
16mg/kg	<i>A. laevis</i>	13.2±1.48 ^{de}	61.88±2.30 ^f	25.32±2.34 ^{def}	16.20±0.43 ^{ef}	5.00±0.27 ^f	8.32±0.21 ^f	4.48±0.37 ^e	94.8±6.61 ^f	73.14±1.95 ^e	2.6±0.89 ^e
	G + P	21.0±2.91 ^{ab}	84.96±2.55 ^a	32.26±1.21 ^a	22.54±2.12 ^a	7.12±0.31 ^a	12.98±0.94 ^b	6.28±0.66 ^b	133.2±5.35 ^b	87.82±2.12 ^b	4.8±0.83 ^b
Medium	A + P	23.2±2.86 ^a	78.46±2.29 ^c	26.42±2.66 ^{de}	21.50±1.00 ^b	6.08±0.23 ^{cd}	11.42±0.74 ^c	5.68±0.39 ^c	112.4±5.50 ^d	75.84±1.64 ^{de}	4.4±0.54 ^b
	G + A + P	19.4±1.14 ^{bc}	82.66±1.50 ^{bc}	32.68±1.14 ^a	22.08±1.71 ^{ab}	6.30±0.21 ^c	14.38±1.16 ^a	6.86±0.47 ^a	142.8±4.60 ^a	90.22±3.53 ^a	6.2±0.83 ^a
Conc.	Control	6.4±1.40 ^{ik}	32.88±2.02 ^k	13.28±1.331	8.74±0.65 ^k	1.22±0.14 ^k	3.94±0.23 ^{kl}	1.60±0.231	17.2±3.70 ⁿ	16.22±1.381	1.0±0.70 ^q
	<i>G. mosseae</i>	9.4±1.94 ^{hi}	53.84±1.55 ^{gh}	23.24±2.23 ^{fg}	15.90±0.36 ^{fg}	4.58±0.21 ^g	6.30±0.26 ^{gh}	3.40±0.29 ^{gh}	81.4±4.92 ^g	57.46±2.63 ^f	1.4±0.54 ^{de}
32mg/kg	<i>A. laevis</i>	10.2±2.28 ^{gh}	60.34±1.09 ^f	20.14±1.52 ^{hi}	13.20±1.13 ^g	4.02±0.16 ^h	5.48±0.51 ^{ij}	2.52±0.43 ^h	70.4±2.30 ^h	49.80±1.61 ^h	0.4±0.54 ^{de}
	G + P	13.6±1.81 ^{de}	81.00±1.93 ^b	24.34±0.37 ^{ef}	18.84±1.25 ^c	5.74±0.41 ^e	6.96±0.45 ^e	4.00±0.49 ^f	98.6±4.77 ^e	55.32±7.42 ^e	1.6±0.54 ^d
High	A + P	12.8±2.28 ^{ef}	72.46±1.88 ^d	28.28±1.18 ^{ce}	17.18±1.84 ^{de}	4.94±0.28 ^f	6.86±0.23 ^e	3.88±0.55 ^{ef}	71.6±4.27 ^e	55.98±3.14 ^e	0.8±0.83 ^{de}
	G + A + P	17.8±1.48 ^c	75.14±2.05 ^d	29.80±0.55 ^b	22.10±0.71 ^{ab}	6.66±0.32 ^b	10.92±1.17 ^d	5.72±0.23 ^c	129.0±5.74 ^{bc}	81.88±2.62 ^c	2.6±0.54 ^c
Conc.	Control	4.4±1.14 ^k	27.56±0.551	16.20±1.37 ^k	3.94±0.301	0.74±0.201	3.64±0.581	1.48±0.39 ^m	10.4±1.81 ^e	9.18±1.34 ^m	-
	<i>G. mosseae</i>	6.8±1.92 ^k	45.08±1.81 ⁱ	21.44±2.69 ^{gh}	9.96±0.451	3.18±0.28 ^{hi}	4.38±0.31 ^{kl}	1.98±0.31 ^{ij}	46.8±3.11 ^j	36.42±2.131	-
64mg/kg	<i>A. laevis</i>	5.4±1.941	51.96±1.78 ^h	18.30±0.831	9.46±0.231	3.02±0.191	4.06±0.35 ^{klm}	1.74±0.20 ^k	35.4±4.151	30.10±2.721	-
	G + P	9.6±1.81 ^{hi}	70.70±1.71 ^e	19.96±1.40 ^{ij}	12.18±0.44 ^{hi}	4.06±0.30 ^h	4.64±0.32 ^{kl}	2.28±0.14 ^{hi}	51.8±4.021	49.00±2.69 ^h	0.4±0.54 ^{hi}
F values	A + P	8.2±1.09 ^{ij}	62.30±2.51 ^f	23.68±1.13 ^f	10.06±0.30 ^f	3.06±0.30 ^f	5.20±0.46 ^{jk}	2.52±0.25 ^h	40.6±1.67 ^k	35.44±4.36 ⁱ	0.6±0.44 ^{hi}
	G + A + P	11.8±1.78 ^{fg}	72.62±1.26 ^d	26.78±2.19 ^{cd}	14.92±0.39 ^e	4.48±0.34 ^e	6.62±0.27 ^e	3.04±0.33 ^{gh}	58.2±3.19 ⁱ	49.66±4.74 ^h	0.8±0.83 ^{de}
L.S.D(P≤0.05)		2.397	2.351	2.965	1.297	0.346	0.810	0.476	5.472	3.423	0.786
ANOVA (F)		40.819	426.807	50.314	141.572	207.549	127.117	100.591	486.919	426.247	40.990
Fertilizer (f)		340.985	5330.531	140.133	579.085	5994.225	439.892	195.537	682.013	6675.046	371.349
Parameter(p)		122.005	2181.621	52.926	411.632	526.231	346.172	203.526	2031.607	666.113	21.479
(f x p)		4.062	16.606	24.473	5.538	17.484	14.185	10.306	101.312	32.101	10.221

G = *Glomus mosseae*, A-*Acaulospora laevis*, P-*Pseudomonas fluorescens*, ± -Standard deviation, *The mean difference is significant at 0.5 level. Mean value followed by differ alphabets within a column do not differ significantly over one other at P≤0.05 lead by Duncan's Multiple Range Test Indicates the level of significance at p≤0.001 level

Table 2. Effect of AM fungi, *P. fluorescens* and superphosphate on phosphorus uptake and phosphatase activity of *Gerbera jamesoni* at flowering stage.

Concentration	Treatments	% Phosphorus		Phosphatase activity (IU/g FW)	
		Shoot P	Root P	Alkaline Phosphatase	Acidic Phosphatase
Low Conc. 16mg/kg	Control	0.414±0.047 ^k	0.456±.044 ^j	0.184±.006 ^j	0.205±.005 ^h
	<i>G. mosseae</i>	0.904±.051 ^d	1.026±.067 ^d	0.336±.009 ^d	0.347±.006 ^d
	<i>A. laevis</i>	0.836±.082 ^{de}	0.946±.057 ^c	0.323±.008 ^c	0.334±.004 ^c
	G + P	1.136±.056 ^b	1.235±.055 ^b	0.384±.009 ^b	0.405±.006 ^b
	A + P	1.067±.056 ^c	1.151±.034 ^c	0.367±.003 ^c	0.392±.006 ^c
Medium Conc. 32mg/kg	G + A + P	1.269±.036 ^a	1.314±.031 ^a	0.404±.005 ^a	0.422±.004 ^a
	Control	0.342±.040 ^{lm}	0.364±.041 ^k	0.155±.0111	0.166±.005 ⁱ
	<i>G. mosseae</i>	0.741±.026 ^{fg}	0.881±.051 ^f	0.306±.011 ^f	0.332±.006 ^c
	<i>A. laevis</i>	0.573±0.45 ^{ij}	0.596±.049 ⁱ	0.261±.005 ^h	0.296±.005 ^g
	G + P	0.782±.056 ^{ef}	0.889±.036 ^{ef}	0.329±.006 ^{de}	0.355±.007 ^d
High Conc. 64mg/kg	A + P	0.619±.049 ^{hi}	0.661±.033 ^h	0.291±.006 ^f	0.318±.005 ^f
	G + A + P	1.022±0.56 ^c	1.200±.033 ^{bc}	0.378±.007 ^b	0.394±.006 ^c
	Control	0.281±.041 ^m	0.281±.0281	0.119±.005 ^m	0.109±.0071
	<i>G. mosseae</i>	0.414±.045 ^k	0.478±.064 ^j	0.165±.007 ^k	0.143±.007 ^j
	<i>A. laevis</i>	0.380±.040 ^{kl}	0.387±.053 ^k	0.138±.0031	0.124±.003 ^k
F values	G + P	0.672±.073 ^{gh}	0.729±.053 ^g	0.207±.005 ⁱ	0.200±.006 ^h
	A + P	0.532±.035 ^j	0.562±.034 ⁱ	0.178±.005 ^j	0.163±.005 ⁱ
	G + A + P	0.684±.030 ^g	0.836±.040 ^f	0.271±.011 ^g	0.293±.008 ^g
	L.S.D(P≤0.05)	0.632	0.581	0.0097	0.0078
	ANOVA (F)	168.869	245.217	735.765	1438.837
	Fertilizer (f)	434.533	2154.069	3848.300	2753.508
	Parameter(p)	591.074	379.808	1059.482	831.358
	(f x p)	15.322	25.394	102.315	75.287

G = *Glomus mosseae*, A-*Acaulospora laevis*, P-*Pseudomonas fluorescens*, ± -Standard deviation, *The mean difference is significant at 0.5 level.

Mean value followed by differ alphabet/s within a column do not differ significantly over one other at P≤0.05 lead by Duncan's Multiple Range Test Indicates the level of significance at p≤0.001 level.