



Efficacy of microbial inoculants on reducing the phosphatic fertilizer in chrysanthemum

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Abstract: The present investigation was conducted to investigate the potential effect of biofertilizers (mycorrhiza and PSB) and different levels of phosphorus (0, 10, 15 and 20 g/m²) on growth, yield and quality of chrysanthemum. The interaction effect of phosphorus levels and biofertilizers on plant spread was found to be significant in first year, while it was non-significant in second year. In first year (2011-12), the maximum plant spread (19.12 cm) was recorded with the application of PSB in combination of phosphorus 15 g/m². The interaction effect of phosphorus levels and biofertilizers on number of branches/plant, number of days taken for first flowering, number of days taken for 50% flowering and fresh weight of flower were found to be non-significant in both the years of investigation. The interaction effect of phosphorus levels and biofertilizers on duration of flowering was found to be significant in first year, whereas, it was non-significant in second year. The longest flowering duration (45.67 days) was obtained with PSB in combination of phosphorus 15 g/m² in first year. The interaction effects of phosphorus levels and biofertilizers on flower size was observed significant in first year, whereas, it was non-significant in second year. In first year (2011-12), the biggest flower (4.50 cm) was obtained with PSB + phosphorus 20 g/m², which remained at par with PSB + phosphorus 15 g/m² (4.41 cm). The maximum flower yield per plant (61.96 and 61.31 g) was recorded with PSB application along with phosphorus 15 g/m², however, in first year, it was at par with mycorrhiza along with phosphorus 15 g/m² (57.92 g).

Key words: Biofertilizers, Chrysanthemum, Growth, Flowering, Mycorrhiza, Phosphorus, PSB

Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) also known as 'Guldaudi' or Autumn Queen, belongs to the family Asteraceae. It is grown for its attractive and most showy flowers of huge form, dazzling colour and varying size with long vase life. The growth and development of a plant, generally, depends on their judicious feeding right from the beginning. Continuous application of imbalanced and excessive nutrients had led to decline in nutrient-use efficiency making fertilizer consumption uneconomical and producing adverse effects on atmosphere and groundwater quality, causing health hazards (Pandey *et al.*, 2010). Moreover, these chemical fertilizers are not only short in supply but costly too and produced at the cost of irreparable loss of non-renewable energy. This situation emphasized the need for developing alternate production systems that are friendlier to the environment and is more judicious in managing soil health. Biofertilizers containing beneficial bacteria and fungi improve soil chemical and biological characteristics, phosphate solutions and agricultural production (Yosefi *et al.*, 2011). Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al.* 2002). VA mycorrhizal fungi have shown their potentiality to increase the uptake of phosphorus, zinc, copper, sulphur, potassium, calcium and water, while on the other hand, phosphate solubilizing microorganisms, which solubilizes bound phosphate have also proved to be as efficient tool for improving the plant growth and development. Hence, the present study was undertaken to investigate the synergistic effect of *Bacillus* and *Pseudomonas* to improve the performance of chrysanthemum and to minimize chemical fertilizer use.

Materials and Methods

The present experiment was laid out in completely randomized design with three replications at the screen-house of the Department of Horticulture, College of Agriculture, CCS Haryana Agricultural University during the year 2011-12 and 2012-13. Hisar is situated at 29° 10' North latitude and 75° 46' East longitude with an elevation of 215.2 meters above mean sea level. The tract falls in the semi-arid subtropical region having the characteristic extremes of weather conditions with hot dry winds during summers and severe cold in winters. For experimental purpose, soil was collected from pure sand dune near to Hisar and mixed thoroughly. Each pot was lined with polythene sheet and filled with 5 kg of soil. The experimental soil was sandy in texture having 0.19% organic carbon, 95.00 kg/hectare available nitrogen, 10.00 kg/hectare available phosphorus and 102.00 kg/hectare available potassium. One month old rooted cuttings of *Chrysanthemum morifolium* Ramat. cv. 'Dolly Orange' having almost equal size (5-7 cm) and vigour were transplanted in the centre of pot in the month of September. Soil was firmly pressed around the plant and light watering was done immediately. Soil was firmly pressed around the plant and light watering was done immediately. The biofertilizers used in the experiment were procured from the Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India during both the years of investigation. Four levels of phosphorus [P₀ - Control, P₁ - 10 g/m² or 50 ppm, P₂ - 15 g/m² or 75 ppm and P₃ - 20 g/m² or 100 ppm], two biofertilizers [BF₀ - Control, MY- Mycorrhiza (*Glomus* sp.) and PSB- P36 (*Pseudomonas* sp.)] and their combination were applied. In addition to above treatments, nitrogen @ 30 g/m² or 150 ppm and potash @ 20 g/m² or 100 ppm were also applied. Half dose of nitrogen

and full dose of phosphorous and potash were applied as a basal dose just before planting of rooted cuttings, while the remaining half of the nitrogen was applied after 30 days of planting by top dressing method. *Pseudomonas* strain was applied to rhizosphere of the plant after 6 days of plantation as per treatments. For adding the mycorrhizal inoculum, the upper layer of soil up to a depth of 5 cm was removed and mycorrhizal inoculum consisting of root pieces and spores was spread as a layer over the surface. Data on various growth, yield and quality parameters *viz.*, plant spread, number of branches per plant, number of days taken for first flowering from bud initiation, number of days taken for 50% flowering, duration of flowering (days), size of flower (cm), fresh weight of flower (g) and flower yield per plant (g) were recorded and average data were analyzed statistically as per method suggested by Panse and Sukhatme (1978).

Results and Discussion

Plant spread (cm): It is inferred from the data presented in table-1 that the plant spread influenced significantly by different phosphorous levels over the control. The maximum plant spread (18.13 and 18.63 cm) was recorded with the application of phosphorus 15 g/m² during the year 2011-12 and 2012-13, respectively. The phosphorus application improved growth due to stimulation in root growth because of which more absorption of water and mineral nutrients from the soil took place, thus, ultimately led to better plant growth and development. The present results are in confirmation with the findings of Liu *et al.* (2010) who found that application of phosphorus fertilizer could significantly promote the growth parameters.

It is further clear from the table that biofertilizers application significantly influenced the plant spread. The maximum plant spread (17.05 and 16.87 cm) was observed with the application of PSB during both the years, respectively. It might be due to their ability to produce growth-promoting substances such as IAA, gibberellins like substances, vitamin B₁₂, riboflavin, *etc.* The effect of PSB on growth might be due to the activity of phosphorus solubilizing bacteria and increased availability of nutrients (Kumar *et al.*, 2006). PSB stimulated the nutrients uptake and biosynthesis of plant growth regulators thereby improved the growth of plant. An interaction between phosphorous levels and bio-fertilizers was found to be significant in first year, while it was non-significant

Table-1: Response of biofertilizers in combination with phosphorous on plant spread (cm) in chrysanthemum

Year Treatments	2011-12					2012-13				
	Levels of phosphorus (g/m ²)					Levels of phosphorus (g/m ²)				
	0	10	15	20	Mean	0	10	15	20	Mean
Control	10.90	13.92	16.77	15.42	14.25	10.85	12.92	16.77	15.75	14.07
Mycorrhiza	11.18	15.83	18.50	17.53	15.76	12.83	14.95	19.33	16.83	15.99
PSB	13.95	16.58	19.12	18.67	17.05	13.23	15.58	19.78	18.87	16.87
Mean	12.01	15.44	18.13	17.20		12.31	14.48	18.63	17.15	
C.D. at 5%	Phosphorous				0.60					0.86
	Biofertilizers				0.52					0.75
	Phosphorous x Biofertilizers				1.04					NS

Table-2: Response of biofertilizers in combination with phosphorous on number of branches in chrysanthemum

Year Treatments	2011-12					2012-13				
	Levels of phosphorus (g/m ²)					Levels of phosphorus (g/m ²)				
	0	10	15	20	Mean	0	10	15	20	Mean
Control	5.67	6.67	7.67	7.33	6.84	5.00	5.67	7.33	7.00	6.25
Mycorrhiza	5.67	7.00	8.33	7.00	7.00	5.67	6.67	8.00	7.67	7.00
PSB	6.67	7.33	8.67	7.67	7.58	6.67	7.33	8.33	7.67	7.50
Mean	6.00	7.00	8.22	7.33		5.78	6.56	7.89	7.44	
C.D. at 5%	Phosphorous				0.63					0.49
	Biofertilizers				0.55					0.42
	Phosphorous x Biofertilizers				NS					NS

Table-3: Response of biofertilizers in combination with nitrogen on number of days taken for first flowering from bud initiation in chrysanthemum

Year Treatments	2011-12					2012-13				
	Levels of phosphorus (g/m ²)					Levels of phosphorus (g/m ²)				
	0	10	15	20	Mean	0	10	15	20	Mean
Control	20.33	17.00	14.33	16.67	17.08	18.67	17.00	13.33	15.33	16.08
Mycorrhiza	19.67	17.33	15.67	17.67	17.59	20.00	16.33	14.00	15.33	16.42
PSB	18.00	16.33	13.33	14.67	15.58	18.67	15.67	12.33	13.00	14.92
Mean	19.33	16.89	14.44	16.33		19.11	16.33	13.22	14.56	
C.D. at 5%	Phosphorous				0.91					0.73
	Biofertilizers				0.78					0.63
	Phosphorous x Biofertilizers				NS					NS

Table-4: Response of biofertilizers in combination with phosphorous on number of days taken for 50% flowering in chrysanthemum

Year Treatments	2011-12					2012-13				
	Levels of phosphorus (g/m ²)					Levels of phosphorus (g/m ²)				
	0	10	15	20	Mean	0	10	15	20	Mean
Control	113.67	106.67	96.33	102.33	104.75	115.67	112.00	102.33	107.67	109.42
Mycorrhiza	109.00	103.00	93.67	97.00	100.67	113.33	107.33	96.33	104.33	105.55
PSB	104.00	97.67	91.67	96.00	97.33	108.33	106.33	94.67	101.00	102.58
Mean	108.89	102.44	93.89	98.44		112.44	108.56	97.78	104.33	
C.D. at 5%	Phosphorous				2.76					2.66
	Biofertilizers				2.39					2.30
	Phosphorous x Biofertilizers				NS					NS

in second year. Similar findings were reported by Prasad *et al.* (2012) in chrysanthemum and Pushkar and Rathore (2011) in marigold.

Number of branches per plant: The data presented in table-2 reveal that the maximum number of branches/plant (8.22 and 7.89) was observed with the application of phosphorus 15 g/m² during both the years, respectively. Phosphorus is a component of complex nucleic acid structure of plants, which regulates protein synthesis. Phosphorus is, therefore, important in cell division and development of new tissue. It is also associated with complex

energy transformations in the plant. The tendency of decreasing number of branches/plant beyond certain dose of phosphorus was also reported by Dorajeerao *et al.* (2012) in garland chrysanthemum and Nath *et al.* (2010) in China aster. Plant spread was also closely dependent on number of branches/plant which indicated that higher number of branches/plant was produced by more spreading plants and *vice versa*.

The response of biofertilizers on number of branches/plant was found to be significant with the maximum number of branches/plant (7.58 and 7.50) in the application of PSB during both the years of investigation, respectively. It was observed that because of proper nutrition of phosphorus there might be more number of branches. Biofertilizers improve the nutrients availability in soil, which promote plant growth. The results of present experimentation are in accordance with the findings of Airadevi and Mathed (2012) in chrysanthemum. The interaction effect of phosphorous levels and biofertilizers on number of branches/plant was found to be non-significant in both the years of investigation.

Number of days taken for first flowering from bud initiation: A close view of data shown in table-3 indicates that the minimum number of days taken for flowering from bud initiation (14.44 and 13.22 days) was recorded with phosphorus 15 g/m² during the year 2011-12 and 2012-13, respectively. Application of phosphorus caused advancement in bud initiation, because of the positive influence of phosphorus on growth parameters. The results are in line with the findings of Nath *et al.* (2011) in China aster.

The response of biofertilizers to number of days taken for first flowering from bud initiation was found to be significant. The minimum number of days taken for flowering from bud initiation (15.58 and 14.92 days) was recorded with PSB application in the year 2011-12 and 2012-13, respectively, irrespective to phosphorous. The reason might be due to the balanced absorption and solubilization of phosphorus by PSB (Prasad *et al.*, 2012). The results of present experimentation are in accordance with the findings of Kumar *et al.* (2006) who reported that the days taken for flower formation decreased with the inoculation of all biofertilizers over control, except *Azospirillum* and it was recorded minimum in plants inoculated with FYM + PSB in marigold. Interaction effect of phosphorous levels and biofertilizers on number of days taken for first

Table-5: Response of biofertilizers in combination with phosphorous on duration of flowering (days) in chrysanthemum

Year Treatments	2011-12					2012-13				
	Levels of phosphorus (g/m ²)					Levels of phosphorus (g/m ²)				
	0	10	15	20	Mean	0	10	15	20	Mean
Control	30.33	32.00	36.67	35.00	33.50	30.67	33.67	35.00	33.33	33.17
Mycorrhiza	29.67	36.67	41.67	40.00	37.00	28.33	35.33	40.00	38.67	35.58
PSB	33.00	34.33	45.67	40.67	38.42	32.33	36.33	42.33	39.00	37.50
Mean	31.00	34.33	41.33	38.56		31.50	35.11	39.11	37.00	
C.D. at 5%	Phosphorous				1.80					1.50
	Biofertilizers				1.56					1.30
	Phosphorous x Biofertilizers				3.12					NS

Table-6: Response of biofertilizers in combination with phosphorous on size of flower (cm) in chrysanthemum

Year Treatments	2011-12					2012-13				
	Levels of phosphorus (g/m ²)					Levels of phosphorus (g/m ²)				
	0	10	15	20	Mean	0	10	15	20	Mean
Control	3.84	4.13	4.21	4.27	4.11	3.67	4.07	4.07	4.20	4.00
Mycorrhiza	4.04	4.16	4.21	4.12	4.13	3.97	4.08	4.20	4.27	4.13
PSB	4.16	4.27	4.41	4.50	4.34	4.15	4.20	4.37	4.20	4.23
Mean	4.01	4.19	4.28	4.30		3.93	4.12	4.21	4.22	
C.D. at 5%	Phosphorous				0.06					0.14
	Biofertilizers				0.05					0.10
	Phosphorous x Biofertilizers				0.11					NS

Table-7: Response of biofertilizers in combination with phosphorous on fresh weight of flower (g) in chrysanthemum

Year Treatments	2011-12					2012-13				
	Levels of phosphorus (g/m ²)					Levels of phosphorus (g/m ²)				
	0	10	15	20	Mean	0	10	15	20	Mean
Control	1.64	1.79	2.01	1.97	1.85	1.55	1.60	1.99	1.76	1.73
Mycorrhiza	1.86	1.92	2.05	2.01	1.96	1.61	1.82	2.05	2.00	1.87
PSB	1.89	1.94	2.14	2.00	1.99	1.86	1.97	2.10	2.07	2.00
Mean	1.79	1.88	2.06	1.99		1.67	1.80	2.05	1.94	
C.D. at 5%	Phosphorous				0.16					0.10
	Biofertilizers				NS					0.08
	Phosphorous x Biofertilizers				NS					NS

Table-8: Response of biofertilizers in combination with phosphorous on flower yield per plant (g) in chrysanthemum

Year Treatments	2011-12					2012-13				
	Levels of phosphorus (g/m ²)					Levels of phosphorus (g/m ²)				
	0	10	15	20	Mean	0	10	15	20	Mean
Control	21.25	30.99	44.95	37.33	33.63	22.15	26.15	44.46	31.70	31.11
Mycorrhiza	27.84	32.38	57.92	48.29	41.61	25.31	41.08	52.73	40.60	39.93
PSB	33.37	45.31	61.96	52.66	48.33	32.14	46.03	61.31	55.21	48.67
Mean	27.49	36.23	54.94	46.09		26.54	37.75	52.83	42.50	
C.D. at 5%	Phosphorous				3.18					2.51
	Biofertilizers				2.75					2.17
	Phosphorous x Biofertilizers				5.64					4.34

flowering was found to be non-significant in both the years of investigation.

Number of days taken for 50% flowering: The data on number of days taken for 50% flowering as influenced by the levels of phosphorous and biofertilizers and their interaction are presented in table-4. It is further evident from the data that the application of phosphorus 15 g/m² recorded the minimum number of days taken to 50% flowering (93.89 and 97.78 days) during the year 2011-12 and 2012-13, respectively. It is clear from the data that the minimum number of days taken to 50% flowering (97.33 and

102.58 days) was recorded with the application of PSB during both the years, respectively. Meshram *et al.* (2008) also reported that days to 50% flowering significantly increased with the treatments receiving 80% NPK + *Azospirillum* sp. + *Azotobacter* sp. + PSB @ 5 kg/ha each followed by the treatment 80% NPK + *Azospirillum* sp. + PSB at 5 kg/ha each over control and rest of the treatments. The interaction effect of different levels of phosphorous and biofertilizers on number of days taken for 50% flowering was found to be non-significant during both the years of investigation.

Duration of flowering (days): The data presented in table-5 indicate that duration of flowering was longest (41.33 and 39.11 days) with phosphorus 15 g/m² during the year 2011-12 and 2012-13, respectively. The increased duration of flowering due to the application of phosphorus could have resulted because of the increased availability of phosphorus in the root zone, leading to optimum uptake of water and nutrients from the soil for a longer period causing the extended duration of flowering. This fact is supported by the earlier findings of Prakash *et al.* (2006), who reported that duration of flowering progressively extended with the addition of phosphorus in liliium. Likewise, Beniwal *et al.* (2005) also noticed that nitrogen and phosphorus each @ 20 g/m² significantly improved the duration of flowering in chrysanthemum. The effect of biofertilizers was found significant with respect to duration of flowering during both the years. The longest flowering duration (38.42 and 37.50 days) was observed with PSB application in both the years, respectively. However, it remained at par with mycorrhiza (37.00 days) application in first year. The results are in accordance with the findings of Kumar *et al.* (2006), who observed that inoculation of PSB and *Azotobacter* increase the duration of flowering in marigold. The interaction effect of phosphorous levels and biofertilizers was found to be significant in first year, whereas, it was non-significant in second year.

Size of flower (cm): The data regarding flower size have been presented in table-6. In the year 2011-12, the biggest flower (4.30 cm) was recorded with the application of phosphorus 20 g/m², which remained at par with phosphorus 15 g/m². In the next year, the biggest flower (4.22 cm) was obtained with phosphorus 20 g/m², which remained at par with phosphorus 15 g/m² and 10 g/m². Phosphorus application increased the flower size due to stimulation in root growth, which helped in better root development, resulting in more absorption of water and nutrients from soil and ultimately the flower size was increased. Such a positive effect was in line with the findings of Satar *et al.* (2012) in annual chrysanthemum and Nath *et al.* (2011) in China aster. The effect of biofertilizers on flower size was also found to be significant in both the years. In the year 2011-12 and 2012-13, the biggest flower (4.34 and 4.23 cm) was found with PSB application, but in the second year, it was at par with application of mycorrhiza (4.13 cm). The improvement in flower size might be due to increased availability of nutrients for flower development because of better absorption and greater solubility, better root proliferation and greater uptake. The results are in confirmation with the findings of Hashemabadi *et al.* (2012) in marigold. The interaction effects of phosphorous levels and biofertilizers was significant in first year, whereas, it was non-significant in second year.

Fresh weight of flower (g): The data on different levels of phosphorous, biofertilizers and their interaction on fresh weight of flower are furnished in table-7. It is clear from the data that the levels of phosphorous had significant effect on fresh weight of flower. The maximum fresh weight of flower was recorded with the application of phosphorus 15 g/m² in both the years. However, in the first year, it was at par with phosphorus 20 g/m². Phosphorous is associated with energy rich compounds like ATP, ADP, NADH and NADPH. These energy rich metabolites ultimately increased weight of flower. The above findings are supported by the views of Satar *et al.* (2012) in annual chrysanthemum and Singh and Sangama (2000) in China aster. The influence of biofertilizers on fresh weight of flower was found to be non-significant in first year, whereas, it was significant in second year. The improvement in flower weight might be due to increased availability of nitrogen and phosphorus for flower development because of better absorption and greater solubility, better root proliferation and greater uptake of nutrients. Further, the interpretation of data reveals that interaction effect of different levels of phosphorous and biofertilizers on fresh weight of flower was found to be non-significant in both the years.

Flower yield per plant (g): It is obvious from the data presented in table-8 that the maximum flower yield per plant (54.94 and 52.83 g) was recorded with phosphorus 15 g/m² during the year 2011-12 and 2012-13, respectively. The increased flower yield per plant as a result of phosphorus application could have been due to the combined influence of increased number of flowers per plant and average weight of flowers. The results are in agreement with previous studies conducted by Satar *et al.* (2012), Liu *et al.* (2010) and Beniwal *et al.* (2006) in chrysanthemum.

The interpretation of data has further reveals that biofertilizers significantly influenced the flower yield per plant. The maximum flower yield per plant (48.33 and 48.67 g) was recorded with PSB application during the year 2011-12 and 2012-13, respectively irrespective of phosphorous. PSB 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 helping bacteria because they promoted higher root colonization rate and spore number of AMF, which helped in solubilization of mineral phosphate and contributed to the phosphorus cycling, promoting sustainable nutrients supply to the crop plants for higher yield. Prasad *et al.* (2012) reported similar results in chrysanthemum. The interaction between different levels of phosphorous and biofertilizers was found to be significant in both the years of investigation. The maximum flower yield per plant was recorded with PSB application along with phosphorus 15 g/m², however, in first year; it was at par with mycorrhiza along with phosphorus 15 g/m². The findings are in accordance with the results of Chandra *et al.* (2009), who reported that VAM improved the mycorrhizal intensity in roots and spore population in root rhizosphere of the plants provided with phosphorous 15 g/m².

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