Research Article

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Mode of application influences the biofertilizing efficacy of cyanobacterial biofilm formulations in chrysanthemum varieties under protected cultivation

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Abstract: Availability of nutrients in soil plays an important role in the productivity and quality of flowers in chrysanthemum. A set of novel biofilm inoculants- *Anabaena-Azotobacter*, *Anabaena-Pseudomonas fluorescens* (*An-Psf*) and *Anabaena-Trichoderma* (*An-Tr*) were applied as carrier based dry formulation or soil drench and their performance compared in two varieties of chrysanthemum (White Star and Zembla), in a climate-controlled greenhouse. Both the *An-Psf* and (*An-Tr*) inoculants enhanced glomalin related soil proteins in the rhizosphere of White Star, while in terms of polysaccharide content of soil, both these inoculants performed better in Zembla variety. Significant increases in the availability of selected macro and micronutrients in rhizosphere soil samples, in both chrysanthemum varieties were recorded, particularly when the inoculants were applied as soil drench. Principal Component analysis illustrated the significant interaction among soil and plant parameters, more specifically, the distinct effect of the inoculants, as compared to the application of carrier alone or control treatment. This investigation demonstrated the varietal effects on soil biological activities and significance of

mode of application of microbial inoculants in influencing plant growth and rhizospheric metabolic activities.

Keywords: biofilms; nutrient availability; microbial activity; plant growth; soil drench

1 Introduction

Plants and microbes are known to communicate through transkingdom signalling systems, which occur during different stages of development, and most of the research has focussed on the physical, biochemical and ecological characteristics of the rhizosphere (Lugtenberg et al. 2002). It has been estimated that around 20-40% of the photosynthetically fixed carbon is given out in the form of root exudates; this excess emphasizes the relevance assigned by the plant to support the establishment of beneficial microbial communities and interact with them for better water and nutrient availability (Aira et al. 2010; Bais et al. 2006).

Crops require requisite nutrients to sustain growth, reproduce and produce high quality grains or flowers or fruits. Fertility management of flower crops is particularly expensive as they are heavy consumers of fertilizers, particularly K/N ratios which improve photosynthetic canopy, after first flush, and stimulate the next flowering event. Chrysanthemum is an important commercial flower crop, which is exacting in its nutritional requirements and day length control, and needs to be supplied with a healthy mix of macro and micronutrients (Handreck and Black 1994). Among plant growth promoting (PGP) bacterial inoculants which are used for biofertilization, phytostimulation and biocontrol, cyanobacteria have become important inputs (Mandal et al. 1999; Gupta et al. 2013; Velmourougane et al. 2017); from their traditional

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use only in water-inundated paddies as diazotrophs, these organisms are now important contributors to soil fertility and crop productivity in diverse crops and agroecologies (Venkataraman 1981; Prasanna et al. 2014). The last decade has seen their potential as biocontrol agents for several soil borne or foliar diseases (Chaudhary et al. 2013; Dukare et al. 2010; Prasanna et al. 2016b; Thapa et al. 2018). Recent innovations in the microbial inoculants include the use of biofilms developed using the mucilaginous matrices of cyanobacteria and fungi as nutrient-rich niche for agriculturally beneficial heterotrophs, which have been employed in a wide range of crops (Seneviratne et al. 2008, 2011; Prasanna et al. 2011; Triveni et al. 2013; Velmourougane et al. 2017). In our earlier studies in chrysanthemum, the significance of these inoculants in enhancing soil fertility and plant growth has been demonstrated (Prasanna et al. 2016; Kanchan et al. 2018). But as this crop is grown mostly under protected cultivation and with fertigation, the present investigation was aimed towards comparing the biofertilizing efficacy of soil drench mode of application with the traditional dry formulation in two varieties of chrysanthemum. The two varieties are both popular standard cut flower type, with White Star being amenable to both greenhouse/polyhouse or field mode of cultivation and Zembla being most suitable for polyhouse cultivation.

2 Materials and Methods

2.1 Experimental site

The experiment was performed in the plastic semi-climate-controlled greenhouse at the Centre of Protected Cultivation Technology, ICAR-Indian Agricultural Research Institute (IARI) (latitude 28°38' N, longitude 77°12'E and altitude 228.4 m; monthly temperature and humidity ranged from 14 to 21°C and 62 to 75% respectively, with solar radiation of 240-285 watt m⁻²), New Delhi, between September 2016 - February 2017. Analyses of the physico-chemical characteristics of soil (0-20 cm depth) revealed concentrations of available nitrogen, phosphorus, and organic carbon as 110.57 kg ha⁻¹, 9.86 kg ha⁻¹, and 0.22% respectively on the day of inoculation. The initial concentration of total polysaccharide content was 1.69 mg g⁻¹ soil. Two standard flowering chrysanthemum varieties (Zembla and White Star) were planted on 1.0 m wide raised soil beds with a planting density of 36 plants m⁻² in a randomized block design with three replications each; each replication comprised 108 plants. The levels of N, P and K varied from 75-90, 40-60 and 60-80 ppm

respectively, during the study period. EC and pH were maintained as 1.3-1.5 dS/m and 7.5 for irrigation, 1.8 - 2.2 dS/m and 7.0 for fertigation respectively during the study period.

The number of irrigations (fertigation) ranged from 4-8 and the quantity of irrigation water was 200-300 litres per 50 m² area, supported with 16:2:30 twin-drip laterals per bed. The sampling for rhizosphere soil and chrysanthemum plants was done at flowering stage, 60 days after transplantation (60 DAT) of the plantlets. Low pressure drip fertigation system was used to regulate the supply of water and nutrients as per the experimental requirement. A water tank of 1000 L capacity was placed at the height of 3.0 meter platform to control the area of 1000 m² semi-climatic controlled greenhouse. 120 mesh/130 micron disc filters, 40 mm main line of HDPE, 16 mm drip lateral lines equipped with dripper of 2 L per hour were used for drip fertigation system. Major nutrient sources for fertigation were urea phosphate (17:44:0), potassium sulphate (0:0:50) and potassium nitrate (13:0:46) for controlling the desired ppm for N, P and K. The other major nutrients - calcium and magnesium were maintained in the range of 80-100 ppm and 40-48 ppm respectively. Micro nutrients - iron, manganese, zinc, copper, boron and molybdenum were maintained at the level of 1.2, 0.30, 0.03, 0.01, 0.4 and 0.005 ppm respectively, during fertigation.

2.2 Growth and maintenance of microorganisms

Azotobacter sp. (CBD15) and *Pseudomonas fluorescens* (PF5) obtained from the germplasm of the Division of Microbiology, IARI, New Delhi were grown using the Jensen's and King's B medium respectively, on a shaker (Scigenics Bioech Private Ltd., Model Orbitek 400LFIL) at 150 rpm at 30 ± 2°C (Subba Rao 1999). *Trichoderma viride* (ITCC 2211) of Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi, was grown as a static culture in Potato Dextrose medium at 30 ± 2°C. The cyanobacterial strain (BF1 of *Anabaena torulosa*) from the germplasm of the Division of Microbiology, IARI, New Delhi was grown using in the 4 L Haffkine flasks with the N free BG-11 medium (Stanier et al. 1971) under the light-dark cycles (16:8 hrs) with white light (50-55 µmoles photons m⁻¹s⁻¹) at 28 ± 2°C.

Both *Anabaena torulosa* and *Trichoderma viride* were used as matrices for developing dual species biofilms as reported earlier (Prasanna et al. 2011, 2013; Triveni et al. 2012; Swarnalakshmi et al. 2013). The biofilms of *Anabaena*

torulosa – *T. viride* (An-Tr), *A. torulosa* – *Azotobacter* sp. (An-Az), and *Anabaena torulosa* – *Pseudomonas fluorescens* (An-Psf) were harvested, washed thoroughly using distilled water to remove adhering media constituents, and mixed with vermiculite (hydrous phyllosilicate mineral): compost (1:1) as the carrier. The paddy straw compost (with pH 7.34, C/N 16.22 and humus 13.8%), was used in this experiment. The cyanobacterial, fungal and bacterial colony forming units (CFU) of inoculants were 10^4 , 10^5 , and 10^8 per g carrier, respectively as optimized in earlier studies (Prasanna et al. 2015, 2016; Kanchan et al. 2018). The treatments for both chrysanthemum varieties (Zembla and White Star) included- Control, Carrier, An-Az biofilm, An-Psf biofilm and An-Tr. The formulations were applied at the rate of 1g per plug at the time of transplanting or as soil drench, 2-3 d after transplanting. Sets of 30 days old self rooted plug plants (to facilitate the establishment of the plantlets) were used. Control treatment refers to the one not receiving any microbial inoculants, but nutrients and other fertigation practices are similar to the other treatments.

Soil drench was prepared by the addition of 100 g of each specific formulation/carrier to 1 L of tap water, followed by incubation for 7 days at room temperature, with intermittent manual shaking. The extracts were diluted to 10 L and then filtered through a muslin cloth, as described earlier (Prasanna et al. 2016) and applied adjacent to the plantlet roots, at the rate of 2 mL/plantlet.

2.3 Plant growth and biometric parameters

The plant biometric parameters evaluated include plant height, number of leaves/plant, stem diameter, internode length, days to bud initiation and flower opening, and diameter /size of flowers were observed on 90 DAT (days after transplanting) of plantlets.

The concentrations of photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) were measured using fresh leaves collected 90 DAT (days after transplanting) of plantlets by extraction with DMSO (dimethyl sulphoxide). The measurements were taken at 480, 510, 645 and 663 nm in a UV VIS spectrophotometer (Model Evolution 300, Thermo Scientific) and calculated using the equations of Arnon (1949). All measurements were done with a set of three replicate plant samples.

2.4 Soil nutrient parameters

Soil samples were also collected 90 DAT (days after transplanting) of plantlets. Available nitrogen and

phosphorus of soils were estimated by the alkaline permanganate method (Subbiah and Asija 1956) and the method of Olsen et al. (1954), respectively. The concentrations of soil organic carbon were measured, and values were expressed as per cent carbon (Hesse 1971). The concentrations of available copper, manganese, and zinc were measured using atomic absorption spectrophotometer (Lindsay and Norvell 1956). Total polysaccharides were estimated based on the method of Lin (2005), using known standard solutions of glucose, and the contents were expressed in mg g⁻¹ soil. Extraction and determination of GRSPs (Glomalin-like Soil Proteins) in the soil samples, using 0.10 g of soil, following the method, as given by Wright and Upadhyaya (1996). The protein content in the crude extract was determined by Bradford assay, with bovine serum albumin as the standard.

2.5 Statistical analysis

Data were analysed by using Windostat 8.5 statistical package and Analysis of variance (ANOVA) was allowed to calculate critical differences (CD) at a probability level of 0.05%; these values, as provided in the Tables. Factorial/Multivariate analysis was done using XLSTAT software (version 2014.5.03), including Principal Component Analysis (PCA) to correlate the soil, microbiological and plant parameters. Significant differences were delineated at $P \leq 0.05$, after ANOVA, followed by the single means differentiated by Tukey's honestly significance difference (HSD) test at $P \leq 0.05$ and designated using lowercase alphabets. ANOVA was done with fixed and random effects model; the fixed effects are two varieties (Zembla and White Star) and the random effects are the treatments (Control, Carrier, An-Az, An-Psf An-Tr) and application methods (Dry formulation and Soil drench). Significance of varieties, inoculants, application mode (dry/soil drench) and their interactions are depicted in the Tables, as CD (Critical Difference). Standard deviation (SD; $n=3$) values were depicted in graphs as error bars using Microsoft Excel, in Figures 1 and 2. Pearson's correlation analyses were performed using Microsoft Excel package.

Soil micronutrients availability (Zn, Cu and Mn), glomalin content and soil polysaccharides were analysed using two factorial (RBD) Analysis of variance (ANOVA), while plant photosynthetic pigments and macro nutrient status was analysed in a three factorial (RBD) ANOVA.

Ethical approval: The conducted research is not related to either human or animal use.

Figure 1: Influence of cyanobacterial biofilm formulations on the micronutrient content in soil. a. Zinc, b. Copper, c. Manganese. The treatments include- Control, Carrier, *A. torulosa* - *Azotobacter* sp. (An-Az) biofilm, *Anabaena torulosa* - *Pseudomonas fluorescens* (An-Psf) biofilm, and *Anabaena torulosa* - *T. viride* (An-Tr) biofilm, applied as dry formulation or soil drench. NS, Non-significant. Based on ANOVA, rankings are denoted as lower case alphabets, in the Figure

3 Results

3.1 Plant growth and biometrical attributes

Significant differences were recorded among the treatments (Table 1), in terms of leaf chlorophyll *a*, with An-Az recording highest values in Zembla, when applied as dry or soil drench (0.33 and 0.30 mg/g fresh weight). In White Star, however, An-Tr recorded significantly higher, almost three-fold higher values over control in the dry mode of application. Chlorophyll *b* content was generally

much lower and An-Az recorded highest values among all the treatments, when applied as dry formulation in White Star, followed closely by An-Tr in Zembla. In terms of carotenoid content, An-Az performed better than other treatments, across varieties. ANOVA illustrated that the treatments, inoculants, varieties were significant, as well as their interactions.

In terms of plant height and number of leaves (Supplementary Table 1), although the biofilm inoculants performed better (in particular, An-Tr which showed to 10-12% enhancement over control, though not always significant). In terms of internode length or stem diameter,

Figure 2: Influence of cyanobacterial biofilm formulations on a. glomalin (GRSPs - Glomalin-like Soil Proteins) content and b. soil polysaccharide. The treatments include- Control, Carrier, *A. torulosa* - *Azotobacter* sp. (An-Az) biofilm, *Anabaena torulosa* - *Pseudomonas fluorescens* (An-Psf) biofilm, and *Anabaena torulosa* - *T. viride* (An-Tr) biofilm, applied as dry formulation or soil drench. Based on ANOVA, rankings are denoted as lower case alphabets, in the Figure

no distinct differences could be observed (data not shown), although the treatments, inoculants, varieties were significant, as well as their interactions. However, in terms of number of days to bud initiation and flower opening, both An-Tr and An-Psf reduced the values only by a day, in White Star, when used as soil drench (Supplementary Table 2); however, the treatments and ANOVA illustrated the significance of treatments and varieties and their interactions. Flower diameter was stimulated by addition of inoculants, particularly in Zembla, with soil drench and using both modes of application in White Star, with minor increases of 2- 5%. Stem diameter was significantly higher in all the three biofilm inoculants receiving treatments (data not shown). In general, the interactions were significant for all the plant related attributes, as shown in the Tables.

3.2 Soil nutrient and microbiological parameters

The available nitrogen values ranged from 106 to 212 kg/ha, with An-Tr recording highest values in Zembla, with both modes of application (dry formulation/soil drench), and as soil drench in White Star (Table 2). An increment of 40-50% was recorded in White Star, over all other treatments with An-Az application as dry formulation recording highest values among all the treatments. Interestingly, White Star variety also recorded much higher values of available N, as compared to Zembla. All the inoculants, varieties and application mode, as well as their interactions recorded significant differences.

On the other hand, the available P values were much higher in most treatments in Zembla, ranging from 13

Table 1: Evaluation of plant photosynthetic pigments in leaf samples of Chrysanthemum varieties, as influenced by mode of inoculation of cyanobacterial formulations

Treatment/ Variety/ Formulations	Chlorophyll <i>a</i> (mg g ⁻¹ f wt)			Chlorophyll <i>b</i> (mg g ⁻¹ f wt)			Carotenoids (mg g ⁻¹ f wt)		
	Zembla		White Star	Zembla		White Star	Zembla		White Star
	Dry formulation	Soil drench	Dry formulation	Dry formulation	Soil drench	Dry formulation	Dry formulation	Soil drench	Dry formulation
Control	0.16±0.02	0.23±0.04	0.08±0.01	0.17±0.03	0.05±0.01	0.07±0.01	0.03±0.00	0.16±0.03	0.22±0.03
Carrier	0.24±0.04	0.27±0.04	0.15±0.03	0.13±0.02	0.08±0.02	0.09±0.00	0.04±0.01	0.19±0.01	0.14±0.05
An-Az	0.33±0.05	0.30±0.01	0.14±0.04	0.15±0.03	0.06±0.02	0.08±0.02	0.04±0.01	0.22±0.01	0.18±0.02
An-Psf	0.23±0.02	0.24±0.03	0.16±0.06	0.16±0.02	0.06±0.01	0.07±0.02	0.05±0.00	0.19±0.02	0.18±0.01
An-Tr	0.23±0.05	0.28±0.05	0.25±0.09	0.17±0.04	0.16±0.02	0.09±0.02	0.06±0.02	0.22±0.00	0.16±0.01
SE (m)	0.02	0.02	0.03	0.02	0.01	0.01	0.01	0.01	0.02
CD (<i>p</i> <0.05)	*	*	*	*	*	*	*	*	*

All the values are mean of three replications; An, *Anabaena torulosa*; Az, *Azotobacter* sp. (CBD15); Psf, *Pseudomonas fluorescens* (PF5); Tr, *Trichoderma viride*;
SE (m), standard error of mean; CD, critical difference; *, significant at 5% probability.
Chlorophyll *a* (CD (*p*<0.05)): Varieties (A)=0.02; Inoculation (B)= 0.02; Treatments (C) = 0.03; A x B= 0.03; A x C=0.05; B x C=0.05; A x B x C=0.07
Chlorophyll *b* (CD (*p*<0.05)): Varieties (A)= 0.01; Inoculation (B)= 0.02; Treatments (C) = 0.02; A x B= 0.01; A x C=0.02; B x C=0.02; A x B x C=0.03
Carotenoids (CD (*p*<0.05)): Varieties (A) = 0.01; Inoculation (B)= 0.01; Treatments (C) = 0.02; A x B= 0.02; A x C=0.03; B x C=0.03; A x B x C=0.04

Table 2: Macronutrient status of rhizosphere soil of Chrysanthemum varieties, as influenced by mode of inoculation of cyanobacterial formulations

Treatment Variety/ Formulations	Available nitrogen (kg ha ⁻¹)			Available phosphorus (kg ha ⁻¹)			Organic carbon %		
	Zembla	White Star		Zembla	White Star		Zembla	White Star	
	Dry formulation	Soil drench	Dry formulation	Dry formulation	Soil drench	Dry formulation	Dry formulation	Soil drench	Dry formulation
Control	106.65 ± 6.25	119.20 ± 18.80	108.73 ± 7.22	129.60 ± 7.27	13.44 ± 4.48	20.91 ± 6.84	11.95 ± 2.59	16.43 ± 2.59	0.17 ± 0.02
Carrier	125.87 ± 25.06	121.27 ± 28.98	118.87 ± 6.27	131.43 ± 31.35	16.43 ± 2.59	22.40 ± 8.96	11.95 ± 5.17	17.92 ± 4.48	0.34 ± 0.12
An-Az	138.33 ± 25.11	126.60 ± 25.13	212.62 ± 37.67	163.05 ± 37.65	17.92 ± 4.48	23.89 ± 6.84	13.44 ± 4.48	20.91 ± 6.84	0.38 ± 0.07
An-Psf	137.97 ± 12.55	125.43 ± 12.55	131.70 ± 6.30	146.37 ± 14.49	19.41 ± 6.84	32.85 ± 6.84	11.95 ± 2.59	25.39 ± 6.84	0.46 ± 0.09
An-Tr	156.20 ± 31.42	141.85 ± 21.33	131.43 ± 18.81	175.67 ± 12.55	26.88 ± 4.48	28.37 ± 9.33	13.44 ± 4.48	19.41 ± 6.84	0.79 ± 0.13
SE (m)	12.76	12.75	11.27	13.71	2.75	4.53	2.31	3.34	0.05
CD (p<0.05)	*	*	*	*	*	*	*	*	*

All the values are mean of three replications; An, *Anabaena torulosa*; Az, *Azotobacter* sp. (CBD15); Psf, *Pseudomonas fluorescens* (PF5); Tr, *Trichoderma viride*;

SE (m), standard error of mean; CD, critical difference; *, significant at 5% probability.

Available nitrogen (CD (p<0.05)): Varieties (A)= 11.38; Inoculation (B)= 17.99; A x B= 16.09; A x C=25.44; B x C=25.44; A x B x C=35.98

Available phosphorus (CD (p<0.05)): Varieties (A)= 2.99; Inoculation (B)= 2.99; Treatments (C) = 4.72; A x B= 4.23; A x C=6.68; B x C=6.68; A x B x C=9.45

Organic carbon % (CD (p<0.05)): Varieties (A)= 0.04; Inoculation (B)= 0.04; Treatments (C) = 0.06; A x B= 0.05; A x C=0.09; B x C=0.09; A x B x C=0.12

to 32 kg/ha, with An-Tr and An-Psf recording almost twofold enhancement over control, when used as dry or soil drench formulation respectively. In White Star, highest values were recorded with An-Psf, applied as soil drench. Organic carbon (%) recorded two to threefold enhancements due to the application of inoculants. An-Tr was the top ranked treatment in Zembla, both as dry or soil drench in White Star. An-Az performed significantly better, when applied as dry formulation in White Star. For both the varieties, highest values were recorded with soil drench mode of application.

Micronutrient availability in soil showed significant differences as a result of inoculation of biofilm inoculants and varieties (Figure 1a-c). The soil-available Zn, Cu and Mn values were almost 2- to 3-fold higher in the samples from White Star rhizosphere soil samples. All the three biofilm inoculants significantly enhanced the Zn availability, when applied as dry/soil drench in both varieties, although to differing extent; values ranged from 0.59 to 9.10 ug/g soil. Cu availability showed a similar trend, with values of 0.73-0.98 ug/g soil in control, as against 0.83-2.03 ug/g soil in the biofilm inoculant receiving treatments. Highest values in Zembla were obtained using An-Tr inoculant, while in White Star, An-Psf inoculant proved most promising, both as dry powder or soil drench. Mn availability also showed enhancement of 50-75% in the biofilm treatments, vis a vis control; however, varietal differences were not so distinct. Micronutrient availability was significantly influenced by varieties, inoculants, mode of application and their interactions.

GRSPs exhibited distinct differences in the soil samples from various treatments (Figure 2a), particularly as a result of mode of application (dry powder/soil drench). Soil drench mode of application exhibited distinctly higher values in Zembla, but not so well-defined in White Star samples. The highest values of 538.14 and 558.56 ug/g soil were recorded in An-Tr treated samples, as soil drench in Zembla and White Star respectively. Polysaccharide content in soil samples from various treatments were markedly different in the biofilm inoculated treatments, particularly with An-Tr and An-Psf (Figure 2b). Highest values were recorded with An-Tr, both as dry or soil drench application in Zembla, while a similar observation as recorded with An-Psf inoculant in White Star. Soil drench, in general, led to higher values. The mode of application, inoculants and varieties, and their interactions were significant.

3.3 Correlation analyses and PCA

In order to understand the nature of interactions among the major soil and plant growth parameters, correlation analyses were undertaken (Supplementary Table 3). Organic carbon (OC, %) positively enhanced polysaccharide in both the varieties, but had a significant effect on Zembla. Organic carbon also had positive correlation on glomalin, Chl *a*, Chl *b* and carotenoids in Zembla, but showed negative correlation for these parameters in White star except glomalin. Except glomalin in Zembla and Chl *a* in White Star, N showed a positive correlation for other parameters. P had positive correlation with all the parameters in Zembla, but significant with polysaccharides. However, P showed negative correlation for polysaccharide and Chl *a* and *b* in White Star. Cu showed positive correlation for all the parameters in Zembla, but had significant effect on polysaccharide in Zembla. However, Cu showed negative correlation for other parameters except glomalin and Chl *b* in White Star. Mn had positive correlation for all parameters except glomalin in Zembla, while it showed significant and positive correlation for glomalin in White Star.

PCA illustrated the treatments had a significant effect on the plant and soil parameters investigated (Figure 3). Inoculant effect was specific; treatment An-Tr as dry formulation (P) and as soil drench (SD) grouped together, while, Control treatment as dry formulation (P) and as soil drench (SD) along with Carrier treatment (P) and soil drench (SD) formed a separate group. The other inoculants and their soil drench counterparts -An-Az as dry formulation (P) and as soil drench (SD) as a group, while An-Psf as dry formulation (P) and as soil drench (SD) also formed a close group.

4 Discussion

Interactions between plants and their associated microbial communities or applied inoculants are never unidirectional; rather there is a mutualistic exchange of signals and nutrients. Orchestration of these events is mainly through molecular dialogues eliciting highly coordinated cellular processes; these help to decide the final outcome of their relationship - ranging from symbiosis to pathogenesis (Ortiz-Castro et al. 2009). Plants produce a variety of organic compounds, including sugars, organic acids, which can serve as signalling molecules, while microorganisms release volatiles or phytohormones. This cross talk directly or indirectly activates plant immunity, thereby growth and morphogenesis and has

Figure 3: Principal component analysis (PCA) depicting the interactions among the plant growth attributes, soil microbiological and macro/micronutrient parameters with the treatments in the rhizospheres of Chrysanthemum var. White Star (WS) and Zembla (Zem). The treatments include- Control, Carrier, *A. torulosa* - *Azotobacter* sp. (An-Az) biofilm, *Anabaena torulosa* - *Pseudomonas fluorescens* (An-Psf) biofilm, and *Anabaena torulosa* - *T. viride* (An-Tr) biofilm, applied as dry /powder formulation (P) or soil drench (SD) Circles illustrate the closely related treatments. Chl a, Chlorophyll a; Chl b, Chlorophyll b; Carot, Carotenoids; SOC, Soil organic carbon; Polysac, Soil Polysaccharides; Glomalin, GRSPs (Glomalin-like Soil Proteins); N, Soil Nitrogen; P, Soil Phosphorus; Zn, Soil Zinc; Cu, Copper; Mn, Soil- Manganese

been the subject of research since decades to increase plant productivity and quality (Bais et al. 2004). In our earlier investigations with chrysanthemum varieties and microbial inoculants, the promise of these biofertilizing options and ability to colonise the rhizosphere and roots was established, using both DGGE and qPCR analyses of selected genes, supported by soil/plant parameters (Prasanna et al. 2016a; Kanchan et al. 2018). The present investigation explores the comparative efficacy of dry formulations and soil drench preparations of these cyanobacterial biofilm inoculants in providing nutrients in a sustainable manner and enhancing crop growth and soil fertility.

Although chrysanthemum cultivation is possible all around the year, in several temperate countries, light becomes a limiting factor influencing both flower quality and nutritional status (Carvalho et al. 2002). Breš and

Jerzy (2008) observed a significant effect of solar radiation on the nutrient concentrations in chrysanthemum leaves, with highest correlations with P, Ca, followed by N and K. They also discussed the practice of modulating fertigation strategies based on solar radiation, in order to provide optimal doses of nutrients and generating healthy chrysanthemum plants. In our study, a significant enhancement in the leaf pigment concentrations and number of leaves was recorded, which correlated well with the micro- and macronutrient content of soil. One of the most limiting elements in flower growing is often oxygen, as plants are known to not grow and function properly below 5% partial pressure (Morgan 2000). It can be hypothesized, that the photosynthetic nature and nutrient-mobilising potential of cyanobacterial inoculants, brings about an enhancement in plant growth and nutritional attributes.

Significant increases in the availability of N and P and fold increase in organic carbon, accretion in polysaccharide content and glomalin illustrate the potential of these cyanobacterial formulations, particularly An-Tr. Maintenance of critical N at different developmental stages leads to firstly a good canopy, followed by better flower production, and K/N ratios above 2.0 are known to stimulate flower production (Handreck and Black 1994). Available P was remarkably higher in treatments receiving soil drench, with both An-Tr and An-Psf, and maintained at optimal levels, as suggested by Handreck and Black (1994) who investigated the sensitivity of flower crops to P levels.

The role of organic matter, glomalin (GRSP) and polysaccharides in soil is established in soil fertility as it helps to bind soil particles and represents a reservoir of nutrients. Walley et al. (2014) concluded from their studies that the proteinaceous material extracted, and characterized in the literature, as GRSP is not exclusively of Arbuscular Mycorrhizal Fungi (AMF) origin, and highlighted that GRSP is important to soil quality, and C and N storage, irrespective of origin. Earlier studies have shown the ecological significance of GRSP, and being highly responsive to ecosystem perturbations, even in short-term experiments, through its contributions to soil aggregate formation and stabilization due to its stability and hydrophobic nature (Rillig et al. 2003; Wright and Upadhayaya 1996; Wang et al. 2017). In our earlier studies, with pea grown as a field crop (Verma et al. 2015), several-fold enhancement in glomalin was recorded, and attributed to cyanobacterial inoculation.

Micronutrients are another important requirement for effective growth and flower quality in flowers, particularly chrysanthemum, which is known to be regulated by plant-microbe interactions (Maathius 2009). In the present investigation, micronutrient availability was found to be significantly enhanced and positively correlated with other soil nutrient/microbiological and plant growth parameters. It was interesting to note that White Star treatments showed comparatively higher values for all micronutrient data, particularly with cyanobacterial biofilm treatments; all three biofilms recorded significantly higher values for both zinc (Zn) and copper (Cu), while Mn was at par in both varieties. In our earlier studies, using these inoculants, in cereal crops, including rice, wheat and maize (Adak et al. 2015; Rana et al. 2015; Prasanna et al. 2015a, b) significant enhancement in the availability of these micronutrients in soil and their translocation to grains/seed has been recorded. A similar report from other researchers in various crops is also documented (de Santiago et al. 2011). Zinc and iron are important micronutrients implicated in promoting height of plants, fresh weight, early flowering and increased

and flower diameter and longevity (Handreck and Black 1994). In our earlier investigation (Kanchan et al. 2018), Fe was found to most influential in terms of both soil and plant attributes analysed. Fe is a redox-active metal involved in major metabolic processes, including respiration, photosynthesis, nitrogen assimilation, osmoprotection and elicitation of defense machinery, and its deficiency in soil leads to interveinal chlorosis in several plants. Zinc supplementation is known to enhance floral attributes, as it is a cofactor for several enzymes, as shown by Naveen kumar et al. (2009) in chrysanthemum. Balanced ratios of application, combination sprays of micronutrients have been successfully resorted for floricultural crops, however, in our investigation, minimal supplementation of micronutrients was given through fertigation. This further illustrates the significance of microbe-mediated micronutrient mobilisation in soil and its improved availability to the crop.

In our earlier investigations (Kanchan et al. 2018; Prasanna et al. 2016), the use of cyanobacterial inoculants was found to bring about quantitative changes (based on qPCR quantification of 16S rRNA abundance) of bacteria, archaea and cyanobacteria in the rhizosphere soils, revealed the var. Zembla showed stronger influences of these inoculants. The distinct influence of An-Tr inoculants is supported by our previous observations, in which we evaluated two varieties-White Star and Golden Ball. Aira and co-workers (2010) employed PLFA based analyses of rhizosphere communities in maize genotypes and their interactions with different levels of fertilizers. They also hypothesized that differences in the composition/quality of exudates may be the result of complex interactions of plants and fertilizers.

Future research is proposed to decipher the chemical and biological activity of the root exudates from different chrysanthemum varieties and relate to the signalling with the microbial inoculants.

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