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Response of single and co-inoculation of plant growth promoting rhizobacteria on growth, flowering and nutrient content of chrysanthemum

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A pot experiment was conducted in the screen-house of Department of Horticulture, College of Agriculture, CCS Haryana Agricultural University, Hisar during the two successive seasons of 2011-12 and 2012-13 to investigate the potential effect of different strains of *Bacillus* (BS_1- SYB101, BS_2- SB155 and BS_3- SB127), *Pseudomonas* (PS_1- WPS73, PS_2- CPA152 and PS_3-P20) and their combination on growth, flowering and nutrient content of chrysanthemum. Strains of *Bacillus* and *Pseudomonas* significantly influenced the observed parameters of chrysanthemum. Maximum plant height and number of branches per plant were recorded in plants inoculated with PS_2 strain of *Pseudomonas* (CPA152) and BS_3 strain of *Bacillus* (SB127) in both the years. The minimum number of days taken to bud initiation, days for first flowering from bud initiation and days taken for 50% flowering were recorded in plants inoculated with PS_2 strain of *Pseudomonas* (CPA152) and BS_3 strain of *Bacillus* (SB127). The maximum flower size was noticed with PS_3 strain of *Pseudomonas* (P20) in the first year whereas, in second year, the response of *Pseudomonas* strains was found non-significant. Among *Bacillus* strains, the plants inoculated with BS_3 (SB127) recorded maximum flower size. Maximum flower yield/plant was recorded in plants inoculated with PS_2 strains of *Pseudomonas* (CPA152) and BS_3 strains of *Bacillus* (SB127). The N content of plant was recorded maximum with PS_2 (CPA152) and PS_3 (P20) strains of *Pseudomonas* and BS_3 (SB127) strains of *Bacillus*, whereas, P content of plant was noticed maximum with PS_3 strains of *Pseudomonas* (P20) in the first year, while it was found non-significant in second. The response of *Pseudomonas* strains to K content of plant was found non-significant in both years, while K content of plant was influenced significantly by *Bacillus* strains.

Key words: Chrysanthemum, PGPR, rhizosphere bacteria, *Bacillus*, *Pseudomonas*, nutrient content.

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) which occupies a prominent place in ornamental horticulture is one of the commercially exploited flower crops. It is mainly grown for cut and loose flowers for garland making, general decoration, hair adornments and religious function. Increased flower production, quality of flowers and perfection in the form of plants are the important objectives to be reckoned in commercial flower production. Though the quality of flowers is primarily a varietal trait, it is greatly influenced by climatic, geographical and nutritional factors among which nutrition play major role (Laishram et al., 2013). At present, these

nutrients are supplied through chemical fertilizers. The use of chemical fertilizers has 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.

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Saharan and Nehra, 2011). They are a heterogeneous group of bacteria that 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 (Glick, 1995). Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoots growth. They not only promote plant growth but also help in sustainable agricultural development and protecting the environment (Das et al., 2013). It is well established that only 1 to 2% of bacteria promote plant growth in the rhizosphere (Antoun and Kloepper, 2001). The mechanisms by which PGPR promote plant growth are not fully understood, but it is believed that the plant growth promoting rhizobacteria enhance plant growth and yield either by direct or indirect mechanisms (Glick, 1995). The direct promotion of plant growth by PGPR entails either providing the plant with a compound that is synthesized by the bacterium, for example plant growth regulators, or facilitating the uptake of certain nutrients from the environment (Glick, 1995). The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. This can happen by producing antagonistic substances or by inducing resistance to pathogens (Glick, 1995). A particular PGPR may affect plant growth and development by using any one, or more, of these mechanisms. PGPR, as biocontrol agents, can act through various mechanisms, regardless of their role in direct growth promotion, such as by known production of auxin phytohormone (Patten and Glick, 2002), decrease of plant ethylene levels (Glick et al., 2007) or nitrogen fixing associated with roots (Dobereiner, 1992). PGPR and their interactions with plants are exploited commercially and hold great promise for sustainable agriculture. Thus the aim of this study was to determine the effect of plant growth promoting rhizobacteria on growth, flowering and nutrient content of chrysanthemum.

MATERIALS AND METHODS

The present experiment was carried out in the screen-house of the Department of Horticulture, College of Agriculture, CCS Haryana Agricultural University, Hisar, India during the year 2011-2012 and 2012-2013. Hisar is situated at 29° 10′ North latitude and 75° 46 East longitude with an elevation of 215.2 m 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, 47.5 ppm available nitrogen, 5 ppm available phosphorus and 51 ppm 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. 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. Three strains of *Bacillus* (BS_1- SYB101, BS_2- SB155 and BS_3- SB127), three strains of *Pseudomonas* (PS_1- WPS73, PS_2- CPA152 and PS_3-P20) and their combination were applied in three replications having CRD experimental design. In addition to the above treatments recommended dose of fertilizers that is NPK 30, 20, 20 g/m^2 or 150, 100, 100 ppm were also applied. Nitrogen, phosphorus and potassium were applied through urea (46% N), single super phosphate (16% P_2O_5) and murate of potash (60% K_2O), respectively. 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. The culture of *Bacillus* and *Pseudomonas* strains were grown in LB broth media for three days. About 20 ml suspension was applied to rhizosphere of the plant after 6 days of plantation as per treatments. Data on various growth, yield and quality parameters *viz*., plant height, number of branches per plant, number of days taken for bud initiation, number of days taken for first flowering from bud initiation, number of days taken for 50% flowering, size of flower (cm), flower (capitulum) yield per plant (g), and nutrient content in chrysanthemum plant (%) were recorded and average data were analyzed statistically as per method suggested by Panse and Sukhatme (1978). Estimation of nitrogen was done by Nessler's reagent method as per standard procedure (Jackson, 1967). Phosphorus content in plant sample was determined by 'Vanado-Molybdate method' (Jackson, 1967). The intensity of yellow colour was read at 430 nm in Elico spectrometer model CL-24. Potassium content of plant tissue was determined by flame photometer method. Readings were taken in Elico flame photometer; model C-140 after digesting the samples with triacid mixture (Jackson, 1967).

RESULTS AND DISCUSSION

Plant height (cm)

The data recorded on the response of different strains of biofertilizers and their interactions on plant height are presented in Table 1. It is apparent from the data that plant height was significantly influenced by different strains of *Pseudomonas* (Figure 1) and the maximum plant

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Table 1. Response of single and co-inoculation of PGPR on plant height (cm) in chrysanthemum.

Treatment	2011-12					2012-13				
	Biofertilizer strains					Biofertilizer strains				
	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean
BS_0-Control	21.13	22.50	23.97	26.50	**23.53**	19.21	24.60	27.47	25.20	**24.12**
BS_1-SYB101	22.63	23.43	28.40	25.53	**25.00**	21.63	23.63	26.50	25.63	**24.35**
BS_2-SB155	24.13	24.93	30.10	24.20	**25.84**	24.43	25.83	29.73	27.50	**26.88**
BS_3-SB127	25.13	24.70	29.13	31.80	**27.69**	27.80	24.07	27.97	28.10	**26.99**
Mean	23.26	23.89	27.90	27.01		23.27	24.53	27.92	26.61	
C.D. at 5%										
Pseudomonas			1.01					1.61		
Bacillus			1.01					1.61		
Pseudomonas x *Bacillus*			2.01					3.22		

Figure 1. Effect of different *Pseudomonas* strains on chrysanthemum.

height (27.90 and 27.92 cm) was recorded with inoculation of PS_2 treatment, which remained at par with PS_3 (27.01 and 26.61 cm), whereas, it was recorded minimum (23.26 and 23.27 cm) in control during the year 2011-2012 and 2012-2013, respectively. It is inferred from the data that the effect of *Bacillus* strains was also found to be significant for plant height (Figure 2). The maximum plant height (27.69 and 26.99 cm) was observed with the application of BS_3 treatment in the year 2011-2012 and 2012-2013, respectively, but in the year 2012-2013, it was at par with BS_2 application with the value of 26.88 cm. The minimum plant height (23.53 and 24.12 cm) was recorded in control during both the years, respectively.

Among the interactions of *Bacillus* and *Pseudomonas* strains, the maximum plant height (31.80 cm) was recorded with the inoculation of BS_3PS_3, which was at par with inoculation of BS_2PS_2 (30.10 cm) in the year 2011-2012, whereas, in the year 2012-2013, the tallest plant (29.73 cm) was recorded with application of BS_2PS_2, which was at par with BS_3PS_3 (28.10 cm), BS_3PS_2 (27.97 cm), BS_3PS_0 (27.80 cm), BS_2PS_3 (27.50 cm) and BS_0PS_2 (27.47 cm) treatments. The smallest plant (21.13 and 19.21 cm) was observed in control during both the years, respectively. Indole acetic acid (IAA) is one of the naturally occurring auxins with broad physiological effects of plants. Many rhizosphere bacteria including *Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillum*, *etc*. were found to have the ability to produce IAA or related auxins (Salma et al., 2013). Auxins have been implicated in initiation of lateral and adventitious roots, in stimulation of cell division and elongation of stems and roots (Malamy and Benefy, 1997). Increased of plant height with the application of *Bacillus* (SYB101) and *Pseudomonas* (CPS63) strain in gladiolus was also reported by Singh

Figure 2. Effect of different *Bacillus* strains on chrysanthemum.

Table 2. Response of single and co-inoculation of PGPR on number of branches per plant in chrysanthemum.

Treatment	2011-12					2012-13				
	Biofertilizer strains					Biofertilizer strains				
	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean
BS_0-Control	5.33	5.67	7.33	5.67	6.00	5.67	6.00	6.67	6.00	6.08
BS_1-SYB101	5.67	6.33	6.67	7.67	6.58	5.33	5.67	6.00	7.67	6.17
BS_2-SB155	5.33	5.67	7.33	6.00	6.08	6.00	7.00	7.67	7.33	7.00
BS_3-SB127	6.67	7.00	7.33	7.67	7.17	6.67	7.33	7.67	7.67	7.33
Mean	5.75	6.17	7.17	6.75		5.92	6.50	7.00	7.17	
C.D. at 5%										
Pseudomonas			0.45					0.50		
Bacillus			0.45					0.50		
Pseudomonas x *Bacillus*			0.90					NS		

(2009). Dua and Sindhu (2012) reported that in wheat single inoculation of *Pseudomonas* strain WPS3 or WPS90 resulted in increased plant growth as compared to control. Such a positive effect was in line with the findings of Prasad et al. (2012) in chrysanthemum and Jayamma et al. (2008) in jasmine.

Number of branches per plant

The data regarding number of branches per plant are given in Table 2, which reveal that the number of branches per plant was influenced significantly by different strains of biofertilizers. In the year 2011-2012, the maximum number of branches per plant (7.17) was observed with the inoculation of PS_2 treatment, which remained at par with PS_3 (6.75), whereas, in the year 2012-2013, it was observed maximum with PS_3 (7.17), which remained at par with PS_2 (7.00). The minimum number of branches per plant (5.75 and 5.92 cm) was observed with control during both the years, respectively. It is inferred from the data that among the *Bacillus* strains, the number of branches per plant was noted maximum with application of BS_3 strain (7.17 and 7.33) in both the years, respectively, which was at par with the treatment of BS_2 (7.00) in the year 2012-13. The minimum number of branches per plant (6.00 and 6.08) was found with control during both the years, respectively.

The interaction effects of different strains of *Bacillus* and *Pseudomonas* on number of branches per plant was found significant in first year, whereas, it was non-significant in second year. During the year 2011-2012, BS_3PS_3 and BS_1PS_3 treatment combination recorded the maximum number of branches per plant (7.67), which was at par with BS_2PS_2 and BS_3PS_2 (7.33) and BS_3PS_1

Table 3. Response of single and co-inoculation of PGPR on number of days taken for bud initiation in chrysanthemum.

Treatment	2011-2012					2012-2013				
	Biofertilizer strains					Biofertilizer strains				
	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean
BS_0-Control	76.00	74.00	63.33	67.00	76.00	74.33	72.33	64.67	69.00	70.08
BS_1-SYB101	71.00	74.33	69.67	63.33	71.00	73.33	70.33	71.00	61.67	69.08
BS_2-SB155	72.00	69.33	64.67	68.67	72.00	71.00	68.67	63.33	67.33	67.58
BS_3-SB127	69.67	64.00	62.00	63.33	69.67	69.00	66.33	62.00	64.00	65.33
Mean	72.17	70.42	64.92	65.58		71.92	69.11	65.25	65.50	
C.D. at 5%										
Pseudomonas			2.39				1.70			
Bacillus			2.39				1.70			
Pseudomonas x *Bacillus*			4.78				3.40			

(7.00) treatment combinations, whereas number of branches per plant was recorded minimum (5.33) in control. The increase in number of branches per plant could be attributed to increased uptake of nutrients and increased activity of hormones like auxins, cytokinins, *etc*. in biofertilizers-inoculated plants. They also help in the absorption of relatively immobile nutrients such as P, Zn, Cu, Mn, Fe, *etc*. Increased number of primary and secondary branches per plant in fenugreek was also reported by Shivran et al. (2013) with the application of different bioformulations. Similar findings have also been reported by Prasad et al. (2012) in *Chrysanthemum indicum* and Jayamma et al. (2008) in jasmine.

Number of days taken for bud initiation

The data regarding number of days taken for bud initiation are presented in Table 3. It is apparent from the data that number of days taken for bud initiation was significantly reduced by the inoculation of *Pseudomonas* strains. The minimum number of days taken for bud initiation (64.92 and 65.25 days) was recorded with PS_2 inoculation, which was at par with PS_3 (65.58 and 65.50 days) in the year 2011-2012 and 2012-2013, respectively. The maximum number of days taken for bud initiation (72.17 and 71.92 days) was observed with control during both the years, respectively. The effect of *Bacillus* strains was also found to be significant with respect to number of days taken for bud initiation. The minimum number of days taken for bud initiation (69.67 and 65.33 days) was recorded with the inoculation of BS_3 treatment in the year 2011-2012 and 2012-2013, respectively, but in first year, it was at par with BS_1 (71.00 days) and BS_2 (72.00 days) treatments. The maximum number of days taken for bud initiation (76.00 and 70.08 days) was found in control during both the years, respectively.

The interaction between *Bacillus* and *Pseudomonas* strains was found to be significant during both the years. In first year, the minimum number of days taken for bud initiation (62.00 days) was recorded with inoculation of BS_3PS_2, which was at par with treatment of BS_0PS_2, BS_1PS_3, and BS_3PS_3 (63.33 days), BS_3PS_1 (64.00 days) and BS_2PS_2 (64.67 days). In second year, the minimum number of days taken for bud initiation (61.67 days) was recorded with inoculation of BS_1PS_3, which was at par with BS_3PS_2 (62.00 days), BS_2PS_2 (63.33 days), BS_3PS_3 (64.00 days), and BS_0PS_2 (64.67 days) treatment combinations. The maximum number of days taken for bud initiation (76.00 and 74.33 days) was found with control during both the years, respectively. The earliness of bud initiation in biofertilizers inoculated plants may be ascribed to easy uptake of nutrients and simultaneous transport of growth promoting substances like cytokinins to the axillary buds, resulting in breakage of apical dominance (Jayamma et al., 2008). The present results are in confirmation with the findings of Singh (2009) who reported that combined application of *Bacillus* (SYB101) and *Pseudomonas* (CPS63) strains caused early spike initiation in gladiolus. The results are also in line with the findings of Salma et al. (2013) and Pandey et al. (2013) in gladiolus.

Number of days taken for first flowering from bud initiation

A close examination of data shown in Table 4 indicates that number of days taken for first flowering from bud initiation was significantly influenced due to different strains of *Pseudomonas*. The minimum number of days taken for first flowering from bud initiation (13.92 and 13.67 days) was recorded with PS_2 application and it was recorded maximum (18.17 and 17.48 days) in control during both the years, respectively. The response of

Table 4. Response of single and co-inoculation of PGPR on number of days taken for flowering from bud initiation in chrysanthemum.

Treatment	2011-2012					2012-2013				
	Biofertilizer strains					Biofertilizer strains				
	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean
BS_0-Control	20.67	17.67	16.00	17.67	18.00	19.67	17.33	14.00	16.67	16.92
BS_1-SYB101	19.33	16.67	15.00	13.33	16.08	19.57	16.67	17.00	14.00	16.81
BS_2-SB155	18.00	15.00	12.33	15.67	15.25	16.33	15.00	12.00	17.00	15.08
BS_3-SB127	14.67	16.33	12.33	13.33	14.17	14.33	15.67	11.67	12.33	13.50
Mean	18.17	16.42	13.92	15.00		17.48	16.17	13.67	15.00	
C.D. at 5%										
Pseudomonas	0.78						0.84			
Bacillus	0.78						0.84			
Pseudomonas x *Bacillus*	1.56						1.69			

Table 5. Response of single and co-inoculation of PGPR on number of days taken for 50% flowering in chrysanthemum.

Treatment	2011-2012					2012-2013				
	Biofertilizer strains					Biofertilizer strains				
	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean
BS_0-Control	112.67	109.33	99.67	103.67	106.33	113.33	111.67	100.67	106.67	108.09
BS_1-SYB101	111.00	107.67	99.00	106.33	106.00	111.00	108.00	97.00	99.33	103.83
BS_2-SB155	108.67	106.00	97.33	100.67	103.17	108.67	106.33	97.33	105.00	104.33
BS_3-SB127	106.33	105.00	95.67	100.00	101.75	104.00	106.67	93.67	99.33	100.92
Mean	109.67	107.00	97.92	102.67		109.25	108.17	97.17	102.58	
C.D. at 5%										
Pseudomonas	2.38					1.67				
Bacillus	2.38					1.67				
Pseudomonas x *Bacillus*	NS					3.33				

Bacillus to number of days taken for flowering from bud initiation was also found significant in both the years. The minimum number of days taken for first flowering from bud initiation (14.17 and 13.50 days) was recorded with BS_3 application, whereas, the maximum number of days taken for flowering from bud initiation was seen in control (18.00 and 16.92 days) during both the years, respectively.

Among the various combinations of *Bacillus* and *Pseudomonas* strains, the minimum number of days taken for first flowering from bud initiation (12.33 days) was recorded with BS_2PS_2 and BS_3PS_2 treatment combinations in first year, which was at par with BS_1PS_3 and with BS_3PS_3 (13.33 days) treatments. In the second year, it was recorded minimum (11.67 days) with application of BS_3PS_2, which were at par with BS2PS2 (12.00 days) and BS3PS3 (12.33 days) treatment combinations. The maximum number of days taken for first flowering from bud initiation (20.67 and 19.67 days) was found with control during both the years, respectively. The probable reason for earlier flowering may be that the hormone secretion by *Bacillus* and *Pseudomonas* strains which enhance early bud initiation and flowering. Singh et al. (2010) reported that number of days taken for spike initiation reduced with the application of *Bacillus* and *Pseudomonas* strains in gladiolus. Barman et al. (2003) also observed that application of *Bacillus* reduced days required for flower opening in tuberose.

Number of days taken for 50% flowering

The data pertaining to response of different strains of *Pseudomonas* and *Bacillus* and their interaction on number of days taken for 50% flowering are presented in Table 5. The observation reveals that the minimum number of days taken to 50% flowering (97.92 and 97.17 days) was found in PS_2 inoculation and maximum

Table 6. Response of single and co-inoculation of PGPR on size of flower (cm) in chrysanthemum.

Treatment	2011-2012					2012-2013				
	Biofertilizer strains					Biofertilizer strains				
	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean
BS_0-Control	3.91	4.36	4.55	4.51	4.33	3.87	4.33	4.42	4.40	4.26
BS_1-SYB101	4.01	4.28	4.37	4.43	4.27	4.06	4.22	4.38	4.54	4.30
BS_2-SB155	4.23	4.34	4.57	4.54	4.42	4.12	4.28	4.53	4.32	4.31
BS_3-SB127	4.22	4.19	4.62	4.72	4.44	4.27	4.31	4.46	4.41	4.36
Mean	4.09	4.29	4.53	4.55		4.08	4.28	4.45	4.42	
C.D. at 5%										
Pseudomonas			0.07					NS		
Bacillus			0.07					0.09		
Pseudomonas x *Bacillus*			0.14					0.19		

in control (109.67 and 109.25 days) during the year 2011-12 and 2012-2013, respectively. It is evident from the data that the response of *Bacillus* strain was found to be significant and the minimum number of days taken to 50% flowering (101.75 and 100.92 days) was recorded with BS_3 inoculation in the year 2011-2012 and 2012-2013, respectively, but in the year 2011-2012, it was at par with application of BS_2 (103.17 days). The maximum number of days taken for 50% flowering (106.33 and 108.09 days) was found with control during both the years, respectively.

The interaction effect of different strains of biofertilizers on number of days taken for 50% flowering was found to be non-significant in the first year, while it was significant in the second year, and the minimum number of days taken for 50% flowering (93.67 days) was recorded with inoculation of BS_3PS_2 in the year 2012-2013, which was at par with BS_1PS_2 (97.00 days) inoculation. The maximum number of days required for 50% flowering (112.67 and 113.33 days) was found in control in the year 2011-2012 and 2012-2013, respectively. The results of our experiment are in confirmation with the findings of Shivran et al. (2013) who reported that application of PGPR significantly decreased the number of days taken to 50% flowering fenugreek. Jayamma et al. (2008) also observed that plant receiving 50 per cent recommended dose of NPK fertilizers + biofertilizers took significantly lesser number of days for 50 per cent flowering as comparison to 100 per cent recommended dose of fertilizers.

Size of flower (cm)

The data on size of flowers as influenced by different strains of *Bacillus* and *Pseudomonas* and their interactions are furnished in Table 6. The biggest flower (4.55 cm) was recorded with the application of PS_3 treatment, which was at par with PS_2 (4.53 cm) in the year 2011-2012, whereas, in the year 2012-2013, the response of *Pseudomonas* strains was found to be non-significant. The smallest flower (4.09 and 4.08 cm) was recorded with control during both the years, respectively. Further, it is cleared from the data that application of BS_3 recorded the biggest flower (4.44 and 4.36 cm) in the year 2011-2012 and 2012-2013, respectively, which was at par with BS_2 application (4.42 cm) in first year, whereas, in second year, it was at par with BS_2 (4.31 cm) and BS_1 (4.30 cm) treatments. The smallest flower (4.27 cm) was recorded in BS_1 inoculation during first year, whereas, in second year, it was recorded smallest (4.26 cm) with control.

The interaction effect of *Bacillus* and *Pseudomonas* strains on flower size was found to be significant in both the years. In the year 2011-2012, the biggest flower (4.72 cm) was recorded with treatment receiving BS_3PS_3 strains, which was at par with BS_3PS_2 (4.62 cm), while in second year, it was biggest with BS_1PS_3 (4.54 cm) treatment combination, which remained at par with BS_2PS_2 (4.53 cm), BS_3PS_2 (4.46 cm), BS_0PS_2 (4.42 cm), BS_3PS_3 (4.41 cm), BS_0PS_3 (4.40 cm) and BS_1PS_2 (4.38 cm) combinations. The smallest flower (3.91 and 3.87 cm) was recorded with control during both the years, respectively. The increased flower size might be due to the increased availability of nitrogen and phosphorus for flower development as a result of greater solubility and absorption of nutrients. Singh et al. (2010) also reported beneficial effect of different biofertilizers and their strains on floret diameter of gladiolus. These findings are in line to that of Pandey et al. (2013) who observed that application of *Bacillus subtilis* + vermicompost registered maximum diameter of floret in gladiolus.

Flower yield per plant (g)

The data pertaining to flower yield per plant have been presented in Table 7. The perusal of data elucidated significant differences amongst the different strains of

Table 7. Response of single and co-inoculation of PGPR on flower yield per plant (g) in chrysanthemum.

Treatment	2011-2012					2012-2013				
	Biofertilizer strains					Biofertilizer strains				
	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean
BS_0-Control	17.69	24.57	47.65	39.60	32.38	20.22	27.20	42.93	34.21	31.14
BS_1-SYB101	28.27	39.00	47.28	50.93	41.37	27.60	38.23	51.00	59.87	44.18
BS_2-SB155	32.40	41.10	69.07	51.58	48.54	47.17	50.00	67.23	54.00	53.10
BS_3-SB127	44.07	47.80	60.63	61.67	53.53	47.20	55.77	65.43	70.93	54.83
Mean	30.61	38.12	56.16	50.95		34.05	42.80	56.65	54.75	
C.D. at 5%										
Pseudomonas		3.13					3.64			
Bacillus		3.13					3.64			
Pseudomonas x *Bacillus*		6.27					7.27			

Figure 3. Effect of different combinations of *Bacillus* and *Pseudomonas* strains on chrysanthemum.

Pseudomonas and *Bacillus.* The maximum flower yield per plant (56.16 and 56.65 g) was recorded with PS_2 inoculation in both the years, respectively, which was at par with PS_3 (54.75 g) in second year. The minimum flower yield per plant (30.61 and 34.05 g) was recorded with control during the year 2011-12 and 2012-13, respectively. Amongst the *Bacillus* strains, the maximum flower yield per plant was obtained with inoculation of BS_3 (53.53 and 54.83 g) during both the years, respectively, which was at par with BS_2 (53.10 g) application in the year 2012-2013. The minimum flower yield per plant (32.38 and 31.14 g) was obtained in control during both the years, respectively.

The interaction effects of *Pseudomonas* and *Bacillus* strains were found to be significant in both the years and the maximum flower yield per plant (69.07 g) was recorded with treatment receiving (Figure 3) BS_2PS_2 in the year 2011-2012, whereas in the year 2012-2013, it was maximum with BS_3PS_3 (70.93g), which was at par with BS_2PS_2 (67.23 g) and BS_3PS_2 (65.43g) treatment combinations. The minimum flower yield per plant (17.69 and 20.22 g) was recorded with control during both the years, respectively. A large body of evidence suggests that PGPR enhance the growth and crop yield, and contribute to the protection of plants against certain pathogens and pests (Kaushal et al., 2011; Dey et al., 2004; Kloepper et al., 2004; Herman et al., 2008 and Minorsky, 2008).

Nutrient content of plant

Nitrogen content (%) in plant (g)

The data on N content are presented in Table 8, which reveal that the different strains of *Bacillus* and

Table 8. Response of single and co-inoculation of PGPR on N content (%) in chrysanthemum plant.

Treatment	2011-2012					2012-2013				
	Biofertilizer strain					Biofertilizer strain				
	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean	PS_0-Control	PS_1-WPS73	PS_2-CPA152	PS_3-P20	Mean
BS_0-Control	0.51	1.74	2.20	2.33	1.69	0.92	2.50	2.34	2.51	2.07
BS_1-SYB101	2.07	2.85	3.45	2.79	2.79	1.94	2.34	2.61	3.15	2.51
BS_2-SB155	2.51	3.46	3.29	3.51	3.19	2.51	2.90	2.05	2.60	2.51
BS_3-SB127	2.77	3.23	3.15	2.94	3.02	2.90	2.17	2.46	2.59	2.53
Mean	1.96	2.82	3.02	2.89		2.07	2.48	2.36	2.71	
C.D. at 5%										
Pseudomonas			0.53					0.35		
Bacillus	0.53					0.35				
Pseudomonas x *Bacillus*	NS					0.69				

Pseudomonas have significant effect on N content in plants. In the year 2011-2012, the maximum N content (3.02 %) was recorded with application of PS_2, which was at par with PS_1 (2.82%) and PS_3 (2.82 %) treatments, and the minimum N content (1.96%) was found with control. In the year 2012-13, the maximum N content (2.71%) was recorded with PS_3, which was at par with PS_1 (2.48%) and PS_2 (2.36%) applications. Goel et al. (2002) reported that co-inoculation chickpea with *Pseudomonas* strains increase the nitrogen content in plants.

It is inferred from the table that the maximum N content (3.19%) was observed with treatment receiving BS_2 in first year, which remained at par with BS_3 (3.02%) and BS_1 (2.79%) treatments, whereas, in second year, the maximum N content (2.53%) was recorded with BS_3 treatment, which remained at par with BS_1 and BS_2 (2.51%). The minimum N content (1.69 and 2.07%) was found in control during both the years, respectively. The increase in concentration of nitrogen in the plant is due to the uptake of nitrogen by plants. The findings of Singh (2009) also revealed that nitrogen content in plant increased significantly with application of PGPR. The interaction effect was found to be non-significant in first year, whereas, it was significant in second year. The maximum N content (3.15%) was found with BS_1PS_3, which was at par with PS_0BS_3 and BS_2PS_1 (2.90%), BS_1PS_2 (2.61%), BS_2PS_3 (2.60%), BS_3PS_3 (2.59%), BS_0PS_3 and PS_0BS_2 (2.51%) and BS_3PS_2 (2.46%) treatment combinations, whereas, the minimum N content (0.92%) was noticed in control in the year 2012-2013.

Phosphorus content (%) in plant

The data pertaining to P content (%) are presented in Table 9. The perusal of data elucidates significant differences amongst the *Pseudomonas* strains for the year 2011-2012 but non-significant for the year 2012-2013. The maximum P content (0.36%) was recorded with PS_3 treatment, which was at par with PS_1 and PS_2 (0.32%), whereas, it was minimum (0.25%) in control during the year 2011-2012. The response of different strains of *Bacillus* on P content was found to be non-significant for both the year. The interaction effect of different strains of *Bacillus* and *Pseudomonas* was found to be significant and the maximum P content (0.43%) was recorded with the treatment combination of BS_1PS_3, which was at par with BS_0PS_2 (0.37%) and BS_3PS_1 (0.35%) in the year 2011-2012, whereas, it was non-significant in the second year. The minimum N content (0.20%) was recorded in control in the year 2011-2012. The beneficial role by biofertilizers may be ascribed as the cumulative activity of P-solubilization and phyto-hormones production. Barman et al. (2003) observed that NPK + FYM + PSB increased phosphorus content in leaves of tuberose from 0.04 to 0.06%. Similarly, Karishma et al. (2013) also reported that gerbera plants inoculated with mix culture of *Glomus mosseae* + *Acaulospora laevis* + *Pseudomonas fluorescens* showed maximum phosphorus content at lower concentration of superphosphate.

Potassium content (%) in plant

The data of K content in plants as influenced by different strains of *Pseudomonas* and *Bacillus* and their interactions are presented in Table 10, which reveals that the effect of *Pseudomonas* was found to be non-significant for both the years, whereas the effect of *Bacillus* strains on K content was found to be significant for both the years. The maximum K content (1.69 and

Table 9. Response of single and co-inoculation of PGPR on P content (%) in chrysanthemum plant.

Treatment	2011-2012					2012-2013				
	Biofertilizer strains					Biofertilizer strains				
	PS_0-Control	**PS_1-WPS73**	**PS_2-CPA152**	**PS_3-P20**	**Mean**	**PS_0-Control**	**PS_1-WPS73**	**PS_2-CPA152**	**PS_3-P20**	**Mean**
BS_0-Control	0.20	0.33	0.37	0.38	0.32	0.22	0.43	0.42	0.34	0.35
BS_1-SYB101	0.28	0.30	0.32	0.43	0.33	0.33	0.35	0.37	0.46	0.38
BS_2-SB155	0.25	0.30	0.34	0.29	0.29	0.27	0.35	0.39	0.34	0.34
BS_3-SB127	0.28	0.35	0.28	0.33	0.31	0.33	0.36	0.38	0.38	0.36
Mean	0.25	0.32	0.32	0.36		0.29	0.37	0.39	0.38	
C.D. at 5%										
Pseudomonas			0.04					NS		
Bacillus			NS					NS		
Pseudomonas x *Bacillus*			0.08					NS		

Table 10. Response of single and co-inoculation of PGPR on K content (%) in chrysanthemum plant.

Treatment	2011-2012					2012-2013				
	2011-2012					2012-2013				
	PS_0-Control	**PS_1-WPS73**	**PS_2-CPA152**	**PS_3-P20**	**Mean**	**PS_0-Control**	**PS_1-WPS73**	**PS_2-CPA152**	**PS_3-P20**	**Mean**
BS_0-Control	0.28	1.29	1.41	2.12	**1.27**	0.33	1.34	1.51	2.21	**1.34**
BS_1-SYB101	1.39	1.89	1.86	1.61	**1.69**	1.89	2.39	2.01	2.11	**2.10**
BS_2-SB155	1.71	1.55	1.62	1.15	**1.51**	2.21	1.85	1.77	1.30	**1.78**
BS_3-SB127	1.39	1.66	1.67	1.56	**1.57**	1.54	1.86	1.87	1.81	**1.77**
Mean	**1.19**	**1.60**	**1.64**	**1.61**		**1.49**	**1.86**	**1.79**	**1.86**	
C.D. at 5%										
Pseudomonas			NS					NS		
Bacillus			0.35					0.49		
Pseudomonas x *Bacillus*			NS					NS		

2.10%) was recorded with treatment BS_1 which was at par with BS_2 (1.51 and 1.78%) and BS_3 (1.57 and 1.77%) for both the year, respectively. According to Kohler et al. (2007), inoculation with *Bacillus subtilis* increased significantly the urease, protease and phosphatase activities of the rhizosphere soil of the lettuce plants and increased foliar P and K contents. The interaction effect of *Pseudomonas* and *Bacillus* strains on K content was found to be non-significant during both the years of investigation.

Conclusion

The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable flower cultivation. Among different strains of *Bacillus* and *Pseudomonas*, SB127 (BS_3) and CPA152 (PS_2) were found effective in increasing growth, flowering and yield parameters. The BS_2PS_2 (SB155 + CPA152), BS_3PS_2 (SB127 + CPA152) and BS_3PS_3 (SB127 + P20) combinations showed best result with respect to growth, flowering and yield parameters of chrysanthemum.

Conflict of interests

The authors did not declare any conflict of interest.

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