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Response of arbuscular mycorrhizal fungi along with *Trichoderma viride* and *Pseudomonas fluorescens* on the growth, biochemical attributes and vase life of *Chrysanthemum indicum*

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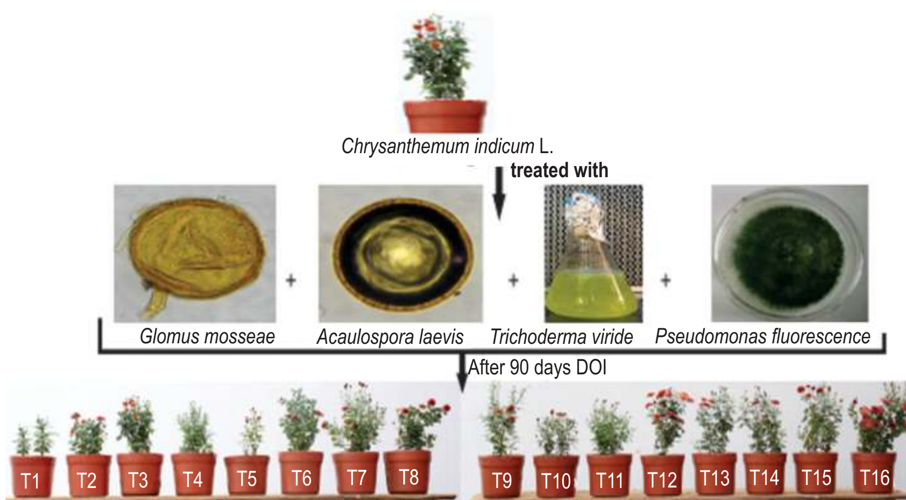
Abstract

Aim : The present study aimed to investigate the effects of two dominant arbuscular mycorrhizal (AM) fungi along with *Trichoderma viride* and *Pseudomonas fluorescens* on the growth and vase life of *Chrysanthemum indicum* ('Garden Mum'- Kathleen Dark Red).

Methodology : An experiment was conducted to evaluate the effect of *G. mosseae* (G) and *A. laevis* (A) along with *T. viride* (T) and *Pseudomonas fluorescens* (P) on the growth and vase life of *C. indicum* L. under polyhouse conditions. The experiment was laid in a randomized block design with five replicates.

Results : AM fungi along with other bioinoculants showed maximum root colonization leading to increased water absorption and various important nutrients, especially phosphorous, thereby enhancing the growth and different biochemical attributes. For the vase life experiment, bioinoculants treated plants showed better result with minimum peroxidase activity, thereby delaying flower senescence.



T1: Control, T2: *G. mosseae.*, T3: *A. laevis.*, T4: *T. viride.*, T5: *P. fluorescence.*, T6: G+A, T7: G-T, T8: GIP, T9: A+T, T10: A+P, T11: T+P, T12: G+A+T, T13: G+T+P, T14: G+P+A, T15: A+T+P and T16: G+A+T+P

Interpretation : AMF inoculation should be recommended at nursery level as biofertilizers are cost effective and also a substitute for chemical fertilizers.

Key words: Arbuscular mycorrhizal fungi, Growth parameters, *Pseudomonas fluorescens*, Vase life

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Introduction

Flowers not only beautify the landscape where they are grown but are used in various ceremonial activities like worship, marriages, social and religious functions. Flowers and floral products are also used as a source of medicine, fragrance and as a flavouring agent. Floriculture has become a source of livelihood for millions of people all over the world. Demand of cut flowers including *Chrysanthemum*, Rose, *Gladiolus*, Tulip, Marigold, Carnation, Lily, etc. are increasing day by day at international level (Zaidi *et al.,* 2016). To meet this demand, growers use chemical fertilizers to increase the productivity. Prolonged use of chemical fertilizers have resulted in uneven distribution of nutrients in the soil affecting the growth of non-target microorganisms (Younis *et al.,* 2013). Thus, the floriculture industry is developing into a specialized profession and has made ample progress in last few years. In India, commercial cultivation of cut flower, especially *Chrysanthemum* is very expensive due to high input of chemical fertilizers for increasing vase life of cut flowers. Similarly, large number of chemical growth regulators are also used in enhancing the vase life of cut flowers. Nearly 20% of fresh flowers decline in quality during harvesting, packaging, transportation and marketing and a large deal of remaining flowers are sold at low quality conditions, dissatisfying the consumer (Asfanani *et al.,* 2008).

The vase life of cut flowers is a commercially important trait. When flowers are cut from the mother plant, they undergo water stress due to an imbalance between transpiration and water uptake affecting the vase life. There are number of growth regulators *viz.,* silver thiosulphate, gibberellins and cytokinins, accel, thidiazuron, salicylic acid and malic acid used to increase the vase life and to maintain good quality of cut flowers (Ferrante *et al.,* 2002; Mutui *et al.,* 2003; Jamshidi *et al.,* 2012).There is an urgent need to develop a low cost technology which may be eco-friendly and sustainable.

Arbuscular Mycorrhizal Fungi (AMF) have been recognized as a promising biofertilizers to improve the growth yield, flowering and vase life of majority of flowering plants (Karishma *et al.,* 2013). *Trichoderma viride* and *Pseudomonas fluorescens* also act as promising growth enhancers in some plants such as *Dianthus* and *Gladiolus* (Lo and Lin, 2002). The use of AMF to enhance growth has been reported on many flowers namely China aster (Nowak, 2009), *Zantedeschia* (Janowska *et al.,* 2013), *Tagetes patula* and *Salvia splendens* (Janowska and Andrzejak, 2017) etc. There is little information available regarding the use of AMF, *T. viride* and *P. fluorescens* as biofertilizers either alone or in combination on growth and vase life aspects of 'Garden Mum-Kathleen Dark Red' variety of *Chrysanthemum indicum.*

Keeping in view the above information, the present investigation was carried out to compare the efficiency of different bioinoculants (*G.mosseae, A. laevis,T. viride* and *P. fluorescence*) alone and in combination on growth and floral response of *C. indicum.* The effect of these bioinoculants and other growth regulators was also compared on the vase life of *C. indicum.*

Materials and Methods

Soil preparation: A pot experiment was conducted in the polyhouse of Department of Botany, Kurukshetra University Kurukshetra, Haryana, India at 25±5°C and 50-70% relative humidity. The soil used in this experiment consisted of 64.2% sand, 21.8% silt, 3.9% clay, 0.042% N, 0.017% available P, 0.06% organic carbon and pH 6.5. A ratio of sand and soil (3:1) was sieved through a 2 mm sieve and autoclaved (twice) at 121°C to eliminate indigenous microflora.

Experimental design

Growth and biochemical attributes: Inoculum preparation-Pure culture of *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *A. laevis* Gerd. & Trappe were used. Spores of selected AM fungi species were isolated from the rhizosphere of *C. indicum* plants grown in Kurukshetra University, Kurukshetra. Identification of spores was done by using the key of Walker (1983). After preparation of a starter inoculum using the Funnel technique of Menge and Timmer (1982), these spores (*Glomus mosseae* and *Acaulospora laevis*) were multiplied in standard pot culture using maize as a host because AMF are obligate biotrophs.

Mass multiplication of *T. viride* Pers. was done by a modified method using wheat bran-saw dust medium (Mukhopadhyay *et al.,* 1986), which was earlier isolated by using Soil Dilution Plate method (Johnson *et al.,* 1959) on Potato Dextrose Agar medium and identified using the manual of Leslie and Summerell (2006). *P. fluorescens* (MTCC No. 103) culture was procured from Institute of Microbial Technology, Chandigarh, India and multiplied using a nutrient broth medium which was then incubated at 32°C for 48 hrs to obtain a concentration of 1×10^{-9} colony ml^{-1}.

The experiment was laid out in a randomized block design with five replicates of each treatment. The earthenware pots were taken and filled with 2 kg soil: sand. To each pot, 10% (w/w) of AMF alone or in combination was added to the soil. The roots of maize with about 85% AM colonization were chopped up along with soil containing AM spores (650-680 per 10 g of inoculum) as AM inoculum. *T. viride* with density 3.4×10^{8} cfu g^{-1} inoculum was added per treatment. Treatment of *P. fluorescens* was given by dipping the roots of *C. indicum* in *P. fluorescens* medium having cfu 1×10^{-9} ml^{-1}. The seedlings of *C. indicum* ('Garden Mum- Kathleen Dark Red') were transplanted in each pot. Pots were regularly watered to maintain the moisture. After every 15 days, Hoagland solution (without KH_2PO_4 source) was also given to the plants.

The following treatments (Tt) were maintained for growth experiment: Tt_1: Control (without any bio-inoculants); Tt_2: *G. mosseae* (G); Tt_3: *A. laevis* (A); Tt_4: *T. viride* (T); Tt_5: *P. fluorescens*

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(P); Tt_6: *G. mosseae* + *A. laevis* (G+A); Tt_7: *G. mosseae* + *T. viride* (G+T); Tt_8: *G. mosseae* + *P. fluorescens* (G+P); Tt_9: *A. laevis* + *T. viride* (A+T); Tt_{10}: *A. laevis* + *P. fluorescens* (A+P); Tt_{11}: *T. viride* + *P. fluorescens* (T+P); Tt_{12}: *G. mosseae* + *A. laevis* + *T. viride* (G+A+T); Tt_{13}: *G. mosseae* + *A. laevis* + *P. fluorescens* (G+A+P); Tt_{14}: *G. mosseae* + *T. viride* + *P. fluorescens* (G+T+P); Tt_{15}: *A. laevis* + *T. viride* + *P. fluorescens* (A+T+P); Tt_{16}: *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* (G+A+T+P).

For vase life study: In the second set of experiment, the flowers were assessed for 'vase life'. When all the pots were at flowering stage, flowers head were harvested with a sharp blade in the morning and then the scapes were put in bucket water immediately to avoid cavitation. These scapes were given a slant cut again to make all scapes almost of equal length. Scapes of Tt_1-Tt_{16} were immersed in conical flask containing 100 ml of distilled water. For treatment Tt_{17}-Tt_{22}, flowers from the control plants were placed in conical flask containing 100 ml of different solutions of nutrient and growth regulators *viz.,* kinetin (37.5 µM and 3.75 µm), salicylic acid (37.5 µM and 3.75 µM), sucrose (0.1mM) and sodium chloride (0.1mM).

The following treatments (Tt) were for vase life experiment: Tt_1: Control (without any bio-inoculants); Tt_2: *G. mosseae* (G); Tt_3: *A. laevis* (A); Tt_4: *T. viride* (T); Tt_5: *P. fluorescens* (P); Tt_6: *G. mosseae* + *A. laevis* (G+A); Tt_7: *G. mosseae* + *T. viride* (G+T); Tt_8: *G. mosseae* + *P. fluorescens* (G+P); Tt_9: *A. laevis* + *T. viride* (A+T); Tt_{10}: *A. laevis* + *P. fluorescens* (A+P); Tt_{11}: *T. viride* + *P. fluorescens* (T+P); Tt_{12}: *G. mosseae* + *A. laevis* + *T. viride* (G+A+T); Tt_{13}: *G. mosseae* + *A. laevis* + *P. fluorescens* (G+A+P); Tt_{14}: *G. mosseae* + *T. viride* + *P. fluorescens* (G+T+P); Tt_{15}: *A. laevis* + *T. viride* + *P. fluorescens* (A+T+P); Tt_{16}: *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* (G+A+T+P); Tt_{17}: Kinetin (37.5 µM); Tt_{18}: Kinetin (3.75 µM); Tt_{19}: Salicylic acid (37.5 µM); Tt_{20}: Salicylic acid (3.75 µM); Tt_{21}: Sucrose (0.1mM); Tt_{22}: NaCl (0.1mM).

Experimental analysis

For growth and biochemical parameters: Plants were harvested after 90 days and length of shoot and root were measured with a measuring tape(cm). Their fresh shoot and root weight was measured in grams (g). For dry weight (g), samples were oven dried at 70°C overnight until a constant dry weight was obtained. Leaf area was measured by using leaf area meter (Systronics 21, Ahmedabad, India).When all the plants were in flowering stage, flowers were harvested and head diameter was measured in centimeter (cm). Harvested flower heads were also weighed (g) using a simple balance. Life span of flowers is the time (in days) between flower bloom and the day when flowers dies. The numbers of buds per plant were counted per treatment.

Chlorophyll and carotenoid content were estimated following the method of (1949) and expressed in mg g^{-1} f.wt. Anthocyanin content was analyzed by Tsushida and Suzuki (1995) method in mg per $100g^{-1}$. f.wt. Total leaf protein was analysed by Bradford method (1976) in mg per $100mg^{-1}$. f.wt. Acid and alkaline phosphatase activity was analysed using the method of Tabatabai and Bremner (1969) in IU g^{-1}. Phosphorous (P) content of shoot and root was estimated by 'vanadomolybdo phosphoric yellow colour method' (Jackson, 1973). Total sugar content was analyzed by the method of Hart and Fisher (1971). Quantification of AM spore number was done by Grid Line Intersect Method of Adholeya and Gaur (1994). Mycorrhizal root colonization was done by using 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970).

For vase life analysis : During the course of vase life experiment, freshness and morphological symptoms of flowers were noticed daily. Head diameter was calculated as mean of two perpendicular measurements across 0th, 7th, 14th and 21st day. The vase life of flower was counted from the day of transfer of scapes in conical flask to the day of termination, when senescence symptoms were visible. The volume of water absorbed by the scapes was calculated by measuring the absorbed volume at the end of experiment and subtracting it from the initial volume *i.e.,* 100 ml. The weight of the flower was measured by electrical weighing balance on 0th day and at the end when the last flower showed senescence (21st day).The peroxidase activity was measured at 0th, 7th, 14th and 21st day by the method of Maehly (1954).

Statistical analysis: For statistical analysis of data, one-way ANOVA was performed using Duncan's Multiple Range Test for comparison of variance and means separated with Least Significant Difference test.

Results and Discussion

After 90 days of inoculation with AMF, *T. viride* and *P. fluorescens* (alone or in combination) on *C. indicum,* a significant increase in the morphological, floral, biochemical parameters as well as mycorrhization pattern was observed (Table 1-3). Among the different treatments, G+A+T+P bloomed first followed by G+A+T, G+A+P and G+A. The consortium treatment was found to be the best treatment for enhancing different morphological parameters. These augmentations may be attributed with the enhancement of nutrients uptake such as N, P, K, Mg and Zn by AMF and PSB (Bhatia *et al.,* 2016). *P. fluorescence* also has a synergistic effect with AMF in better water absorption and enriching different quality parameters of host plant (Imperiali *et al.,* 2017). Combined application of G+A+T treatment proved outstanding for increasing the life span of flower (Table1). Maximum number of buds were encountered with G+A+T+ P treatment, which could be due to synthesis of more hormones (Bhatia *et al.,* 2016).

Among the different biochemical attributes studied, total chlorophyll and carotenoid content were apparently highest in the G+A+P treatment (Table 2). Rani and Mahesh (2015) also noticed more accumulation of total chlorophyll content in AMF treated *Centella asiatica.* Anthocyanin content was found to be maximum

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Table 1 : Effect of different bioinoculants on various morphological parameters of *Chrysanthemum indicum*

Treatments	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Leaf area (cm^2)	Number of buds	Life span of flower on plant (days)	Head diameter (cm)	Head dry weight (g)
Control	28.99 ± 0.77^{j}	18.04 ± 0.32^{k}	8.4 ± 0.47^{m}	3.8 ± 0.57^{k}	0.19 ± 0.001^{n}	0.09 ± 0.005^{l}	12.19 ± 0.16^{j}	7.2 ± 0.83^{j}	20.6 ± 1.14^{m}	2.6 ± 0.008^{l}	0.365 ± 0.038^{h}
Glomus mosseae (G)	34.01 ± 0.14^{g}	26.35 ± 0.31^{g}	18.2 ± 0.45^{f}	13.6 ± 0.63^{d}	1.02 ± 0.043^{e}	0.91 ± 0.023^{e}	26.36 ± 0.23^{b}	21.4 ± 2.3^{f}	30 ± 0.7^{def}	4.19 ± 0.008^{f}	1.112 ± 0.124^{ab}
Acaulospora laevis (A)	40 ± 0.13^{f}	20.68 ± 0.25^{i}	19.2 ± 0.48^{cd}	14.6 ± 0.63^{c}	0.81 ± 0.01^{h}	0.7 ± 0.004^{g}	24.05 ± 0.04^{c}	16.6 ± 1.14^{h}	29.4 ± 0.54^{efg}	3.59 ± 0.008^{h}	0.705 ± 0.083^{e}
Trichoderma viride (T)	32.17 ± 0.27^{i}	22.81 ± 0.22^{h}	12 ± 0.42^{k}	7.8 ± 0.47^{i}	0.56 ± 0.016^{k}	0.45 ± 0.075^{j}	23.78 ± 1.67^{c}	22.2 ± 1.3^{ef}	27.8 ± 0.83^{ij}	3.29 ± 0.008^{i}	0.402 ± 0.056^{g}
Pseudomonas fluorescence (P)	31.84 ± 0.26^{i}	19.4 ± 0.48^{j}	10±0.2l	5.6 ± 0.62^{j}	0.52 ± 0.009^{l}	0.42 ± 0.027^{j}	23.55 ± 0.05^{c}	12 ± 1.58^{i}	23.4 ± 0.54^{l}	2.8 ± 0.008^{k}	0.444 ± 0.123^{g}
GA	51.2 ± 0.21^{d}	38.67 ± 0.48^{b}	19.9 ± 0.15^{c}	14.9 ± 0.37^{c}	1.72 ± 0.008^{b}	1.62 ± 0.005^{b}	19.07 ± 0.04^{i}	24.2 ± 0.83^{d}	35 ± 0.7^{b}	5.11 ± 0.01^{b}	0.989 ± 0.187^{bc}
GT	55.03 ± 0.09^{c}	34.32 ± 0.6^{d}	22.2 ± 0.87^{b}	17.7 ± 0.55^{b}	1.06 ± 0.006^{d}	0.97 ± 0.023^{d}	23.5 ± 0.01^{c}	39.2 ± 1.3^{b}	28.6 ± 0.89^{ghi}	4.6 ± 0.01^{e}	1.08 ± 0.115^{abc}
GP	41.24 ± 0.33^{de}	35.98 ± 0.69^{c}	15.4 ± 0.64^{ij}	10.7 ± 0.29^{g}	0.84 ± 0.016^{g}	0.73 ± 0.04^{g}	21.24 ± 0.03^{ef}	23.2 ± 0.83^{def}	27.4 ± 1.14^{j}	3.6 ± 0.01^{h}	0.77 ± 0.106^{de}
AT	55.37 ± 0.27^{c}	27.9 ± 0.69^{f}	16.2 ± 0.77^{h}	11.2 ± 0.58^{f}	0.65 ± 0.006^{j}	0.55 ± 0.041^{i}	20.07 ± 0.06^{g}	24.8 ± 0.83^{d}	33.8 ± 1.3^{c}	3.7 ± 0.012^{g}	0.714 ± 0.106^{e}
AP	57.79 ± 0.1^{b}	27.56 ± 0.46^{f}	16.9 ± 0.59^{g}	12.2 ± 0.36^{e}	0.65 ± 0.006^{j}	0.56 ± 0.012^{i}	22.33 ± 0.02^{d}	15.8 ± 0.83^{h}	29 ± 0.7^{fg}	3.69 ± 0.019^{g}	0.789 ± 0.126^{de}
TP	33.93 ± 0.12^{h}	26.3 ± 0.46^{g}	16 ± 0.14^{hi}	11 ± 0.58^{fg}	0.31 ± 0.009^{m}	0.218 ± 0.007^{k}	21.38 ± 0.01^{ef}	32.4 ± 1.51^{c}	28.2 ± 0.44^{hij}	3.1 ± 0.017^{j}	0.666 ± 0.169^{f}
GAT	39.24 ± 0.3^{f}	36.48 ± 0.49^{c}	22 ± 0.64^{b}	17.3 ± 0.42^{b}	1.66±0.01c	1.55 ± 0.03^{c}	25.85 ± 0.03^{b}	22.2 ± 1.3^{ef}	36 ± 0.7^{a}	5.11 ± 0.013^{b}	1.067 ± 0.246^{abc}
GAP	40.82 ± 0.77^{e}	36.22 ± 0.64^{c}	19.1 ± 0.74^{d}	14.3 ± 0.41^{c}	0.88 ± 0.005^{f}	0.77 ± 0.007^{f}	20.93 ± 0.1^{f}	15 ± 0.7^{h}	29.2 ± 0.44^{fgh}	5.09 ± 0.011^{c}	1.122 ± 0.246^{ab}
GTP	41.66 ± 0.37^{d}	20.28 ± 0.64^{i}	17.8 ± 0.54^{f}	12.6 ± 0.44^{e}	0.81 ± 0.006^{h}	0.7 ± 0.007^{g}	21.6 ± 0.08^{e}	19.6 ± 1.14^{g}	30.6 ± 0.54^{d}	4.9 ± 0.007^{d}	1.115 ± 0.16^{ab}
ATP	57.98 ± 0.22^{b}	30.48 ± 0.67^{e}	14.7 ± 0.44^{j}	9.7 ± 0.35^{h}	0.71 ± 0.01^{i}	0.61 ± 0.008^{h}	19.67 ± 0.03^{h}	$23.4 \pm 1.51d^{e}$	26.4 ± 0.54^{k}	4.1 ± 0.008^{g}	0.882 ± 0.148^{cd}
GATP	60.72 ± 0.53^{a}	40.3 ± 0.56^{a}	24.2 ± 0.71^{a}	18.5 ± 0.57^{a}	2.29 ± 0.011^{a}	2.13 ± 0.008^{a}	27.56 ± 0.34^{a}	47.8 ± 2.38^{a}	$30.4 \pm 0.54d^{e}$	5.59 ± 0.008^{a}	1.23 ± 0.159^{a}
LSD ($P \leq 0.05$)	0.2359	0.6627	0.7089	0.6367	0.0183	0.0359	0.502	1.7243	0.9868	0.0138	0.18924
ANOVA (15, 64)	4166.35	995.29	298.61	341.77	7220.23	1788.57	339.13	270.87	124.83	3482.89	17.24

Values are mean of replicates ±SD; Values in column followed by same letter are not significantly different, $p \leq 0.05$, LSD

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Table 2 : Effect of different bioinoculants on chlorophyll, carotenoids, anthocyanin and AMF colonization of *Chrysanthemum indicum*

Treatments	Chlorophyll a (mg g^{-1} f.wt.)	Chlorophyll b (mg g^{-1} f.wt.)	Total chlorophyll (mg g^{-1} f.wt.)	Total carotenoids (mg g^{-1} f.wt.)	Total anthocyanin (mg $100g^{-1}$ f.wt.)	AM spore number	AM root colonization (%)
Control	0.68 ± 0.01^{m}	0.19 ± 0.005^{l}	0.877 ± 0.016^{l}	0.017 ± 0.002^{l}	40.55 ± 0.65^{l}	0 ± 0^{j}	0 ± 0^{h}
Glomus mosseae (G)	1.01 ± 0.008^{h}	0.513 ± 0.007^{g}	1.528 ± 0.015^{g}	0.058 ± 0.004^{g}	47.32 ± 0.23^{f}	67.2 ± 2.07^{d}	75.2 ± 3.96^{b}
Acaulospora laevis (A)	0.952 ± 0.007^{i}	0.553 ± 0.008^{f}	1.505 ± 0.015^{g}	0.051 ± 0.006^{h}	45.4 ± 0.24^{h}	70.8 ± 1.64^{c}	72 ± 2.54^{bc}
Trichoderma viride (T)	0.86 ± 0.011^{k}	0.363 ± 0.006^{j}	1.229 ± 0.017^{j}	0.039 ± 0.004^{jk}	42.35 ± 0.15^{j}	0 ± 0^{j}	0 ± 0^{h}
Pseudomonas fluorescence (P)	0.8 ± 0.008^{l}	0.351 ± 0.006^{k}	1.152 ± 0.014^{k}	0.035 ± 0.006^{k}	41.84 ± 0.62^{k}	0 ± 0^{j}	0 ± 0^{h}
GA	1.12 ± 0.006^{f}	0.516 ± 0.007^{g}	1.627 ± 0.014^{f}	0.165 ± 0.004^{a}	49.77 ± 0.62^{d}	44.2 ± 2.58^{h}	70 ± 4.3^{cd}
GT	1.18 ± 0.006^{d}	0.817 ± 0.008^{c}	2.172 ± 0.015^{d}	0.077 ± 0.004^{de}	48.33 ± 0.37^{e}	78 ± 2.54^{b}	66 ± 3.16^{de}
GP	0.81 ± 0.006^{l}	0.582 ± 0.006^{e}	1.398 ± 0.012^{h}	0.071 ± 0.004^{ef}	48.28 ± 0.31^{e}	60 ± 2.54^{e}	84.2 ± 3.76^{a}
AT	0.91 ± 0.007^{j}	0.373 ± 0.007^{i}	1.288 ± 0.015^{i}	0.098 ± 0.005^{c}	45.72 ± 0.14^{h}	59.4 ± 0.89^{e}	71 ± 4.12^{bc}
AP	1.04 ± 0.005^{g}	0.434 ± 0.005^{h}	1.476 ± 0.011^{gh}	$0.047 \pm 0.004h^{i}$	45.65 ± 0.43^{h}	55 ± 2.91^{f}	62 ± 3.16^{ef}
TP	1.11 ± 0.007^{f}	0.521 ± 0.006^{g}	1.634 ± 0.011^{f}	0.042 ± 0.007^{ij}	43.04 ± 0.19^{i}	0 ± 0^{j}	$0 \pm 0h$
GAT	1.22 ± 0.007^{c}	0.991 ± 0.009^{a}	2.211 ± 0.004^{b}	0.108 ± 0.004^{b}	51.93 ± 0.52^{b}	81.2 ± 1.92^{a}	85 ± 4.18^{a}
GAP	1.99 ± 0.009^{a}	0.821 ± 0.009^{c}	2.81 ± 0.013^{a}	0.109 ± 0.004^{b}	50.87 ± 0.34^{c}	40.4 ± 2.07^{i}	52 ± 3.16^{g}
GTP	1.13 ± 0.007^{e}	0.819 ± 0.006^{c}	1.96 ± 0.018^{d}	0.08 ± 0.005^{d}	48.13 ± 0.26^{e}	56 ± 1.58^{f}	60.2 ± 3.96^{f}
ATP	1.149 ± 0.006^{e}	0.672 ± 0.009^{d}	1.811 ± 0.015^{e}	0.068 ± 0.005^{f}	46.44 ± 0.35^{g}	48.4 ± 2.07^{g}	55 ± 4.3^{g}
GATP	1.262 ± 0.007^{b}	0.865 ± 0.005^{b}	2.127 ± 0.013^{c}	0.113 ± 0.006^{b}	52.54 ± 0.51^{a}	57 ± 2.91^{f}	61 ± 3.67^{f}
LSD ($P \leq 0.05$)	0.0101	0.00805	0.01605	0.0068	0.4812	2.4324	4.0831
ANOVA (15, 64)	6776.43	4521.34	5497.68	249.3	444.75	1124.61	478.66

Values are mean of replicates ±SD; Values in column followed by same letter are not significantly different, $p \leq 0.05$, LSD

in G+A+T+P treated plants (Table 2). AM fungi increases the secondary metabolite pathway many folds resulting in the expression of secondary metabolite contents in the flowers of *Hypercium perforatum* (Lazzara *et al.,* 2017). Total leaf protein content was noticed to be highest in G+A+T followed by G+A (Table 3). AMF colonized plants expand the extra-radical hyphae in the soil around roots, providing many important nutrients (P, Zn, Cu, Mn, Fe, Ca, K and NH_4), accelerating protein synthesis in leaves (Smith and Read, 2008). Ratti *et al.* (2010) also noticed the effect of *Glomus* sp. on *Catharanthus roseus* in increasing the protein content along with phosphatase activity. Acid and alkaline phosphatase activity were found to be maximum in G+A+T+P followed by G+A+P (Table 3). Karishma *et al.* (2013) observed the positive effect of AMF and *P. fluorescens* in increasing the acid, as well as alkaline phosphatase in *Gerbera*.

Phosphorous content (both shoot and root) was examined maximal in G+A+T (Table 3). Phosphorous is one of the diffusion limited essential nutrient for plant growth, less available for roots to absorb from the soil (Smith and Smith, 2011). Application of AMF and PSB increases the absorption of phosphorous. On solubilisation, PSB release H_2PO_4 ions from unsolubilized form, expanding the root area resulting in greater absorption of nutrients (Bagyaraj *et al.,* 2015). Total sugar content was found utmost in A+T+P (Table 3). As discussed above, AMF inoculated plants accumulate more chlorophyll content leading to increased photosynthesis, which eventually leads to high total sugar in plants (Rani and Mahesh, 2015). Highest AM spore number and percent AM colonization were observed to be best in G+A+T treatment. AMF infected plant increase the production of AMF infectious propagules in *Zinnia,* when inoculated with *Gigaspora* and *Glomus* (Long *et al.,* 2010). During vase life study, shrinkage in flower diameter was first observed in control on 7th day followed by NaCl on 10th day (Table 4). After 14 days, almost all treatments showed drooping, except for G+A+T, G+A+P and A+P treatments. Flowers of G+A+T showed very little shrinkage and showed delayed senescence and hence proved to be the best treatment for increasing the vase life of *Chrysanthemum* in terms of days. Mycorrhizal symbiosis significantly increased the flower vase life by decreasing ethylene production (Besmer and Koide, 1999). As the number of days increased, senescence increased but the diameter of flower heads decreased (Table 4). AMF pre-treated flower heads showed slower rate of senescence over growth regulators and control. After 14th day, G+A+T+P showed maximum head diameter followed by G+A+T and G+A+P. The flower head diameter and stem diameter of *Chrysanthemum* decreased gradually with an increase in the number of days using various holding solutions due to loss of moisture content and tissue degradation (Karishma *et al.,* 2011).

In general, *Chrysanthemum* had a vase life of 14±2 days but it can be increased by giving some extra treatments (Table 5). In the present study, G+A+T proved to be the best treatment for increasing the vase life followed by G, Kinetin (37.5 μM) and A alone. Bhalla *et al.* (2006) also observed an improvement in the overall vase life in the flowers of *Gladiolus* using AMF, PSB and

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Table 3 : Effect of bioinoculants on different biochemical processes of *Chrysanthemum indicum*

Treatments	P content % Shoot	Root	Total sugar (mg 100mg^{-1} f.wt.)	Total protein (mg 100mg^{-1} f.wt.)	Phosphatase (IU g^{-1} f.wt.) Alkaline	Acidic
Control	0.015±0.005^{g}	0.022±0.005^{i}	0.107±0.01^{j}	1.155±0.003^{o}	0.115±0.019^{k}	0.288±0.024^{k}
Glomus mosseae (G)	0.045±0.004^{b}	0.077±0.005^{b}	0.24±0.005^{h}	1.762±0.002^{e}	0.506±0.025^{f}	0.6±0.02^{f}
Acaulospora laevis (A)	0.036±0.04^{cde}	0.072±0.003^{c}	0.257±0.005^{g}	1.706±0.003^{f}	0.427±0.03^{g}	0.518±0.021^{g}
Trichoderma viride (T)	0.031±0.002^{e}	0.06±0.003^{e}	0.274±0.011^{f}	1.595±0.003^{h}	0.297±0.037^{i}	0.401±0.019^{i}
Pseudomonas fluorescence (P)	0.035±0.004^{de}	0.066±0.003^{d}	0.324±0.004^{c}	1.495±0.003^{l}	0.244±0.057^{j}	0.361±0.03^{j}
GA	0.04±0.006^{bcd}	0.08±0.005^{b}	0.315±0.005^{cd}	2.065±0.003^{b}	0.623±0.026^{d}	0.762±0.026^{d}
GT	0.042±0.005^{bc}	0.075±0.003^{bc}	0.237±0.005^{gh}	1.623±0.003^{g}	0.533±0.02^{ef}	0.649±0.026^{e}
GP	0.033±0.003^{e}	0.045±0.003^{gh}	0.325±0.004^{c}	1.585±0.003^{i}	0.56±0.037^{e}	0.677±0.024^{e}
AT	0.022±0.004^{f}	0.043±0.002^{h}	0.357±0.006^{b}	1.577±0.003^{j}	0.443±0.018^{g}	0.542±0.015^{g}
AP	0.029±0.004^{e}	0.05±0.004^{fg}	0.366±0.006^{b}	1.626±0.003^{g}	0.521±0.027^{ef}	0.644±0.026^{e}
TP	0.035±0.004^{de}	0.044±0.003^{h}	0.305±0.006^{d}	1.527±0.003^{k}	0.355±0.032^{h}	0.476±0.019^{h}
GAT	0.056±0.004^{a}	0.085±0.002^{a}	0.225±0.003^{h}	2.217±0.003^{a}	0.704±0.014^{c}	0.9±0.028^{c}
GAP	0.022±0.005^{f}	0.042±0.002^{h}	0.369±0.006^{b}	1.373±0.003^{m}	0.748±0.041^{b}	0.941±0.027^{b}
GTP	0.032±0.002^{e}	0.054±0.002^{f}	0.386±0.005^{a}	1.778±0.004^{d}	0.528±0.025^{ef}	0.646±0.019^{e}
ATP	0.03±0.006^{e}	0.062±0.003d^{e}	0.387±0.026^{a}	1.356±0.004^{n}	0.55±0.039^{ef}	0.662±0.046^{e}
GATP	0.041±0.006^{bcd}	0.065±0.003d^{e}	0.291±0.022^{e}	1.909±0.011^{c}	0.88±0.05^{a}	1.032±0.038^{a}
LSD ($P \leq 0.05$)	0.0061	0.0049	0.0134	0.005	0.0425	0.0343
ANOVA (15, 64)	20.66	98.46	154.13	18209.33	163.55	289.61

Values are mean of replicates ±SD; Values in column followed by same letter are not significantly different, $p \leq 0.05$, LSD

Table 4 : Effect of senescence on flower head diameter and peroxidase activity of *Chrysanthemum indicum*

Treatments	Flower head diameter (cm)				Peroxidase activity (mg min^{-10})			
	0 day	7th day	14th day	21st day	0th Day	7th day	14th day	21st day
Control	2.6±0.008l	2.47±0.015^{n}	2.13±0.054l	1.84±0.089^{o}	0.276±0.002^{jk}	0.445±0.002^{l}	0.633±0.012^{n}	0.749±0.058^{j}
Glomus mosseae (G)	4.19±0.008^{fg}	4.17±0.015^{e}	4.03±0.032^{e}	4±0.07^{e}	0.065±0.003^{e}	0.111±0.005^{de}	0.251±0.01^{f}	0.297±0.023^{e}
Acaulospora laevis (A)	3.59±0.008^{h}	3.57±0.016^{h}	3.36±0.114^{f}	3.36±0.089^{h}	0.066±0.004^{e}	0.14±0.022^{g}	0.34±0.014^{j}	0.567±0.038^{hi}
Trichoderma viride (T)	2.802±0.008^{k}	2.77±0.015^{k}	2.59±0.072^{h}	2.5±0.007^{l}	0.081±0.002^{h}	0.143±0.002^{g}	0.272±0.009^{gh}	0.333±0.038^{e}
Pseudomonas fluorescence (P)	3.29±0.01^{i}	3.27±0.015^{i}	3.1±0.075^{g}	3±0.07^{j}	0.057±0.004^{d}	0.124±0.002^{ef}	0.164±0.015^{c}	0.204±0.024^{bc}
GA	4.6±0.01^{e}	5.05±0.02^{b}	5±0.07^{b}	4.94±0.054^{b}	0.082±0.003^{h}	0.172±0.003^{h}	0.181±0.01^{cde}	0.252±0.015^{d}
GT	3.6±0.01^{h}	4.57±0.015^{d}	4.34±0.089^{d}	4.3±0.07^{d}	0.079±0.004^{gh}	0.103±0.001^{cd}	0.119±0.013^{e}	0.155±0.026^{a}
GP	3.71±0.012^{g}	3.57±0.015^{h}	3.34±0.114^{f}	3.2±0.07^{i}	0.074±0.002^{f}	0.098±0.003^{cd}	0.165±0.013^{c}	0.215±0.016^{bc}
AT	3.69±0.019^{g}	3.68±0.013^{g}	3.38±0.083^{f}	3.5±0.07^{g}	0.052±0.003^{de}	0.113±0.003^{de}	0.189±0.013^{de}	0.261±0.016^{d}
AP	3.7±0.019^{g}	3.67±0.015^{g}	3.37±0.048^{f}	3.46±0.054^{g}	0.047±0.003^{c}	0.105±0.004^{cd}	0.127±0.012^{b}	0.199±0.027^{b}
TP	3.1±0.017^{j}	3.07±0.01^{j}	3±0.07^{g}	2.8±0.07^{k}	0.044±0.003^{c}	0.131±0.005^{fg}	0.194±0.016^{de}	0.241±0.015^{cd}
GAT	5.11±0.013^{b}	5.06±0.02^{b}	5.01±0.007^{b}	4.96±0.068^{b}	0.032±0.003^{a}	0.041±0.003^{a}	0.103±0.019^{a}	0.127±0.022^{a}
GAP	5.09±0.011^{c}	5.05±0.02^{b}	4.98±0.044^{b}	4.89±0.008^{b}	0.036±0.04^{b}	0.067±0.008^{b}	0.2±0.013^{e}	0.251±0.021^{d}
GTP	4.9±0.007^{d}	4.87±0.015^{c}	4.66±0.296^{c}	4.69±0.016^{c}	0.075±0.002^{fg}	0.091±0.006^{c}	0.173±0.018^{cd}	0.231±0.015^{bcd}
ATP	4.2±0.008^{f}	4.07±0.015^{f}	4±0.01^{e}	3.9±0.001^{f}	0.091±0.005^{i}	0.133±0.006^{fg}	0.259±0.011^{fg}	0.304±0.027^{e}
GATP	5.59±0.008^{a}	5.56±0.039^{a}	5.38±0.13^{a}	5.4±0.07^{a}	0.082±0.006^{h}	0.102±0.012^{cd}	0.281±0.015^{h}	0.371±0.029^{f}
Kinetin-1 (37.5μM)	2.44±0.005^{o}	2.43±0.008^{m}	2.16±0.054^{k}	2.15±0.07^{n}	0.282±0.001^{lm}	0.297±0.005^{i}	0.31±0.011^{i}	0.376±0.026^{f}
Kinetin-2 (3.75μM)	2.49±0.005^{o}	2.48±0.013^{m}	2.26±0.054^{jk}	2.2±0.007^{mn}	0.283±0.001	0.304±0.003^{i}	0.398±0.019^{k}	0.436±0.009^{h}
Salicylic acid-1 (37.5μM)	2.54±0.005^{n}	2.46±0.013^{m}	2.24±0.089^{j}	2.26±0.017^{m}	0.285±0.003^{lm}	0.327±0.004^{j}	0.381±0.023^{k}	0.452±0.02^{h}
Salicylic acid-2 (3.75μM)	2.47±0.004	2.46±0.008^{m}	2.32±0.047^{jk}	2.2±0.07^{mn}	0.275±0.002^{jk}	0.305±0.003^{i}	0.378±0.01^{k}	0.401±0.028^{f}
Sucrose (0.1M)	2.56±0.005^{n}	2.54±0.008^{lm}	2.4±0.004^{ik}	2.26±0.049^{m}	0.28±0.003k^{l}	0.37±0.007^{j}	0.461±0.02^{l}	0.51±0.033^{h}
NaCl(0.1M)	2.57±0.005^{m}	2.41±0.044^{m}	2.22±0.044^{j}	2.08±0.089^{no}	0.279±0.001^{jkl}	0.465±0.039^{k}	0.515±0.021^{m}	0.625±0.024^{i}
LSD ($P \leq 0.005$)	0.0246	0.0438	0.236	0.1542	0.0109	0.0276	0.0386	0.069
ANOVA (21, 88)	57822.01	18131.65	663.59	1661.96	4184.97	666.74	405.02	188.05

Values are mean of replicates ±SD; Values in column followed by same letter are not significantly different, $p \leq 0.05$, LSD

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Table 5 : Effect of senescence on vase life, holding solution uptake and weight of flower head of *Chrysanthemum indicum* L.

Treatments	Vase life (days)	Volume/Solution uptake (ml 100ml^{-1} used)	Weight of flower head (g) 0 day	21st day
Control	16±1.22^{n}	9.22±0.549^{i}	0.56±0.01^{ij}	0.341±0.015^{m}
Glomus mosseae (G)	28.6±0.54^{b}	12.01±0.048^{a}	1.28±0.062^{b}	1.054±0.004^{b}
Acaulospora laevis (A)	27.6±0.54^{bc}	11.68±0.13^{ab}	0.81±0.027^{e}	0.604±0.019^{f}
Trichoderma viride (T)	23±0.7^{jk}	9.88±0.077^{h}	0.62±0.015^{h}	0.402±0.001^{j}
Pseudomonas fluorescence (P)	20±0.7^{m}	10.96±0.167^{c}	0.68±0.025^{g}	0.492±0.019^{h}
GA	25.6±0.89^{def}	10.16±0.103^{efg}	0.77±0.023^{f}	0.564±0.005^{g}
GT	26.4±1.14^{c}	11.86±0.013^{ab}	0.76±0.025^{f}	0.557±0.003^{g}
GP	25±0.7^{efg}	11.45±0.013^{b}	0.75±0.02^{f}	0.562±0.015^{g}
AT	25±0.7^{efg}	10.33±0.015^{def}	0.77±0.012^{f}	0.563±0.016^{g}
AP	24.8±1.09^{fgh}	10.36±0.013^{def}	0.78±0.01^{ef}	0.569±0.011^{g}
TP	24.4±0.89	10.61±0.123^{cd}	0.7±0.019^{g}	0.472±0.009^{i}
GAT	30.4±0.54^{a}	12.06±0.134^{a}	1.22±0.015^{c}	1.011±0.018^{c}
GAP	24.4±0.54^{gh}	11.47±0.071^{b}	1.18±0.022^{d}	0.964±0.023^{de}
GTP	26±0.7^{cde}	10.64±0.042^{cd}	1.19±0.022^{cd}	0.979±0.016^{d}
ATP	23.2±0.44^{ij}	9.98±0.109g^{h}	1.17±0.15^{d}	0.962±0.016^{e}
GATP	26.2±0.44^{cd}	10.09±0.255^{fg}	1.32±0.012^{a}	1.121±0.004^{a}
Kinetin-1 (37.5μM)	28.2±0.83^{b}	11.76±0.181^{ab}	0.55±0.015^{ij}	0.45±0.006i^{j}
Kinetin-2 (3.75μM)	23.8±0.83^{hi}	10.66±0.008^{cd}	0.58±0.015^{hi}	0.387±0.003^{jk}
Salicylic acid-1 (37.5μM)	24.8±1.3^{fgh}	10.8±0.836^{c}	0.59±0.016^{hi}	0.399±0.004^{j}
Salicylic acid-2 (3.75μM)	23±0.7^{jk}	10.6±0.34^{cd}	0.6±0.015^{hi}	0.389±0.004^{jk}
Sucrose (0.1M)	22.8±0.44^{l}	10.13±0.09^{efg}	0.57±0.016^{hi}	0.361±0.014^{l}
NaCl (0.1M)	19.6±0.54^{m}	10.4±0.547^{de}	0.59±0.015^{hi}	0.379±0.005k^{l}
LSD (P≤0.005)	1.989	0.686	0.0568	0.0324
ANOVA (21, 88)	80.34	40.89	697.85	2134.81

Values are mean of replicates ±SD; Values in column followed by same letter are not significantly different, p≤0.05, LSD

Azospirilum to the growing media through better nutrient uptake. AMF act as bio-stimulant, thereby increasing the production of several plant hormones like auxin, zeatin, gibberellin and jasmonic acid (He *et al.,* 2017). An increased vase life could also be due to increase in water use efficiency through better vascular tissues formation (Shekoofeh *et al.,* 2012). Mycorrhizal root systems influence the source to sink balance by utilizing photosynthate supplied as a results higher photosynthesis in leaves and a considerable proportion of carbon assimilation (Smith and Read, 2008).

AMF pre-treated flowers showed more water absorption over other holding solutions *viz.,* kinetin, salicylic acid, sugar and salt. Water absorption was more during initial days of experiment but it decreased consistently as the number of days passed, which might be due to blockage in vascular tissues. Ghaderi and Nazarideljou (2018) reported an increased shelf life of *Gerbera jamesonii* due to reduced transpiration using *G. mosseae* and *G. intradices.* A consistent decrease in flower heads weight was also observed using different treatments as the number of days passed (Table 5). After 21 days, G+A+T+P and G treatments showed lowest decrease in the head weight over other treatments. The larger size of florets and higher number of floret layers in AMF treated plants might be the reason for increased weight. It was due to more absorption of water and nutrients, especially P along with N, Zn, Mg and Ca that plays an important role in enhancing photosynthesis rate resulting in an increased accumulation of photosynthate (Sikes *et al.,* 2010; Syafruddin and Arabia, 2016). AM fungi influences both primary and secondary metabolites in host plant leading to accumulation of carotenoids and polyphenols (Schliemann *et al.,* 2008). Chaudhary *et al.* (2013) also observed a significant improvement in the floral characters of *Gladiolus* inoculated with PSB and *Azotobacter.* Increase in peroxidase activity is one of the most reliable indicators of maturity in plants, as peroxidase activity increases, the level of peroxides and free radicals that react with cellular constituent also increases, ultimately leading to senescence (Vranova *et al.,* 2002). A fast increase in peroxidase activity was observed in control. Among all the holding solution, kinetin was found to be the most effective hormone in decreasing the peroxidase activity, and among AMF pre-treated plants G+A+T proved to the be best in decreasing peroxidase activity (Table 4). AM fungi accelerates several scavenging enzymes such as superoxide dismutase, ascorbate peroxidase, dehydroascorbate reductase, catalase, monodehydroascorbate reductase and guaiacol peroxidase as free radicals increase, which can be regarded as antioxidants (Essahibi *et al.,* 2018). The roots colonized with AMF accumulate these anti-oxidative enzymes and carotenoids (Fester and Hause, 2005).

It can be concluded that all bioinoculants enhanced plant growth and floral response and vase life of *Chrysanthemum indicum* over control by enriching nutrient

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uptake, especially phosphorous, enhancing the growth hormones and resistant to root-borne pathogens. Therefore, it is advised that use of bioinoculants, especially the arbuscular mycorrhizal fungi are more productive and eco-friendly.

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