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Effects of Chemical and Biological Fertilizers on Morpho-Physiological Traits of Marigold (*Calendula officinalis* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AA, GRZ, MHS and JA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Fertilizer management is an important factor for a successful growth of officinal plants and the identity of suitable fertilizers in plants could have the desirable effects on quantitative and qualitative indices. In order to study the effects of biological fertilizers and NPK fertilizer on growth characteristics, chemical composition of marigold.
Methods: This experiment was conducted in 2012 at the research green house of Birjand University, Iran in a completely randomized in factorial design with three replications.
Treatments included biological fertilizers (without biofertilizer, *Psedomonas fluorescence* 187, *P. fluorescence* 178, *P. Fluorescence* 169, *P. putida* 159, *P. Fluorescence* 36) with different NPK fertilizer rates (0, 25, 50, 100%).
Results: Results show that by increasing NPK rates up to 100% dose, number of branches, number of flowers, number of leaf, capitulum diameter, capitulum, bracket diameter, phosphorus

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and potassium content were significantly increased when compared with the zero NPK. The combined treatment of biofertilizer and chemical fertilizer significantly increased the flowering stem height, chlorophyll index, nitrogen and flavonoids content. The highest content and the concentration of flavonoids were obtained when plant was treated with *P. fluorescence* 36 strain.
Conclusion: The results showed that just usage of biological fertilizers or in combination with chemical fertilizers had the positive effects on physiological traits of *Calendula officinalis* L., and using the biological potential instead of continious usage of chemical fertilizers can improve the stability of agriculture and certify the quality of officinal plants.

Keywords: Growth; flavonoids; Psedomonas fluorescence; NPK.

1. INTRODUCTION

Marigold (*Calendula officinalis* L.) is one of the major medicinal plants grown on field plantations. Its cultivation is stimulated not just by the pharmaceutical industries but also by the dynamically developing manufacturing of cosmetics. The pharmaceutical industry uses either ligulate florets (*Flos Calendulae*) or completes composite flowers of calendula (*Flores Calandulae cum Calycibus*) which are deep orange in colour and have a specific smell and bitter taste. With its rich chemical composition, the calendula plant material contains a whole range of active substances, including essential oil, triterpene saponins (*calendulosides*), flavonoids, carotenoids, saponins, polyacetylenes, sterols, carbohydrates, vitamin C, mucilages and minerals, e.g. manganese, which makes the plant an important source of preparations with therapeutic effects used in pharmacy and medicine [1].

For a sustainable agriculture system, it is imperative to utilize renewable inputs which can maximize the ecological benefits and minimize the environmental hazards [2]. Chemical fertilizers have contributed significantly toward the pollution of water, air and soil. Therefore the current trend is to explore the possibility of supplementing chemical fertilizers with organic ones that are ecofriendly and cost-effective [3]. Using biofertilizer and selection of the best microbial strains have vital role when integrating human society with vulnerable ecosystems. Biological fertilizers, which are calledbio-fertilizers, may be used in a way of to maintain soilfertility and soil improvement. Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutrionally important elements (N, P, …) form unavailable to available from through biological process such as Nitrrogen fixation and solubilization of rock phosphate. One possible way of achieving biofertilizers is to decrease dependence on use of chemical Nitrogen fertilizers by harvesting the atmospheric nitrogen through biological processes. The use of growth promoting bacteria, like asymbiotic N2 fixing bacteria, is known as bio-fertilizers and has recently gained importance in crop production. It is well known that these bacterial species, mostly those associated with the plant rhizosphere, are able to exerta beneficial effect upon plant growth. Therefore, their use asbio-fertilizers for agriculture improvement has been a focus of numerous studies recently [2]. Fertilization is the most important and controllable factor affecting the nutritional value of vegetables. The type and value of fertilizer and the level of application directly influence the level of nutrients available in plants and indirectly influence plant physiology and the biosynthesis of secondary compounds in plants. Secondary compounds in plants are known as secondary metabolites or phytonutrients [4].

Biofertilizers differ from chemical and organic fertilizers in that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. Some microorganisms have positive effects on plant growth promotion, including the plant growth promoting rhizobacteria (PGPR) such as Azospirillum, Azotobacter, Pseudomonas fluorescens, and several gram positive Bacillus spp. [5].Various Pseudomonas species have shown to be effective in controlling pathogenic fungi and stimulating plant growth by a variety of mechanisms, including production of siderophores, synthesis of antibiotics, production of phytohormones, enhancement of phosphate uptake by the plant, nitrogen fixation, and synthesis of enzymes that regulate plant ethylene levels [6]. Good soil fertility management ensures adequate nutrient availability to plants and increases yields. High above-ground biomass yield is obviously accompanied by an active root system, which releases an array of organic compounds into the rhizosphere [7].

Positive response of nitrogen fertilizers has been reported by Koul [8], and Omer [9]. Sharma [10] observed that addition of nitrogen fertilizer increased plant height. Increase in plant height resulted in an increase in leaf number per plant as reported by Akintoye [11].

Several workers studied the response of various medicinal and aromatic plants to nitrogen-fixing bacteria. The role of bio-fertilizers containing symbiotic or non-symbiotic nitrogen-fixing bacteria in augmenting vegetative growth characters, yield and yield components, essential oil productivity and/or chemical composition (including chlorophyll a, b and carotenoids and/or N, P and K leaf percent and content) was revealed by Kandeel et al. [12] on fennel, Nofal et al. [13] on Ammivisnaga, Abdou and El-Sayed [14] on caraway, Safwat and Badran [15] on cumin, Abd El-Kader and Ghaly [16] on coriander, Badran and Safwat[17] on fennel. Many studies were executed to explore the effectiveness of phosphorein bio-fertilizer (phosphate dissolving bacteria) on different vegetative growth traits, seed yield and essential oil productivity of some medicinal plants. In this respect, Soliman [18]; Abd El-Kader and Ghaly [16] and Abdou et al. [19], they showed that phosphore in biofertilizer succeeded in enhancing vegetative growth parameters, seed yield and essential oil productivity of Nigella sativa , fennel , coriander, anise and fennel plants, respectively.

The nutritional requirements of (NPK) for Umbilliferae Family and other plants were reported by many researchers. In this concern, El-Sakov et al. [20] worked on some medicinal and aromatic plants, Kozera and Nowak [21] on *Silybum marianum*, Niakan et al. [22] on Menthapiperita, Lee et al. [23] on Chrysanthemum boreale, Gomaa and Youssef [24]; on fennel and lovage plants concluded that NPK fertilizers had an important physiological and biochemical functions on structure of photosynthetic pigments, metabolism of carbohydrates and protein and these effects were observed with significant increase in growth, vegetative and seed yield and essential oil content of the different plant species. Very few researches were done in relation to how biological and chemical fertilizers had an effect on increasing the amount Flavonoids in plants. Current study was aimed to investigate the effect of chemical and biological fertilizers on morpho-physiological traits of marigold.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted in form of randomize complete design with three replications in the research greenhouse of agricultural faculty, Birjand University, Iran in 2012. *Calendula officinalis* seeds were obtained from Farm of Faculty of Agriculture, Ferdowsi University.

2.2 Soil Analysis

Soil sample was collected from site and passed through a 2 mm sieve in order to give uniform sample. Samples were analyzed at the laboratory. The chemical and physical properties of the collected soils are given in Table 1.

2.3 Experimental Design and Treatments

The first factor was kinds of biological fertilizer (*Psedomonas* fluorescence187, *P. fluorescence* 178, *P. fluorescence* 169, P.putida159, *P. fluorescence* 36) associated with control (without biofertilizer) and the second factor was chemical fertilizer NPK with four levels including:0, 25, 50, 100% NPK(full dose of NPK fertilizer = 150:75:50 mg/kg). Microorganisms were obtained from the soil & water research institute, Ministry of Agriculture, Iran. Treatments were added to the soil in each pot. The pots were randomized placed in a wire house. Initially 10 seeds were planted in each pot, but after confidence of germination, three plants were maintained per pot after thinning. Pots irrigation was done according to the field capacity every day. The average temperature during the experiment was between 15°C to 23°C.

2.4 Parameters Measured

2.4.1 Morphological traits

After full maturity, all plants of each plot harvested for determining flowering stem height, number of branches, number of flowers, leaf number, diameter and the diameter of the capitulum and capitulum Bracket.

Table 1. Characteristics of soil in experiment plot before treatment applications

Field capacity (%)	Sp (%)	pH	Organic matter (%)	Cl (meq)	Ca (meq)	Mg (meq)	Na (meq)	EC (ds/m)	Soil texture	Clay (%)	Silt (%)	Sand (%)
13%	33	8	0.3	6.6	4.8	4.4	2.6	1.5	Sandy loam	5	39	56

2.4.2 Content of nitrogen, phosphorus and potassium

Content of nitrogen, phosphorus and potassium of leaves, were measurement with AOAC Washington DC [25] Method.

2.4.3 Total flavonoid content (TFC)

Total flavonoid content was spectrophotometrically determined by the aluminum chloride method using quercetin as standard [26]. After incubation at room temperature, samples were measured at 512 nm and expressed as mg quercetin equivalents (QE)/g fresh weight.

2.5 Statistical Analysis

All the data were analyzed with analysis of variance (ANOVA) procedures using the mstatc. Least significant difference test ''LSD'' at 5% level of probability was used to compare means of the treatments.

3. RESULTS AND DISCUSSION

3.1 Morphology Traits

Data presented in Table 2 reveal that flowering stem height was affected significantly by the application of NPK fertilization. The highest value of flowering stem height was 14.23 cm with 100% NPK fertilization. The increase in plant height with NPK fertilization can be attributed to the fact that Macro elements, particularly nitrogen promotes plant growth increases the number and length of the internodes which results in progressive increase in plant height. Similar results were reported by Turkhede and Rajendra [27], Saigusa et al. [28] and Gasim [29]. Also, results indicated that plant height was affected significantly by the interaction between NPK fertilization rates and biofertilizer. With increasing NPK fertilization rates, flowering stem height increased either in the all biofertilizer treatments. But inoculation with *P. fluorescens* 169 + 100% NPK and *P. fluorescens* 187 + 100% NPK showed good results for this trait, i.e. 124.00 and 122.43 cm, respectively (Fig 1). These PGPR inhabit plant roots and affect plant growth promotion by mechanisms such as increased solubilization and uptake of nutrients and/or production of plant growth regulators [30,31].

Number of leaf number of pot marigold showed significantly increases from 60.17 (zero NPK) to 101.76, 115.28 and 196.11 with 25%, 50% and 100% NPK fertilization respectively, (69, 91 and 225% increase) compared to those of zero NPK. The increase in the number of leaves could possibly be ascribed to the fact that nitrogen often increases plant growth and plant height and this resulted in more nodes and internodes and subsequently more production of leaves. In this respect, Okajina et al. [32], Sawi [33] and Jhones et al. [34] found that nitrogen fertilization, significantly increased the number of leaves and they suggested that the increasing in number of leaves may be as a result of increasing number of nodes. The results indicate NPK fertilization showed significant effect on number of branches and number of flower of marigold plants. The application of NPK fertilizers significantly increased number of branches in marigold from 1.44 (zero NPK) to 19.16 (100% NPK). Also, the highest value of number of flowers was 19.5 with 100% NPK fertilization.

Letchamo [35] noted that the addition of nitrogen increased number of flower. These increments in number of flower may be due to the increases in plant height, number of branches, and number of leaves as nitrogen application rates increased.

From the data presented in Table 2 it is clear that capitulum diameter and capitulum bracket diameter were affected significantly by the application of NPK fertilization but no different significant in between treatments of NPK fertilization. Generally, the reasons of increasing traits like height, the number of secondary branches, the number of flower and leaf, the diameter of capitulum, and capitulum's brackets of marigold are the role of nitrogen elements, phosphorus, and potassium in physiological, biochemical and metabolic process of carbohydrates and proteins and supply of these elements by consuming chemical fertilizer in this experiment.

3.2 Physiology Traits

Physiological parameters of marigold were greatly increased by the use of NPK fertilizer and bacterial inoculation (Table 3 and 4). As shown in Table 3 the phosphorus content is significantly increased when fertilized with different NPK rates. The highest and lowest values of phosphorus content were 0.007 gr and 0.001 gr with 100% and zero NPK fertilization, respectively. Also, result showed that with the increasing amount of NPK fertilization, the leaf potassium concentration had decreased from 0.76% in zero NPK to 0.52% in 100% NPK treatment, whereas potassium contentin marigold significantly increased from 0.012 gr (zero NPK) to 0.02 gr (100% NPK).

Table 2. Effect of NPK fertilizer and biofertilizer on morphology traits of marigold

Treatment	Flowering stem height (cm)	No. of branches	No. of flowers	No. of leaf	Capitulum diameter (cm)	Capitulum bracket diameter(cm)
Zero	7.61 c	1.44 c	2.27 c	60.17 c	4.13 b	1.86 b
25% NPK	12.23 b	5 bc	6 b	101.7 b	6.68 a	3.14 a
50% NPK	11.89 b	6.88 b	8.22 b	115.28 b	7.11 a	3.02 a
100% NPK	14.23 a	19.16 a	19.5 a	196.11 a	7.1 a	3.01 a
ANOVA						
NPK	**	**	**	**	**	**
Bio	NS	NS	NS	NS	NS	NS
NPK* Bio	*	NS	NS	NS	NS	NS

*NS: not significant, * significant at the 0.05 level, ** significant at the 0.01 level*

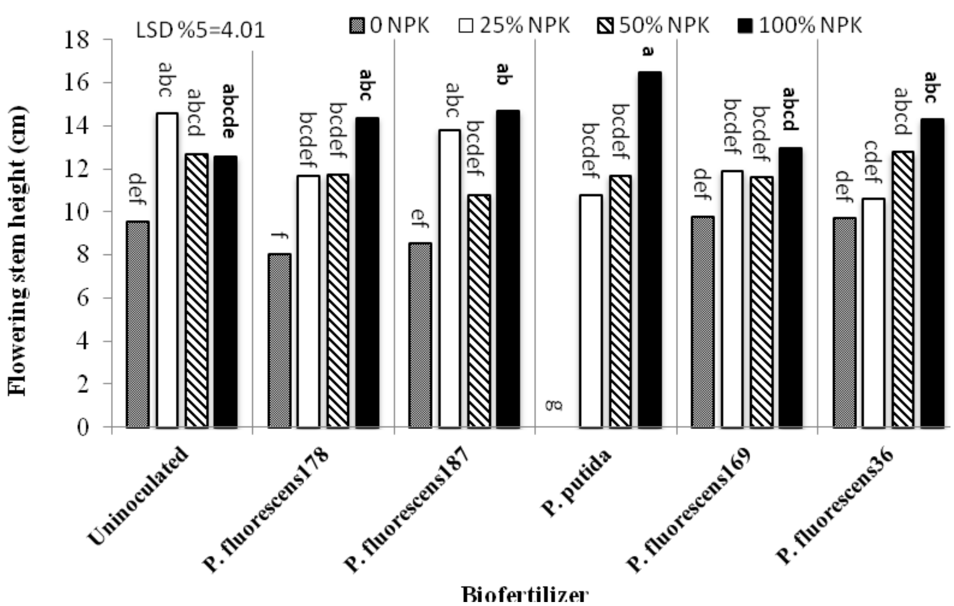

Fig. 1. Effect of biology and chemical fertilizer on flowering stem height of marigold

Table 3. Effect of NPK fertilizer on phosphorus (p) and potassium (K) traits of marigold

Treatment	Concentrations (%)		Content (gr)	
	P	K	P	K
Zero NPK	0.11 a	0.76 a	0.001 b	0.012 c
25% NPK	0.13 a	0.65 ab	0.003 ab	0.018 b
50% NPK	0.10 a	0.56 bc	0.002 b	0.016 bc
100% NPK	0.15 a	0.52 c	0.007 a	0.02 a
ANOVA				
NPK	NS	**	*	**
Bio	NS	NS	NS	NS
NPK* Bio	NS	NS	NS	NS

*NS: not significant, * significant at the 0.05 level, ** significant at the 0.01 level*

Table 4. Effect of NPK fertilizer and biofertilizer on physiology traits of marigold

Biofertilizers	NPK	Concentrations (%) N	Concentrations (%) F	Content (gr) N	Content (gr) F	Chlorophyll index
Uninoculated	Zero	2.09 a	10.20 p	0.033 cde	4.05 m	24.33 ab
	25%	1.22 b	10.63 op	0.075 ab	10.19 h	32.97 a
	50%	0.66 b	10.82 op	0.021 de	13.81 f	32.43 a
	100%	0.80 b	11.43 o	0.037 cde	22.05 cd	30.70 a
P. fluorescens 178	Zero	0.82 b	15.10 n	0.014 e	4.26 m	8.37 b
	25%	0.98 b	15.93 n	0.023 cde	12.98 fg	30.27 a
	50%	2.70 a	19.90 m	0.085 a	12.87 fg	35.50 a
	100%	0.68 b	23.68 k	0.037 cde	25.76 b	33.37 a
P. fluorescens 187	Zero	0.70 b	20.85 lm	0.011 e	4.89 lm	9.03 b
	25%	1.01 b	21.60 l	0.028 cde	8.65 i	23.60 ab
	50%	1.05 b	27.65 ij	0.027 cde	18.92 e	29.27 a
	100%	0.81 b	27.09 j	0.035 cde	22.99 c	35.30 a
P. putida	Zero	0.88 b	28.60 i	0.016 de	5.80 kl	21.87 ab
	25%	0.79 b	32.38 h	0.018 de	7.49 j	30.23 a
	50%	0.92 b	37.49 g	0.030 cde	21.70 d	28.20 a
	100%	0.95 b	40.70 f	0.044 cd	22.16 cd	32.07 a
P. fluorescens 169	Zero	0.65 b	38.24 g	0.012 e	1.89 n	31.13 a
	25%	1.20 b	41.27 f	0.025 cde	10.89 h	31.60 a
	50%	0.73 b	45.24 e	0.020 de	19.04 e	28.67 a
	100%	0.83 b	46.94 d	0.050 bc	22.92 c	33.97 a
P. fluorescens 36	Zero	0.99 b	47.51 d	0.015 e	6.29 k	28.07 a
	25%	0.89 b	57.72 c	0.023 cde	12.41 g	9.23 b
	50%	0.94 b	60.37 b	0.029 cde	22.48 cd	33.50 a
	100%	0.81 b	61.51 a	0.036 cde	29.44 a	34.93 a
ANOVA						
NPK		NS	*	**	*	**
Bio		NS	*	*	*	NS
NPK* Bio		**	*	**	*	*

*NS: not significant, * significant at the 0.05 level, ** significant at the 0.01 level, N: nitrogen, F: flavonoids*

The effect of fertilizer' treatments on leaf nitrogen concentration and nitrogen content of marigold is shown in Table 4. The highest nitrogen concentration (2.7%) and nitrogen content (0.085 gr) of leaf were obtained by a mixture of *P. fluorescens* 178 + 50% NPK (Table 4). In this context, Hossein et al. [36] reported that Biological fertilizer on marigold increases NPK in leaves, stem, and root. Concentration and content of nitrogen has increased probably due to releasing nitrogen and more phosphorus in soil by the strains of bacteria [37] and increasing in attracting them [38], and also the access of plant to the nitrogen.

Data of Table 4 showed that combination between biofertilizer and use of NPK fertilizer gave significant increases in chlorophyll index, as compared with treatments of NPK fertilizer without. In the between treatments without of NPK fertilizer, the highest chlorophyll index of leaf was 28.06 in *P. fluorescens* 36 strain (Table 4). Pseudomonas bacterium provide mineral elements inaccessible and also organic compounds accessible to the plant [39] and consequently, more effective attraction of nutrient elements, nitrogen and important components of photosynthesized pigments and the amount of chlorophyll has been increased.

From the data presented in Table 1 it is clear that the flavonoids content and flavonoids concentration of flower of Pot marigold was significantly increased as a result of NPK and biofertilizer treatments. With increasing NPK fertilization rates, flavonoids content and flavonoids concentration of flower increased either in the all biofertilizer treatments.

The highest value of flower flavonoids concentration was 61.5 mg/g extract in pseudomonas fluorescens 36 +100%NPK treatment. But the best treatment were that of pseudomonas fluorescens 36 +50%NPK and pseudomonas fluorescens 36 +25%NPK which gave 60.37 and 57.72 mg/g extract, respectively, from the economic and environmental sides. In addition, the highest value of flower flavonoids content was 29.44 mg/g DW in pseudomonas fluorescens 36 +100%NPK treatment (Table 4). Sharma et al. [40] remarked that the amount of chlorophyll has increased in vetch mixed with Pseudomonas than vetch without mixing with Pseudomonas.

Increasing the access to nitrogen elements and phosphorus in soil due to the activity of Pseudomonas bacterium and due to increasing their attraction by plant has caused to increase nitrogen and probably caused to increase the initial substrate of related reactions by producing secondary metabolitesuch as flavonoids. Increasing the efficiency of nitrogen attraction due to increasing two fertilizers on each other can be another research of increasing Flavonoids in the experiment.

4. CONCLUSION

In conclusion, dual inoculation with pseudomonas fluorescens improved the growth parameters and nutrient contents of marigold plant using the recommended doses of N,P,K and enhanced environmental sustainability.

Considering the experiment results, Suitable strain to increase the quality and the amount of flavonoids content of marigold, *P. fluorescence* 36 (22/89 mg) strain is recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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