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Effect of microbial biofilm inoculants on growth attributes of chrysanthemum under protected cultivation

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Abstract
Protected cultivation of flowers becomes less profitable with excessive use of fertilizers in commercial ventures. In the present experiment, the influence of microbial biofilm inoculants (Anabaena–Azotobacter, Anabaena–Trichoderma, and Trichoderma–Azotobacter) as field application and nursery treatment on the growth attributes in two varieties of chrysanthemum (var. White Star and Zembla) was studied. Varietal-specific responses in various growth attributes were recorded. All the growth attributes were highest in var. White Star. There are non-significant differences among biofilm treatments for all the attributes except for plant height in var. Zembla. Field application of biofilm and nursery treatment with Anabaena–Trichoderma and Trichoderma–Azotobacter showed a mixed response in both varieties. The Anabaena– Trichoderma and Trichoderma–Azotobacter biofilm inoculants proved superior in both chrysanthemum varieties.

Keywords: Protected cultivation, chrysanthemum, trichoderma, treatment and biofilm

Introduction
Protected cultivation of chrysanthemums in the greenhouse offers several benefits, including the supply of high-quality flowers year-round. However, continuous cultivation year after year can cause significant soil problems, especially those related to nutrient deficiencies, loss of microbial diversity, and greater propensity to attack by diseases and pests (Castilla 2013) [3]. Agronomic practices including soil management practices and environmental factors govern the ecology, diversity, and abundance of microbial communities (Miller *et al*. 1989; Hoitink & Boehm 1999) [14, 7], with scanty reports of biofertilizers use in protected cultivation (Abbasi & Yousra 2012) [1]. Few reports suggest that biofertilizers significantly affected fresh weight in chrysanthemum under field conditions (Kumari *et al.* 2014; Prasanna *et al.* 2016) [10, 18]. Different types of biofertilizers including diazotrophs and P solubilizers – Azotobacter, Azospirillum, and AM fungi are used in the cultivation of ornamentals and flower crops (Chaudhary 2010) [4] including carnation, marigold, aster, rose, tuberose and jasmine.

Plant nutrition and photosynthetic efficiency are critical to obtaining high-quality chrysanthemum flowers in both pots and fields (Barbosa 2003) [2]. Though applying chemical fertilizers is an easy method of nutrient supply, they can adversely influence vegetative growth, by increasing the susceptibility of plants to herbivorous insects and soil health in the long term (Scheirs *et al.* 2003) [23]. Microbial biofilms can be a promising option, as polysaccharides in the bacteria/fungi/cyanobacterial matrices provide hospitable conditions for colonization by other microbes in nature, besides facilitating their establishment and "greening" of diverse extreme habitats. The nutrient-rich mucilage of the plant growth-promoting filamentous cyanobacterium *Anabaena torulosa* and filamentous fungus *Trichoderma viride* have been explored as matrices for agriculturally important rhizospheric bacteria and fungi, to develop laboratory-constructed novel multi-functional and two-membered biofilms (Seneviratne 2003; Prasanna *et al.* 2011, 2014, 2015; Triveni *et al.* 2012) [24, 5, 21, 16, 28]. Biofilms can perform better as inoculants and improve the soil nutrient availability mediated by better colonization in the rhizosphere and roots, and positively influence crop growth and yields, as shown in crops such as chickpeas, wheat, maize, etc., (Prasanna *et al.* 2014, 2015, 2016; Swarnalakshmi *et al.* 2013) [21, 16, 18, 27]. The relationship (s) existing between the plant and soil biological functions, including the changes in the structure of rhizosphere microbial communities and variety-specific responses of biofilm inoculants in chrysanthemums is also examined.

Materials and Methods

The study was conducted between October and February at the Centre of Protected Cultivation Technology, Indian Agricultural Research Institute, New Delhi. The initial concentrations of available nitrogen, phosphorus, and organic carbon were 104.57 kg/ha, 8.63 kg/ha, and 0.2%, respectively. The initial concentration of total polysaccharide content was 1.492 mg/gm soil. The average monthly temperature and humidity ranged from 18 and 21 °C and 62 and 73%, respectively, while the average minimum and maximum solar radiation were found to be 240 and 285 W /m^2 inside the greenhouse. Two chrysanthemum varieties (White Star and Zembla) were planted on the raised beds of 15 m × 1.25 m with four plants m^2 (with three rows) in a completely randomized design with four replications each; each replication comprised 20 plants. The number of irrigations (fertigation) was six during the study period while the quantity of irrigation water was 200–300 litres per 50 m^2 area with the major nutrients (N, P, and K at the rate of 80, 50, and 60 ppm, respectively).

Azotobacter sp. (CBD15) and cyanobacterial strain (BF1 *Anabaena torulosa*) were procured from the germplasm of the Division of Microbiology, IARI, New Delhi, and *Trichoderma viride* (ITCC 2211) from the Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi. Both *Anabaena torulosa* and *Trichoderma viride* were grown under the respective optimized conditions (Prasanna *et al.* 2011, 2015) [5, 16] and used as matrices for developing dual-species biofilms (Prasanna *et al.* 2011, 2013; Triveni *et al.* 2012; Swarnalakshmi *et al.* 2013) [5, 17, 28, 27]. Harvested biofilms were mixed with vermiculite (hydrous phyllosilicate mineral): compost (1:1) as the carrier, keeping the cyanobacterial, fungal, and bacterial colony forming units (CFU) of inoculants as 104, 105, and 108 per g carrier, respectively, as optimized in earlier studies (Prasanna *et al.* 2015, 2017) [16, 20].

Available nitrogen and phosphorus of soils were estimated by the alkaline permanganate method (Subbiah & Asija 1956) [26] and the method of Olsen *et al*. (1954) [15], respectively. The concentrations of soil organic carbon were expressed as percent carbon (Hesse 1971) [6]. Total polysaccharides were estimated based on the method of Lin (2005) [11], using known standard solutions of glucose, and the contents were expressed in mg/g soil.

The plant growth attributes such as total plant height, fresh weight, dry weight, number of leaves, stem dia. and internodal length were observed on 60 DAT of plantlets. All measurements were done with a set of three replicate plant samples.

Results and Discussion

Improved nutrient uptake by plants through the enhanced release of growth hormones and bioactive molecules leading to better quality of produce with savings in the cost of production are the major benefits obtained through the use of biofertilizers. Cyanobacteria are well-established biofertilizers not only for rice but also bring about plant growth promotion and mobilization of macro- and micronutrients for several other crops (Venkataraman 1972; Mandal *et al.* 1998; Karthikeyan *et al.* 2007) [29, 12, 9]. Biofilm biofertilizers are a novel means of combining the beneficial characteristics of two organisms and our earlier findings showed that their inoculation in different cereals and vegetables grown under field conditions lead to positive effects, including plant growth promotion and improving the availability of macro and micronutrients, besides disease suppression (Dukare *et al.* 2011; Prasanna *et al.* 2013) [5, 17].

Field application of biofilm

All the treatments were found to be non-significant for all the characters under study in both varieties except for the plant height in cv. Zembla. However, field application of T2 (Anabaena–Trichoderma) produced maximum plant height, number of leaves, stem diameter, and fresh weight in cv. Zembla whereas field application of T3 (Trichoderma–Azotobacter) generated maximum internodal length and dry weight in the same variety. Chrysanthemum variety White Star responded maximum to the field application of T3 for plant height, number of leaves, stem diameter, and internodal length. Fresh weight and dry weight were maximum in field application of T2 for the same variety.

Nursery treatment with biofilm

Chrysanthemum Nursery of cv Zembla treated with T3 (Trichoderma–Azotobacter) produced maximum plant height, stem diameter, and internodal length. The number of leaves was maximum in nursery-treated cv. Zembla with T2 (Anabaena–Trichoderma). Chrysanthemum cv. White Star produced maximum plant height, number of leaves, fresh weight, and dry weight when nursery treated with T2 whereas stem diameter and internodal length were highest in nursery treated with T3.

In the present study, the biofilm inoculants were tested for their promise in chrysanthemum, grown under protected cultivation which has emerged as a promising option globally, especially for cultivating high-value crops, particularly flowers. Chrysanthemum, a popular flower crop, suffers from excessive vegetative growth and increased susceptibility to phytophagous insects when yield enhancement is attempted through the application of more than the recommended quantity of chemical fertilizers (Kanchan *et al.* 2018) [8]. Although there are several advantages to the protected mode of cultivation, continuous cultivation year after year is known to lead to major soil problems, especially related to nutrient deficiencies and loss of microbial diversity. Verma *et al.* (2011) [30] observed that the use of Azospirillum and PSB (Phosphate Solubilizing Bacteria), along with vermicompost and 50% of the recommended dose of NPK fertilizers gave better flower yield and plant biometrical parameters in chrysanthemum.

The growth and productivity of crop plants are often dictated by the availability of nutrients, particularly, N, P, and Fe at the soil–root interface, which is mediated by the biological activities of both roots and micro-organisms (Zhang *et al.* 2010; Marschner 2011) [31, 13].

The promise of cyanobacterial inoculation in enhancing plant growth parameters has been reported by several workers. Riahi *et al.* (2013) [22] recorded the stimulatory effect of cyanobacteria on the vegetative and reproductive growth of the medicinal plants – *Matricaria chamomilla* L. (chamomile) and *Satureja hortensis* L. (garden savoury). Similar observations in *Mentha* sp. were recorded by cyanobacterial inoculation, leading to a significant increase in the root, stem, and leaf growth parameters (Shariatmadari *et al.* 2015) [25].

The biofilms *viz*. Anabaena– Trichoderma, Trichoderma–Azotobacter proved superior in terms of plant biometrical parameters, including the fresh weight of plants and flowers. The beneficial effects of biofilm inoculants included increased nutrient availability in soils, improved growth, enzyme activities, and yield of chrysanthemum, mediated by rhizosphere microbial community modulation (Kanchan *et al.* 2018) [8]. These analyses also served to illustrate the significant contributions of soil nutrient availability and microbiological activities in enhancing plant growth and flower yields, irrespective of variety.

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