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Effect of bioinoculants on morphological and biochemical parameters of *Zinnia elegans* Jacq.

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Abstract

The dominant AMF *Glomus mosseae* (G) and *Acaulospora laevis* (A) were isolated from the rhizosphere soil of *Z. elegans* Jacq**.** and mass produced in laboratory for further studies. A pot experiment was performed to see the interactive potential of arbuscular mycorrhizal fungi (*G. mosseae* and *A. laevis*) alone or in combination with *Trichoderma viride* (T) and *Pseudomonas fluorescens* (P) on *Z. elegans* under glass house conditions. The experiment were conducted in a factorial arrangement based completely randomized design with five replicates. Various morphological (plant height, shoot biomass, root biomass, root length, leaf area, flower number, diameter) and biochemical attributes (chlorophyll, carotenoids, flower anthocyanin content, P content, total sugar, starch and protein content) were measured after 60 days. The results indicated a variation in growth response of *Z. elegans* with different treatments. AMF alone or in combination helped in increasing the different parameters of *Z. elegans.* The combination of G+A showed maximum increase of growth parameters followed by G+T and G+A+P. Consortium inoculation of bioinoculants plants with G+A+T+P treatment proved to be the best treatment for total proteins and total chlorophyll content while flower anthocyanin content was best in G+A treatment. AMF promotes higher AM colonization and spore number enhancing nutrient acquisition especially phosphorus, producing plant growth hormones resulting in an improvement of rhizospheric condition of soil, altering the physiological and biochemical properties of *Z. elegans*. Based on different parameters studied, *G. mosseae* was found to be an efficient bioinoculant as compared to *A. laevis* for growth enhancement of *Z. elegans*.

Key words: *Zinnia elegans*, AM fungi, *Pseudomonas fluorescens*, *Trichoderma viride*, interaction

Introduction

Zinnia elegans Jacq**. (**family: Asteraceae**)** is well known garden plant cultivated worldwide, and used as cut flowers and flowering potted plant. It has attractive capitula with a wide variety of brightly, bi-colored ray florets and long bloom period (Metcalf and Sharma, 1971). *Z. elegans* 'Liliput' and 'Thumbelina' are dwarfs and low growing varieties (Andersen and Andersen, 2000).

There are several chemical fertilizers that can increase the growth of flowers but the use of AM fungi as biofertilizers is very important and beneficial. The symbiotic relationship between mycorrhiza and plant is one of the most common symbiotic activities in plant kingdom. The improved nutritional status assist the flowering plant to produce higher biomass which is primarily attributed to the uptake of immobile nutrient especially phosphate. It is now acknowledged that mycorrhizal fungi provide around 80% of plant phosphorous and other micronutrients (Heijden *et al.*, 2015). The benefit of combined association of AM fungi and *P. fluorescence* is comparatively more to the host plants, than their single associations with the host plants. Inoculation with phosphate solubilizing microorganisms *i.e. P. fluorescence* may help to solubilize native soil phosphate. Released soluble phosphate can actively taken up by mycorrhizal roots. In addition to this, *Trichoderma* also prevent plant from other pathogens.

Combined inoculation of bioinoculants results in significant enhancement of soil fertility leading to improved growth parameters (Govindan and Thirumurgan, 2003). Absorption of insoluble nutrients by the plants can be achieved better through the symbiotic association of AMF, *Trichoderma* sp. and phosphate solublizing bacteria (PSB). The main objective of this work was to reduce the use of chemical fertilizers using microbes especially AMF, which are beneficial for the plant growth and development. *Z. elegans* is relatively sensitive to environmental stress conditions and weeds. Therefore, the present investigation was carried out to evaluate the effect of different bioinoculants on growth and physiological parameters of *Z. elegans*.

Materials and methods

Mass multiplication of selected AM fungi: The two dominant AM fungi, *G. mosseae* and *A. laevis* were raised by Funnel Technique of Menge and Timmer (1982). The starter inoculums were raised using maize as a host for three months. These spores were then multiplied in earthen funnels followed by big earthen pots and used for the further experiment.

Isolation and multiplication of *Trichoderma viride*: *T. viride* was isolated from soil by using Soil Dilution Plate method (Johnson *et al.*, 1959) on Potato Dextrose Agar medium. It was purified and identified by using the manual of Leslie and Summerell (2006) and then mass multiplication was done on wheat bran: sawdust: water in ratio 3:1:4 for 25 -30 days.

Multiplication of *Pseudomonas fluorescens* culture: *P. fluorescens* was procured from Institute of Microbial Technology (IMTC), Chandigarh, India and was multiplied in nutrient broth medium (beef extract: 3 g L^{-1}, peptone: 5 g L^{-1} and: NaCl: 5g L^{-1}), incubated at 32 ^{o}C for 48 hours for proper growth of bacteria.

Preparation of pot mixture: The experiment was laid down

in a complete randomized block design with five replicates per treatment. Top soil from the Botanical Garden of Botany Department, Kurukshetra University, Kurukshetra was collected and mixed with sand to form soil: sand ratio 3:1, which was then sterilized in autoclave at 121°C and 15 psi for 20 minutes (2 times) to avoid previous AM spores and mycelia. The required concentrations of inoculums were added to the earthen pots (25×25 cm). 10% of AM inoculums (having 350-400 AM spores/100 g and chopped AM colonized root pieces with an infection level of around 94%) were added to each pot as a band below the top soil layer in the pot. *T. viride* inoculum with density 3.4×10^8 cfu g^{-1} was added per treatment. *P. fluorescens* was also added and different combinations were made. The different treatments were as follows:

1) Control (without any bio-inoculants)
2) *G. mosseae* (G)
3) *A. laevis* (A)
4) *Trichoderma viride* (T)
5) *Pseudomonas fluorescens* (P)
6) G+A
7) G+T
8) G+P
9) A+T
10) A+P
11) T+P
12) G+A+T
13) G+A+P
14) G+T+P
15) A+T+P
16) G+A+T+P

Seedlings of *Z. elegans* were planted in each pot. The variety taken for experiment was Liliput. The roots were dipped in *P. fluorescens* having cfu $1x10^{-9}$ mL^{-1} for 5-6 minutes and were planted. The plants were watered regularly and supplied with Hoagland solution after every 15 days during the experiment.

Harvest and plant analysis: Plants were harvested at the flowering stage (after 60 days) when almost all the plants had flowers. Different growth and physiological parameters were analyzed. Chlorophyll and carotenoids content were estimated using the formula of Arnon (1949). Leaf protein and anthocyanin content were estimated by using the method of Bradford (1976) and Tsushida and Suzuki (1995), respectively. Acid and alkaline phosphatase activity was estimated by the modified method of Bergmeyer (1974). Leaf area was assessed by using leaf area meter (Systronics 21, Ahmedabad, India).

Identification and quantification of the AM spores number and root colonization: Isolation of AM spores was done by using Wet Sieving and Decanting Technique of Gerdman and Nicolson (1963). Identification of AM spores (*G. mosseae* and *A. laevis*) was done as per Walker (1983); Scheneck and Perez (1990) and Aggarwal *et al.* (2012). Quantification of the number of AM spores was done using 'Grid Line Intersect Method' of Adholeya and Gaur (1994). 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970) was used to determine mycorrhizal root colonization.

Percent AM colonization of roots was calculated as:

$$\text{AM colonization of roots (\%)} = \frac{\text{Number of root segments colonized}}{\text{Number of root segments studied}} \times 100$$

Statistical analysis: Data were subjected to analysis of variance and means separated using the least significant difference test in the Statistical Package for Social Sciences (ver.11.5, Chicago, IL, USA).

Results

After 60 days of inoculation with different bioinoculants, alone or in combination on *Z. elegans,* it was found that there was an increase in different morphological parameters (shoot length, root length, shoot and root dry weight, leaf area, number of leaves), biochemical parameters (chlorophyll content, carotenoids content, leaf proteins) and mycorrhization percent as compared to non inoculated control plants (Table 1-4).

The shoot length and root length were found to be maximum in consortium treatment (G+A+T+P) followed by double treatment (G+T). The shoot biomass and root biomass were also maximum in consortium followed by triple treatment of G+T+P. The leaf area and number of leaves per plant were also found to be higher in G+A+T+P treatment followed by *G. mosseae* (alone) in case of leaf area and triple treatment of A+T+P in case of number of leaves (Table 1).

The floral parameters of plants also showed great variation in response to various treatments. The flower head diameter was found to be largest in G+A+T followed by G+T. Number of flower heads per plant was found to be maximum in G+A+T+P treatment followed by G+A and G+A+T. Number of AM spores and percentage AM colonization of roots were higher in plants treated with G+A+T+P. In all the treatments, the plant inoculated with P were less efficient in comparison with AM fungi but better than control one (Table 2).

After 60 days of inoculation, protein and chlorophyll content were higher in the plants inoculated with G+A+T+P treatment followed by G+A+T (Table 3 and 4). The flower anthocyanin content was found to be maximum in consortium (G+A+T+P) treatment and dual inoculation treatment of G+A followed by G+A+T. Maximum carotenoids content was found in G+A treatment followed by G+A+T+P, G+A+P and G+A+T. However, difference in carotenoids content of flowers with different treatments was not significant (Table 3).

Total sugar and starch content were higher in consortium treatment *i.e.* G+A+T+P. For phosphorous uptake, consortium treatment (G+A+T+P) resulted in best in both shoot and root (Table 4).

Discussion

In the present investigation, dual and consortium inoculation showed better results in comparison to individual inoculation. AMF along with other microbes (*T. viride* and *P. fluorescence*) act as biofertilizer and assist the plant in better nutrient uptake. AMF also act as phytostimulators by promoting plant growth usually by producing several hormones like zeatin, auxin, salicylic acid, jasmonic acid etc. AM Fungi act as biocontrol agent against plant pathogens, thus improving the plant growth. Padmadevi *et al.* (2004) also reported better growth and early flowering in *Anthurium* by the application of PSB and AMF.

Z. elegans plants inoculated with different bioinoculants including AM fungi showed better morphological parameters, it may be due to better uptake of nutrients especially phosphorous by AMF. Cruz (2008) also reported that an increase in the production of cytokinins and gibberellins using AM fungi. It was also

Table 1. Effect of bioinoculants on different morphological parameters of *Zinnia elegans*

Treatments	Shoot length (cm)	Root length (cm)	Leaf area (cm^2)	Dry shoot weight (g)	Dry root weight (g)	No. of leaves (per plant)
Control	41.85±0.84^{n}‡	21.6±0.3^{l}	12.31±0.37^{m}	13.14±0.63^{i}	1.92±0.07^{g}	18.8±2.2^{j}
G. mosseae (G)	54.03±0.21^{j}	31.3±0.39^{h}	25.1±0.21^{b}	16.18±0.21^{f}	2.03±0.12^{fg}	25.6±1.6^{i}
A. laevis (A)	49.07±0.1^{k}	28.7±0.33^{j}	16.02±0.18^{l}	15.46±0.39^{g}	1.99±0.07^{fg}	24.8±1^{i}
T. viride (T)	62.6±0.34^{d}	35.4±0.37^{e}	19.45±0.3^{g}	15.24±0.44^{gh}	1.98±0.13^{fg}	29.6±1.6^{h}
P. fluorescence (P)	49.46±0.34^{k}	25.2±0.26^{k}	17.11±0.11^{k}	14.74±0.28^{h}	1.95±0.04^{fg}	23.6±1.6^{i}
G+A	61.71±0.13^{e}	35±0.28^{f}	23.24±0.24^{c}	17.07±0.64^{e}	2.53±0.31^{cd}	36±1.4^{ef}
G+T	68.04±0.08^{b}	50.5±0.36^{b}	21.47±0.22^{e}	17.17±0.65^{e}	2.32±0.3^{de}	38±1.4^{de}
G+P	61.61±0.21^{e}	35.6±38^{e}	20.79±0.15^{f}	17.95±0.41^{d}	2.22±0.21^{ef}	32.4±1.6^{g}
A+T	55.84±0.08^{i}	31.5±0.37^{h}	18.27±0.01^{i}	18.01±0.31^{cd}	2.51±0.23^{d}	35.2±2.2^{f}
A+P	66.06±0.09^{c}	48.7±0.48^{c}	17.48±0.1^{j}	18.02±0.31^{cd}	2.44±0.07^{de}	32±1.4^{g}
T+P	47.56±0.3^{m}	31.2±0.31^{h}	18.87±0.11^{h}	16.72±0.86^{ef}	2.31±0.2^{de}	34±1.4^{fg}
G+A+T	58.78±0.5^{f}	35.5±0.33^{e}	22.01±0.05^{d}	18.25±0.3^{bcd}	2.77±0.19^{bc}	40.4±1.6^{bc}
G+A+P	57.73±0.21^{h}	34.5±0.37^{g}	19.37±0.08^{g}	18.66±0.38^{abc}	2.86±0.2^{b}	37±1.4^{ef}
G+T+P	48.36±0.39^{l}	29.2±0.26^{i}	20.75±0.03^{f}	18.81±0.24^{ab}	2.93±0.21^{ab}	38.4±1.6^{cd}
A+T+P	58.15±0.39^{g}	40.5±035^{d}	21.4±0.02^{e}	17.22±0.6^{e}	2.78±0.26^{bc}	42±1.4^{b}
G+A+T+P	69.73±0.27^{a}	51.4±0.32^{a}	26.49±0.13^{a}	19.19±0.56^{a}	3.14±0.12^{a}	44.4±1.6^{a}
LSD (P≤0.005)	0.4379	0.4422	0.2271	0.618	0.2452	2.0761

†G- *G. mosseae,* A- *A. laevis,* T- *T. v*iride, P- *P. fluorescen*ce; FW- Fresh weight ; ±- Standard deviation
‡values in column followed by the same letter are not significantly different, $P \leq 0.05$, LSD

Table 2. Effect of bioinoculants on different floral parameters of *Zinnia elegans*

Treatments	No. of flower heads per plant	Diameter of flower head (cm)	Flower dry weight (g)	Flower age (days)	AM root colonization (%)	AM spore number
Control	2.6±0.54^{h}‡	1.49±0.013^{i}	0.48±0.022^{g}	7.6±0.5^{k}	0±0^{j}	0±0^{i}
G. mosseae (G)	4±0.7^{fg}	2.11±0.053^{fg}	0.94±0.008^{cd}	13±0.7^{g}	61.6±0.65^{f}	122.2±2.58^{e}
A. laevis (A)	4.6±0.54^{ef}	1.86±0.045^{h}	0.82±0.014^{e}	11.6±0.5^{hi}	37.84±0.21^{i}	65.2±1.92^{h}
T. viride (T)	4.2±0.83^{fg}	1.8±0.014^{hi}	0.76±0.011^{e}	11.4±0.5^{hi}	0±0^{j}	0±0^{i}
P. fluorescence (P)	3.8±0.44^{g}	1.78±0.013^{hi}	0.6±0.006^{f}	10±1^{j}	0±0^{j}	0±0^{i}
G+A	6.4±0.54^{a}	2.5±0.038^{c}	1.52±0.013^{a}	26±1.2^{a}	86.18±0.47^{b}	144±3.3^{b}
G+T	6.2±0.44^{ab}	3.02±0.04^{ab}	1.02±0.015^{c}	26.2±0.8^{a}	62.13±0.9^{e}	122.6±2.07^{e}
G+P	5.6±0.54^{bcd}	2.49±0.008^{cd}	0.98±0.10^{c}	21.8±0.8^{b}	63.39±0.4^{d}	124.8±2.58^{d}
A+T	5.4±0.44^{cd}	2.19±0.005^{f}	0.86±0.036^{de}	20±0.7^{c}	41.43±0.39^{g}	69±1.58^{g}
A+P	5.2±0.44^{de}	2.02±0.041^{g}	0.83±0.022^{de}	15.8±0.8^{e}	41.44±0.73^{g}	70±1.58^{f}
T+P	5.4±0.54^{cd}	2.29±0.008^{e}	0.81±0.007^{e}	17.2±0.8^{d}	0±0^{j}	0±0^{i}
G+A+T	6.4±0.44^{a}	3.2±0.007^{a}	1.47±0.016^{a}	25.8±0.8^{a}	84.8±1.51^{c}	145.8±2.86^{ab}
G+A+P	6.2±0.44^{ab}	2.49±0.01^{cd}	1.46±0.017^{a}	20.2±0.8^{c}	86.4±1.82^{b}	145±2.23^{b}
G+T+P	6±0^{abc}	2.39±0.005^{d}	1.45±0.033^{a}	14.6±0.5^{f}	62.05±1.83^{e}	122.8±3.83^{e}
A+T+P	5.6± 0.54^{bcd}	2.29±0.005^{e}	0.97±0.017^{c}	12± 0.70^{h}	38.73±0.44^{h}	68.2±1.92^{g}
G+A+T+P	6.6± 0.54^{a}	2.96±0.054^{b}	1.29±0.324^{b}	10.6±0.54^{ij}	86.58±0.57^{a}	146±3.53^{a}
LSD (P≤0.005)	0.6701	0.037	0.1053	0.9838	0.5196	2.4589

†G- *G. mosseae,* A- *A. laevis,* T- *T. v*iride, P- *P. fluorescen*ce; FW- Fresh weight ; ±- Standard deviation
‡values in column followed by the same letter are not significantly different, $P \leq 0.05$, LSD

Table 3. Effect of bioinoculants on chlorophyll, carotenoids and anthocyanin content of *Zinnia elegans*

Treatments	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Total carotenoids (mg/g FW)	Total anthocyanin (mg/100g FW)
Control	0.532 ±0.067^{j}‡	0.246±0.035^{j}	0.77±0.103^{i}	0.029±0.002^{j}	23.47±0.028^{l}
G. mosseae (G)	0.738±0.046^{fg}	0.596±0.024^{ef}	1.33±0.071^{f}	0.063±0.003^{f}	30.7±0.564^{d}
A. laevis (A)	0.618±0.029^{hi}	0.379±0.022^{i}	0.99±0.051^{h}	0.051±0.002^{gh}	28.3±0.02^{h}
T. viride (T)	0.673±0.038^{gh}	0.463±0.03^{h}	1.13±0.068^{g}	0.046±0.003^{hi}	24.97±0.024^{k}
P. fluorescence (P)	0.546±0.04^{ij}	0.401±0.012^{i}	0.94±0.053^{h}	0.04±0.001^{i}	27.13±0.033^{i}
G+A	0.787±0.022^{def}	0.679±0.024^{d}	1.46±0.047^{e}	0.178±0.263^{a}	35.23±0.027^{a}
G+T	0.939±0.067^{c}	0.8±0.02^{b}	1.74±0.087^{c}	0.083±0.005^{de}	32.05±0.024^{b}
G+P	0.76±0.037^{efg}	0.679±0.018^{d}	1.44±0.056^{e}	0.071±0.002^{ef}	30.72±0.027^{d}
A+T	0.717±0.033^{fg}	0.566±0.037^{fg}	1.28±0.071^{f}	0.101±0.005^{c}	29.93±0.027^{g}
A+P	0.745±0.035^{fg}	0.54±0.044^{g}	1.28±0.079^{f}	0.053±0.001^{g}	30.03±0.037^{g}
T+P	0.715±0.027^{fg}	0.621±0.049^{e}	1.33±0.077^{f}	0.048±0.001^{h}	25.74±0.028^{j}
G+A+T	1.028±0.124^{b}	0.88±0.038^{a}	1.9±0.162^{b}	0.118±0.004^{bc}	35.06±0.024^{a}
G+A+P	0.837±0.063^{de}	0.601±0.025^{ef}	1.43±0.089^{e}	0.119±0.017^{b}	31.1±0.027^{c}
G+T+P	0.842±0.04^{de}	0.62±0.023^{e}	1.46±0.063^{e}	0.089±0.002^{de}	30.34±0.019^{f}
A+T+P	0.868±0.035^{cd}	0.742±0.037^{c}	1.61±0.072^{d}	0.077±0.002^{e}	30.32±0.023^{f}
G+A+T+P	1.121±0.127^{a}	0.909±0.048^{a}	2.03±0.176^{a}	0.128±0.002^{ab}	35.24±0.03^{a}
LSD (P≤0.005)	0.077	0.0413	0.091	0.089	0.181

†G- *G. mosseae,* A- *A. laevis,* T- *T. v*iride, P- *P. fluorescen*ce; FW- Fresh weight ; ±- Standard deviation
‡values in column followed by the same letter are not significantly different, $P \leq 0.05$, LSD

Table 4. Effect of bioinoculants on different biochemical attributes of *Zinnia elegans*

Treatments	Total sugar (mg/100mg FW)	Total starch (mg/100mg FW)	Total protein (mg/100mg)	P content % (shoot)	P content % (root)	Phosphatase (IJ g-1 FW)	
						Acidic	Alkaline
Control	28.29±0.35^{n}‡	8.34±0.0581^{l}	28.29±0.35^{n}	0.77±0.049^{l}	0.86±0.034^{n}	0.15±0.002^{o}	0.26±0.005^{p}
G. mosseae (G)	32.22±0.12^{h}	11.59±0.024^{d}	32.22±0.12^{h}	1.59±0.031^{ef}	1.89±0.015^{g}	0.51±0.003^{g}	1.1±0.003^{i}
A. laevis (A)	30.4±0.29^{l}	10.59±0.037^{h}	30.4±0.29^{m}	1.15±0.041^{j}	1.33±0.039^{l}	0.26±0.026^{m}	0.83±0.004^{o}
T. viride (T)	31.61±0.1^{j}	9.62±0.035^{j}	31.61±0.1^{j}	1.51±0.067^{g}	1.81±0.015^{h}	0.28±0.003^{l}	0.9±0.004^{m}
P. fluorescence (P)	30.96±0.16^{m}	8.73±0.14^{k}	30.96±0.16^{l}	1.06±0.032^{k}	1.21±0.022^{m}	0.25±0.003^{n}	0.67±0.006^{n}
G+A	31.94±0.13^{i}	12.46±0.027^{c}	31.94±0.13^{i}	1.7±0.047^{d}	1.98±0.023^{f}	0.55±0.003^{f}	1.12±0.003^{h}
G+T	32.17±0.12^{h}	11.02±0.028^{f}	32.17±0.12^{h}	1.8±0.051^{c}	2.2±0.019^{e}	0.45±0.002^{h}	1.06±0.004^{j}
G+P	33.6±0.13^{e}	10.61±0.031^{h}	33.6±0.13^{e}	2.01±0.085^{b}	2.39±0.019^{b}	0.56±0.002^{f}	1.14±0.004^{f}
A+T	32.95±0.08^{g}	11.24±0.1^{e}	32.95±0.08^{g}	1.54±0.039^{fg}	1.52±0.019^{i}	0.43±0.002^{i}	1.13±0.003^{g}
A+P	31.22±0.11^{k}	10.99±0.064^{f}	31.22±0.11^{k}	1.29±0.025^{i}	1.37±0.02^{k}	0.36±0.001^{j}	1.02±0.002^{k}
T+P	31.45±0.07^{j}	10.04±0.02^{i}	31.45±0.07^{j}	1.41±0.052^{h}	1.42±0.015^{j}	0.35±0.001^{k}	0.99±0.002^{l}
G+A+T	34.54±0.23^{c}	12.55±0.027^{b}	34.54±0.23^{c}	1.63±0.04^{e}	1.96±0.03^{f}	0.95±0.001^{b}	1.2±0.003^{b}
G+A+P	35.02±0.06^{b}	12.53±0.021^{b}	35.02±0.06^{b}	2.17±0.082^{a}	2.3±0.019^{d}	0.59±0.003^{e}	1.17±0.003^{d}
G+T+P	34.03±0.11^{d}	11.64±0.024^{d}	34.03±0.11^{d}	1.81±0.043^{c}	2.22±0.024^{e}	0.82±0.002^{c}	1.18±0.003^{c}
A+T+P	33.27±0.07^{f}	10.7±0.021^{g}	33.27±0.07^{f}	1.76±0.043^{cd}	2.34±0.027^{c}	0.71±0.001^{d}	1.15±0.004^{e}
G+A+T+P	36.49±0.06^{a}	12.86±0.03^{a}	36.49±0.06^{a}	2.22±0.085^{a}	2.77±0.019^{a}	1±0.001^{a}	1.21±0.003^{a}
LSD (P≤0.005)	0.076	0.068	0.206	0.069	0.030	0.009	0.005

†G- *G. mosseae,* A- *A. laevis, T- T. v*iride, P- *P. fluorescen*ce; ±- Standard deviation
‡values in column followed by the same letter are not significantly different, $P \leq 0.05$, LSD

and percentage root colonization which can be due to increased concentration of auxin that plays a vital role in root growth (Smith and Read, 2008; Prasad *et al.*, 2012; Yadav *et al.*, 2013a; Yadav *et al.*, 2013b).

AMF treated plants showed early flowering and bigger flower size, which resulted into increased flower dry weight. Garmendia and Mangas (2012) noticed an early flowering induction in *Rosa hybrid* using AMF. Shamshiri *et al.* (2011) reported an increased flower dry weight using AMF while working on *Petunia.* Varga and Kytöviita (2010) also observed an increase in flower number with AM Fungi.

Biochemical parameters (chlorophyll, anthocyanin, sugar) were higher in AMF inoculated plants. Nowak (2004) also observed a notable enhancement in total sugar content with higher flower production in AMF treated plants of *Geranium*. Aboul-Nasr (1996) noticed a little effect on carotenoids content of AM treated *Zinnia* plants. The increase in concentration of chlorophyll and carotenoids might have resulted due to higher uptake of nutrients by AM fungi (Baslam *et al.*, 2013). It might also be due to the better absorption of water and nutrient especially phosphorous (Bolandnazar *et al.*, 2007). In the present study, both acidic and alkaline phosphatase activity increased with increased colonization thereby causing conversion of organic to inorganic phosphorous. Phosphatase is the main enzyme responsible for the mineralization of organic phosphorous (Amaya-Carpio *et al.*, 2003).

The evaluation of the effect of different bioinoculants on growth and physiological parameters revealed *G. mosseae* as an efficient bioinoculant as compared to *A. laevis* for growth enhancement of *Z. elegans*.

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