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Influence of bio-stimulants on growth and flowering in Chrysanthemum (*Dendranthema grandiflora* T.) cv. local yellow

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Abstract
The present experiment entitled "Influence of Bio-Stimulants on Growth and Flowering of Chrysanthemum" was conducted at ICAR-DFR, Regional Station, Vemagiri in Randomized Block Design (RBD) with ten treatments each replicated thrice. Different bio-stimulant formulations such as *Trichoderma viride* (9, 18 and 36 g per plant), *Pseudomonas fluorescens* (9, 18 and 36 g per plant) and Arbuscular Mycorrhizal Fungi (9, 18 and 36 g per plant) were applied to soil. Significant variations in growth and flowering were noticed after application of bio-stimulants. The plants treated with Arbuscular Mycorrhizal Fungi @18 g per plant recorded maximum plant height (62.36 cm), chlorophyll content (69.90 SPAD units), plant spread (54.83 cm), number of primary branches (13.87), secondary branches (27.65), leaf area (4690.23 cm^2), fresh weight of leaf (64.38 g), dry weight of leaf (6.96 g), fresh weight of shoot (667.06 g), dry weight of shoot (206.14 g), fresh weight of root (39.99 g), dry weight of root (17.99 g), root-shoot ratio (0.087), number of flowers per plant (394.29) and flower yield per plant (925.26 g).

Keywords: *Trichoderma viride*, *Pseudomonas fluorescens*, Arbuscular Mycorrhizal Fungi, growth and flowering parameters

Introduction
Chrysanthemum is one of the most cultivated garden plants primarily for its blossoms (Prasad *et al*. 2012) [21]. It is commonly known as 'Guldaudi' in India and 'Mum' or 'Glory of East' in the USA. It gets its name from the Greek words "chrysos" means golden and "anthos" means flower. It is widely cultivated in many parts across world owing to its economic value, elegance and aesthetic beauty (Navale *et al*., 2010) [20]. Chrysanthemum is widely grown in India for both cut flower and loose flower purpose. The loose flowers are mainly used in religious offerings and for preparing garlands and venis as adornments. It is an important source of essential oils and sesquiterpenoids. Some of the chrysanthemum species are also extensively cultivated for pyrethrum, an important botanical. The flowers are used to prepare herbal infusion known as chrysanthemum tea which has many health benefits including recovery from influenza in some parts of the world.
The quality of chrysanthemum blooms is greatly affected by the quantity and source of nutrients. At present, these nutrients are being supplied through chemical fertilizers. The imbalanced and indiscriminate use of chemical inputs *i.e.*, fertilizers and pesticides over the years resulted in accumulation of toxic chemical residues in the soil, depleting the organic carbon content and native micro flora and fauna. All these factors contribute to lower productivity of chrysanthemum. The use of chemical fertilizers has not only resulted in the deterioration of soil health but also has led to some serious environmental problems. Use of bio-stimulants in crop production helps in sustainable and ecologically safe farming of chrysanthemum through enhancement of growth and flowering by absorption of more water and nutrients, besides being effective in inhibiting the soil borne pathogens by various bio-control mechanisms. Henceforth, the present study was conducted to evaluate the suitable biostimulant formulation for profitable and sustainable chrysanthemum cultivation.

Materials and Methods
The biostimulant formulations at different concentrations *viz*., of T_1: control, T_2: *Trichoderma viride* @ 9 g per plant, T_3: *Trichoderma viride* @ 18 g per plant, T_4: *Trichoderma viride* @

36 g per plant, T_5: *Pseudomonas fluorescens* @ 9 g per plant, T_6: *Pseudomonas fluorescens* @ 18 g per plant, T_7: *Pseudomonas fluorescens* @ 36 g per plant, T_8: Arbuscular Mycorrhizal Fungi @ 9 g per plant, T_9: Arbuscular Mycorrhizal Fungi @ 18 g per plant and T_{10}: Arbuscular mycorrhizal fungi @ 36 g per plant were applied to the soil in three split doses.

One month old rooted cuttings of cv. Local Yellow were transplanted in the main field with a plot size of 3 m × 3 m for each treatment and each plot consisted of 16 plants at a spacing of 40 cm × 40 cm. All the bio-stimulants were applied in split doses at the time of planting, 30 and 60 days after planting. Initial bio-stimulant doses were applied after incubating in neem cake (10 g per plant) for 15 days. The second and third split doses were directly applied to soil without neem cake. Cultural and management practices were followed regularly. Three plants were selected at random and tagged in each treatment, for the purpose of recording observations on various parameters of growth and flowering.

Results and Discussion

The data corresponding to growth and flowering in chrysanthemum as influenced by application of bio-stimulants are presented in Table 1, Table 2, Table 3 and Table 4.

Growth parameters Plant height

Maximum plant height was maximum for T_9 treatment (62.36 cm) followed by T_{10} (59.86 cm) while lowest plant height was registered in T_1 (51.46 cm). This might be due to the production of auxins, gibberellins, cytokinins and vitamins at optimum level in the plants treated by AM fungi. The above compounds could have increased water uptake in treated plants thereby effecting a significant enhancement in plant height through the processes like cell division and cell multiplication at relatively rapid rates. The application of AM fungi @ 36 g per plant was could not be more effective at higher concentrations compared to lower levels. It might be due to the reason that at higher doses, AM fungi acts as parasite to host plant by competing for more extent of carbon for its survival (Garrido *et al*., 2010) [12].

Similar findings were also reported by Prasanna (2007) [22] in Gerbera, Elizabeth George (2012) [10] in Crossandra, Raju and Haripriya (2001) [19] in Gundumalli.

Plant spread

Plant spread was recorded highest in T_9 (54.83 cm) and it was on par with T_{10} (53.89 cm) whereas minimum plant spread was recorded in T_1 (45.46 cm). It might be due to the AMF extending their hyphae through plant roots thereby exploring a more amount of soil volume and enhancing the uptake of nitrogen and phosphorous which in turn expand plant canopy structure and architecture, leading to an increased plant spread.

Similar findings were also reported by Asrar and Elhindi (2011) [2] and Hussain *et al*. (2016) [13] in marigold.

Chlorophyll content (SPAD units)

Chlorophyll content was highly recorded in T_9 (69.90) followed by T_{10} (67.86). The least content of chlorophyll was noticed in T_1 (61.96). It might be due to the symbiotic associations between host and fungi. This association would have led to higher water and nutrient uptake resulting in higher biosynthesis of chlorophyll. More chlorophyll content could be associated with higher nitrogen uptake, since chlorophyll consists of elemental nitrogen (Clapperton and Reid, 1992) [8]. However, the application of AM fungi @ 36 g per plant was could not be more effective at higher concentration compared to lower levels. It might be due to the reason that at higher doses AM fungi acts as parasite to host plant by competing for more extent of carbon for its survival These results are in accordance with Manoharan *et al*. (2010) [17] in *Erythrina variegata* and Nagarajappa *et al.* (2003) [18] in papaya, Asrar and Elhindi (2011) [2] in marigold.

Leaf area (cm^2)

The highest leaf area (4690.23 cm^2) was noticed in plants treated with T_9 which was on par with the T_{10} (4647.78 cm^2). The lowest leaf area (3982.23 cm^2) was recorded T_1. Which might be due to the Application of AMF at an optimum dose *i.e.* 18 g per plant. It might be enhanced the production of cytokinins it could have hastened the cell expansion and division in plants associated with mycorrhiza plants.

Similar findings were also reported in Van Staden and Davey (1979) [23] in grapevines, Ravindra kumar *et al*. (2014) [24] in chrysanthemum.

Table 1: Vegetative parameters of chrysanthemum as influenced by different bio-stimulants

Treatments	Leaf area (cm^2)	Chlorophyll (SPAD units)	Plant height (cm)	Plant spread (cm)
T_1	3982.23	61.96	51.46	45.46
T_2	4324.39	64.36	54.65	48.34
T_3	4635.59	66.40	56.13	51.87
T_4	4620.94	65.50	55.62	49.55
T_5	4069.72	62.80	55.97	50.02
T_6	4266.64	63.70	57.32	53.36
T_7	4154.15	64.90	56.47	52.39
T_8	4463.13	63.50	58.96	51.26
T_9	4690.23	69.90	62.36	54.83
T_{10}	4647.78	67.86	59.86	53.89
Mean	4385.48	69.90	56.88	51.09
S.Em (±)	65.68	0.61	0.75	0.94
CD (5%)	194.86	1.80	2.23	2.73

Number of primary branches

Primary branches per plant was highly recorded in T_9 (13.87) which was on par with T_{10} (13.00) and least was observed in T_1 (7.30).

Number of secondary branches

No. of Secondary branches per plant was recorded highly in was noticed in T_9 (27.65) which was on par with T_{10} (26.99). While lowest number of secondary branches per plant (21.21) was recorded in T_1. It might be due to the application of AM fungi that increased vegetative growth, root colonization, thereby enhancing the uptake of phosphorus through roots and increase in the absorption of nutrients from the soil surface. These factors might have caused cell elongation and multiplication eventually leading to multiple branching from the main stem and main or primary branches.

Similar findings were also reported in Bagyaraj and Powell (1985) [4] in marigold, Kale *et al.* (1987) [14] in Aster and salvias and Cazares and Smith (1996) [6] in *Tagetes erecta* and *Zinnia elegans.*

Fresh and dry weight of leaves per plant (g)

Maximum fresh and dry weight of leaves per plant (64.38 g and 6.96 g) was shown in T_9 and which was on par with T_{10}

(57.83 g and 6.80 g). However, the lowest fresh and dry weight of leaves was registered in T_1 (26.22 g and 3.20 g). This might be due to mycorrhizal symbiotic associations with host plants, assisting in the effective exploration of rhizosphere for water and nutrients. Such a phenomemon could have enhanced the photosynthetic efficiency of plants through an increase in leaf area index thus leading to an increased fresh weight of leaf, eventually resulting in higher dry weight of leaves per plant.
Similar findings were reported by Aboul Nasr (1996) [1] in zinnia, Asrar and Elhindi (2011) [2] in African marigolds.

Table 2: Vegetative parameters of chrysanthemum as influenced by different bio-stimulants

Treatments	No. of primary branches	No. of secondary branches	Fresh weight of leaves per plant (g)	Dry weight of leaves per plant (g)
T_1	7.30	21.21	26.22	3.20
T_2	9.96	22.67	43.68	4.78
T_3	11.07	24.98	53.76	6.72
T_4	10.89	24.07	49.80	4.98
T_5	11.85	24.01	39.52	4.56
T_6	12.93	26.75	47.70	4.87
T_7	12.60	25.69	41.58	4.62
T_8	12.79	26.54	54.08	6.76
T_9	13.87	27.65	64.38	6.96
T_{10}	13.00	26.99	57.88	6.80
Mean	11.63	25.06	47.85	5.43
S.Em (±)	0.48	0.52	2.25	0.44
CD (5%)	1.43	1.54	6.51	1.27

Fresh and dry weight of shoot (g)
The fresh weight of shoot was highly observed in T_9 (667.06 g) followed by T_{10} (600.03 g) however, lowest was recorded in T_1 (528.09 g).
The maximum shoot dry weight was observed in T_9 (206.14 g) next by T_{10} (192.75 g) however lowest was shown in T_1 (138.06 g). Which may be due to AM fungi application which increased the rate of sucrose translocation from expanded leaves since they had a higher photosynthetic surface *i.e.* leaf area. Such increase in photo assimilation may be due to a corresponding increase in dry and fresh weights of shoot or more biomass per plant.
Similar findings were reported by Ravindra kumar *et al.* (2014) [24] in chrysanthemum, Boyer *et al.* (2014) [5] in strawberry.

Fresh and dry weight of root (g)
The studies reveal that significantly highest fresh weight of root was recorded in T_9 (39.99 g) which was on par with T_{10} (38.57 g). While T_1 recorded minimum fresh weight of root (24.09 g).
The dry weight of root was maximum in T_9 (17.99 g) and it was on par with T_{10} (16.34 g), a minimum was observed in T_1 (8.21 g). This might be due to the reason that mycorrhizal association could expand the root hairs and total root volume subsequently leads to enhancement of root biomass and also the symbiotic association between plant and mycorhizae resulted in an increase in water and phosphorous absorption from more volume of soil by the hyphae leading to increase in fresh and dry weight of plant roots (Chandra *et al*., 2010) [7].
Similar results were reported in Ravindra Kumar *et al*. (2014) [24] in chrysanthemum, Bagheri *et al.* (2018) [3] in zinna.

Root-shoot ratio
The highest root-shoot ratio was recorded in T_9 (0.087) and it was on par with T_{10} (0.086) however minimum was registerd in T_1 (0.059). It might be due to more relative development of root system as compared to shoot system, facilitating efficient nutrient absorption (Clapperton and Reid 1992) [8].
Similar reports were found by Khade *et al*. (2009) [15] in papaya, Bagheri *et al.* (2018) [3] in zinnia.

Table 3: Vegetative parameters of chrysanthemum as influenced by different bio-stimulants

Treatments	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)	Root - shoot ratio
T_1	528.09	138.06	24.09	8.21	0.059
T_2	539.89	157.06	26.75	9.76	0.062
T_3	572.01	173.06	35.07	14.16	0.082
T_4	567.01	171.09	33.17	12.90	0.075
T_5	569.06	172.39	35.06	14.30	0.083
T_6	579.03	179.70	37.09	14.62	0.085
T_7	574.07	175.06	36.33	14.50	0.083
T_8	589.56	180.06	37.89	15.20	0.084
T_9	667.06	206.14	39.99	17.99	0.087
T_{10}	600.03	192.75	38.57	16.34	0.086
Mean	578.58	174.54	34.40	13.79	0.079
S.Em (±)	9.41	4.60	1.29	0.72	0.003
CD (5%)	27.95	13.66	3.83	2.15	0.008

Number of flowers per plant and Flower yield per plant (g)
The highest number of flowers per plant was recorded in T_9 (394.29) which was on par with T_{10} (378.06) while minimum was observed in T_1 (264.06).

Flower yield per plant (g)
The maximum flower yield per plant (925.26 g) was registered in T_9 and which was statistically on par with T_{10} (890.23 g) whereas, T_1 recorded lowest flower yield per plant (624.15 g). The promotion of flowering and quality by mycorrhizal inoculation may be due to improvement in nutrient concentrations like K, P, Zn and S and hormonal effect caused by fungal colonization. It helps in more photosynthesis and the production of efficient carbohydrates. This could have supported higher sink numbers thus increasing the retention of a greater number of flowers, with higher mean weight and multiple pickings in the same duration of flowering and also the enhanced flowering of plants and led to increases the flower yield.
The results are in accordance with, Garmendia and Mangas (2012) [11] in Rose. Long *et al.* (2010) [16] in zinnia, Dufault *et al*. (1990) [9] in gerbera

Table 4: Vegetative parameters of chrysanthemum as influenced by different bio-stimulants

Treatments	Number of flowers per plant	Flower yield per plant (g)
T_1	264.06	624.15
T_2	337.46	800.90
T_3	368.26	880.65
T_4	349.39	818.53
T_5	324.81	757.54
T_6	352.54	821.09
T_7	339.89	795.73
T_8	367.63	863.31
T_9	394.29	924.26
T_{10}	378.06	890.23
Mean	347.64	817.54
S.Em (±)	8.98	21.20
CD (5%)	26.69	62.99

Conclusion

Application of Arbuscular Mycorrhizal Fungi @ 18 g per plant was found best in plant height, plant spread, chlorophyll content, leaf area, number of primary branches, number of secondary branches, fresh weight of leaf, dry weight of leaf, fresh weight of shoot, dry weight of shoot, fresh weight of root, dry weight of root, root-shoot ratio that led to highest flower yield per plant.

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