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# Impact of different levels of superphosphate using arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on *Chrysanthemum indicum* L.

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**Abstract**

A pot experiment was conducted to investigate the potential effect of arbuscular mycorrhizal fungi (*Glomus mosseae* & *Acaulospora laevis*) and phosphate solubilizing bacteria (*Pseudomonas fluorescens*) with different levels of superphosphate on *Chrysanthemum indicum* L. After 100 days, different plant growth parameters such as mycorrhization's characteristics, phosphatase activity and phosphorus uptake were measured. The obtained results revealed that the inoculation of plants with biofertilizers and recommended dose of superphosphate significantly improved the growth parameters. Inoculation with *A. laevis* + *P. fluorescens* at medium concentration of superphosphate showed maximum height, fresh and dry root weight, AM root colonization, AM spore count, alkaline phosphatase activity, acidic phosphatase activity and the percent phosphorus uptake in shoot and root whereas root length was maximum in *G. mosseae* + *A. laevis* + *P. fluorescens*. Leaf area and fresh and dry shoot weight were maximum in the treatment (*G. mosseae* + *A. laevis* + *P. fluorescens*) at low concentration of superphosphate. The use of AMF increased nutrient acquisition from an organic fertilizer source by enhancing acidic phosphatase (ACP) and alkaline phosphatase (ALP) activity, thus facilitating P acquisition and improving plant growth.

**Keywords:** Superphosphate; *Glomus mosseae*; *Acaulospora laevis*; *Pseudomonas fluorescens*; phosphatase; *Chrysanthemum indicum* L.

## 1. Introduction

*Chrysanthemum indicum* L. commonly known as Gul-e-Daudi or 'Glory of the East' belongs to the family Compositeae (Asteraceae). It is one of the most widely cultivated garden flowers and ranks probably next to the rose in popularity. Extracts of the plants (stem and flower) have been shown to have a wide variety of potential medicinal properties, including anti-HIV, antibacterial and antimycotic. Despite of large demand of this flower, their production is quite low. The production constraints in *Chrysanthemum* cultivation is poor soil fertility, high cost of chemical fertilizers, traditional system of crop management etc.

Current development in sustainability involves a rational exploitation of soil microbial activities and the use of less expensive source of plant nutrients like superphosphate, which may be made available to the plants by microbiologically mediated process. Microbial world particularly the beneficial microbes associated with plant roots are of paramount importance in horticulture which includes nitrogen fixers, phosphorus solublizers, growth enhancers and biocontrol agents. One such beneficial interaction is Arbuscular Mycorrhizae (AM). AM fungi have been found to increase plant growth (Mortimer *et al*., 2008), increase chlorophyll content (Demir, 2004), phosphorus content (Gaur *et al*., 2000), increase resistance to cultural and environmental stresses, and consequently improves plant growth (Smith and Read, 1997). Hyphae of AMF grow into the soil from roots and greatly improve access to immobile nutrients especially phosphorus. Phosphorus exists in soil as inorganic orthophosphate, as inert complexes with iron phosphate ($FePO_4$), aluminum phosphate ($AlPO_4$), and inorganic molecules such as phytate. Plant roots colonized with intra and extra-radical AMF hyphae can utilize sources of P in soil that are not readily available to non AMF roots. This is thought to involve an increase in the rate of solubilization of insoluble inorganic phosphorus (Pi) and/or hydrolysis of organic phosphorus (Po) (Smith and Read, 1997). Organic phosphorus is made available to plants largely after its mineralization or when hydrolyzed into Pi. Mineralization and hydrolysis of Po is mediated by the enzymatic activity of phosphatase. Phosphatase activity in the rhizosphere of colonized plants originates from plant roots, AMF, and bacteria. Greater enhancement of enzymatic acid phosphatase (ACP) and alkaline phosphatase (ALP) activity occurred with AMF roots compared to non AMF roots.

The mycorrhizal helper bacteria such as phosphate solublizing bacteria (PSB) are known to stimulate mycelial growth of arbuscular mycorrhizal fungi. The microbiologically solubilized phosphate could, however be taken up a mycorrhizal mycelium, thereby developing a synergistic microbial interaction. The role of PSB as a biofertilizer is unique in making the fixed soil phosphorus available to plants. PSB produce plant growth regulating substances, which promote root growth which ultimately improve the growth of the plant. So, now days, application of AMF as a biofertilizers in crop production is recommended with aim of increasing productivity and reducing fertilizers use. Because chemical fertilizers are expensive, produce short term benefits, and above all, their use may contribute to environmental pollution.

Thus, the present study was carried out to evaluate the effectiveness of AMF along with *Pseudomonas fluorescens* on growth parameters, root ACP and ALP activity and to determine the relationship of root phosphatase activity in phosphorus acquisition of *Chrysanthemum* at different levels of superphosphate in pot condition.

## 2. Materials and methods

### *2.1 Collection of soil sample*

Composite soil sample from rhizospheric soil of *C. indicum* L. was collected for isolation of dominant AM fungi. 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.

### *2.2 Isolation and identification of AM spores*

Isolation of dominant AM spores i.e., *Glomus mosseae* and *Acaulospora laevis* were done by using 'Wet Sieving and Decanting Technique' of Gerdemann and Nicolson (1963). The quantification of dominant AM spores was done by 'Grid line intersect 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) and Scheneck and Perez (1990**),** on the basis of conventional morphological characters i.e. colour, size, shape, wall structure, surface ornamentations of spores and size of subtending hyphae, bulbous suspensor, the number and arrangement of the spores in the sporocarps.

### *2.3 Mycorrhizal root colonization*

Mycorrhizal root colonization was done by 'Rapid clearing and Staining method' of Philips and Hayman (1970). The percent AM root colonization was calculated by using the formula:

$$\text{Percent AM root colonization} = \frac{\text{Total no. of root segments colonized}}{\text{Total no. of root segments examined}} \times 100$$

### *2.4 Mass productio of AM spores*

Dominant AM spores i.e. *A. laevis* (Gerd. and Trappe) and *G. mosseae* (Nicol. and Gerd.) isolated from rhizosphere of *C. indicum*, were mass produced using wheat as host plant and sand soil (1:3) as substrate.

### *2.5 Mass culture of Pseudomonas fluorescens*

*P. fluorescens* was multiplied in nutrient broth medium for 24 hrs for proper growth of bacteria.

### *2.6 Different concentrations of Super Phosphate*

Granules of super phosphate were grinded using pestle and mortar to make it a fine powder. Then, different concentrations of super phosphate i.e., low, medium and high were used. Medium concentration is recommended one (40kg $ha^{-1}$), lower is half of the recommended (20kg $ha^{-1}$) and higher is the double dose of recommended one (80kg $ha^{-1}$).

*2.7 Soil description and pot experimental set up*

Earthern pots (30 × 30 cm) were taken and amended with air-dried sterilized soil: sand mixture (300: 1500 gm) having total N 0.024%, available P 0.017%. To this different levels of superphosphate were applied i.e. low (20 kg $ha^{-1}$), medium (40kg $ha^{-1}$) and high (80kg $ha^{-1}$) concentrations. Different treatments were maintained i.e., Control (non-inoculated), *Glomus mosseae*, *Acaulospora laevis*, *G. mosseae* + *P. fluorescens*, *A. laevis* + *P. fluorescens* and *G. mosseae* + *A. laevis* + *P. fluorescens*. For mycorrhizal inoculation, 10% inoculum of each AMF (*G. mosseae* and *A. laevis*) consisting of roots and spores as single inoculation was placed 2 cm below the soil surface. Later, 10 ml of culture suspension of *Pseudomonas fluorescens* was mixed in the soil having cfu $1x10^{-9}$ $ml^{-1}$. A set of control was also maintained in similar condition without any inoculum. Now, double seedlings of Chrysanthemum were planted in each pot. The experiment was carried out in a polyhouse, where humidity was approximately 55-70%, temperature 25–30°C and *p*H 6.8±0. Light was provided by cool white fluorescent lamps (8000 lux) under a 16-h photoperiod. The polyhouse also received sunlight. Five replicates were maintained of each treatment. Then, the consortium effect of these bioinoculants and different levels of superphosphate were recorded on different growth parameters of *C. indicum* after 100 days of inoculation

*2.8 Measurement of growth, root and shoot weight (fresh and dry)*

After 100 days, plant height, leaf area was measured. For root and shoot weight (fresh and dry), roots and shoots were harvested, weighed for their fresh weight and then, oven dried at 70˚C for dry weight.

*2.9 Phosphorus estimation*

Estimation of plant (shoot and root) phosphorus was done by 'Vanadomolybdo phosphoric yellow color method' (Jackson, 1973) with percent (%) as unit.

*2.10 Phosphatase activity*

Phosphatse activity was measured in plant roots and 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 were used. Phosphatase activity was measured in terms of IU $g^{-1}$ FW.

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 $p \leq 0.005$ level of significance using Duncan's Multiple Range Test for comparison.

## 3. Results

*3.1 Change in height*

In the present investigation, it was found that mycorrhizal inoculation (*G. mosseae* and *A. laevis*) with superphosphate and *P. fluorescens* increased the height of the plant in comparison to the control. The most effective results were observed in medium concentra-

tion of superphosphate where various bioinoculants were applied to the plant. It is evident from Table 1 that after 100 days of inoculation, plant height was maximum in the dual combination of *A. laevis* + *P. fluorescens* (57.2±2.38 cm ) followed by *G. mosseae* + *P. fluorescens* and then, in triple combination of *G. mosseae* + *A. laevis* + *P. fluorescens* in medium concentration. It is also evident from table-1 that in low concentration, maximum plant height was observed in triple combination of *G. mosseae* + *A. laevis* + *P. fluorescens* (50.80±2.38 cm). Same combination was also effective in increasing the height (45.00±2.54 cm) in high concentration of superphosphate.

*3.2 Root Length*

Inoculation of *C. indicum* with significant bioinoculants (AM fungi, *P. fluorescens*) and superphosphate significantly increased the root length after 100 days of inoculation (Table 1). The medium concentration of superphosphate was found to be more effective for root length increment. Maximum root length increment in medium concentration was observed in combination of *G. mosseae* + *A. laevis* + *P. fluorescens* (24.301±1.94 cm) followed by dual combination of *G. mosseae* + *P. fluorescens* and then *G. mosseae* + *A. laevis* respectively. Similarly *G. mosseae* + *A. laevis* + *P. fluorescens*, significantly increased the maximum root length (22.72±2.04 cm, 19.85±0.97 cm) in low and high concentration of superphosphate respectively.

*3.3 Leaf area*

Bioinoculants (AM and PSB) with superphosphate significantly increased the leaf area of all treated plants as compared to control plant (Table 1). It was found that after 100 days of inoculation, the maximum leaf area (38.12±1.98) was found in the medium concentration of superphosphate with the combination of *G. mosseae* + *A .laevis* + *P. fluorescens* followed by *G. mosseae* + *P. fluorescens* and *A. laevis* + *P. fluorescens*. In low concentration maximum leaf area (35.80±1.51 $cm^2$) was observed in the dual combination of *A. laevis* + *P. fluorescens* but in high concentration of superphosphate *G. mosseae* + *A .laevis* + *P. fluorescens* showed maximum leaf area (30.50±1.85 $cm^2$).

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**Table1.** Consortium Effect of AM fungi, *P. fluorescens* and superphosphate on growth parameters of *C. indicum* after 100 days of inoculation

| Concentration | Treatments | Change in height (cm) | Root length (cm) | Leaf area (sq.cm) | Fresh shoot weight (gm) | Dry shoot weight (gm) | Fresh root weight (gm) | Dry root weight (gm) | AM Spore number/ 10 gm. of soil | AM Root colonization (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Low Conc. (20 kg h$^{-1}$)** | Control | 24.00±3.53$^{ef}$ | 12.73±1.75$^{e}$ | 16.92±2.83$^{ef}$ | 11.20±0.98$^{ef}$ | 1.10±0.30$^{ef}$ | 2.99±0.50$^{e}$ | 0.57±0.05$^{e}$ | 24.4±6.02$^{e}$ | 21.59±2.54$^{e}$ |
| | *G. mosseae* | 40.00±2.54$^{cd}$ | 21.05±3.03$^{b}$ | 22.54±2.34$^{e}$ | 18.10±0.67$^{bc}$ | 2.57±0.31$^{bc}$ | 4.21±0.60$^{c}$ | 0.89±0.07$^{c}$ | 85.8±4.20$^{c}$ | 74.83±3.83$^{c}$ |
| | *A. laevis* | 32.20±5.26$^{de}$ | 20.05±1.82$^{bc}$ | 22.46±1.95$^{e}$ | 16.81±1.49$^{cd}$ | 1.96±0.18$^{cd}$ | 3.74±1.28$^{d}$ | 0.72±0.98$^{d}$ | 73.6±3.20$^{d}$ | 54.39±4.35$^{d}$ |
| | G + P | 33.00±3.53$^{de}$ | 20.24±1.37$^{bc}$ | 27.46±2.34$^{cd}$ | 13.86±1.62$^{de}$ | 1.91±0.22$^{de}$ | 4.04±0.66$^{c}$ | 0.75±0.04$^{c}$ | 77.8±6.37$^{cd}$ | 63.07±3.03$^{cd}$ |
| | A + P | 46.00±2.54$^{c}$ | 22.58±1.98$^{ab}$ | 35.80±1.51$^{ab}$ | 18.70±0.41$^{bc}$ | 2.90±0.23$^{bc}$ | 4.41±0.72$^{c}$ | 0.91±0.05$^{c}$ | 90.6±4.15$^{bc}$ | 83.40±4.92$^{b}$ |
| | G + A.+ P | 50.80±2.38$^{bc}$ | 22.72±2.04$^{ab}$ | 32.48±1.64$^{b}$ | 22.49±0.46$^{a}$ | 4.25±0.30$^{a}$ | 5.44±0.83$^{b}$ | 1.08±0.28$^{b}$ | 90.8±3.56$^{bc}$ | 79.25±4.11$^{bc}$ |
| **Medium Conc. (40 kg h$^{-1}$)** | Control | 28.80±2.86$^{de}$ | 16.20±1.81$^{de}$ | 16.04±2.11$^{ef}$ | 12.33±0.42$^{e}$ | 1.74±0.36$^{e}$ | 3.19±0.61$^{d}$ | 0.61±0.03$^{d}$ | 23.0±3.53$^{ef}$ | 22.46±1.93$^{de}$ |
| | *G. mosseae* | 52.00±2.54$^{b}$ | 18.71±2.33$^{cd}$ | 34.32±1.76 $^{b}$ | 19.46±0.41$^{b}$ | 2.51±0.42$^{b}$ | 5.17±0.71$^{b}$ | 0.94±0.12$^{b}$ | 92.6±4.92$^{b}$ | 86.57±3.93$^{ab}$ |
| | *A. laevis* | 40.00±2.54$^{cd}$ | 20.55±2.50$^{bc}$ | 29.74±1.75$^{c}$ | 16.66±0.79$^{cd}$ | 2.31±0.51$^{cd}$ | 4.95±0.60$^{c}$ | 0.79±0.14$^{c}$ | 76.0±4.63$^{cd}$ | 65.73±2.89$^{cd}$ |
| | G + P | 54.20±3.19$^{ab}$ | 23.50±1.94$^{ab}$ | 36.00±.80$^{ab}$ | 20.17±1.12$^{ab}$ | 3.58±0.33$^{ab}$ | 5.34±0.86$^{b}$ | 1.04±0.21$^{b}$ | 95.0±3.39$^{ab}$ | 86.74±2.17$^{ab}$ |
| | A + P | 57.20±2.38$^{a}$ | 22.89±2.54$^{ab}$ | 38.12±1.98$^{a}$ | 20.98±0.44$^{ab}$ | 3.82±0.37$^{ab}$ | 6.40±1.21$^{a}$ | 1.09±0.23$^{a}$ | 101.8±6.97$^{a}$ | 93.48±2.95$^{a}$ |
| | G + A + P | 51.00±2.91$^{bc}$ | 24.30±1.94$^{a}$ | 31.32±2.07$^{ab}$ | 19.05±0.71$^{b}$ | 3.03±0.26$^{b}$ | 5.24±1.50$^{b}$ | 0.89±0.06$^{b}$ | 81.2±5.49$^{c}$ | 63.91±2.45$^{cd}$ |
| **High Conc. (80 kg h$^{-1}$)** | Control | 21.80±2.38$^{f}$ | 12.35±1.41$^{e}$ | 13.56±1.81$^{f}$ | 9.42±0.40$^{f}$ | 1.15±0.51$^{f}$ | 2.56±0.38$^{f}$ | 0.49±0.07f | 18.9±2.30$^{f}$ | 21.35±2.33$^{e}$ |
| | *G. mosseae* | 38.40±2.01$^{d}$ | 17.26±1.61$^{d}$ | 23.92±3.05$^{de}$ | 12.10±0.34$^{e}$ | 2.04±0.39$^{e}$ | 4.26±1.30$^{c}$ | 0.86±0.15$^{c}$ | 76.8±4.65$^{cd}$ | 60.70±3.80$^{d}$ |
| | *A. laevis* | 42.60±3.04$^{cd}$ | 19.42±1.87$^{c}$ | 29.64±1.82$^{c}$ | 18.95±0.21$^{bc}$ | 2.64±0.29$^{bc}$ | 3.59±0.47$^{d}$ | 0.71±0.03$^{d}$ | 64.8±3.27$^{de}$ | 63.01±3.96$^{cd}$ |
| | G + P | 38.20±3.34$^{d}$ | 18.09±1.51$^{cd}$ | 29.04±2.37$^{c}$ | 17.51±0.27$^{c}$ | 2.47±0.22$^{c}$ | 3.75±0.67$^{d}$ | 0.79±0.04$^{d}$ | 63.2±3.56$^{de}$ | 74.92±3.94$^{e}$ |
| | A + P | 37.60±2.88$^{d}$ | 18.46±0.80$^{cd}$ | 25.26±1.29$^{d}$ | 14.38±0.29$^{d}$ | 2.04±0.34$^{d}$ | 5.26±1.25$^{b}$ | 1.19±0.42$^{b}$ | 82.8±7.62$^{c}$ | 79.17±2.29$^{bc}$ |
| | G+ A + P | 45.00±2.54$^{c}$ | 19.85±0.97$^{c}$ | 30.50±1.85$^{bc}$ | 19.51±0.52$^{b}$ | 2.84±0.62$^{b}$ | 4.11±0.60$^{c}$ | 0.81±0.10$^{c}$ | 73.6±2.88 $^{d}$ | 76.48±2.94$^{e}$ |

G-*Glomus mosseae*, A-*Acaulospora laevis*, P- *Pseudomonas fluorescens*, ± Standard desviation,
*The mean difference is significant at 0.5 level.
Mean value followed by different alphabet/s within a column do not differ significantly over one other at $p \leq 0.05$ lead by Duncan's Multiple Range Test

*3.4 Shoot and Root fresh & dry weight*

As shown in Table 1 that biomass of all the inoculated plants of *C. indicum* increased significantly in terms of fresh and dry shoot weight after 100 days of inoculation. Low concentration where half of recommended dose of superphosphate used with various bioinoculants showed best results. Maximum increase in shoot biomass (fresh & dry) was recorded in the triple combination of *G. mosseae* + *A. laevis* + *P. fluorescens* (22.49±0.46 gm, 4.25±0.30 gm) in the low concentration followed by *G. mosseae* + *P. fluorescens* in medium concentration. After 100 days, the increase in root biomass was observed maximum with the combination of *A. laevis* + *P. fluorescens* in medium concentration. Second most effective result was observed in low concentration of the treatment *G. mosseae* + *A. laevis* + *P. fluorescens*.

*3.5 Root Colonization and AM spore number*

After 100 days of inoculation, percent mycorrhizal root colonization and AM spore number also increased in all treated plants over control (Table 1). Maximum percent root colonization was present in combination of *A. laevis* + *P. fluorescens* (93.48±2.95 %) followed by *G. mosseae* + *P. fluorescens* and *G. mosseae* in medium concentration was applied to the plants. Similarly *A. laevis* + *P. fluorescens* was found most significant in increasing the root colonization (83.40± 4.92 %, 79.17±2.29 %) in low and high concentration respectively.

*3.6 Phosphorus content and phosphatase activity*

The amount of phosphorus in shoot and roots significantly increased in inoculated plants as compared to control after 100 days of inoculation (Table 2). In the medium concentration, phosphorus content in shoots was found to be maximum in the plants treated with *A. laevis* + *P. fluorescens* (1.489±0.138 %). Second most effective results were observed in the combination *G. mosseae* + *P. fluorescens* in low concentration where half of the recommended dose of superphosphate was applied to the plant. Similarly, in roots, maximum P uptake was observed in *A. laevis* + *P. fluorescens* (1.767±0.109 %) followed by *G. mosseae* + *P. fluorescens* in medium concentration. Plants inoculated with AM fungi either *G. mosseae* or *A. laevis* along with *P. fluorescens* and with recommended dose of superphosphate significantly increased both root surface acid phosphatase and alkaline phosphatase activity. After 100 days of inoculation, maximum acid phosphatase activity was observed in the dual combination of *A. laevis* + *P. fluoresence* followed by *G. mosseae* + *P. fluoresence* and then, in the treatment of *G. mosseae* in the recommended concentration of superphosphate. The activity of alkaline phosphatase also follows the same pattern. Maximum alkaline phosphatase activity was seen in *A. laevis* + *P. fluoresence* (0.132±0.006 IU/g FW) in recommended dose of superphosphate. However high concentration does not showed significant results.

**Table 2.** Effect of AM fungi, *P. fluorescens* and superphosphate on phosphorus uptake and phosphatase activity of *C. indicum* after 100 days of inoculation.

| Concentration | Treatments | % Phosphorus | | Phosphatase activity (IU g-1 FW) | |
|---|---|---|---|---|---|
| | | Shoot P | Root P | Alkaline Phosphatase | Acidic Phosphatase |
| Low conc. (20 kg $h^{-1}$) | *Control* | 0.44±0.097$^{e}$ | 0.60±0.097$^{e}$ | 0.10±0.016$^{e}$ | 0.39±0.012$^{e}$ |
| | *G. mosseae* | 0.80±0.145$^{c}$ | 0.95±0.115$^{c}$ | 0.18±0.012$^{c}$ | 0.73±0.013$^{c}$ |
| | *A. laevis* | 0.59±0.126$^{d}$ | 0.74±0.133$^{d}$ | 0.15±0.009$^{de}$ | 0.60±0.009$^{de}$ |
| | *G + P* | 0.80±0.102$^{c}$ | 0.91±0.099$^{c}$ | 0.16±0.011$^{d}$ | 0.71±0.010$^{d}$ |
| | *A + P* | 1.04±0.136$^{bc}$ | 1.16.±0.088$^{bc}$ | 0.19±0.012$^{bc}$ | 0.76±0.013$^{bc}$ |
| | G + A.+ P | 1.14±0.011$^{ab}$ | 1.23±0.091$^{ab}$ | 0.20±0.008$^{b}$ | 0.76±0.009$^{b}$ |
| Medium conc. (40 kg $h^{-1}$) | *Control* | 0.45±0.151$^{de}$ | 0.67±0.066$^{de}$ | 0.08±0.008$^{ef}$ | 0.42±0.009$^{de}$ |
| | *G. mosseae* | 0.99±0.144$^{b}$ | 1.42±0.127$^{b}$ | 0.21±0.010$^{b}$ | 1.00±0.013$^{b}$ |
| | *A. laevis* | 0.80±0.125$^{c}$ | 1.20±0.130$^{c}$ | 0.15±0.009$^{de}$ | 0.69±0.008$^{de}$ |
| | *G + P* | 1.13±0.108$^{b}$ | 1.53±0.077$^{b}$ | 0.23±0.010$^{ab}$ | 1.04±0.010$^{ab}$ |
| | *A + P* | 1.48±0.138 $^{a}$ | 1.76±0.109$^{a}$ | 0.25±0.011$^{a}$ | 1.21±0.004$^{a}$ |
| | G + A+ P | 0.95±0.121$^{b}$ | 1.28±0.094$^{b}$ | 0.17±0.008$^{cd}$ | 0.92±0.010$^{c}$ |
| High conc. (80 kg $h^{-1}$) | *Control* | 0.39±0.109$^{f}$ | 0.36±0.081$^{f}$ | 0.05±0.008$^{ef}$ | 0.22±0.010$^{ef}$ |
| | *G. mosseae* | 0.62±0.106$^{d}$ | 0.69±0.106$^{d}$ | 0.10±0.007$^{e}$ | 0.39±0.012$^{e}$ |
| | *A. laevis* | 0.76±0.118$^{cd}$ | 0.96±0.066$^{cd}$ | 0.17±0.011$^{cd}$ | 0.56±0.008$^{c}$ |
| | *G+P* | 0.71±0.106$^{cd}$ | 0.80±0.093$^{cd}$ | 0.16±0.008$^{cd}$ | 0.48±0.013$^{cd}$ |
| | *A+P* | 0.60±0.098$^{d}$ | 0.78±0.085$^{d}$ | 0.12±0.009$^{de}$ | 0.43±0.010$^{de}$ |
| | G+ A+P | 0.90±0.157$^{bc}$ | 1.07±0.067$^{bc}$ | 0.19±0.022$^{bc}$ | 0.66±0.009$^{bc}$ |

G- *Glomus mosseae*, A-*Acaulospora laevis*, P- *Pseudomonas fluorescens* ± Standard deviation,
*The mean difference is significant at 0.5 level.
Mean value followed by different alphabet/s within a column do not differ significantly over one other at $p \leq 0.05$ lead by Duncan's Multiple Range Test

## 4. Discussion

The height increment registered could be as a result of enhanced inorganic nutrient absorption (Cooper, 1984) and greater rate of photosynthesis (Allen *et al*., 1981). *P. fluorescens* was found to be common effective bioinoculant which increased the height of the plant in recommended dose of superphosphate. The reason may be due to the balanced absorption and solubilization of phosphorus by *P. fluorescens*. The results are also in close conformity with Gaur *et al*., (2000) who found increase in vegetative growth of *Petunia hybrida*, *Callistephus chinensis* and *Impatiens balsamina* while inoculating with AM fungi and recommended dose of chemical fertilizers. El-Khateeb *et al*. (2010) also observed increase in height of *Chamedora elegans* by AM fungi and NPK.

The effectiveness of lower concentration of superphosphate in increasing the root length may be due to the direct effect of superphosphate fertilizers or indirectly through the microbial propagation activation. AMF enhanced nutrient uptake by increasing surface area of roots with the development of an extensive extra-radical hyphae network (Smith and Read, 1997).

The higher increment of leaf area registered with inoculated plant could be as a result of increased phosphorus uptake due to which biosynthesis processes are enhanced, determining a faster growth and development, which leads to a greater leaf area (Schmedit *et al*., 2010). Similarly, Chaitra (2006) also observed increase in leaf area and flower yield per plant with the application of biofertilizers + vermicompost + 50 percent recommended dose of NPK fertilizers in China aster.

Higher concentration of superphosphate markedly reduced the shoot and root biomass as compared to medium concentration. Earlier studies have also shown that biomass of *Geranium* plant increased significantly when plant is inoculated with *P. fluorescence* and AM fungi (Shivakumar *et al*., 2002). Padamadevi *et al*. (2004) reported that application of phosphate solubilizing bacteria and AMF along with inorganic nutrients (NPK) brought about significantly higher effects on growth in *Anthurium* sp. The application of NPK at 45:45:37 mg per kg of soil along with inoculation of AM exhibited the increment in biomass might be due to the indirect effect of more number of branches as stimulated and developed by the influence of inorganic fertilizers along with bioinoculants. AMF with PSB stimulated the nutrient uptake and biosynthesis of plant growth regulators, there by improving the growth and developmental process of plant.

Lower concentration (half of recommended dose of phosphorus) also showed higher mycorrhizal root colonization and spore number as compared to high concentration (double of recommended dose of phosphorus). It may be that high soil phosphate level determines the reduction of hyphal growth and spore production of arbuscular mycorrhizal fungi. Results are also in accordance of Guillemin *et al*. (1995) who demonstrated that in P-sufficient soils mycorrhizal infection were reduced by phosphate fertilization, meanwhile in P- deficient soil fungal infection is not modified. The soil used was sterilized, even after that AM spore number and AM colonization were observed in uninoculated plants which implies that contamination might have occurred due to aerial mycorrhizal spores at some stage of the experiment in polyhouse (Stottlemer *et al*., 2008). Other possible sources of contamination are seedling roots adhering the soil particles and growing mixture which ultimately becomes the reason for the presence of spore number and root colonization in uninoculated plants.

The acid phosphatase activity was much greater than alkaline phosphatase activity. Bhadraiah *et al*. (1999) also noted positive correlation between acid phosphatase activity and phosphorus uptake. The increased phosphorus uptake has been attributed to extended AM hyphae, which explore a larger volume of soil and P solubilization by AM root exudates. Effective P acquisition by the external hyphae is related to the formation of polyphosphates in the hyphae and the small hyphal diameter leading to a relatively larger volume delivering P per unit surface compared to the root surface area (Jungk and Classen, 1989; Jakobsen *et al*., 1992). 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. Alkaline phosphatase activity is also closely linked to both mycorrhizal stimulation and the arbuscular phase of the colonization and there is strong evidence that it is of fungal origin (Gianinazzi- Pearson and Gianinazzi, 1978). Gianinazzi *et al*. (1979) revealed that alkaline

phosphatase activity was localized in the vacuoles of mature arbuscules. It is believed that ACP was involved in the increased uptake of phosphorus from the soil, while ALP may be linked to active phosphate assimilation or transport in mycorrhizal roots. Results are also in accordance of Garcia-Gomez *et al*. (2002) who reported that soluble and extractable root acid phosphatase activity was higher in *Glomus claroideum* inoculated *Carica papaya* plants. *A. laevis* was found to be the better strain as compared to the *G. mossese* in enhancing most of the studied parameters alone and even in combination with *P. fluorescence* and thus demonstrated biological specificity between AM fungal strain and host plant species (Bever *et al*., 1996).

Most growth parameters and P-uptake, significantly increased in the medium concentration was amended as compared to low and high concentration of superphosphate. As some amount of P fertilizer is required for the establishment and growth of the AM fungal strains and this fungus is also inhibited as the concentration of phosphoric fertilizer increases and even if it is present in low concentration. Thus mycorrhization was highest in the standard organic system in which the recommended dose of P fertilizer was added (Abbott *et al*., 1984)

Phosphorus solubilizing microorganism (*P. fluorescens*) might have influenced the root exudation of host plant that resulted in the stimulation of AM spores in the rhizosphere and thus behaved as mycorrhiza helper bacteria because they promoted higher root colonization rate and spore number of AMF which helps in solubilization of mineral phosphate and contribute to the P cycling, promoting a sustainable nutrient supply to the crop plants for higher yield. Similar interactive effect of AM fungi and rhizobacteria was observed by Boer *et al*. (2005) that root colonization by *G. intraradices* alone was about 20%, while co-inoculation with *P. fluorescens*, or *Agrobacterium rhizogenes* enhanced fungal colonization up to 40%, 50% and 60%, respectively.

High soil superphosphate concentration resulted in the reduction of hyphal growth and spore production of arbuscular mycorrhizal fungi that ultimately reduced the phosphatase secretion which are actually responsible for the conversion of bound P into available form and hence lesser P-uptake in high superphosphate concentration. Similarly, the overall level of AM colonization and spore number decreases with increasing availability of soluble P (Azcon-Aguilar and Barea, 1997). Mycorrhizal roots acquire P more efficiently than non-mycorrhizal roots, especially at low soil fertility levels. High P may be detrimental to mycorrhizal colonization and limit the phosphorus uptake (Hu *et al*., 2009).

## 5. Conclusions

This work clearly indicates the beneficial effects of coinoculation of *P. fluorescens* and AM fungi with different levels of superphosphate on the growth and biomass of economically important *C. indicum.* The coinoculation of *A. laevis* and *P. fluorescens* with medium dose of superphosphate had better growth effect and phosphorus uptake in comparison to other treatments. This is due to the mutual positive action of *P. fluorescens* and AM fungi strains that helped to absorb more phosphorus from soil. On the basis of results it can be concluded that this practice can be exploited in field condition for commercial production of *Chrysanthemun*.

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