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Efficacy of bio-control agent on growth and flowering in chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Marigold

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Abstract

Present investigation was carried out to examine the efficacy of different bio-agents on vegetative and flowering parameters of chrysanthemum at Department of Floriculture and Landscaping, College of Horticulture and Forestry, Jhalawar. For this formulations of different bio-agents like *Pseudomonas fluorescens* (125g and 175g and 250g/plot area), *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma longibrachiatum*, *Bacillus subtilis* used as root application. *Pseudomonas fluorescens* (250g) treatment was found effective and significantly affected growth and flowering parameters. Plant treated with 250g *Pseudomonas fluorescens* had maximum plant height (35.27 cm), number of leaves per plant (161.20), leaf length (9.80 cm), leaf width (7.44 cm), main stem diameter (1.22 cm), number of primary branches per plant (7.83) plant fresh weight (281.65 g), plant dry weight (87.67 g). While, flower diameter (7.34 cm), fresh weight of flower (31.56 g), dry weight of flower (4.06 g), *In-situ* life of flower (19.80 days) were recorded in *Trichoderma viride* 250g/plot area.

Keywords: *Pseudomonas fluorescens*, *Trichoderma viride*, growth, chrysanthemum, bio-agent

Introduction

Chrysanthemum also called as mums or chrysanth is flowering plant belongs family Asteraceae. They have native to East Asia and Northeastern Europe. Most species originated from East Asia and center of diversity is in China. About 160 species of this Chrysanthemum exist in the world. Mostly annual chrysanthemum in commercial cultivation in India. In India chrysanthemum ranks second in area and production among the loose flowers. The major chrysanthemum growing states are Tamil Nadu, Karnataka, Andhra Pradesh, Madhya Pradesh, Himachal Pradesh, West Bengal, Maharashtra, Assam, Jammu & Kashmir and Telangana etc. In India, the present area under chrysanthemum cultivation is 25.76 lakh ha. with a production of cut flower 14.85 lakh and loose flower 464.41 MT (Anonymous, 2017-18) [2]. In Rajasthan region, chrysanthemum is mostly growing in winter season. The loose flowers are mainly used in social and religious ceremonies. Flowers are frequently used as cut flowers for bouquets, vases and flower arrangements for interior decorations and as loose flowers for garlands, gajra, rangoli and veni making along with uses in worshipping, flower showers in receptions, wedding etc. However, the growers are unaware of suitable bio-control agents for their location and selecting based on fellow farmer recommendation. The bio-control agent (BCAs) can inhibit the growth of soil borne pathogens through various bio-control mechanisms such as ability to grow much faster than them for space and nutrients, producing many powerful plant degrading enzymes such as lytic enzymes, proteolytic enzymes and more than 200 types of antibiotics which are highly toxic to any macro- and microorganism. The ability to produce multiple antibiotics probably helps to suppress diverse microbial competitors, some of which are likely to be plant pathogens and thus enhance biological control. The *Pseudomonas fluorescens* belong to Plant Growth Promoting Rhizobacteria (PGPR), the group of bacteria that play a major role in plant growth promotion, induced systemic resistance, biological control of pathogens etc. *Trichoderma* species is considered as promising biological control agents against numerous phytopathogenic fungi since it is able to inhibit the phytopathogenic fungi either by including resistance and plant defense reaction or by direct confrontation

through mycoparasitism and competition or by producing antibiotics.

Materials and Methods

The present investigation was carried out during winter season of the year 2019-20 in the college of Horticulture and Forestry, Jhalawar. The district is situated between 23°45'20" and 24°52'17" North latitudes and 75°27'35" and 76°56'46" East longitudes at 317 meter above mean sea level. The experimental field was laid out in Randomized Block Design with three replications. The materials utilized for the present study consisted of sixteen different doses of different bio-control agents. In field trials with Sixteen treatments viz. T₀ (Control), T₁ (*Pseudomonas fluorescens* 125g/ plot area), T₂ (*Pseudomonas fluorescens* 175g/ plot area), T₃ (*Pseudomonas fluorescens* 250g/ plot area), T₄ (*Trichoderma harzianum* 125g/ plot area), T₅ (*Trichoderma harzianum* 175g/ plot area), T₆ (*Trichoderma harzianum* 250g/ plot area), T₇ (*Trichoderma viride* 125g/ plot area), T₈ (*Trichoderma viride* 175g/ plot area), T₉ (*Trichoderma viride* 250g/ plot area), T₁₀ (*Trichoderma longibrachiatum* 125g/ plot area), T₁₁ (*Trichoderma longibrachiatum* 175g/ plot area), T₁₂ (*Trichoderma longibrachiatum* 250g/ plot area), T₁₃ (*Bacillus subtilis* 125g/ plot area), T₁₄ (*Bacillus subtilis* 175g/ plot area), T₁₅ (*Bacillus subtilis* 250g/ plot area). The rooted cuttings of chrysanthemum were procured from Department of Floriculture and Landscaping, College of Horticulture, Arabhavi (Karnataka). One month old, healthy, vigorous and uniform seedlings were selected and transplanted during June-July. All treatments were replicated thrice with a plot size of 2.4 m x 2.4 m for each treatment and replication and each plot consisted of 25 plants and they were transplanted at a spacing of 40 x 40 cm. Five plants were selected at random and tagged in each treatment, for the purpose of recording observations on various parameters of growth and flowering parameters. The mean value of the data observed was taken to represent a particular variety with respect to character. The growth and flowering characters viz. plant height, number of leaves per plants, leaf length, leaf width, main stem diameter, number of primary branches, plant fresh weight, plant dry weight, flower diameter, fresh weight of flower, dry weight of flower and *In-situ* life of flower were recorded at first harvesting stage.

Result and Discussion

Data with respect to plant height, number of leaves per plants, leaf length, leaf width, main stem diameter, number of primary branches, plant fresh weight, plant dry weight, flower diameter, fresh weight of flower, dry weight of flower and *In-situ* life of flower in different treatments of chrysanthemum are presented in Table 1, Table 2 and Table 3.

Vegetative parameters

Plant height (cm)

The results revealed (Table .1) that there were highly significantly differences among the treatments of plant height.

The maximum plant height recorded (35.27 cm) in treatment T₃ (*Pseudomonas fluorescens* 250 g per plot area), while the minimum plant height (28.10 cm) was recorded in T₀ (control) at 120 DAT (Figure 1.a). This may be due to use of bio-control agent *Pseudomonas fluorescens* also worked as phosphate solvent, produced IAA, ACC-deaminase enzyme, and siderophore (Alishahi *et al.*, 2013). It might also due to soil application of *Pseudomonas fluorescens* that controlled the *F. oxysporum* f. sp. *ciceri*. Growth and increased the vegetative growth. (Rangeswaren *et al.*, 2000) [20]. The results are in conformity with the finding of Jagtap (2002) [12] in chilli seedlings and Muthukumar *et al.* (2005) [15] in tuberose.

Number of leaves per plant

The results revealed in (Table .1) that there were highly significantly differences among the treatments of number of leaves per plant. The maximum number of leaves per plant (161.20) was recorded in T₃ (*Pseudomonas fluorescens* 250 g per plot area), while the minimum (132.40) was recorded in T₀ (control) at 120 DAT (Figure 1.a). The more number of leaves may be due the plant promoting features of *Pseudomonas fluorescens*, which might have positively regulated nutrient uptake or resulted enhanced production of endogenous phytohormone (Jimtha John *et al.*, 2017 and Banchio *et al.*, 2008) [13, 4]. The results are in conformity with the finding of Singh *et al.* (2014) [28] chick pea and Dubey *et al.* (2008) [8] in gladiolus.

Leaf length (cm)

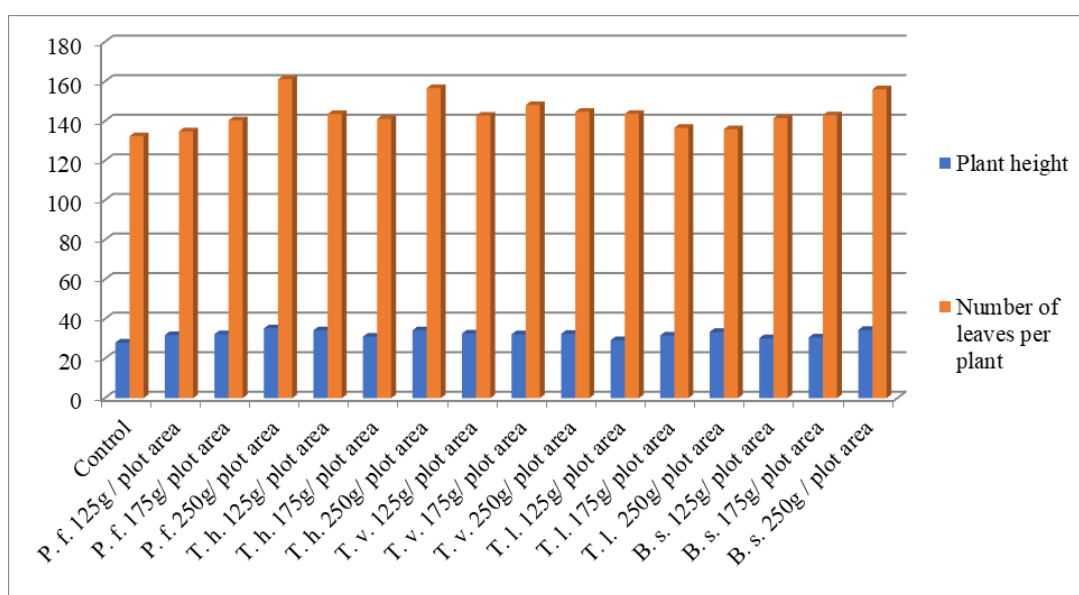
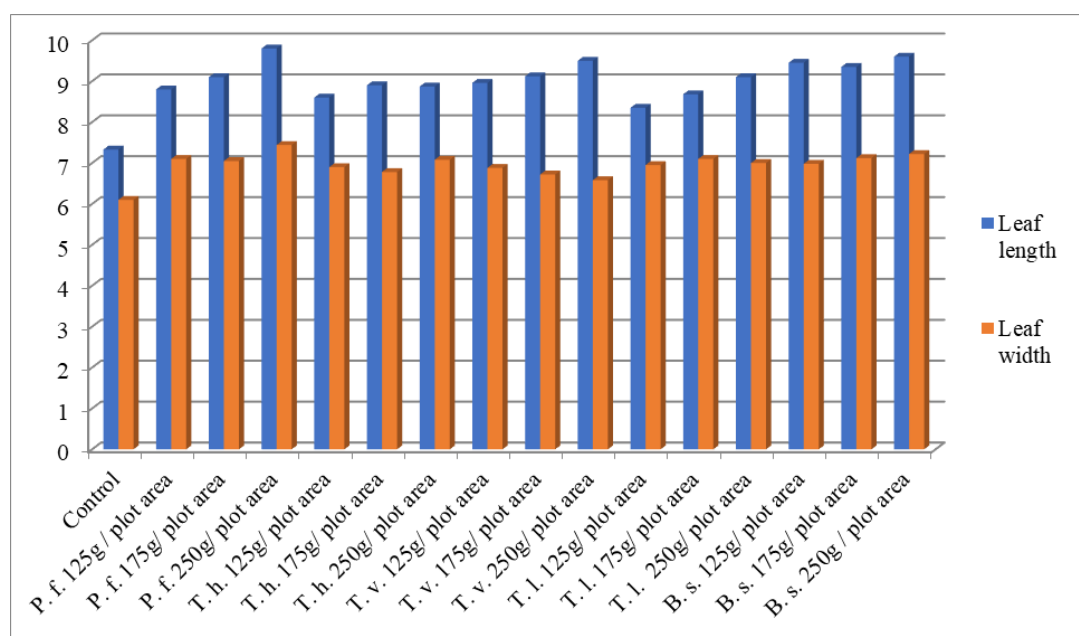
Highly significant difference were showed by the treatments in leaf length with the maximum leaf length (9.80 cm) recorded in T₃ (*Pseudomonas fluorescens* 250 g per plot area) and the minimum leaf length (7.33 cm) recorded in treatment T₀ (control) at 120 DAT (Table .1) (Figure 1.b). This may be due to use *Pseudomonas fluorescens*, with a possibility of enzymatic degradation of starch to fructose and glucose, provide more energy to the plants and stimulate plant leaf length (Kumar *et al.*, 2011) [14]. The results are in conformity with the finding of Dubey *et al.* (2008) [8] in gladiolus, Mohamed *et al.* (2000) [16] in gladiolus and Sharaf-Eladin *et al.* (2008) [26] in saffron.

Leaf width (cm)

The results elucidated in (Table .1) that there were highly significantly differences among the treatments of leaf width. The maximum leaf width (7.44 cm) was recorded in T₃ (*Pseudomonas fluorescens* 250 g per plot area). While, the minimum leaf width (6.10 cm) recorded in T₀ (control) at 120 DAT (Table .1) (Figure 1.b). This may be due to use *Pseudomonas fluorescens*, with a possibility of enzymatic degradation of starch to fructose and glucose, provide plants more energy and stimulate plant leaf length (Kumar *et al.*, 2011) [14]. The results are in conformity with the finding of Dubey *et al.* (2008) [8] in gladiolus, (Gore and Altin, 2006) in chrysanthemum and dahlia and Rodriguez-Romero *et al.* (2005) [21] in banana.

Table 1: Mean performance of different bio-control agents for vegetative parameters.

Treatments	Plants height (cm)	Number of leaves per plants	Leaf length (cm)	Leaf width (cm)
T ₀ (Control)	28.10	132.40	7.33	6.10
T ₁	31.89	134.87	8.80	7.10
T ₂	32.29	140.43	9.10	7.05
T ₃	35.27	161.20	9.80	7.44
T ₄	34.28	143.60	8.60	6.90
T ₅	31.09	141.17	8.90	6.78
T ₆	34.28	156.70	8.87	7.08
T ₇	32.69	142.80	8.96	6.88
T ₈	32.29	148.17	9.12	6.72
T ₉	32.49	144.73	9.50	6.58
T ₁₀	29.30	143.67	8.35	6.95
T ₁₁	31.69	136.67	8.68	7.10
T ₁₂	33.48	135.93	9.10	7.00
T ₁₃	30.30	141.47	9.45	6.98
T ₁₄	30.70	143.00	9.35	7.12
T ₁₅	34.48	156.13	9.60	7.22
S.Em (±)	0.19	5.48	0.11	0.11
CD (5%)	0.53	15.60	0.31	0.33

**Fig 1a:** Effect of different bio-control agents on plant height (cm) and number of leaves per plant of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Marigold.**Fig 1b:** Effect of different bio-control agents on leaf length (cm) and leaf width (cm) of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Marigold.

Main stem diameter (cm)

The result revealed that there were highly significant differences among the treatments with respect to main stem diameter. Treatment T₃ (*Pseudomonas fluorescens* 250 g per plot area) recorded the maximum main stem diameter (1.22 cm), while the minimum main stem diameter recorded (0.87 cm) in T₀ (control) at 120 DAT (Table .2) (Figure 2.a). This may be due to increase plant growth, hormonal level and auxin production in some parts of plants, resulting in cell elongation, cell division, consequently caused the increase in stem diameter (Rolfe *et al.*, 1997) ^[22]. The results are in conformity with the finding of Naserzadeh *et al.* (2018) in *Carthamus tinctorius* L.

Number of primary branches per plant

The number of primary branches per plant had significantly increased with root application of different bio-control agents. The T₃ treatment (*Pseudomonas fluorescens* 250 g per plot area) recorded the maximum number of branches per plant (7.83) and T₀ (control) recorded the minimum number of primary branches per plant (5.18) at 120 DAT (Table .2) (Figure 2.a). This may be bacterial genes of *Pseudomonas spp.* with deaminase-ACC enzyme resulted in increase in the number of branches (Cheng *et al.*, 2007) ^[7], also may be due to increased number of branches could result in increased plant spread. The plant growth pronounced in response to PGPR (*Pseudomonas fluorescens*) application which might be due to multiple direct and indirect mechanisms of actions like increasing nutrient availability, synthesis of phytohormones and suppression of harmful microbes in rhizosphere (Saharan and Nehra, 2011) ^[24]. This result was supported from previous work by Gooma *et al.* (2000) ^[9] in dahlia.

Plant fresh weight (g)

All treatments from data (Table .2) of root application of different doses of biocontrol agent increased the plant fresh weight as compare to control. The maximum plant fresh weight (281.64 g) was recorded in treatment T₃ (*Pseudomonas fluorescens* 250 g per plot area) and the minimum (146.83 g) in T₀ (control) at 120 DAT (Figure 2.b). This might be due to the ability of biofertilizers (*Pseudomonas fluorescens*) to produce growth promoting substance such as IAA and gibberellins like substances vitamins, riboflavin *etc.* which might have helped in increasing plant growth (Sukhda, 1999). The results are in conformity with the finding of Mohamed *et al.* (2000) ^[16] in gladiolus and Parsad *et al.* (2012) ^[18] in chrysanthemum and Hashemabadi *et al.* (2012) ^[11] in African marigold.

Plant dry weight (g)

The results indicated that there were highly significantly differences in plant dry weight among the treatments. The maximum (87.67 g) plant dry weight was recorded in T₃ treatment (*Pseudomonas fluorescens* 250 g per plot area). While, the minimum (51.73 g) plant dry weight observed in T₀ (control) at 120 DAT (Table .2) (Figure 2.b). Pathogenecity might cause little damage and this was also recorded in case of plant dry weight. The five bacteria were evaluated to determine their promoting effect on the plant dry weight, mature healthy plants (Gravel *et al.* 2007) ^[10]. This might be due to the ability of biofertilizers (*Pseudomonas fluorescens*) to produce growth promoting substance such as IAA and gibberellins like substances vitamins, riboflavin *etc.* which increases the plant growth (Sukhda, 1999). The results are in conformity with the finding of Mohamed *et al.* (2000) ^[16] in gladiolus, Parsad *et al.* (2012) ^[18] in chrysanthemum and Hashemabadi *et al.* (2012) ^[11] in African marigold.

Table 2: Mean performance of different bio-control agents for vegetative parameters.

Treatments	Main stem diameter (cm)	Number of primary branches per plant	Plant fresh weight (g)	Plant dry weight (g)
T ₀ (Control)	0.87	5.18	146.82	51.73
T ₁	1.03	6.76	214.65	65.17
T ₂	1.02	6.55	181.81	74.31
T ₃	1.22	7.83	281.64	87.67
T ₄	0.89	6.31	237.48	85.17
T ₅	0.96	6.95	181.31	76.00
T ₆	1.06	6.47	235.81	60.81
T ₇	0.96	6.27	210.98	67.17
T ₈	0.99	6.81	273.64	82.50
T ₉	0.91	7.00	277.81	79.48
T ₁₀	1.15	6.20	275.63	72.00
T ₁₁	1.10	6.89	168.65	57.17
T ₁₂	1.09	6.46	253.48	68.50
T ₁₃	0.97	6.68	158.00	56.67
T ₁₄	0.98	6.80	216.14	67.33
T ₁₅	1.00	6.98	244.31	53.83
S.Em (±)	0.01	0.11	1.80	0.86
CD (5%)	0.04	0.32	5.12	2.45

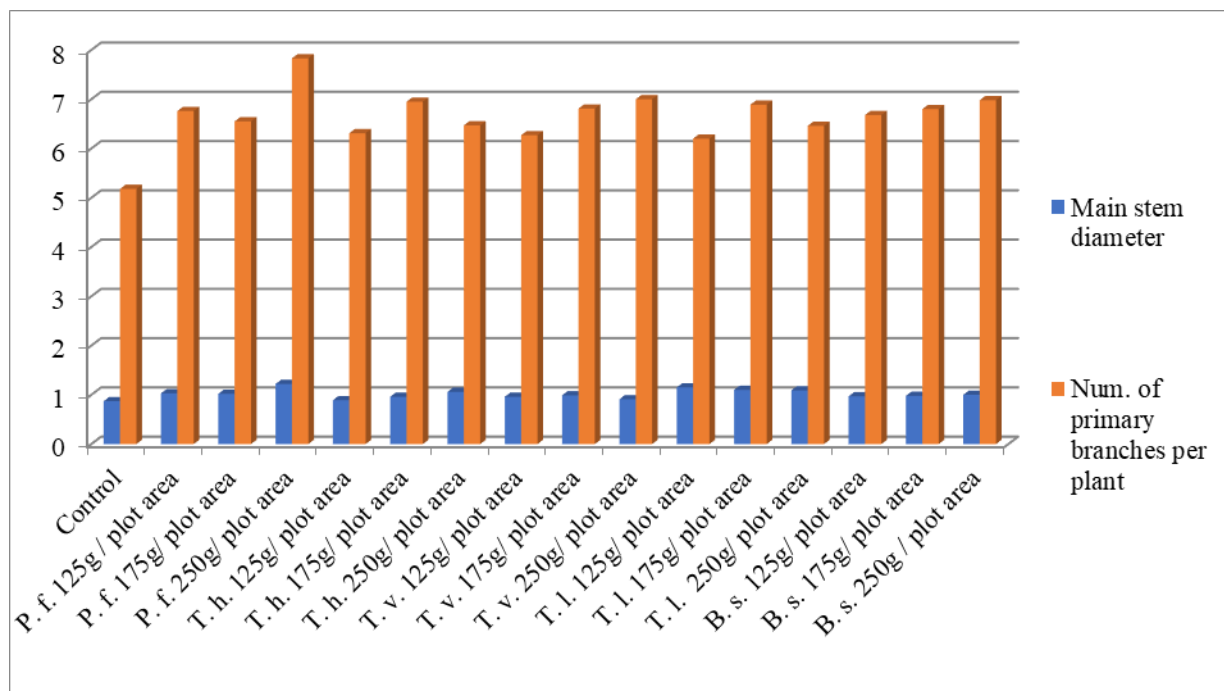


Fig 2a: Effect of different bio-control agents on main stem diameter (cm) and number of primary branches per plant of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Marigold.

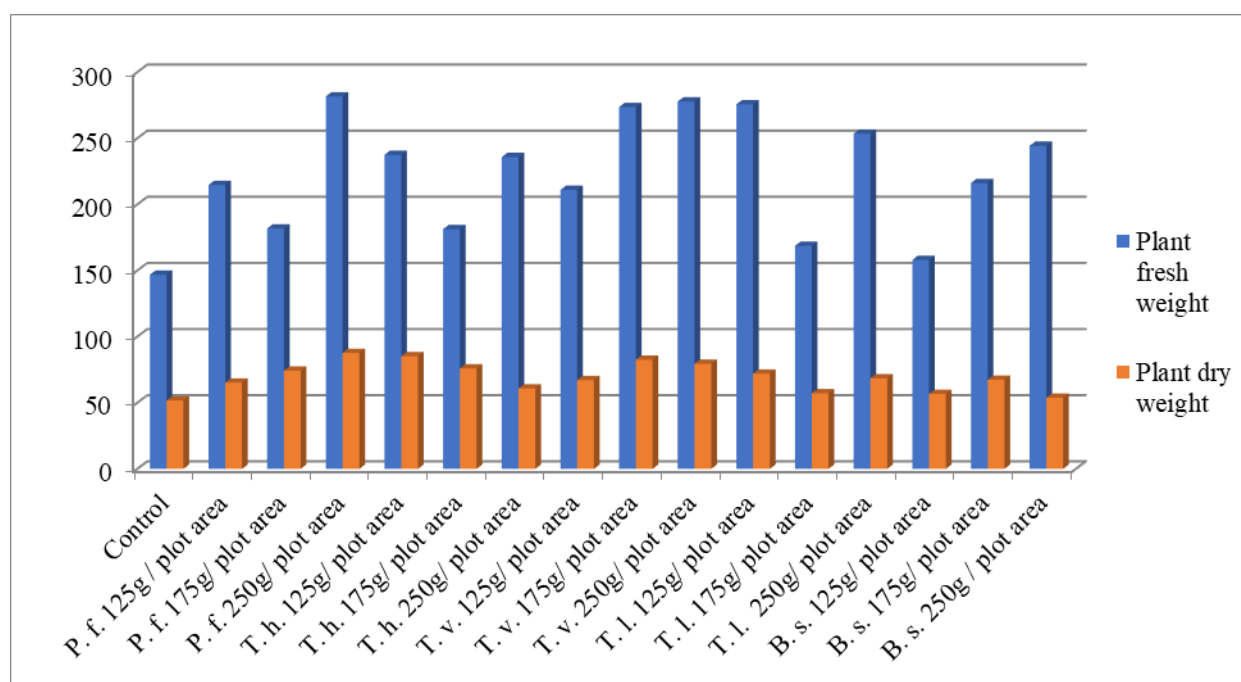


Fig 2b: Effect of different bio-control agents on main plant fresh weight (g) and plant dry weight (g) of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Marigold.

Flowering parameters

Flower diameter (cm)

The flower diameter had significantly influenced through different treatments application. The maximum flower diameter was (7.34 cm) reported in T₉ treatment (*Trichoderma viride* 250 g per plot area). While, the minimum flower diameter was reported in T₀ (control) (5.72 cm) (Table .3) (Figure 3.a). This may be due to *Trichoderma* spp. this access nutrients, increased solubility of insolubly compounds as well as increased availability of micronutrients by result of increased plant growth and flower diameter (Altmore *et al.* 1999 and Yedidia *et al.* 2001) ^[1, 32] The results are in conformity with the finding of Anusuya *et al.* (2003) ^[3]

in carnations, Sirin (2011) in lilium, Dubey *et al.* (2008) ^[8] in gladiolus and (Sharma and Chandel, 2013) in carnation.

Fresh flower weight (g)

The maximum fresh flower weight of flower (31.56 g) was recorded in T₉ treatment (*Trichoderma viride* 250 g per plot area) while, the minimum fresh flower weight (5.72 cm) was recorded in T₀ (control) (Table .3) (Figure 3.a). This might be due to direct relation with the flower diameter and number of ray florets, smaller the flower size might reduce the weight of fresh flower (Saniewski *et al.*, 2014). The difference in fresh weight of flower might affected due to the flower weight in marigold. The results are in conformity with the finding of Preethi, D.M. *et al.* (2016) ^[19] in tuberose, Cardenas *et al.*

(2007) ^[5] in marigold and Chang *et al.* (1986) ^[6] in chrysanthemum.

Dry flower weight (g)

It is evident from the results that all the treatments of bio-control had significantly improved the dry weight as compared to control. The maximum dry flower weight (4.06 g) was recorded in treatment T₉ (*Trichoderma viride* 250 g per plot area) and the minimum (2.61 g) in control (Table .3) (Figure 3.b). This might be due to direct relation with the flower diameter, smaller the flower size might reduce the weight of fresh flower (Saniewski *et al.*, 2014). This result was supported from previous work by Mohamed *et al.* (2000) ^[16] in gladiolus and Gooma *et al.* (2000) ^[9] in dahlia

In-situ life of flower (days)

The performances of the *in-situ* life of flower that highly significantly differences among the treatments. The maximum *in-situ* life of flower (19.80 days) was recorded in T₉ treatment (*Trichoderma viride* 250 g per plot area). While, the minimum *in-situ* life of flower (14.00 days) was recorded in T₀ (control) (Table .3) (Figure 3.b). The might be due to *Trichoderma* has a strong capacity to mobilize and take up soil nutrients and increase plant life, flower showed very little shrinkage and showed delayed senescence and increasing the vase life of chrysanthemum in terms of days (Saini *et al.*, 2018) ^[25]. The similar results were also obtained by Srivastava *et al.* (2013) ^[30] in tuberose and Roopa *et al.* (2018) ^[23] in chrysanthemum.

Table 3: Mean performance of different bio-control agents for flowering parameters.

Treatments	Flower diameter (cm)	Fresh flower weight (g)	Dry flower weight (g)	In-situ life flower (days)
T ₀ (Control)	5.72	23.69	2.61	14.00
T ₁	6.20	27.64	3.38	15.30
T ₂	6.27	24.11	3.69	16.80
T ₃	6.38	29.18	3.48	17.20
T ₄	6.38	28.14	3.46	14.58
T ₅	6.31	29.46	3.18	16.60
T ₆	6.36	26.28	3.28	17.92
T ₇	6.54	27.79	3.19	18.00
T ₈	6.38	27.99	3.45	18.56
T ₉	7.34	31.56	4.06	19.80
T ₁₀	6.31	27.80	3.69	14.80
T ₁₁	6.46	27.98	3.48	15.64
T ₁₂	6.40	26.01	2.87	17.30
T ₁₃	6.22	27.60	3.19	15.00
T ₁₄	6.41	29.04	3.46	16.68
T ₁₅	6.07	28.21	3.69	17.95
S.Em (±)	0.21	0.81	0.10	0.23
CD (5%)	0.60	2.31	0.28	0.65

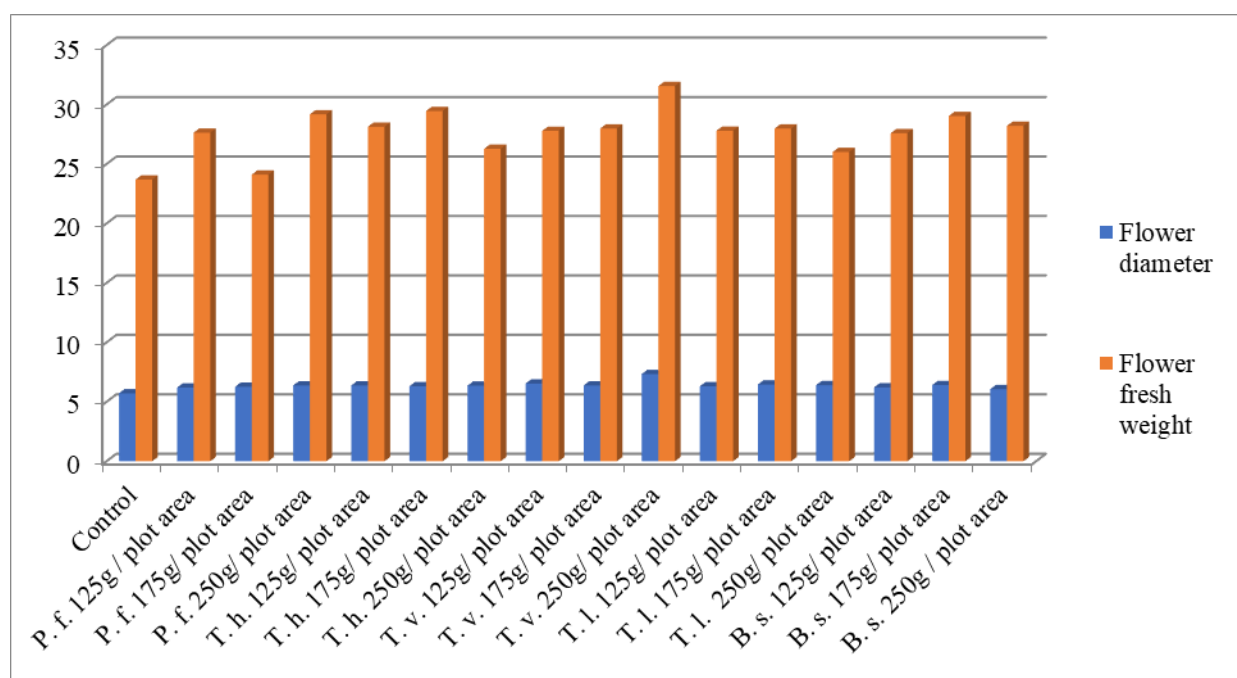


Fig 3a: Effect of different bio-control agents on flower diameter (cm) and flower fresh weight (g) of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Marigold.

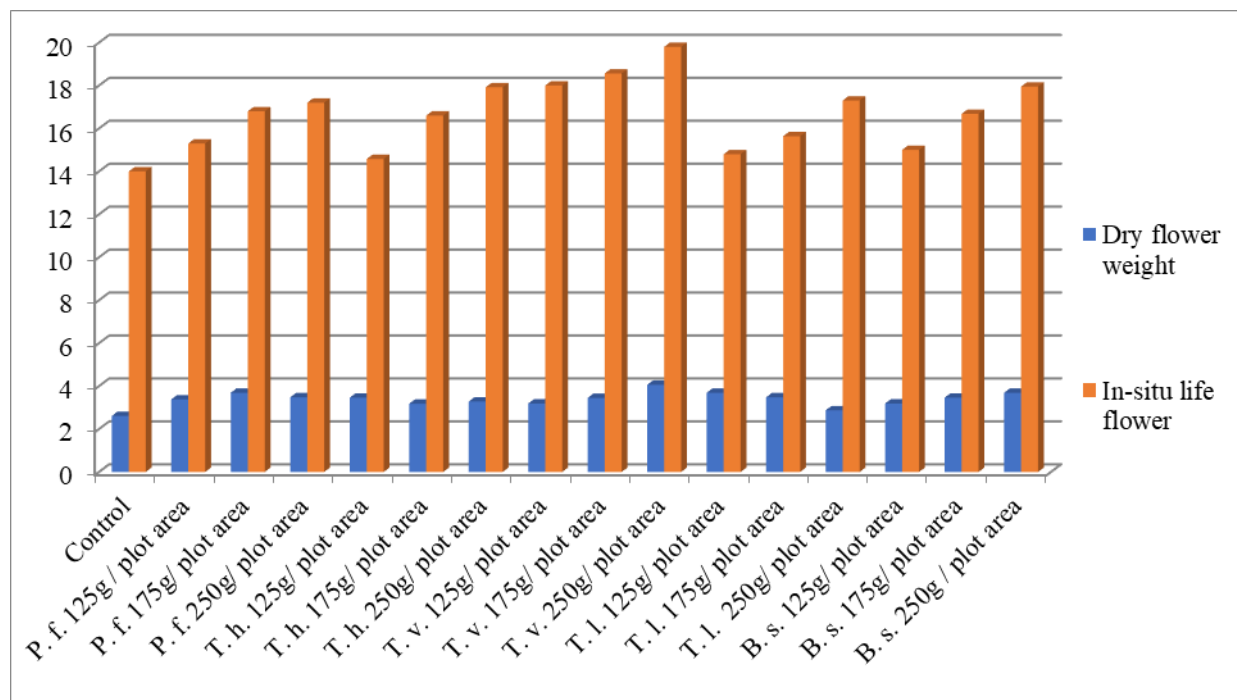


Fig 3b: Effect of different bio-control agents on dry flower weight (g) and In-situ life flower of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Marigold.

Conclusion

Therefore, based on the present investigation, it may be concluded that the treatments T₃ was found better in vegetative parameters viz. maximum plant height (35.27 cm), number of leaves per plant (161.20), leaf length (9.80 cm), leaf width (7.44 cm), main stem diameter (1.22 cm), number of primary branches per plant (7.83) plant fresh weight (281.65 g), plant dry weight (87.67 g) and treatments T₉ was found better in flowering parameters viz. flower diameter (7.34 cm), fresh weight of flower (31.56 g), dry weight of flower (4.06 g) and *In-situ* life of flower (19.80 days) compare than all other treatments.

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