Biological Forum – An International Journal **15(10): 1375-1379(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Influence of Bio-Stimulants on Flowering, Yield and Disease Incidence in Chrysanthemum (*Dendranthema grandiflora* T.) cv. Local Yellow

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(Received: 24 August 2023; Revised: 28 September 2023; Accepted: 08 October 2023; Published: 15 October 2023)
(Published by Research Trend)

ABSTRACT: The present experiment was conducted to study the influence of bio-stimulants on flowering, yield and disease incidence in chrysanthemum cv. Local yellow. 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 flowering and wilt disease incidence were noticed with application of bio-stimulants. The plants treated with *T. viride* (18 g per plant) recorded earlier days to flower bud formation (62.66 days) and flowering (82.20 days), maximum duration of flowering (54.55 days), highest catalase activity (0.85 units/mg protein/min), peroxidase activity (0.99 units/mg protein/min) and lowest disease incidence (18.00 %) and percent disease index (16.66 %). Arbuscular Mycorrhizal Fungi (18 g per plant) recorded maximum shelf life (5.10 days), capitulum diameter (6.21 cm), flower yield per plot (14.14 kg) and number of pickings (10.12).

Bio-stimulants in horticulture has been the focus of scientific interest for quite some time now. As the need for sustainable, eco-friendly and innovative horticulture solutions grows, more and more studies are being done on the efficacy of bio-stimulants.

Keywords: *Trichoderma viride*, *Pseudomonas fluorescens*, Arbuscular Mycorrhizal Fungi, Flowering and *Fusarium* wilt.

INTRODUCTION

Chrysanthemum is grown in many parts of the world owing to its excellence, aesthetic beauty and economical values (Navale *et al.*, 2010). In India, chrysanthemum is being grown for pot plant, loose flower and cut flower production. The loose flowers are being used for making venis, garlands and religious offerings. Some of its species are also cultivated as a source of pyrethrum, an important botanical insecticide. It is eaten as delicacy after frying and the flowers are used to prepare a sweet drink known as chrysanthemum tea which has many medicinal uses including recovery from influenza in some parts of the world. The bio-stimulants serves a great purpose and ensures the agricultural sustainability in those areas which possess agricultural lands with less availability of nutrients. These products when applied at low concentrations are pretty much benefecial to the plant but, when applied in high cocentration there will be noticeable fatality responses shown by the plants (Carolina *et al*., 2019). The important diseases that are affecting the chrysanthemum are leaf spot (*Septoria chrysanthemella*), leaf blight (*Alternaria alternata*), wilts (*Verticillium* and *Fusarium* spp.), root rot (*Phytophthora* spp., *Pythium* spp.), powdery mildew (*Golovinomyces chrysanthemi*), dry root rot (*Rhizoctonia solani*), brown rust (*Puccinia chrysanthemi*), viral stunt, mosaic, and nematodes (Pradeepkumar *et al*., 2008). *Fusarium* wilt is a serious disease in chrysanthemum (Singh *et al*., 2014). Wilt of Chrysanthemum incited by *Fusarium oxysporum* f sp. *chrysanthemi* (Singh and Kumar 2011), causes damage in both green house and field conditions (Garibaldi *et al*., 2009). Biological control has been probably the most useful in plant disease management as agro-chemicals cause damage to the environment and

threaten the ecological balance. Biological control is gaining importance because of its plant growth promotion, multiple modes of action, natural control mechanisms, environmental safety and sustained ecological balance. Henceforth, the present study was conducted to evaluate the suitable bio-stimulant formulation for profitable and sustainable chrysanthemum cultivation.

MATERIAL AND METHODS

The biostimulant formulations at different concentrations *viz*., of T_1: control, T_2: *T. viride* @ 9 g per plant, T_3: *T. viride* @ 18 g per plant, T_4: *T. viride* @ 36 g per plant, T_5: *P. fluorescens* @ 9 g per plant, T_6: *P. fluorescens* @ 18 g per plant, T_7: *P. 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.

$$\text{Per cent Disease Incidence} = \frac{\text{Number of plants showing symptoms}}{\text{Total Number of plants observed}} \times 100$$

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 three 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. Leaf samples were also taken from the tagged plants for determining the defense enzymes activity. Wilt incidence was observed plot wise and per cent disease incidence was calculated using the formula as follows

A. Assay of Catalase (units/mg protein/ min)

Fresh leaf sample of chrysanthemum weighing 500 mg was ground using 10 ml of sodium phosphate buffer (pH-6.8). The sample was centrifuged at 3000 rpm for 10 min at 4°C. One ml of the above supernatant was taken in five different beakers. Five ml of 1.5 % sodium per borate and 1.5 ml of phosphate buffer were added. Ten ml of 2 N sulphuric acid was later added at one, two, three- and four-min. interval after adding enzyme extract in first four beakers respectively. In final beaker 10 ml of 2 N sulphuric acid was added before addition of enzyme extract and kept as blank for comparison. The content in the beaker was titrated against 0.5 N $KMnO_4$. Development of pink colour persisted for 30 sec. was considered as end point and volume of $KMnO_4$ consumed was noted. The method for estimation of catalase was adapted from Luck (1974)

$$\text{Catalase activity} = \frac{\text{Amount of } 0.5\text{N } KMnO_4 \text{ consumed} \times 0.85}{1 \times 0.5} \mu\text{g of } H_2O_2 \text{ g}^{-1} \text{ min}^{-1}$$

B. Assay of Peroxidase (units/mg protein/ min)

Peroxidase activity in chrysanthemum leaf samples were estimated as per the method suggested by Mallik and Singh (1980). Chrysanthemum leaf sample weighing 500 mg was ground using 10 ml of sodium phosphate buffer (pH-7.0). The sample was centrifuged at 5000 rpm for 15 min at 4°C. One ml of the above supernatant was taken in a test tube and three ml of 0.05 M pyrogallol was added. The content was transferred to cuvette and read as blank in UV-spectrometer @ 430 nm. A 0.5 ml of H_2O_2 was added as substrate and changes were recorded for 2 min at every 30 sec intervals. The average of the differences (A) recorded in the different time interval readings was worked out and expressed as $min^{-1}g^{-1}$

$$\text{Peroxidase activity (units/mg protein/ min)} = \frac{A \times 60 \times 10 \times 1000}{1 \times 30 \times 500} \text{ min}^{-1}\text{g}^{-1}$$

RESULTS AND DISCUSSION

The data corresponding flowering, yield and disease incidence in chrysanthemum as influenced by application of bio-stimulants are presented in Table 1, Table 2 and Table 3.

A. Days to flower bud formation

Minimum number of days to flower bud formation was recorded with the application of *T. viride* @18 g per plant (T_3) (62.66 days) and it was statistically on par with *T. viride* @ 36 g per plant (T_4) (64.63 days). Whereas the maximum number of days to flower bud formation was recorded in the control plants (T_1) (72.66 days) (Table 1).

B. Days to first flowering

Application of *T. viride* @18 g per plant (T_3) recorded the minimum days to first flowering (82.20 days) which was on par with the application of *T. viride* @ 36 g per plant (T_4) (84.10 days). The maximum days to first flowering was recorded in the control plants (T_1) (95.66 days) (Table 1). *T. viride* exhibited the earliest flowering. It might have helped in uptake of micronutrients which are essential components of dehydrogenase, proteinase, peptidase and promotes growth hormones. All these factors contributed to cell multiplication, cell division and cell differentiation and that their effect on floral primordia might lead to early flowering. The results are in conformity with the finding of Roopa *et al*. (2018) in chrysanthemum, Shabnam (2017) in china aster, Nosir (2016) in tuberose, Srivastava *et al*. (2013) in tuberose, Sharma and Chandel (2013) in carnation.

C. Duration of flowering (days)

The longest duration of flowering was recorded in the plants that were treated with *T. viride* @18 g per plant (T_3) (54.55 days) and it was on par with the application of *T. viride* @ 36 g per plant (T_4) (52.91 days). The lowest duration of flowering was recorded in the non-treated plants (T_1) (46.91 days) (Table 1). This might be due to lower disease incidence percentage, percent disease index and optimum level of soil nutrients

(organic acid, phosp hates, micro nutrients and minerals) mobilized by root application of *T. viride* and taken up by the plants which led to the increased duration of flowering (Vinale *et al*., 2014).

D. Shelf life (days)

The maximum shelf life of the florets recorded was 5.10 days which was observed in the plants that were treated with Arbuscular mycorrhizal fungi 18 g per plant (T_9) and it was on par with the application of Arbuscular mycorrhizal fungi 36 g per plant (T_{10}) (4.99 days) whereas the treatment control (T_1) recorded the minimum shelf life of florets (3.89 days). Maximum shelf life was recorded with the application of AM fungi (Table 1). It might be due to increased flower stalk length and overall food status of flowers due to increased nutrient uptake by mycorrhiza associated plants and greater development of water conducting tissue which influences flower longevity. These results are in agreement with the findings of Saini *et al.* (2019) in chrysanthemum, Bellubbi *et al*. (2015) in gerbera.

E. Capitulum diameter (cm)

The diameter of the capitulum was recorded highest with the application of Arbuscular Mycorrhizal Fungi 18 g per plant (T_9) (6.21 cm) which was on par with the application of Arbuscular Mycorrhizal Fungi @ 36 g per plant (T_{10}) (6.15 cm) while the lowest diameter of capitulum was recorded with the control plants which were not treated with bio-stimulants (T_1) (4.83 cm) (Table 2). AM fungi significantly influenced the capitulum diameter might be due to it increased uptake of phosphorus it transfers the energy from one part to another and increases the overall food status of the flower hence resulted in higher diameter of flowers. Results were in accordance with the findings of Abdel *et al*. (2018) in damask rose, Dhiraj Kumar *et al*. (2009) in marigold and Renukaradya (2005) in carnation

F. Number of pickings

Application of Arbuscular Mycorrhizal Fungi @ 18 g per plant (T_9) recorded the maximum number of pickings (10.12) which was on par with the application of Arbuscular Mycorrhizal Fungi @ 36 g per plant (T_{10}) (10.00). The minimum number of pickings was recorded in the control plants which were not treated with bio-stimulants (T_1) (8.33) (Table 2).

G. Flower yield per plot (kg)

Application of Arbuscular Mycorrhizal Fungi @ 18 g per plant (T_9) recorded the maximum flower yield per plot (14.14 kg) which was on par with the application of Arbuscular Mycorrhizal Fungi @ 36 g per plant (T_{10}) (13.52 kg). The minimum flower yield per plot was recorded in the control plants which were not treated with bio-stimulants (T_1) (9.24 kg) (Table 2). The reason for such efficient photosynthetic assimilation by leaves might be because of greater leaf area and chlorophyll content as recorded by AM treated plants. Superiority of this treatment in terms of increase in number of branches and total plant bio-mass, might had resulted in corresponding enhancement with respect to number of leaves and total leaf area per plant; which in turn was responsible for carbohydrate production and greater nutrient uptake. In view of merits in these parameters, number of flowers per plant, mean flower weight and number of pickings could have increased, ultimately leading to greater flower yield in AM treated plants as compared to control Asrar and Elhindi (2011) in marigold

H. Disease incidence (%)

Application of *T. viride* @18 g per plant (T_3) recorded the minimum disease incidence (18.00%) which was on par with the application of *T. viride* @ 36 g per plant (T_4) (18.76 %). The maximum disease incidence was recorded in the control plants which were not treated with bio-stimulants (T_1) (48.75%) (Table 3). The results confirmed that the disease incidence (%) and per cent disease index were minimum by using the bio-stimulants. Among them, *Trichoderma viride* had the advantage of fast growth and vigorous vitality and occupied the growing space quickly absorbing the required nutrients. At the same time, it secrete cell wall degrading enzymes like chitinases, cellulases, xylanases, glucanases and proteinases, which can degrade microbial cells in the rhizosphere thus changing the structure of microbial community (Zhang *et al*., 2015).

I. Catalase activity (units/mg protein/min)

The catalase activity was recorded maximum with the application of *T. viride* 18 g per plant (T_3) (0.85 units/mg protein/min) on par with *T. viride* 36 g per plant (T_4) (0.83 units/mg protein/min). Whereas the minimum activity of catalase was recorded with the non-treated plants (T_1) (0.53 units/mg protein/min) (Table 3).

J. Peroxidase activity (units/mg protein/min)

T. viride 18 g per plant (T_3) was recorded highest peroxidase activity (0.99 units/mg protein/min) on par with *T. viride* 36 g per plant (T_4) (0.96 units/mg protein/min). The lowest activity of catalase was recorded in the control plants (T_1) (0.49 units/mg protein/min) (Table 3).

T. viride could improve defense enzymes in plants (Gajera *et al.*, 2016). Thus, effective activation of these enzymes enhanced stressed plant tolerance, prevented damages caused by abiotic and biotic stresses. Plants had advantage of the antioxidative enzyme defense systems such as catalase and peroxidase activities to protect themselves from adverse effects of ROS by detoxify H_2O_2 to H_2O and O_2 (Apel and Hirt 2004).

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

Treatments	Days to flower bud formation	Days to first flowering	Duration of flowering (days)	Shelf life (days)
T_1	72.66	95.66	46.91	3.89
T_2	66.33	85.97	51.56	4.66
T_3	62.66	82.20	54.55	4.89
T_4	64.63	84.10	52.91	4.76
T_5	69.23	89.23	47.43	4.43
T_6	66.00	87.25	49.42	4.64
T_7	68.60	88.23	47.24	4.59
T_8	66.46	85.33	47.75	4.87
T_9	65.97	84.99	51.30	5.10
T_{10}	66.26	85.00	50.99	4.99
Mean	66.98	86.79	50.01	4.68
S.Em(±)	0.66	0.93	0.66	0.09
CD (5%)	1.97	2.76	1.97	0.25

Table 2: Flower yield parameters of chrysanthemum as influenced by different bio-stimulants.

Treatments	Capitulum diameter (cm)	Number of pickings	Flower yield per plot (kg)
T_1	4.83	8.33	9.24
T_2	5.95	9.33	12.04
T_3	6.11	9.78	13.41
T_4	5.86	9.66	12.37
T_5	5.79	8.66	11.49
T_6	5.91	9.54	12.47
T_7	5.83	9.43	12.05
T_8	5.69	9.33	13.12
T_9	6.21	10.12	14.14
T_{10}	6.15	10.00	13.52
Mean	5.83	9.42	12.38
S.Em(±)	0.10	0.14	0.34
CD (5%)	0.29	0.42	1.02

Table 3: Wilt incidence and induction of defense enzymes as influenced by different bio-stimulants.

Treatments	Disease Incidence (%)	Catalase activity (units/mg protein/min)	Peroxidase activity (units/mg protein/min)
T_1	48.75	0.53	0.49
T_2	20.00	0.67	0.75
T_3	18.00	0.85	0.99
T_4	18.76	0.83	0.96
T_5	21.87	0.62	0.76
T_6	19.37	0.67	0.86
T_7	20.09	0.64	0.82
T_8	23.06	0.61	0.64
T_9	21.50	0.66	0.71
T_{10}	22.15	0.64	0.68
Mean	23.35	0.67	0.76
S.Em(±)	3.02	0.02	0.03
CD (5%)	8.74	0.07	0.10

CONCLUSIONS

Application of *Trichoderma viride* @ 18 g per plant was found to be best for early flowering, duration of flowering, induction of catalase and peroxidase activity and reduce wilt incidence. Arbuscular Mycorrhizal Fungi @ 18 g per plant was the best treatment with respect to shelf life of flowers, capitulum diameter, higher number of flower pickings and highest flower yield per plot.

FUTURE SCOPE

Effect of bio-stimulants among different seasons or months of application in chrysanthemum for maximising their effectiveness under local conditions. Efficiency of bio stimulants may be analysed under different incubation methods. Combination of bio-stimulants can be tested for their effectiveness in flower crops of local region.

Acknowledgement. The authors are thankful to ICAR-DFR Regional Station and Dr. YSRHU for their constant support and guidance for conduct of the experiment. I hereby take the opportunity to tell my heartfelt thanks to my family who stood by me through my research.
Conflict of Interest. None.

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How to cite this article: Sivalakshmi B., Madhavan S., Sirisha T., Sai Mohan A., Raju D.V.S., Dorajee Rao A.V.D. and Ravindra Kumar K. (2023). Influence of Bio-Stimulants on Flowering, Yield and Disease Incidence in Chrysanthemum (*Dendranthema grandiflora* T.) cv. Local Yellow*Biological Forum – An International Journal, 15*(10): 1375-1379.