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Interaction effect of bio-inoculants on modulating growth and yield of chrysanthemum (*Dendranthema grandiflora*) cv. yellow gold

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Abstract
Drawbacks of intensive farming practices, environmental hazards and costs of chemical fertilizers have renewed interest in bio-inoculants. Microorganisms playing an important role in improving the plant growth and yield are generally referred as beneficial microbes or plant growth promoting rhizo-microorganisms (PGPR). The investigation was carried out in green house at Department of Agricultural Microbiology, UAS, GKVK, Bengaluru during 2016-17 in a Completely Randomized Design (CRD) with three replications and nine treatments. The observations were taken at 30, 60, 90 days after transplanting and at harvest. The treatment received 75% N & P + 100% K + *Azotobacter* isolate (PGPR-24) @ 2kg/ha + *Bacillus* isolate (PGPR-9) @ 2kg/ha recorded significantly highest plant height (41.74 cm), number of branches (8.33/plant), number of leaves (136.33/plant), plant spread (798.27 cm^2), number of suckers (11.67), total dry weight (88.50g/plant) accumulation; yield attributes such as number of flowers (26.33 per plant) and flower weight (101.90g/plant). The native isolates (*Azotobacter* spp. and *Bacillus* sp.) performed well in compare with control as well as reference strains in both single and dual inoculated treatments may be because of positive interaction between plant and native isolates and may be the rhizospheric effect of chrysanthemum on native isolates was good in compare to reference strains

Keywords: PGPR, Bio-fertilizers, *Azotobacter*, *Bacillus megaterium*, PSB, Chrysanthemum

Introduction
Chrysanthemum (*Dendranthema grandiflora* Tzvelev.) also known as "Autumn Queen" or "Queen of East" belongs to the family Asteraceae. It occupies a prominent place in ornamental horticulture; it is one of the commercially exploited and considered as the number one flower crop in many countries, including India, United States, Japan *etc*., It is mainly grown for cut and loose flowers, using in garland making, general decoration, hair adornments and religious functions and also grown in pots for flower shows because of its most attractive and dazzling flower colours with varying in size and long vase life. In India Chrysanthemum has been recognized as one among the five important commercially potent flower crops by the All India Coordinated Floriculture Improvement Project (ICAR) with total production 207.17 thousand tones. Though the Chrysanthemum is one of the important commercial flower crops of Karnataka, its yield and quality levels are low.

Growth and yield of flower crops are directly influenced by the rate and time of application of chemical fertilizers (Beniwal *et al*., 2006) [3]. Chrysanthemum is a heavy feeder of nutrients specially nitrogen and phosphorus (Nalewadi *et al*., 1982) and nutrient management is very important in chrysanthemum to obtain good quality and higher yield of flowers. At present these nutrients are supplied by chemical fertilizers. The use of chemical fertilizers had resulted not only in the deterioration of soil health but also has led to some major environmental problems, such as soil and water pollution and other health related problems, besides increasing the input cost for crop production especially on the marginal farmers. This situation emphasized the need for developing alternate production systems that are friendlier to the environment and are more judicious in managing soil health, crop growth and yield.

Heterogeneous group of naturally occurring soil bacteria aggressively colonize plant roots and owe benefit to plants. They can be found in the rhizosphere, at root surfaces and in association with roots, enhancing the growth of the plant either directly and/or indirectly through nutrient fixation, mobilization and translocation or by producing plant growth hormones and vitamins or by reducing the pathogenic microbial load in rhizospheric region (Glick, 1995) [8]. It is well established that only 1 to 2 per cent of bacteria promote plant growth in the rhizosphere (Antoun and Kloepper, 2001) [2]. They not only promote plant growth but also help in sustainable agricultural development and protecting the environment (Das *et al*., 2013) [6]. With this the present investigation was carried out to check the effect of native PGPR isolates

on growth and yield of chrysanthemum in different combinations.

Material and Method

The present investigation was carried out at the Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru for studying the Effect of Nitrogen fixing and Phosphate solubilizing bacteria on modulation of growth and yield of chrysanthemum (*Dendranthema grandiflora* Tzvelev.) cv. Yellow Gold.

Bacterial isolates used: The Nitrogen fixing and Phosphate solubilizing bacterial isolates were isolated from rhizosphere soil samples of chrysanthemum crop grown in various parts of Bengaluru urban district, Karnataka, India, by standard serial dilution plate method, further the isolates were characterized morphologically and biochemically and screened for plant growth promoting activates like Nitrogen fixation, Phosphate solubilization (Jackson, 1973) [11] and plant growth hormone production (Ivanova *et al.*, 2001) [10] both qualitatively and quantitatively as for standard protocols. The pure cultures of isolates were obtained by repeated streaking on respective media and were maintained under refrigerated condition at 4 °C. The reference strains *Azotobacter chroococcum* and *Bacillus megaterium* were procured from Biofertilizer lab, UAS, GKVK, Bengaluru.

Preparation of biofertilizer

Talc used as carrier material for preparation of powder based inoculum. The pure cultures of *Azotobacter* sp. (PGPR-24) and *Bacillus* spp. (PGPR-9) as well as reference strains (*Azotobacter chroococcum* and *Bacillus megaterium*) were inoculated to 100 ml freshly prepared Luria broth and kept for incubation in rotatory incubator at 100 rpm with 28±2 °C. Once the population reached 1×10^8 cfu ml^{-1} the broth cultures were mixed with sterilized and cooled talcum powder in the ratio of 1:2.5 separately, followed by curing for 24 hr and contents were packed in polythene bags and sealed (Vidhyasekaran and Muthamilan, 1995) [20].

Treatment details

The experiment was laid out in a Completely Randomized Design(CRD) with nine treatments and three repetitions comprising of different combinations of both isolated strains and standard reference isolates nitrogen fixers, phosphate solubilizes and chemical fertilizers.

Treatments	**Details**
T_1	Control (100% RDF)
T_2	75% N & P + *Azotobacter* isolate (PGPR-24)
T_3	75% N & P + *Bacillus* isolate (PGPR-9)
T_4	75% N & P + Ref. strain of *Azotobacter* (*A. chroococcum*)
T_5	75% N & P + Ref. strain of PSB (*B. megatherium*)
T_6	75% N & P + Ref. strain of *Azotobacter* sp. + Ref. strain of PSB
T_7	75% N & P + *Azotobacter* isolate (PGPR-24)+ Ref. strain of PSB (*B. megatherium*)
T_8	75% N & P + *Bacillus* isolate (PGPR-9) + Ref. strain of *Azotobacter* (*A. chroococcum*)
T_9	75% N & P + *Azotobacter* isolate (PGPR-24) + *Bacillus* (PGPR-9)

*For all treatments recommended dose of potash (K) and FYM was common.
N- Nitrogen, P- Phosphate
PSB: Phosphate solubilizing bacteria
RDF: Recommended Dose of Fertilizer

Preparation of pot culture soil: The red sandy loam soil was used as planting medium and it was collected from an uncultivated land at UAS, GKVK Bangalore, Karnataka, India. It was sieved through 2 mm sieve and mixed thoroughly to get homogenous mixture. Initial microbial population *viz.*, Bacteria, Fungi and Actinomycetes 9.5 X 10^6, 3.8 X 10^4 and 1.7 X 10^3 cfu/gm respectively were estimated using standard dilution plate technique and chemical properties like soil pH (6.65), Electrical conductivity (0.25 dS m^{-1}) and Organic carbon (0.47%) were analysed before conducting the experiment. PVC flower pots with the capacity of 8 kg were filled with well homogenized soil along with recommended dose of well decomposed FYM (90 g/pot) 8 days prior to planting and were watered regularly.

Seedling material

Chrysanthemum seedlings of variety Yellow Gold (Marigold) were procured from Nallappa Nursery, Ramsagara village, Chandapura, Anekal, were transplanted by adding chemical fertilizers and talc based PGPR biofertilizers (@ 2 kg/ha as for the treatments mentioned above and watered regularly to maintain moisture at 60 per cent.

Growth parameters

The data on vegetative plant growth parameters *viz.*, plant height (cm) was measured from the base (ground level) to the tip of the growing point, total number of fully opened green leaves per plant were counted and recorded manually, number of lateral branches per plant arose from the main stem were counted and recorded, plant Spread (cm) was measured by recording the maximum length in the north-south and the east-west direction, number of suckers arose from the main stem were counted and recorded at 30, 60, 90 days after transplanting and at harvest.

Statistical analysis:

The data obtained was subjected for statistical analysis by one way analysis of variance using WASP: 2.0 (Web Agri Stat Package) statistical tools (www.icargoa.res.in/wasp/ index. P hp).

Results and Discussion

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads:

Plant growth attributes

Plant height (cm)

Among different treatment combinations treatment T_9 had positive influence on the vegetative growth parameters at different plant growth stages. During initial stage of crop growth (30 DAT) treatment T_1 showed higher plant height (20.00 cm) compare to all other treatments, after 30 DAT treatment T_9 - 75% N & P + *Azotobacter* isolate (PGPR-24) + *Bacillus* (PGPR-9) was shown maximum plant height 31.67, 41.40 and 41.73 cm at 60, 90 DAT and at harvest respectively (Table 1, plate 2). Lowest plant height was observed in T_5 (25.67, 31.00 and 31.33 cm at 60, 90 DAT and at harvest respectively. The increase in the plant height in the treatment T_9 might be due to availability of nutrients and growth promoting substances. The increase in plant growth in the inoculated treatment of Nitrogen fixers and Phosphate solubilizers enhanced N and P nutrient uptake (Kaushik *et al.*, 2013 and Jayamma *et al.*, 2008) [12]. IAA and GA are known

to enhance the shoot length root length and also the plant growth (Brown, 1972, Panchal *et al.*, 2010) [4, 16].

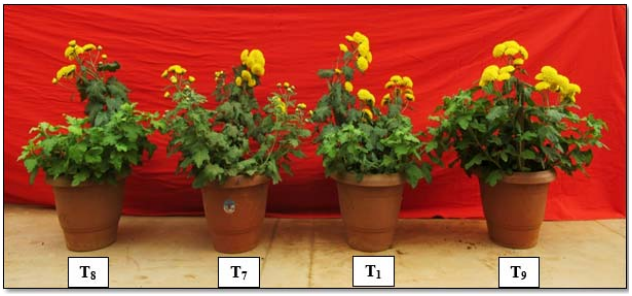

Plate 2: Comparison of growth of chrysanthemum (*Dendranthema grandiflora* Tzvelev.) in different treatments at harvesting stage.

Table 1: Effect of Nitrogen fixing and Phosphate solubilizing bacteria on chrysanthemum (*Dendranthema grandiflora* Tzvelev.) plant height at different plant growth stages.

Treatments	Plant height (cm)			
	30 DAT	60 DAT	90 DAT	At harvest
T_1	20.00	30.33^{a}	39.00^{b}	39.33^{b}
T_2	18.67	26.67^{cd}	33.67^{de}	33.83^{de}
T_3	18.50	25.83^{d}	31.33^{f}	31.67^{ef}
T_4	18.67	26.33^{cd}	32.17^{ef}	32.33^{ef}
T_5	18.33	25.67^{d}	31.00^{f}	31.33^{f}
T_6	18.83	27.00^{c}	35.17^{cd}	35.50^{cd}
T_7	19.00	28.67^{b}	37.43^{b}	38.83^{b}
T_8	19.10	28.33^{b}	37.07^{bc}	37.37^{bc}
T_9	19.90	31.67^{a}	41.40^{a}	41.73^{a}

Notes: The number followed by the same latter are not significantly different at (p<0.05) level of Duncan's test.

Table 2: Effect of Nitrogen fixing and Phosphate solubilizing bacteria on number of leaves of chrysanthemum (*Dendranthema grandiflora* Tzvelev.) at different plant growth stages.

Treatments	Number of leaves			
	30 DAT	60 DAT	90 DAT	At harvest
T_1	33.00	76.33^{a}	128.00^{b}	132.67^{b}
T_2	32.00	$68.33d^{e}$	114.00^{e}	119.00^{e}
T_3	31.67	64.33^{fg}	106.67^{g}	111.33^{f}
T_4	31.33	67.00^{ef}	110.33^{f}	116.00^{e}
T_5	31.33	64.00^{g}	105.00^{g}	109.33^{f}
T_6	32.00	70.00^{d}	119.67^{d}	124.00^{d}
T_7	33.00	74.33^{ab}	127.33^{b}	131.67^{b}
T_8	32.67	72.33^{bc}	123.67^{c}	127.33^{c}
T_9	32.67	76.67^{a}	131.00^{a}	136.33^{a}

Notes: The number followed by the same latter are not significantly different at (p<0.05) level of Duncan's test.

Number of leaves per plant

The numbers of leaves are very important in growth and yield of any crop since they perform photosynthesis (Tanaka, A and Makino, A, 2009) [18]. The data on number of leaves per plant (Table-2) shows that initially seedling respond to chemical fertilizers, resulted in more leaves 33.00 leaves/plant in control but after 30 DAT may be due to loss of applied fertilizers, the results were gradually overtaken by treatment T_9 (76.67, 131.00 and 136.33 leaves/plant at 60, 90 DAT and at harvest respectively). Lower numbers of leaves were observed in treatment received only single procured phosphate solubilizer (Tien *et al.*, 1979 and Wang *et al.*, 1995) [19, 21].

Number of lateral branches per plant and plant spread

Number of branches per plant increases the total plant area and plant spread which in turn decreases the shading effect of one leaf over others and number of branches proportion to number of flowers per plant. Initial two months there were no significant difference among the treatments but after 60 days the treatment T_9 has shown increased lateral branches per plant (8.00 and 8.33 branches per plant) and plant spread (791.67 and 798.27 cm^2) at 90 DAT and at harvesting respectively (Figer-1 and Table-4). This might be due to availability of nutrients throughout the crop growth and growth regulators like Auxins like NAA and Cytokinins released by *Azotobacter* and PSB might have resulted in breaking of apical dominance and accelerated higher number of branches (Airadevi, 2010) [1]. Increase in the numbers of branches per plants due to inoculation of PGPR has been reported by several workers (Subba Rao, 1993 and Hemavathi, 1997) [17, 9].

Table 4: Effect of Nitrogen fixing and Phosphate solubilizing bacteria on Yield parameters of chrysanthemum (*Dendranthema grandiflora* Tzvelev.)

Treatments	Yield parameters	
	Number of flowers per plant	Weight of flower per plant (g)
T_1	25.33^{ab}	99.00^{b}
T_2	22.67^{cde}	90.33^{e}
T_3	21.67^{de}	87.67^{ef}
T_4	22.00^{de}	88.33^{ef}
T_5	21.33^{e}	87.43^{f}
T_6	23.00^{cd}	93.10^{d}
T_7	24.00^{bc}	97.13^{bc}
T_8	23.67^{c}	95.46^{cd}
T_9	26.33^{a}	101.90^{a}

Notes: The number followed by the same latter are not significantly different at (p<0.05) level of Duncan's test.

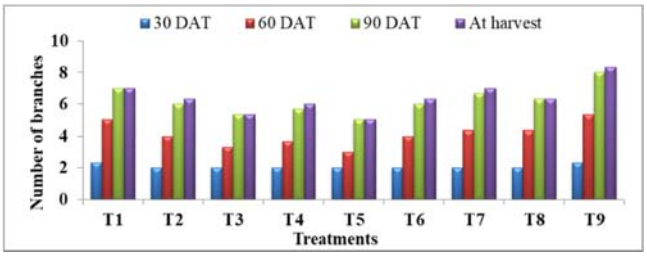

Fig. 1: Effect of Nitrogen fixing and Phosphate solubilizing bacteria on number of lateral branches of chrysanthemum (*Dendranthema grandiflora* Tzvelev.) at different plant growth stages.

Number of suckers per plant

The results on number of suckers per plant in different treatments (Figer-2) reviled that the effect of plant growth promoters (nitrogen fixers and phosphate solubilizers) in treatment T_9 influenced more (10.67 and 11.67 suckers per plant at 90 DAT and at harvesting) compare to other treatments after 60 DAT. This might be due to more fixation of nitrogen, solubilization of phosphate and production of growth hormones by isolates in treatment T_9 compare to other isolates in other treatments.

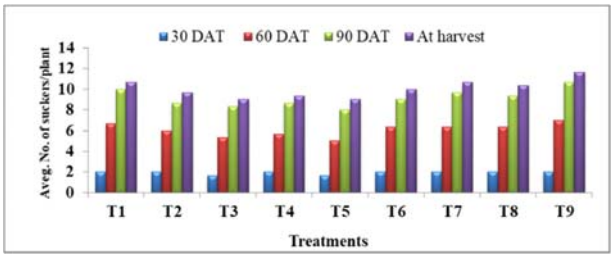

Fig. 2: Effect of Nitrogen fixing and Phosphate solubilizing bacteria on number of suckers of chrysanthemum (*Dendranthema grandiflora* Tzvelev.) at different plant growth stage.

Yield attributes

Number of flowers per plant

Different treatments significantly influenced the flower production in terms of number of flower produced per plant (Table-4). Plants received 75% N & P + *Azotobacter* isolate (PGPR-24) + *Bacillus* isolate (PGPR-9) registered higher (26.33) number of flowers followed by control (25.33). The least number of flowers (21.33) produced per plant was in treatment (T_5) which had 75% N & P + Ref. strain of PSB (*Bacillus megaterium*). Narasimharaju and Haripriya (2001) [15] reported higher flower yield in crossandra with the combination of *Azotobacter* and PSB with 75 per cent NPK. Deshmukh *et al*., (2008) [7] in gaillardia.

Yield of flowers per plant (g)

The data on flower yield per plant was significantly influenced by PGPRs (Table-6). Application of 75% N&P + *Azotobacter* isolate (PGPR-24) + *Bacillus* isolate (PGPR-9) gave significantly maximum flower yield per plant (101.90 g/plant). However, minimum flower yield per plant (87.43 g/plant) was recorded in the treatment (T_5) being the 75% NP + Ref. strain of PSB (*Bacillus megaterium*). This might be due to nutrient availability and translocation by PGPR organisms and consequently early flowering results in higher yields than in late or delayed flowering in control (99.00 flowers /plant) and other treatments. Higher flower yield in chrysanthemum with the combination of *Azotobacter* and PSB with 75 per cent NPK was reported by Mesharam *et al*., (2008) [13] and similar results were found by Panchal *et al*., (2010) [16] and Chandra *et al*., (2007) [5] in chrysanthemum.

Total dry weight (g/plant)

The dry weight accumulation in plants is mainly due to their growth performances, availability of nutrients during plant growth. In the present study, the application of combined PGPR isolates shown significantly more total dry weight (88.50 g/plant) in comparison with control and single inoculated treatment T_4 72.57 and 85.00 g/plant respectively (Table-5).

Table 5: Effect of Nitrogen fixing and Phosphate solubilizing bacteria on plant dry matter of chrysanthemum (*Dendranthema grandiflora* Tzvelev.)

Treatments	Plant dry matter		
	Dry weight of shoot (g/plant)	Dry weight of root (g/plant)	Total dry matter (g/plant) At harvest
T_1	55.17^{b}	30.00^{bc}	85.00^{bc}
T_2	48.10^{ef}	26.33^{fg}	74.87^{f}
T_3	49.67^{de}	28.10^{de}	77.80^{e}
T_4	46.80^{f}	25.50^{g}	72.57^{f}
T_5	48.50^{ef}	27.00^{ef}	74.20^{f}
T_6	51.67^{cd}	28.77^{cd}	80.47^{d}
T_7	53.33^{bc}	29.13^{cd}	83.19^{c}
T_8	55.07^{b}	30.70^{ab}	85.90^{b}
T_9	57.43^{a}	32.00^{a}	88.50^{a}

Notes: The number followed by the same latter are not significantly different at (p<0.05) level of Duncan's test.

Conclusion

In the present scenario use of microorganisms in the field of agriculture to enhance growth, yield and quality of crops as well as to reduce cost of production and maintain ecological harmony is the best way in compared with chemicals. Although bio-inoculants alone will not meet the crop nutritional requirement but they can reduce the usage and also enhances the nutrient use efficiency along with plant growth promotion mechanisms finally, lead to increase in growth and yield of agricultural and horticultural crops. Microbial consortia have better result because of interaction with each other as well as with crop plant instead of using single inoculation.

Plate 1: General view of Chrysanthemum pot culture experiment at different stages of crop growth

T_1 - Control (RDF)	**T_6** - 75% NP + Ref. strain of *Azotobacter* + Ref. strain of PSB
T_2 -75% NP + *Azotobacter* isolate (PGPR-24)	**T_7** - 75% NP + *Azotobacter* isolate (PGPR-24) + Ref. strain of PSB
T_3 - 75% NP + *Bacillus* isolate (PGPR-9)	**T_8** - 75% NP + *Bacillus* isolate (PGPR-**9**)+ Ref. strain of *Azotobacter*
T_4 - 75% NP + Ref. strain of *Azotobacter* (*Azotobacter chroococcum*)	**T_9** - 75% NP + *Azotobacter* isolate (PGPR-24) + *Bacillus* isolate (PGPR-9)
T_5 - 75% NP + Ref. strain of PSB (*Bacillus megaterium*)	

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