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Effect of macronutrients, microbial consortium and biostimulants on growth and yield of African marigold (*Tagetes serecta* L.)

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Abstract
The present study was undertaken to bring out the best combination of microbial consortium on effect of biostimulant for the growth and yield of African marigold (*Tagetes erecta* L.) hybrid L3. The experiment was conducted in open field condition in a randomized block design with 10 treatments combinations and replicated thrice at ICAR-KVK, Bagalkot during the year 2017. Application of bio-control agents *viz.*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis* were applied in different combinations through soil application (SA), seedling treatment (ST), and foliar spray/application (FA) and were evaluated for growth promotion and disease management in marigold. The bio-stimulant, vermiwash was applied as foliar spray at four intervals at 30, 45, 60 and 75 days after transplanting. Observations were recorded on marigold growth, flowering and yield parameters. The growth parameters *viz.,* plant height, number of primary branches per plant, plant spread, number of leaves per plant were recorded highest in treatment T8 compared to other treatments. Days taken for first flowering, days taken for 50% flowering, flower diameter, individual flower weight, flowering duration, number of flowers per plant, flower yield per plant and flower yield per hectare were also recorded highest in treatment T8 which received combined soil application of 50% recommended dose of fertilizers (63:30:30 Kg NPK ha^{-1}) + soil application of bioagents @ 3kg/acre followed by seedling dip and foliar spray of *Trichoderma harzianum* (10ml/litre) +*Pseudomonas fluorescens* (10ml/litre)+ *Bacillus subtilis* (10ml/litre) and vermiwash (0.5%) compared to control. The disease incidence (PDI) of leaf spot and flower blight was observed less in T8 whereas, leaf spot and flower blight was high in control. Hence, treatment T8 was best for growth, development and disease management through organic way in marigold.

Keywords: Bio-fertilizers, bio-control agents, bio-stimulants, marigold, microbial consortium, vermiwash

Introduction
Marigold, a member of the family Asteraceae or Compositae, is a potential commercial flower that is gaining popularity on account of its easy culture, wide adaptability, and increasing demand in the subcontinent [2]. Marigold is one of commercially exploited flower crop. Marigold is grown for cut flowers, garlands, decoration besides used in landscape gardening. It is also valued for extraction of aromatic oil and dried flower petals are used as poultry feed. In marigold the spacing has greater importance in manipulating growth, flowering behaviour and flower and yield. It is also a heavy feeder of nutrients, at present the nutrients are supplied through chemical fertilizers. Indiscriminate and continuous use of chemical fertilizer in intensive cropping system has led to an imbalance of nutrients in soil which has an adverse effect on soil health and also affecting seed yield. Commercial exploitation of the flowers for xanthophylls extraction has made this flower crop much more popular among the flower growers and industrialists. L3 hybrid of marigold is especially grown for extracting natural dye. To reduce the fertilizer cost to safeguard the soil health and to manage the diseases in marigold by organic way by using biocontrol agents this trial with african marigold was taken up using integrated nutrients such as macronutrients, bio control agents and bio stimulants with the objective of increasing the growth, yield and quality of african marigold hybrid L3 and to manage the blight disease organically.

Materials & Methods
The experiment was conducted in open field condition in a randomized block design with 10 treatments combinations and replicated thrice at ICAR-Krishi Vigyan Kendra, Bagalkot during the year 2017. The land was brought to a fine tilth by thorough tillage.

Irrigation channels and bunds were maintained properly. Twenty five days old healthy and uniform L 3 hybrid marigold seedlings were transplanted on 8th June 2017 in ridges and furrows with a spacing of 45 cm x 45 cm. Light irrigation was given after transplanting. Full dose of phosphorus and potassium and half dose of nitrogen as per treatment were applied one week after transplanting. The remaining half dose of nitrogen was supplied in one split doses at 30 days after transplanting. All treatments contain macronutrients *i.e.* 50% recommended dose of fertilizers (63:30:30 Kg NPK ha^{-1}), soil application of bio-control agents @ 3 kg/acre and biostimulants along with the control. *viz.*, *Trichoderma harzianum* (10ml/litre), *Pseudomonas fluorescens* (10ml/litre), *Bacillus subtilis* (10ml/litre)were applied in different combinations through seedling treatment (ST) and foliar spray/application (FA) and were evaluated for growth promotion and disease management in marigold. The bio- stimulant vermiwash (0.5%) was applied as foliar spray at four intervals at 30, 45, 60 and 75 days after transplanting. Observations were recorded on marigold growth, flowering and yield parameters. The growth parameters like plant height, number of primary branches per plant, leaf number per plant and flowering parameters like days to first bud initiation, days to first flowering, days to 50% flowering, number of flowers per plant, flowering duration, flower diameter, individual flower weight and flower yield per plant, flower yield per hectare were recorded from time to time. The observations on leaf spot and flower blight severity was recorded using 0-5 scale [15] for recording disease intensity on leaves and 0-4 scale [7] for disease severity on flowers. After the recording the disease severity on leaves and flowers, the per cent disease index (PDI) was calculated using the formula [26]. The data on these parameters were subjected to statistical analysis to draw logical conclusion.

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{Number of Leaves/flowers X Maximum disease ratings observed}} \text{ X } 100$$

Rating Scale	PDI on Leaves	PDI on Flowers
0	No disease symptom	Healthy (disease free flowers)
1	1 – 5% leaf area covered	up to 25% flower area covered by the disease
2	6 – 10% leaf area covered by the disease	26-50% flower area covered by the disease
3	11 - 25% leaf area covered by the spot	51-75% flower area covered by the disease
4	26 – 50% leaf area covered by the disease	Whole of flower area covered by the disease symptom
5	>50% leaf area covered by disease	

Results & Discussion

All the growth parameters were influenced significantly by application of different dose of macro nutrients, bio control agents and bio stimulants. The growth parameters *viz.*, plant height (104.93 cm), number of primary branches per plant (19.63), plant spread (35.73 cm^2), number of leaves per plant (425.10) were recorded highest in treatment T8 which received combined soil application of 50% recommended dose of fertilizers (63:30:30 Kg NPK ha^{-1}) and soil application of bioagents @ 3kg/acre + foliar spray of *Trichoderma harzianum* (10ml/litre) +*Pseudomonas fluorescens* (10ml/litre)+ *Bacillus subtilis* (10ml/litre) and foliar spray of vermiwash (0.5%) compared to control (Table 1).

Improvement in growth characters might be due to the pronounced effect of the treatments. Sufficient supply of plant nutrients might have shown stimulatory action in terms of cell elongation and thus resulting in increased plant height. Better availability of nutrients would have helped in protein synthesis resulting in production of taller plants with larger leaves and more number of branches [16]. The pronounced effects of these parameters are due to application of nitrogen and phosphorus [21]. Application of N significantly enhanced the plant growth parameters in african marigold [3]. Phosphorus is associated with phosphorylation and is constituent of energy rich compounds like ATP, ADP, NADH and NADPH. Potassium is an activator of many enzymes that are essential for photosynthesis and respiration, and it also activates enzymes needed to form starch and proteins. It also increases water uptake by plants which eventually increased the growth of the marigold plant. Similar experimental findings were also observed [25] in tuberose. *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* have also been known to promote plant growth by causing robust root growth, with some strains having been found to increase root growth to as much as a meter below the soil surface [8]. Therefore the improvement of plant nutritional status might be directly related to a general beneficial growth effect on the root system.

Table 1: Effect of macronutrients, microbial consortium and biostimulants on growth parameters of Marigold

	Treatments details	Plant height (cm)	No. of primary branches/plant	Plant spread (cm^2)	No. of leaves/plant
T1	Trichoderma+ 50% RDF	81.17	15.77	24.87	358.30
T2	Pseudomonas + 50% RDF	81.97	16.23	25.87	355.80
T3	Bacillus + 50% RDF	83.40	17.87	26.27	377.20
T4	Tri+Pseu+ 50% RDF	88.80	16.63	26.37	367.40
T5	Tri+Baci+ 50% RDF	91.90	17.17	29.27	379.30
T6	Pseu+Baci+ 50% RDF	98.67	18.13	34.90	416.20
T7	Vermiwash+ 50% RDF	95.83	17.73	33.47	407.20
T8	Tri + Pseu +Baci + Vermiwash+50% RDF	104.93	19.63	35.73	425.10
T9	50% RDF	81.60	11.37	26.80	291.30
T10	Absolute Control	72.60	11.07	22.73	276.30
	CD (P=0.05)	4.19	2.20	2.51	5.05

*Tri=Trichoderma, Pseu=Pseudomonas, Baci=Bacillus, RDF=Recommended Dose of Fertilizers

The yield parameters *viz*., Days taken for first flowering (57.87 days), days taken for 50% flowering (61.20 days), flower diameter (9.23 cm), individual flower weight (8.83g), flowering duration (49.73 days) were also recorded highest in treatment T8 which received combined soil application of 50% recommended dose of fertilizers (63:30:30 Kg NPK ha^{-1}) + *Trichoderma harzianum*(10ml/litre) +*Pseudomonas fluorescens* (10ml/litre)+ *Bacillus subtilis* (10ml/litre) and foliar spray of vermiwash (0.5%) compared to other treatments (Table 2).

The earliness of bud initiation in bioagents with vermiwash - inoculated plants may be ascribed to easy uptake of nutrients and simultaneous transport of growth promoting substances like cytokinin to the axillary buds, resulting in breakage of apical dominance. Ultimately, this has resulted in a better sink for faster mobilization of photosynthates and early transformation ofplant parts from vegetative to reproductive phase. The higher production of auxin and growth substances by vermiwash at early phase of growth would have contributed to early flowering. A positive significant effect was observed on the number of days taken for fifty percent flowering and number of flowers that remained open at a time due to the application of 0.5 per cent vermiwash spray. Plant-associated Pseudomonas live as saprophytes and parasites on plant surfaces and inside plant tissues. Many plant-associated Pseudomonas promote plant growth by suppressing pathogenic micro-organisms, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance. Plant hormones produced by Pseudomonas include auxin (indole acetic acid, IAA) and cytokinins, as well as volatile signals such as ethylene 2,3 butanediol and acetonin [11, 18]. Pseudomonas may also have indirect effects on hormones and signalling intermediates, for example by secreting cell wall degrading enzymes that release peptides and oligosaccharides that subsequently affect plant development and plant signal transduction [6]. Plant hormone synthesis may serve a similar but less disruptive role in PGPP, which have frequently been shown to stimulate root growth and proliferation [18]. Hormone producing bacteria may also benefit from IAA and cytokinin-stimulated release of saccharides and methanol from the plant cell wall as a local nutrient source, and from the effects of phytohormones on wound and defense signal transduction [12].

The earliness in 50% flowering may be due to continuous supply of nutrientsand PGPR activity from by the soil microbes and uninterrupted supply of water to the plant system would have transformed the vegetative phase to reproductive phase much earlier. The results are in line with the findings in jasmine [23], in garland chrysanthemum [1], in marigold [5] and in crossandra [15]. Increase in flower diameter and weight could be due to the increased photosynthetic activity which, in turn, might have favoured an increased accumulation of dry matter and also efficient partitioning of photosynthates towards the sink. It might be attributed to the nature of interaction of physiological and growth parameters by way of increased dry matter production which is in conform it with findings in marigold [17, 24, 9].

The higher flower yield is a manifestation of other yield contributing characters *viz.* number of flowers per plant, flowering duration, flower diameter and fresh weight of individual flower. Increases in yield contributing characters directly influence the crop yield. Number of flowers per plant (45.63), flower yield per plant (405.40g) and flower yield per hectare (17.6 t/ha) were also recorded highest in treatment T8 compared to control. Increased flower yield per plant and total yield of flowers per hectare is due to increase in available levels of nutrients by the combined effect of inorganic +biocontrol agents+ bio stimulant application. The increase in yield was due to the performance of overall crop growth and yield attributing characters due to better availability of soil moisture, environment and availability of plant nutrients throughout the crop growth period due to the beneficial soil microbes which mobilized the minerals from soil to root tips which is easily translocated into the plant system.

Table 2: Effect of macronutrients, microbial consortium and biostimulants on flowering and yield parameters of Marigold

	Treatments details	Days taken to 1st flowering (days)	Days taken to 50% flowering	Flower diameter (cm)	Individual flower weight (g)	flowering duration (days)	No. of flowers /plant	Flower yield/ plant (g)	Flower yield/ plot (kg)	Flower yield/ ha (t)
T1	Trichoderma+ 50% RDF	68.63	73.67	7.16	6.80	42.80	33.03	224.57	2.11	15.07
T2	Pseudomonas + 50% RDF	66.90	73.40	7.33	6.97	43.40	33.70	235.80	2.43	15.10
T3	Bacillus + 50% RDF	68.97	72.73	7.65	7.67	42.80	35.20	269.10	2.57	15.77
T4	Tri+Pseu+ 50% RDF	67.23	71.50	7.77	7.43	45.67	38.40	282.60	2.87	15.97
T5	Tri+Baci+ 50% RDF	66.43	70.67	8.07	7.17	43.30	38.10	274.93	3.13	16.13
T6	Pseu+Baci+ 50% RDF	61.47	67.10	8.73	8.37	48.63	42.43	356.50	3.40	16.87
T7	Vermiwash+ 50% RDF	59.20	63.97	8.50	8.23	47.57	41.47	339.73	2.87	16.57
T8	Tri+Pseu+Baci+Vermiwash+ 50% RDF	57.87	61.20	9.23	8.83	49.73	45.63	405.40	3.53	17.60
T9	50% RDF	69.37	75.50	7.00	6.10	40.60	28.73	171.40	1.93	14.57
T10	Absolute Control	72.27	79.27	6.80	5.97	36.43	25.63	152.87	1.30	12.70
	CD (P=0.05)	4.12	3.49	0.44	0.34	2.48	3.66	8.61	0.83	1.66

*Tri=Trichoderma, Pseu=Pseudomonas, Baci=Bacillus, RDF=Recommended Dose of Fertilizers

Table 3: Effect of macronutrients, microbial consortium and biostimulants on leaf spot and flower blight in Marigold

Treatments	Treatment Details	Alternaria Leaf Spot	Alternaria Flower Blight
T1	Trichoderma+ 50% RDF	38.14	44.07
T2	Pseudomonas + 50% RDF	37.94	43.17
T3	Bacillus + 50% RDF	33.72	42.67
T4	Tri+Pseu+ 50% RDF	32.67	42.17
T5	Tri+Baci+ 50% RDF	32.41	41.33
T6	Pseu+Baci+ 50% RDF	27.68	40.63
T7	Vermiwash+ 50% RDF	26.67	42.17
T8	Tri + Pseu + Baci + Vermiwash +50% RDF	24.46	34.13
T9	50% RDF	62.58	75.67

T10	Absolute Control	64.78	78.92
	CD (P=0.05)	1.17	1.45

*Tri=Trichoderma, Pseu=Pseudomonas, Baci=Bacillus, RDF=Recommended Dose of Fertilizers

The disease incidence was recorded on 60 days after transplanting, Percent Disease Index of alternaria leaf spot (24.46) and alternaria flower blight (34.13) was observed less in T8 which received combined soil application of 50% recommended dose of fertilizers (63:30:30 Kg NPK ha^{-1}) and bioagents + *Trichoderma harzianum* (10ml/litre) +*Pseudomonas fluorescens* (10ml/litre)+ *Bacillus subtilis* (10ml/litre) and foliar spray of vermiwash (0.5%) whereas, alternaria leaf spot (64.78) and alternaria flower blight (78.92) was observed high in control. This indicates impact of bioagents *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* combined application proved to be effective in alternaria disease management in marigold [10] reported that *Pseudomonas fluorescens* and other plant growth promoting rhizobacteria enhance plant growth by showing antagonisms to potentially deleterious rhizoplane fungal and bacterial pathogens. The direct application of microorganism to seed or other plant part gives them a competitive advantage over pathogen that must compete for nutrients and sites for attachment prior to infection. Reports are on hand indicating that bacteria may promote plant growth by suppression of deleterious root micro flora indicating those not causing obvious disease [4]. The antagonists attack the pathogens by various modes of action *viz*., competition for nutrients, space, antibiotic production, siderophores and also triggering plant originated resistance mechanism called Induced Systemic Resistance [20]. *Trichoderma* was used in strawberry grey mould management [22] *Bacillus subtilis* against brown rot management in strawberry [19].

Conclusion

In a nut shell, it was perceived that, the combined soil application application of 50% recommended dose of fertilizers (63:30:30 Kg NPK ha-1) + *Trichoderma harzianum* (10ml/litre) +*Pseudomonas fluorescens* (10ml/litre)+ *Bacillus subtilis* (10ml/litre) and foliar spray of vermiwash (0.5%) to marigold improved the overall growth and yield in african marigold hybrid 'L 3'.Biological control has emerged as an alternative strategy to combat major diseases. The results of this investigation showed that antagonist activity of bio-control agents of fungi *Trichoderma*, bacteria *Pseudomonas* and *Bacillus* with PGPR activity has minimized the *Alternaria* blight disease on leaves and flowers in marigold.

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