

EFFECT OF BIOINOCULANTS ON GROWTH, FLOWERING AND YIELD OF MARIGOLD (*Tagetes erecta* L.)

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ABSTRACT

Excessive use of inorganic fertilizers degrades the soil, environment, quality of the produce, and adversely affects living beings. The experiment was planned to minimize the cultivation cost by use of bio-inoculants in marigold; which was conducted at CCS Haryana Agricultural University, Regional Research Station, Bawal (Rewari). The marigold seeds were sown in the nursery beds every year during the first week of October. About 25 days after sowing (DAS), seedlings were transferred to polybags, filled with field soil + farmyard manure (FYM) in 1:1 ratio. Just after transplanting, the seedlings were drenched with 1.0 ml of bioinoculants per plant with the concentration of 1×10^8 c.f.u./ml. The observations on growth parameters were recorded 70 days after transplanting (DAT). On the basis of three years average data, maximum plant height (47.0 cm), plant spread (18.8 cm) and stem diameter (7.4 mm) were observed in HT-54 strain. The flowers were initiated earlier in SD-25 (57.7 days), this value was at par with HT-54, SD-97 and SD-99 treatments. However, maximum days taken to initiate the flowers (60.2 days) were recorded in control. Maximum biomass, shoot dry weight (6.60 g) and root dry weight (1.60 g) were also observed in HT-54. Total biomass was also recorded highest (8.20 g) in HT-54. Overall, basis, *Azotobacter chroococcum*, HT-54 was adjudged as the best, followed by *Pseudomonas*, P-36.

Keywords : Bioinoculants, Biomass, Flowering, Growth, Marigold (*Tagetes erecta* L.)

Marigold (*Tagetes erecta* L.) is one of the important Ornamental crops for beautification and is being used as a loose flower for commercial purpose. It produces large-sized attractive blooms which are common in the Indian household for decoration during religious and social functions. The excellent keeping quality of flowers and attractive colour make them popular. It can be grown on a wide range of soils and climatic conditions; however, deep, well-drained fertile soils near to neutral in reaction with good water holding capacity are better for its growing. Being a day neural plant, it can be grown throughout the year but the temperature between 14.5 to 28.6°C is better for flowering while the yield of the flowers is affected adversely when the temperature rises above 28.6°C. This plant can be grown in between/ under the canopy of nematode infested fruit plants to repel the nematode away from the root zone (Kumar, 2002).

The flowering crops are chosen for the aesthetic and beautification purpose but the continuous use of inorganic/ chemical fertilizers affects its productivity and soil health. They also diminish the quality of the produce. The application or inoculation with microbes will be helpful in reducing the load of non-renewable energy resources. Microbial inoculation in marigold

may increase the yield/ size of flowers and shelf life of the flowers. Biofertilizers are cost-effective renewable energy source and play a crucial role in reducing the inorganic fertilizer application along with maintenance of soil fertility. Biofertilizers enrich soil environment by enhancing nutritional status, fixation of nitrogen, solubilization of phosphate and mineralization of other elements, release of plant growth-promoting substances (PGPS), biodegradation of soil organic matter and enhancement of organic carbon (Sinha *et al.*, 2014); reducing the infection of diseases and insect pests, improving resistance against adverse environmental stress and pollutants (Pathak *et al.*, 2017). Multiplication of microorganisms is also involved in nutrient cycling increased crop productivity (Singh *et al.*, 2011). Different bioinoculants have been tested in various field crops like cereals, oilseeds and coarse grain crops. But their impact on floriculture, particularly marigold has not been studied in details. Keeping in view, the commercial importance of the crop, ecofriendly nature of bioinoculants restricting the nematode infection in orchard, the present investigations have been undertaken. Further, marigold cultivation can be one of the alternatives in doubling farmers' income; biofertilization can also enhance the shelf life of flowers and compensate the chemical fertilizers.

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MATERIALS AND METHODS

The seeds of marigold cv. Pusa Narangi were sown in the raised beds nursery at CCS Haryana Agricultural University, Regional Research Station, Bawal (Rewari) every year during the first week of October. Seeds were sown by broadcasting on the raised bed and then covered with fine FYM. This nursery bed was further covered with grasses to maintain the moisture in the bed. When the seedlings attained a height of 12-15 cm i.e. 25 days after sowing i.e. during the last week of October, one seedling/ bag of equal-sized were transplanted in the polythene bags of 15cm x 25cm size, filled with field soil + farmyard manure (FYM) in 1 : 1 ratio,

Just after transplanting, the seedlings were inoculated with 1.0 ml of log phase (Approximately 1×10^8 c.f.u./ml) of each bioinoculant (SD-97, SD-99, SD-25, HT-54, P-36, CK-16, CK-15, CR-10) per plant and untreated polybag was kept as uninoculated control. Five replications were kept for each treatment following a completely randomized block design (CRD) described by Panse and Sukhatme (1985) for interpretation of the experimental results. The bioinoculants SD-97, SD-99 and SD-25 were PGPR isolates isolated from rhizosphere of *Dalbergia sissoo* and biochemical characterization showed them to be belonging to *Bacillus spp.*, HT-54 was the strain from *Azotobacter chroococcum* a high temperature tolerant isolate whose efficiency has been tested in different field crops; P-36 was phosphate solubilizing bacteria *Pseudomonas sp.* strain, while CK-15 and CK-16 were *Rhizobium sp.* cicer isolates, CR-10 isolate was isolated from castor (*Ricinus communis*) and PCR technique showed it as *Klebsiella sp.* having good plant growth-promoting substances.

The growth parameters such as plant height, stem girth and plant spread were observed at 70 days after transplanting. Plant height was measured from the ground level to the tip of the tallest shoot/flower with the help of meter rod. Plant spread, an average of East-West (EW) and North-South (NS) was observed with the help of measuring tape in all the replications and their average was calculated and expressed in centimeters. Stem diameter was measured 2.5 cm above the ground level with the help of digital vernier calliper for all the replications of each treatment and expressed in millimeter. Primary branches were counted on a regular basis with the naked eyes and sum of branches per plant were calculated. Flower initiation was also observed with the naked eyes when the flower bud shows yellowish emerging petal from the bud as the opening of a flower and the days taken to flowering were calculated from the day of transplanting to the emergence of a flower. Flowers per plant were

counted regularly and the total number of flowers were calculated by adding the number of flowers present on the plant with harvested flowers. Plants were irrigated before removal from polybag to avoid flouting of roots. The removed plants were washed for biomass under running tap to remove sand to take weight of root and shoots. The fresh weight of the harvested flowers was also added in the fresh weight of the shoots. The dry weight of root and shoot was taken after drying of fresh root and shoot in oven at $65 \pm 2^\circ\text{C}$, till the constant weight achieved.

All the parameters were observed in three replications. The data was analyzed using completely randomized block design (Panse and Sukhatme, 1985) for interpretation of the experimental results. In order to evaluate comparative performance of the various treatments, the data was analyzed by analysis of variance technique (Fisher, 1958). The data has been analysed using the statistical tool/ programme "opstat" CCS HAU, Hisar (Sheoran *et al.*, 1998). This tool is open for all and available on official website of CCS HAU, Hisar (www.hau.ac.in).

RESULTS AND DISCUSSION

The marigold seedlings were transferred to polythene bags filled with field soil + FYM in 1:1 ratio at 25 DAS and observations on growth parameters were recorded at 70 days of transplanting. The study on effect of different bioinoculants was conducted for three years. The mean data of three years observations was calculated and the results are presented on the basis of average of three years data.

Among the different inoculants on over all mean basis, maximum mean plant height (47.0 cm) was recorded in HT-54, however, lowest mean plant height (31.0 cm) was recorded in inoculation with CK-15 (Table 1). Stem diameter in respect to different treatments was observed as non-significant during 2017-18 and 2018-19. However, stem diameter during 2019-20 was recorded maximum (8.5 mm) in HT-54 and minimum (6.5 mm) in Control. The variation in mean stem diameter in relation to different bioinoculant treatments was also observed as non-significant (Table 1). The maximum average plant spread (EW and NS) was recorded (18.8 cm) in HT-54, being at par with P-36 (18.3 cm), however, lowest plant spread (15.7 cm) was recorded in control and SD-97 (Table 2). Average data of three years showed maximum number of primary branches (14.5) with HT-54 being at par with SD-25 and lowest primary branches (9.4) were recorded in control.

On the basis of average data, minimum days taken to initiation of flowering (57.7 days) were recorded in SD-25, being at par with SD-97, SD-99 and HT-54. However, the maximum days taken to initiation of

Table 1. Effect of different bioinoculants on plant height and stem diameter of marigold (*Tagetes erecta* L.)

Treatment	Plant height (cm)				Stem diameter (mm)			
	2017-18	2018-19	2019-20	Mean	2017-18	2018-19	2019-20	Mean
Control	37.3	35.4	33.6	35.4	6.3	5.2	6.5	6.0
SD-97	35.7	33.6	30.2	33.2	6.0	5.3	6.7	6.0
SD-99	40.6	36.2	33.4	36.7	6.7	6.0	7.7	6.8
SD-25	43.7	41.6	39.2	41.5	7.0	6.3	8.2	7.2
HT-54	48.7	46.3	46.1	47.0	7.3	6.5	8.5	7.4
P-36	44.3	42.5	41.3	42.7	6.3	5.8	8.3	6.8
CK-16	39.0	37.1	36.5	37.5	6.6	5.8	8.2	6.9
CK-15	30.3	32.3	30.5	31.0	7.7	6.1	7.7	7.2
CR-10	36.7	34.8	32.8	34.8	5.6	5.2	7.8	6.2
Mean	39.6	37.8	36.0	37.8	6.6	5.8	7.7	6.7
CD (P=0.05)	4.7	4.4	4.2	4.4	NS	NS	1.2	NS

flowering (60.2 days) were recorded in control (Table 3). Maximum number of flowers per plant were recorded highest (22.5) in HT-54 and lowest (14.0) in control. The fresh weight of shoot was recorded highest (57.8 g) in CK-16, being at par with P-36 and HT-54, however, lowest (33.8 g) in SD-99 (Table 4). The higher growth of the plant may have more fresh weight of shoot as well as the root. Higher fresh weight of shoot may be due to accumulation of food material and more uptake of the nutrients. On the average basis, maximum root fresh weight (11.3 g) was recorded in HT-54, followed by P-36 (11.1g), however, lowest fresh weight (7.7 g) was recorded in control/ uninoculated. The treatment which had more growth and more fresh weight of the plant/ shoot may have higher root weight.

Maximum average shoot dry weight (6.60 g) was recorded in HT-54 and lowest (4.08 g) in SD-99 (Table

5) and maximum root dry weight (1.60g) was also observed in HT-54 and minimum (0.95 g) in control. Plant biomass was recorded maximum (8.20 g) in HT-54 and minimum (5.04 g) in control / uninoculated (Table 6). Maximum biomass, shoot dry weight (6.60 g) and root dry weight (1.60 g) were also observed in HT -54. Total biomass was also recorded highest (8.20 g) in HT-54 and P-36 was adjudged as the second best treatment.

These bioinoculants were not tested earlier in marigold and any other crops, however, some other bioinoculants were studied and the results obtained with the application of different bioinoculants in different crops are discussed here. The inoculation of biofertilizers (*Azotobacter* and phosphate solubilizing bacteria, PSB) increased plant height and plant spread of marigold as compared to uninoculated/ control (Kumar, 2002).

Table 2. Effect of different bioinoculants on plant spread and number of primary branches of marigold (*Tagetes erecta* L.)

Treatment	Plant spread (cm)				No. of primary branches			
	2017-18	2018-19	2019-20	Mean	2017-18	2018-19	2019-20	Mean
Control	16.2	15.4	15.4	15.7	10.0	9.0	9.0	9.4
SD-97	16.0	15.2	15.8	15.7	10.3	9.3	9.4	9.7
SD-99	17.5	16.6	17.3	17.1	13.3	12.0	11.6	12.3
SD-25	18.0	17.1	17.8	17.6	16.3	13.5	12.6	14.2
HT-54	18.6	17.7	20.2	18.8	15.0	14.7	13.8	14.5
P-36	18.1	17.2	19.5	18.3	11.7	10.5	10.6	10.9
CK-16	17.1	16.3	17.8	17.1	14.0	12.7	10.2	12.3
CK-15	17.0	16.2	18.1	17.1	10.3	9.3	9.2	9.6
CR-10	17.4	16.5	16.3	16.8	13.7	12.3	8.2	11.4
Mean	17.3	16.5	17.6	17.1	12.7	11.5	10.5	11.6
CD (P=0.05)	1.2	1.2	1.1	1.2	3.0	2.8	1.2	2.3

Table 3. Effect of different bioinoculants on days taken to flower initiation and number of flowers per plant of marigold (*Tagetes erecta* L.)

Treatment	Days taken to flower initiation				No. of flowers per plant			
	2017-18	2018-19	2019-20	Mean	2017-18	2018-19	2019-20	Mean
Control	55.4	64.3	61.0	60.2	13.2	13.2	15.5	14.0
SD-97	53.5	59.3	62.0	58.3	13.6	14.2	16.5	14.8
SD-99	55.0	60.0	60.0	58.3	16.2	16.8	19.6	17.5
SD-25	57.2	59.0	57.0	57.7	18.8	19.6	22.7	20.4
HT-54	61.5	59.0	55.0	58.5	20.4	21.1	26.0	22.5
P-36	58.0	59.3	62.0	59.8	16.8	17.5	20.4	18.3
CK-16	55.7	60.7	63.0	59.8	15.6	16.2	19.0	16.9
CK-15	51.3	61.3	65.0	59.2	14.4	14.8	17.2	15.5
CR-10	54.5	62.1	62.0	59.5	13.8	14.4	14.9	14.4
Mean	55.8	60.6	60.8	59.0	15.87	16.4	19.1	17.1
CD (P=0.05)	2.1	2.0	1.8	2.0	0.65	1.9	1.3	1.3

More growth under biofertilizers might be due to better root system in bio inoculants treatments (Awasthi *et al.*, 1996b). The growth hormones synthesized by the biofertilizers interacts with mycorrhizal species increased the ability of absorption of nutrients (Awasthi *et al.*, 1996a). Inoculation of *Azotobacter* and phosphate solubilizing bacteria (PSB) increased the plant height and plant spread while there was no effect of *Azospirillum* treatment (Kumar *et al.*, 2006b).

Increased cell metabolism and enzymatic activities due to phosphobacteria enhance the absorption of nitrogen and its direct participation in protein synthesis (Subbiah and Ramanathan, 1982). Phosphobacteria influences the activities of a number of enzymes which led to increasing the cell metabolism and enzymatic activities (Kumar *et al.*, 2006a). The other possible reason is that the availability of phosphorus with the

inoculation of phosphobacteria increased the root biomass of the plant, which in turn increased the absorption of nutrients leads to the overall growth of the plant (Kumar *et al.*, 2014).

The number of days taken to initiation of the flower were recorded lower in biofertilizer inoculation treatments as compared to control (Kumar *et al.*, 2006b). The size of the flowers increased by 9.1 per cent while the yield increased by 51.5 per cent over control with the inoculation of PSB. The growth of marigold enhanced by bioinoculants may be due to their ability to produce growth-promoting substances such as IAA, gibberellin like substances, vit. B12, thiamine, riboflavin (B2) etc (Wange and Patil, 1994). Different biofertilizers such as *Azospirillum*, *Azotobacter* and PSB decreased days taken to first flower bud formation and days taken to first flower formation significantly, whereas, duration

Table 4. Effect of different bioinoculants on shoot fresh weight and root fresh weight of marigold (*Tagetes erecta* L.)

Treatment	Shoot fresh wt. (g)				Root fresh wt (g)			
	2017-18	2018-19	2019-20	Mean	2017-18	2018-19	2019-20	Mean
Control	30.6	39.2	41.4	37.1	8.0	7.7	7.4	7.7
SD-97	44.3	42.3	45.9	44.2	7.2	8.7	8.8	8.3
SD-99	30.3	28.9	42.3	33.8	7.5	9.1	8.3	8.3
SD-25	44.5	42.5	50.6	45.9	6.7	8.1	10.2	8.4
HT-54	55.5	53.9	59.8	56.4	9.9	12.0	11.8	11.3
P-36	56.4	53.0	56.3	55.2	9.9	12.0	11.3	11.1
CK-16	60.5	57.8	54.9	57.8	8.3	10.1	10.9	9.8
CK-15	20.5	48.3	49.2	39.3	13.8	11.7	9.9	11.8
<i>Klebsiella</i> CR-10	51.6	49.3	46.6	49.2	12.2	10.7	8.7	10.5
Mean	43.8	46.2	49.7	46.5	9.3	10.0	9.7	9.7
CD (P=0.05)	3.4	4.5	3.2	3.7	2.2	1.5	1.1	1.6

Table 5. Effect of different bioinoculants on shoot dry weight and root dry weight of marigold (*Tagetes erecta* L.)

Treatments	Shoot dry wt (g)				Root dry wt. (g)			
	2017-18	2018-19	2019-20	Mean	2017-18	2018-19	2019-20	Mean
Control	3.15	4.08	5.04	4.09	0.93	1.01	0.91	0.95
SD-97	4.57	5.87	5.46	5.30	0.76	1.11	1.12	1.00
SD-99	3.14	3.99	5.11	4.08	1.09	1.15	0.98	1.07
SD-25	4.46	6.06	5.74	5.42	0.66	1.06	1.47	1.06
HT-54	5.68	7.53	6.58	6.60	1.29	1.55	1.96	1.60
P-36	5.73	7.46	6.37	6.52	1.39	1.54	1.75	1.56
CK-16	6.41	7.80	5.46	6.56	0.82	1.24	1.54	1.20
CK-15	2.11	6.69	4.27	4.36	1.75	1.26	1.33	1.45
CR-10	5.43	6.70	4.13	5.42	1.50	1.23	1.05	1.26
Mean	4.52	6.24	5.35	5.37	1.13	1.24	1.34	1.24
CD (P=0.05)	0.80	0.85	0.21	0.62	0.31	0.32	0.56	0.40

Table 6. Effect of different bioinoculants on biomass of marigold (*Tagetes erecta* L.)

Treatment	Biomass (g)			
	2017-18	2018-19	2019-20	Mean
Control	4.08	5.09	5.95	5.04
SD-97	5.33	6.98	6.58	6.30
SD-99	4.23	5.14	6.09	5.15
SD-25	5.12	7.12	7.21	6.48
HT-54	6.97	9.08	8.54	8.20
P-36	7.12	9.00	8.12	8.08
CK-16	7.23	9.04	7.00	7.76
CK-15	3.86	7.95	5.60	5.80
CR-10	6.93	7.93	5.18	6.68
Mean	5.65	7.48	6.70	6.61
CD (P=0.05)	1.20	1.82	2.10	

of flowering increased in comparison to control/uninoculated (Awasthi *et al.*, 1996a).

The phosphobacteria enhance the cell division and cell enlargement besides the growth hormone synthesis (Narashma and Harirpiya, 2001). The better performance of flowering parameters might be due to the facts that the biofertilizers produce the growth-promoting substances and other acids like acetic, formic, propionic, lactic, glycolic, fumaric and succinic, which were positively correlated with growth and flowering (Wange and Patil, 1994).

In general, various plant growth parameters viz., plant height, stem girth, number of flowers per plant, minimum days to flower initiation and biomass are positively affected in marigold by the application of different bioinoculants. But the degree of impact varies with the nature of inoculants. Some other characters

like the shelf life of flowers or bioinoculants impact under field conditions need further investigations.

Authors' contribution

Conceptualization of research work and designing of experiments (MK, DVP); Execution of field/lab experiments and data collection (MK, DVP); Analysis of data and interpretation (MK, DVP); Preparation of manuscript (MK, DVP).

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