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Effect of microbial biofilm in the sustainable production of chrysanthemum

Sagar C.T., Vartika Budhlakoti, K. P. Singh, A.K. Tiwari, S. P. Singh[1], Sudhir Kumar[2], Radha Prasanna[3] and Gunjeet Kumar*

Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi-110012, Delhi, India.

ABSTRACT

The study was undertaken to analyze the effect of cyanobacteria biofilm inoculants on plant growth, floral attributes, soil microbial and nutrient parameters of *Chrysanthemum morifolium* Ramat cvs. Pusa Sona and Pusa Chitraksha. Plant spread increased by 49% and 36.1% in Pusa Sona and Pusa Chitraksha over the control (T_1). Treatment T_7 (Anabaena-Trichoderma (An-Tz) two times drench plus 732:1406:375 mg NPK/Pot) showed 25.6% and 56.2% increase over the control for the number of flowers per plant in cvs. Pusa Sona and Pusa Chitraksha, respectively. Available soil nitrogen increased by 74.9% in Pusa Sona and 57.4% in Pusa Chitraksha with the treatment T6 (Anabaena-Nostoc (BF1-4) two times drench along with 732:1406:375 mg NPK/pot) as compared to the uninoculated control. Treatments T_6 and T_7 were particularly promising in most plant and soil-related parameters. In addition, applying biofertilizers saved 25% of nitrogen fertilizers, besides improving soil health.

Keywords: *Chrysanthemum morifolium*, Cyanobacteria, Biofilms, Sustainable, Biofertilizers

INTRODUCTION

Flowers have cultural, aesthetic and economic value. Globalization of the economy also gave impetus to enhanced demand and flower production. Environmental regulations demand economic and sustainable production. Soil nutrient status remarkably affects quality production (Pathak *et al*., 11). Non-judicious use of fertilizers deteriorates soil properties and increases production cost demanding alternate fertilizer sources like organic manures and biofertilizers. Biofertilizers are eco-friendly and cost-effective, enabling sustainable crop production. Applying biofertilizers can replace up to 50 % NPK fertilizers (Jayamma *et al*., 5). Among biofertilizers, application of cyanobacteria is common in rice, wheat, cotton, legumes and vegetables (Prasanna *et al.,*14). Cyanobacteria work as nutrient supplements and improve soil physical properties (Prasanna *et al*., 15). A survey of cyanobacterial diversity from rice rhizosphere revealed that genera *Nostoc* and *Anabaena* comprised 80 % of isolates (Prasanna *et al*., 13). Cyanobacterial biofilmed biofertilizers (CBBs) represent a promising option, as polysaccharides in the cyanobacterial matrices provide hospitable conditions for colonization by other microbes. The nutrient-rich mucilage of cyanobacterium *Anabaena torulosa* and fungus *Trichoderma viride* have been explored to construct two-membered biofilms (Prasanna *et al*. 14; Triveni *et al*. 21). Root colonization by *Trichoderma spp*. solubilize nutrients in the soil and induce biotic resistance (Harman *et al*., 3). Chrysanthemum displays diverse flower colour, shape, and form. It is grown as cut flowers, loose flowers, pot mums and garden displays. Its excellent keeping quality makes it a leading cut flower worldwide and ranks second in global trade after roses (Jaime *et al*., 4). There is significantly less information about cyanobacterial strains in Chrysanthemum. Hence, the present research work aimed to understand cyanobacterial inoculants in improving the vegetative growth, floral attributes, and soil fertility of Chrysanthemum.

MATERIALS AND METHODS

The study was conducted at the research farm of the Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute (IARI), New Delhi (latitude 28°38' N, longitude 77°12'E and altitude 228.4 m) during November 2018-March 2019. Two cultivars, *viz*. Pusa Chitraksha(deep magenta colour pot/garden display variety with spatulate ray floret) and Pusa Sona(early/extremely dwarf yellow bushy type) were used. The experiment was planted in November in earthen pots 10 inches in diameter filled with 4.5 kg garden soil in a randomized block design with 3 replications and 7 treatments, each containing 7 pots. The 30 days old plug plants raised from terminal cuttings were transplanted in media comprising coco-peat, vermiculite and perlite (2:1:1, w/w). During the growth period, the day temperature was 25.6-28.2°C and the night temperature was 5-15°C. The soil was sandy loam with a pH of 6.8. Major nutrients, viz. N, P and K @ 978:1406:375

*Corresponding author: kumar_gunjeet@yahoo.com
[1]Division of Genetics and Plant Breeding, ICAR-IARI, New Delhi-110012, Delhi, India
[2]Division of Plant Physiology, ICAR-IARI, New Delhi-110012, Delhi, India
[3]Division of Microbiology, ICAR-IARI, New Delhi-110012, Delhi, India

mg w/w, respectively, were applied in control pots (T_1), whereas 732:1406:375 mg w/w N, P and K, respectively, were applied in cyanobacterial inoculant treated pots (T_2 to T_7). Two strains of cyanobacterial formulations, *Anabaena-Nostoc* (BF1-4) consortium and *Anabaena-Trichoderma* (An-Tz) biofilm, were procured from the Division of Microbiology, ICAR-IARI, New Delhi and their details are given in earlier investigations (Prasanna *et al.*, 14). Treatments details are mentioned below:

T_1-Recommended Dose of Fertilizer (RDF)-978:1406:375 mg NPK/Pot (control),

T_2-*Anabaena-Nosto* (BF1-4) dry powder + 732:1406:375 mg NPK/Pot,

T_3-*Anabaena-Trichoderma*(An-Tz) dry powder +732:1406:375 mg NPK/Pot,

T_4-*Anabaena-Nostoc*(BF1-4) single drench +732:1406:375 mg NPK/Pot,

T_5-*Anabaena-Trichoderma*(An-Tz) single drench +732:1406:375 mg NPK/Pot,

T_6-*Anabaena-Nostoc*(BF1-4) two times drench +732:1406:375 mg NPK/Pot,

T_7-*Anabaena-Trichoderma*(An-Tz) two times drench + 732:1406:375 mg NPK/Pot.

The observations were recorded on five randomly selected plants. Plant height, spread, and number of primary branches were recorded at 30, 50, and 100 days after transplanting (DAT) in cv. Pusa Sona and 30, 60, and 110 DAT in Pusa Chitraksha. Flowering attributes like days to bud initiation, days to first blooming, length of flower stem and vase life were recorded. Root length (cm) and volume (cm^3) were measured with a root scanner equipped with WinRHIZO Pro software with a specific positioning and lighting system in the scanning area to eliminate shadows and permanent calibration to improve measurement precision. Soil samples were collected at 50 and 100 DAT to estimate the soil parameters. Available soil nitrogen was estimated by the alkaline permanganate method (Subbiah and Asija, 19). The soil organic carbon was measured by the Walkey-Black method (Walkley and Black, 22) and expressed as carbon per cent. Vials filled with 30gm soil samples were injected with 3.5 ml acetylene (10% gas phase) after removing an equal amount of air using plastic disposable syringes to estimate the nitrogen fixation. Acetylene reduction activity (ARA), an index of nitrogen fixation, was measured after incubating for 24 hours at 28°C at 2500 lux light intensity and expressed as nmol C_2H_4/mg chlorophyll/day (Prasanna *et al.,*15). After measuring ARA, the same soil sample was used for soil chlorophyll estimation. Soil samples were flooded with an acetone-DMSO (Dimethyl Sulfoxide) mixture (1:1) at a 1:10 ratio and incubated in the dark for 48 hours with intermittent shaking. Concentrations of soil chlorophyll, an index of biomass accumulation, were determined by optical density at 630, 645, 663 nm using a spectrophotometer and expressed as micrograms of soil chlorophyll/g of soil (Score/UNESCO,1966). Dehydrogenase activity indicating soil microbial activity was measured by using 2-3-5-Triphenyl Tetrazolium Chloride (TTC) reduction technique (Casida *et al.,* 1). Test tube containing 1 gm of fresh soil was added with 0.1 g $CaCO_3$ and 1 ml of 3 % TTC. The mixture was agitated and capped with a rubber stopper before being incubated at 30 °C for 24 hours. After that, 10 ml of methanol was added and left for 30 minutes to see the activity indicated by red or orange colour development. Using a Spectrophotometer, the solution's optical density (OD) was noted at 485 nm and results on dehydrogenase activity are expressed as TPF (triphenylformazan) released/gm/day. The data were subjected to statistical analysis using Randomized Block Design as described by Panse and Sukhatme (10). The treatment differences were tested by F test of significance.

RESULTS AND DISCUSSION

The maximum plant height was recorded for T_7 and the least with T_1 (Fig. 1a, 1b). The plant height increase over T_1 was more in Pusa Chitraksha (54.7% - 68.3%) than in Pusa Sona (29.5%-38.9%) over various growth stages. T7 was better for the number of primary branches in both varieties, whereas T_4 was at par in Pusa Chitraksha at 110 DAT (Table 1). In T_7, Pusa Sona and Pusa Chitraksha showed 49.1% and 36.2% increase in plant spread, respectively over T_1 (Fig. 2). The root length of Pusa Sona and Pusa Chitraksha ranged from 11.9 cm - 18.6 cm and 31.1 cm - 41.4 cm, respectively. The longest root length was recorded with $T_{6,}$ followed by T_7. T_6 also showed the maximum root volume of 9.9 cm^3 and 11.9 cm^3 in Pusa Sona and Pusa Chitraksha, respectively, and T_1 showed the lowest root volume (Table 2). The interaction of microbial population in the rhizosphere significantly affects the growth and yield of cereals, legumes, fruits, vegetables and flowers (Glick, 2). Microorganisms mobilize nutrients facilitating their uptake and increasing root growth, biomass and yield (Manjunath *et al*., 7). Biofertilizers improved plant growth and yield in China aster, and bulb size and yield in bulbous crops may be due to enhanced N availability (Srivastava *et al.,* 18). In the present experiment, all the biofertilizer treatments significantly increased growth over the uninoculated control and $T_{7,}$ and T_6 were better.

In both genotypes, flower bud initiation was the earliest in T_7. Similarly, the earliest flowering was recorded in T_7 and was on par with T_5 in both

Table 1. Influence of cyanobacterial formulations on number of primary branches in var. Pusa Sona and Pusa Chitraksha at different stages of plant growth.

Treatment		Pusa Sona			Pusa Chitraksha		
		30 DAT	50 DAT	100 DAT	30 DAT	60 DAT	110 DAT
T_1		1.3	2	2.5	0.31	1.2	1.9
T_2		1.5	2.2	2.8	0.51	1.3	2.1
T_3		2.1	3.3	3.9	0.81	1.8	2.4
T_4		1.8	2.7	3.2	0.72	1.5	3.5
T_5		2.3	3.4	4.3	0.91	2.2	2.7
T_6		2.1	3.2	3.8	0.85	2.1	2.9
T_7		2.5	3.7	4.6	1.1	2.4	3.1
C.D. (0.05)	F(T)	0.06	0.1	0.54	0.06	0.1	0.54
	F(V)	0.03	0.06	0.29	0.03	0.06	0.29
	F(T*V)	0.09	0.15	0.76	0.09	0.15	0.76

T_1-Recommended Dose of Fertilizer (RDF)-978:1406:375 mg NPK/Pot (control), T_2-*Anabaena-Nosto* (BF1-4) dry powder + 732:1406:375 mg NPK/Pot, T_3-*Anabaena-Trichoderma*(An-Tz) dry powder +732:1406:375 mg NPK/Pot, T_4-*Anabaena-Nostoc*(BF1-4) single drench +732:1406:375 mg NPK/Pot, T_5-*Anabaena-Trichoderma*(An-Tz) single drench +732:1406:375 mg NPK/Pot, T_6-*Anabaena-Nostoc*(BF1-4) two times drench +732:1406:375 mg NPK/Pot, T_7-*Anabaena-Trichoderma*(An-Tz) two times drench + 732:1406:375 mg NPK/Pot.

Table 2. Influence of cyanobacterial formulations on root length and root volume in var. Pusa Sona and Pusa Chitraksha.

Treatment		Root Length (m)		Root Volume (cm^3)	
		Pusa Sona	Pusa Chitraksha	Pusa Sona	Pusa Chitraksha
T_1		11.9	31.1	4.5	7
T_2		14.7	33.9	6.7	9.6
T_3		14.4	32	6.3	7.6
T_4		15.4	34.3	7.1	8.7
T_5		14.5	33.2	5.3	7.8
T_6		18.6	41.4	9.9	11.9
T_7		15.7	39.1	7.8	10.2
C.D. (0.05)	F(T)	0.23		0.13	
	F(V)	0.12		0.07	
	F(T*V)	0.32		0.19	

T_1-Recommended Dose of Fertilizer (RDF)-978:1406:375 mg NPK/Pot (control), T_2-*Anabaena-Nosto* (BF1-4) dry powder + 732:1406:375 mg NPK/Pot, T_3-*Anabaena-Trichoderma*(An-Tz) dry powder +732:1406:375 mg NPK/Pot, T_4-*Anabaena-Nostoc*(BF1-4) single drench +732:1406:375 mg NPK/Pot, T_5-*Anabaena-Trichoderma*(An-Tz) single drench +732:1406:375 mg NPK/Pot, T_6-*Anabaena-Nostoc*(BF1-4) two times drench +732:1406:375 mg NPK/Pot, T_7-*Anabaena-Trichoderma* (An-Tz) two times drench + 732:1406:375 mg NPK/Pot.

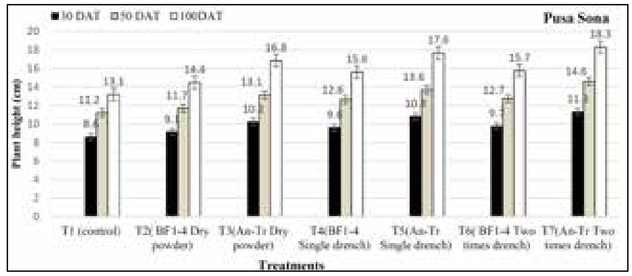

Fig. 1a. Influence of cyanobacterial formulations on plant height in var. Pusa Sona at 30, 50 and 100 DAT.

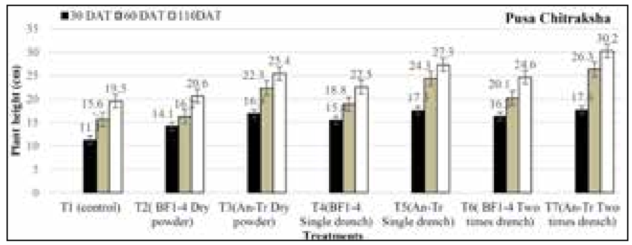

Fig. 1b. Influence of cyanobacterial formulations on plant height in var. Pusa Chitraksha at 30, 60 and 110 DAT.

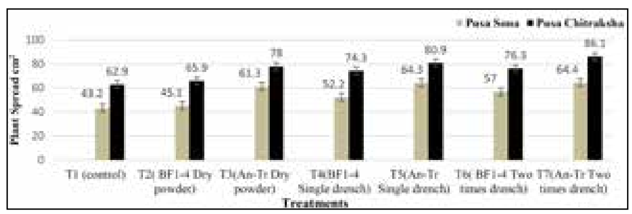

Fig. 2. Influence of cyanobacterial formulations on plant spread in var. Pusa Sona and Pusa Chitraksha.

varieties. Flower stalk length ranged from 6.5-7.5 cm in Pusa Sona and 7.2-8.8 cm in Pusa Chitraksha; the longest stalk length was measured in T_7, followed by T_6 in both varieties. Flower diameter was largest in Pusa Sona (5.1cm) and Pusa Chitraksha (10.2cm), with T_6 followed by T_7 and T_4 (Table 3). The number of flowers per plant was 25.6% higher in Pusa Sona with T_7 over T_1. Similarly, in Pusa Chitraksha, T_7 recorded a 56.2% increase in the number of flowers per plant and was statistically at par with T_6 (Fig. 3). Longest flowering duration was recorded with T_7 in both Pusa Sona and Pusa Chitraksha which was over 31 days and 41 days, respectively. The vase life of Pusa Sona and Pusa Chitraksha stems treated with cyanobacterial formulations was significantly longer in T_7 over other treatments (Table 4). The flowering attributes such as flower yield, size, and stalk length increased significantly with microbial treatments over control, possibly due to the increased availability of micro-nutrients. Kanchan *et al.* (6) observed the

Table 3. Influence of cyanobacterial formulations on the number of days to bud initiation, days to first bloom, stalk length and flower diameter in var. Pusa Sona and Pusa Chitraksha.

Treatment		Days to bud initiation		Days to first blooming		Stalk length (cm)		Flower diameter (cm)	
		PS	PC	PS	PC	PS	PC	PS	PC
T1		64	102	69	107	6.5	7.8	4.3	9.4
T2		63.3	99	68	105	6.6	7.6	4.4	9.5
T3		63	98	67	103	6.7	7.2	4.6	9.6
T4		63	97	67	102.3	7.2	7.3	4.9	9.9
T5		61	96.6	66	101	7.1	8.3	4.2	9.8
T6		62	97	67	102	7.4	8.7	5.1	10.2
T7		59	96	66	101	7.5	8.8	5	10.1
C.D. (0.05)	F(T)	1.42		1.19		0.05		0.02	
	F(V)	0.76		0.63		0.02		0.01	
	F(T*V)	N/A		N/A		0.07		0.04	

PS - Pusa Sona ; PC - Pusa Chitraksha

T_1-Recommended Dose of Fertilizer (RDF)-978:1406:375 mg NPK/Pot (control), T_2-*Anabaena-Nosto* (BF1-4) dry powder + 732:1406:375 mg NPK/Pot, T_3-*Anabaena-Trichoderma*(An-Tz) dry powder +732:1406:375 mg NPK/Pot, T_4-*Anabaena-Nostoc*(BF1-4) single drench +732:1406:375 mg NPK/Pot, T_5-*Anabaena-Trichoderma*(An-Tz) single drench +732:1406:375 mg NPK/Pot, T_6-*Anabaena-Nostoc*(BF1-4) two times drench +732:1406:375 mg NPK/Pot, T_7-*Anabaena-Trichoderma*(An-Tz) two times drench + 732:1406:375 mg NPK/Pot.

highest concentration of iron due to the application of *Anabaena–Trichoderma* in the Chrysanthemum variety Thai Chen Queen. Zinc improves plant and flower attributes, which could be due to enhanced polysaccharides, microbial biomass carbon, dehydrogenase activity and photosynthetic pigments (Kanchan *et al*. 6). Riahi *et al.*(16) recorded the stimulatory effect of cyanobacteria on the vegetative and floral growth in *Matricaria chamomilla* L. and *Satureja hortensis* L.. Kanchan *et al.*(6) reported a significantly higher number of flowers per plant and flower diameter in chrysanthemum var. White Star and Zembla with the *Anabaena–Azotobacter* biofilm inoculants. Significantly enhanced vase life in all the microbial inoculant treatments may be attributed to increased biomass in the flower stem.

Soil microbial biomass commands the build-up and breakdown of organic matter (Prasanna *et al.,* 14). The soil organic carbon of 0.35%-0.37% in control increased to 0.65%-0.67% in T_7 in Pusa Sona and Pusa Chitraksha, respectively (Fig. 4). The available nitrogen ranged from 61.2-107.1 mg

Table 4. Influence of cyanobacterial formulations on flowering duration and vase life in var. Pusa Sona and Pusa Chitraksha.

Treatment		Flowering duration		Vase life	
		PS	PC	PS	PC
T1		26.4	35.4	9.8	11.7
T2		26	35	10.1	13
T3		27.3	36.2	11.5	13.5
T4		27.3	38.5	10.5	12.3
T5		28.4	37.2	11.5	13
T6		29.3	39.4	12.2	13.8
T7		30.4	41.3	13.5	14.5
C.D. (0.05)	F(T)	0.13		0.83	
	F(V)	0.07		0.44	
	F(T*V)	0.18		N/A	

PS - Pusa Sona ; PC - Pusa Chitraksha

T_1-Recommended Dose of Fertilizer (RDF)-978:1406:375 mg NPK/Pot (control), T_2-*Anabaena-Nosto* (BF1-4) dry powder + 732:1406:375 mg NPK/Pot, T_3-*Anabaena-Trichoderma*(An-Tz) dry powder +732:1406:375 mg NPK/Pot, T_4-*Anabaena-Nostoc*(BF1-4) single drench +732:1406:375 mg NPK/Pot, T_5-*Anabaena-Trichoderma*(An-Tz) single drench +732:1406:375 mg NPK/Pot, T_6-*Anabaena-Nostoc*(BF1-4) two times drench +732:1406:375 mg NPK/Pot, T_7-*Anabaena-Trichoderma*(An-Tz) two times drench + 732:1406:375 mg NPK/Pot.

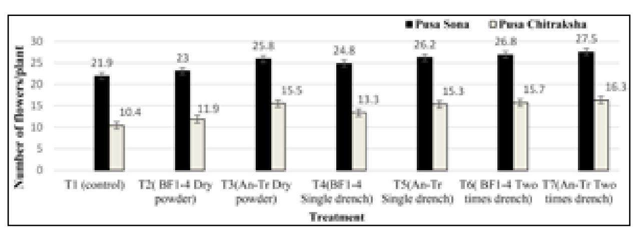

Fig. 3. Influence of cyanobacterial formulations on number of flowers per plant in var. Pusa Sona and Pusa Chitraksha.

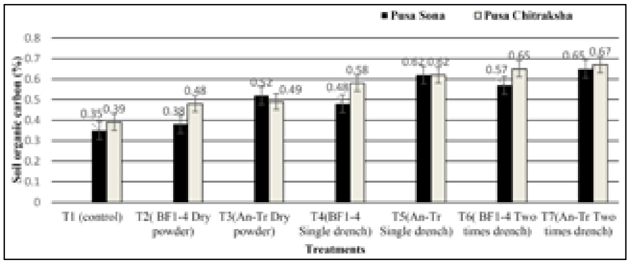

Fig. 4. Influence of cyanobacterial formulations on soil organic carbon in var. Pusa Sona and Pusa Chitraksha.

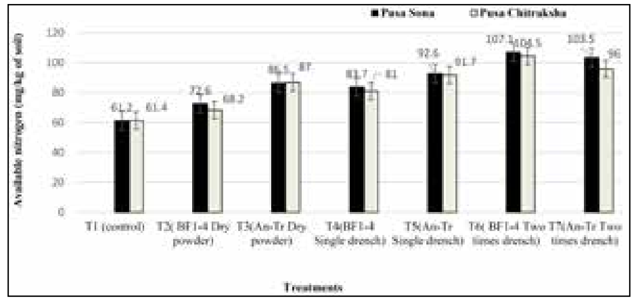

Fig. 5. Influence of cyanobacterial formulations on available nitrogen in var. Pusa Sona and Pusa Chitraksha.

N/kg of soil in Pusa Sona and 66.4-104.5mg N/kg of soil in Pusa Chitraksha. Treatment T_6 recorded the greatest increment in available nitrogen, followed by T_7 and T_5 in both the genotypes (Fig. 5). In Pusa Sona, the highest dehydrogenase activity of 135µg/g/day was recorded at 50 DAT and 138.2 µg/g/day at 100 DAT with T_7. In Pusa Chitraksha, T_6 exhibited the highest dehydrogenase activity at 60 DAT and 110 DAT, which is 142.6µg/g/day and 148.2µg/g/day, respectively (Table 5). In Pusa Sona, the concentration of soil chlorophyll ranged from 32.7-47.7µg/g/day[1] in inoculated treatment, with the highest in T_7 and a similar trend was observed in Pusa chitraksha with the highest value of 46.9µg/g/day. The potential nitrification activity in soil samples of 'Pusa Sona' and 'Pusa Chitraksha' ranged from 25.9-53.1nmol/g/h and 27.8-52.2nmol/g/h, respectively, with the highest value recorded in T_7, followed by T_6 in both varieties (Table 5).

T_7 recorded the highest soil organic carbon percentage in both varieties. Similar observations have been reported in cotton (Triveni *et al.,*20). Biofertilizers led to significantly higher chlorophyll over control. Enhanced chlorophyll indicates increased microflora activity in the sub-surface soils (Nayak *et al*., 9). Cyanobacteria and their biofilm role in nitrogen fixation and sustainable yields are well-established in rice, wheat, maize, vegetables, legumes and cotton (Prasanna *et al*., 12; Manjunath *et al.,*7). In the present study, the available nitrogen increased by 74.9% in Pusa Sona and 57.3% in Pusa Chitraksha with T_6 over control. Cyanobacterial inoculants in rice-wheat cropping systems significantly enhanced soil fertility, crop yields, and micronutrient enrichment in soil (Manjunath *et al*., 8). Heterocystous cyanobacteria (*Nostoc* and

Table 5. Influence of cyanobacterial formulations on Soil chlorophyll and ARA in var. Pusa Sona and Pusa Chitraksha.

Treatment		Soil chlorophyll (µg/g/day)		ARA (nmol/g/h)		Dehydrogenase activity			
		Pusa Sona	Pusa Chitraksha	Pusa Sona	Pusa Chitraksha	Pusa Sona		Pusa Chitraksha	
						50 DAT	100 DAT	60 DAT	110 DAT
T1		18.6	22.5	25.9	27.8	35.1	39.7	42.3	45.2
T2		32.7	33.6	30.3	30.2	93.3	95.4	87.6	89.1
T3		36.2	34.5	33.9	35.1	97.5	99.4	93.4	97.1
T4		37.2	37.8	38	37.1	103.2	105.5	105.5	107.3
T5		37.6	37.1	43	42.1	84.1	133.5	97.5	95.2
T6		43.1	42.2	47.2	46.3	127.4	129.2	142.6	148.2
T7		47.7	46.9	53.1	52.2	135	138.2	133.5	137.1
C.D. (0.05)	F(T)	1.85		0.64		18.55	0.54	18.55	0.54
	F(V)	N/A		N/A		N/A	0.29	N/A	0.29
	F(T*V)	N/A		0.911		N/A	0.76	N/A	0.76

T_1-Recommended Dose of Fertilizer (RDF)-978:1406:375 mg NPK/Pot (control), T_2-*Anabaena-Nosto* (BF1-4) dry powder + 732:1406:375 mg NPK/Pot, T_3-*Anabaena-Trichoderma*(An-Tz) dry powder +732:1406:375 mg NPK/Pot, T_4-*Anabaena-Nostoc*(BF1-4) single drench +732:1406:375 mg NPK/Pot, T_5-*Anabaena-Trichoderma*(An-Tz) single drench +732:1406:375 mg NPK/Pot, T_6-*Anabaena-Nostoc*(BF1-4) two times drench +732:1406:375 mg NPK/Pot, T_7-*Anabaena-Trichoderma*(An-Tz) two times drench + 732:1406:375 mg NPK/Pot.

Anabaena) are known to remarkably improve the surface soil's nitrogen content (Shariatmadari *et al.*, 17). The varietal difference in response to microbial inoculants was also reported by Prasanna *et al*. (14). CBBs supply both photosynthates and nitrogen (Prasanna *et al.,*12). *Anabaena-Trichoderma* biofilm and *Anabaena Nostoc consortium* in their different forms of application, proved superior in terms of higher levels of plant enzymes elicited plant biometrical parameters. In the present investigation, it was seen that T_7 performed better for most of the characters. These analyses illustrate significant contributions of microbiological activities (>60-70%) in enhancing plant growth and flower yields, irrespective of variety. The added advantage is saving 25% of nitrogen fertilizers by replacing them with eco-friendly biofertilizers.

AUTHORS' CONTRIBUTION

Conceptualization of research (KG, SCT), Designing of the experiments (SCT, KG), Contribution of experimental materials (PR, SKP, TAK , KS), Execution of field/lab experiments and data collection (KG, SCT, BV), Analysis of data andinterpretation (KG, SCT, PR, BV, SSP), Preparation of the manuscript (KG, SCT, BV)

DECLARATION

The authors declare that they have no conflict of interest.

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