Journal of Ornamental Plants
Available online on: www.jornamental.iaurasht.ac.ir
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441
Research Paper

Study of Morphological and Biochemical Traits of Marigold as Influenced by Phosphorous Biofertilizer and Zinc

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Received: 27 October 2018 Accepted: 22 October 2019
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Abstract

The application of biofertilizers constitutes one of the main components of nutrient management with a fundamental role in sustainable agriculture and the improvement of plant qualitative traits. The present research assessed the effect of various treatments of phosphate solubilizing bacteria including *Pantoea agglomerans* strain P5 and *Pseudomonas putida* strain P13 (seed inoculation, the application of biofertilizer 2, 4 and 6 weeks after plant emergence, and no inoculation of seeds as control treatment) and the foliar application of $ZnSO_4$ (at 0, 1, 2, and 3 g L^{-1} rates) on morphological and biochemical traits of marigolds. It was found that the highest plant height and flower fresh weight belonged to plants whose seeds were inoculated with biofertilizer and were fertilized with 2 g L^{-1} $ZnSO_4$ and also, in plants treated with biofertilizer 2 weeks after plant emergence and fertilized with 1 and 2 g L^{-1} $ZnSO_4$. Also, the highest flower dry weight and anthocyanin content were obtained from the treatments of biofertilizer 2 and 4 weeks after plant emergence supplemented with 1 and 2 g L^{-1} $ZnSO_4$. The highest P content was seen in the treatments of biofertilizer 4 and 6 weeks after plant emergence × 2 and 1 g L^{-1} $ZnSO_4$. In addition, the highest Zn content was obtained from biofertilizer application 6 weeks after plant emergence and in plants fertilized with 3 g L^{-1} $ZnSO_4$. In contrast, the lowest amount of most parameters was observed at different levels of biofertilizer application without the use of $ZnSO_4$ and with the use of 3 g L^{-1} $ZnSO_4$. Therefore, the foliar application of $ZnSO_4$ and the soil application of phosphate solubilizing fertilizers can influence the biochemical and morphological traits of marigolds.

Keywords: Anthocyanin, Chlorophyll, Fresh weight of flower, Dry weight of flower, Foliar application, Nutrients.

INTRODUCTION

Marigold (*Calendula officinalis* L.), from the family Asteraceae, is an herbaceous, annual or perennial plant that is native to the Mediterranean region with ornamental and medicinal uses. Some of its varieties are used as a cut flower (Azzaz *et al*., 2007). The key biologically active compounds of marigold are terpenoids, flavonoids, coumarins, essential oil, carotenoid, and amino acids (Buntariu and Zepa Cradini, 2012). In general, the quantitative and qualitative yields of plants are dictated by genetic factors, environmental conditions, and nutrition management (Tesfamariam *et al*., 2010; Kamkar *et al*., 2011).

Thus, the improvement of plant growth, as well as its yield and quality, requires an adequate and balanced supply of all nutrients (Parveen *et al*., 2015). Phosphorus (P) is an important element required by plants for growth and development. It involves in processes such as cell division, reproductive organ development, and adenosine triphosphate (ATP) structure, thereby playing a key role in plant metabolism and growth (Gyaneshwar *et al*., 2002; Rajendran *et al*., 2008). Despite the fact that soils are typically rich in P, its uptake by plants is severely limited due to its low solubility and fixation by mineral ions like Al and Fe in acidic soils and by Ca and Mg in alkaline soils (Parent, 2005). Hence, sustainable agriculture can play a critical role in fertility and maintaining bioactivities by its emphasis on the application biofertilizers to reduce or stop the use of chemical inputs (Zaidi *et al*., 2003; Kocabas *et al*., 2010). Accordingly, the use of biofertilizers is an important strategy for the management of nutrients, and today these compounds are considered as a good alternative instate chemical fertilizer to improve soil fertility and structure for crop production, stimulate plant growth, increase crop quantity and quality, and enhance resistance to environmental stresses (Nagananda *et al*., 2010; Taha *et al*., 2011).

On the one hand, some studies have reported that micronutrients, similar to macronutrients, play some crucial and vital roles in the growth and development of plants (Akter *et al*., 2017; Nadeem and Farooq, 2019). Zinc (Zn), as a micronutrient, is used as a component of enzyme structures to build DNA and RNA, increase water use efficiency, and the metabolism of carbohydrates, fats, proteins, and build tryptophan as the precursor of IAA synthesis, which is absorbed as a divalent cation (Zn^{2+}) by plants (Mousavi, 2011; Castillo-Gonzalez *et al*., 2019). On the other hand, in most Iranian soils, solvability of micronutrients is limited by high pH and calcareous soils and this has resulted in the decline of the uptake of these elements (Mousavi, 2011). It has been documented that the foliar application of iron and zinc sulfate enhances photosynthesizing pigments, proteins, and phenols in *Cassia angustifolia* Vahl. significantly (Shitolw and Dhumal, 2012). Also, it has been reported that phosphorus is the most important element that interferes with zinc uptake by plants (Mousavi, 2011) so that numerous studies have been conducted on the interaction of zinc and phosphorus and all have confirmed that excessive accumulation of phosphorus causes zinc deficiency in plants (Das *et al*., 2005; Khorgamy and Farnia, 2009; Salimpour *et al*., 2010).

One of the main challenges of ornamental plants production, particularly cut flowers, in Iran is improper nutrient management arising from producers' lack of scientific and technical knowledge, ignorance of environmental considerations, and the excessive and unbalanced use of chemical fertilizers and herbicides in greenhouses. Given the importance of the safety of the crops produced by different systems in terms of the residual herbicides and chemicals and their impact on human and environment health, it is imperative to consider the production methods and input application. Thus, the present paper assesses the effect of phosphate biofertilizer (a combination of *Pantoea agglomerans* strain P5 and *Pseudomonas putida* strain P13) and zinc sulfate on some morphological and biochemical traits of marigold.

MATERIALS AND METHODS

Plant materials and experimental design

This study was carried out in the research fields of Islamic Azad University of Isfahan (Khorasgan) in the 2017 growing season. An experimental research was conducted using a factorial experiment based on a randomized complete block design with four periods of foliar application of biofertilizer (control (no inoculation), 2, 4, and 6 weeks after plant emergence) as the first factor and four levels of foliar application of $ZnSO_4$ as the second factor. Marigold 'Candeman Yellow' seeds were procured from HEM ZADEN Company (Netherland Co.) and were planted in May 2017 in pots with 15 cm mouth diameter and 18 cm height containing soil culture medium. Phosphate biofertilizer (Barvar 2) was procured from Zist Fannavar Co. and was applied in 2:1000 concentration in different treatments that included control (no biofertilizer inoculation and no application of $ZnSO_4$), seed inoculation with biofertilizer and the application of the biofertilizer 2, 4, and 6 weeks after plant emergence. Also, the plants were sprayed with zinc sulfate ($ZnSO_4$) at four different rates of zero (control), 1, 2, and 3 g L^{-1} twice during the experiment (in 4-leaf and 8-leaf stages of the plants). Also, it should be noted that distilled water was used in both stages of foliar application for control plants.

MEASUREMENTS

Plant height

The plant height was recorded using a ruler 70 days after sowing.

Fresh and dry weight of flowers

To measure fresh and dry weight of flowers, the flowers were harvested 70 days after sowing, they were selected, and their fresh weight was measured using a digital scale with a precision of 0.001 g. After this stage, the samples in each plot were put into a special paper pocket and were placed in an oven (Shimazco model) for 48 hours at 70°C. After the drying of the samples, the dry weight of the flowers was measured by the same digital scale individually.

Chlorophyll a, b and total

Chlorophyll a, b and total were estimated with a spectrophotometer (model D6320) at the wavelengths of 663 and 645 nm. Then, Eq. (1) was applied to determine the concentration of chlorophyll pigments, in which A645 and A663 are the readings at 645 and 663 nm, respectively. Also, V is the acetone volume in mL and W is fresh leaf weight in g (Arnon, 1949).

$$\text{Chl.a (mg g}^{-1}) = [(12.7 \times A_{663}) - (2.6 \times A_{645})] \times V/W \times 1000$$
$$\text{Chl.b (mg g}^{-1}) = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V/W \times 1000$$
$$\text{Total chl.(mg g}^{-1}) = \text{Chl.a Chl.b} \quad (1)$$

Anthocyanin

Anthocyanin content was estimated by Wagner (1979)'s method, with a small modification. So, 0.1 g of plant tissue was completely crashed in 10 cc acidic methanol solution and the extract was centrifuged at 4000 rpm for 10 minutes. Then, the supernatant was placed in darkness at 25°C for 24 hours. Finally, its absorption was read at 550 nm with a spectrophotometer and it was placed in Eq. (2) to assess the anthocyanin content:

$$A = \varepsilon BC \quad (2)$$

in which A is the reading at 550 nm, ε denotes extinction coefficient (equal to 33000 cm^2 mol^{-1}),

B represents cuvette width in cm, and C is anthocyanin concentration (mM g^{-1}).

Minerals

To determine the uptake ratio of N, P, K, and Zn elements, leaf samples were selected from the experimental units and were oven-dried. Then, N content was determined by the Kjeldahl method (Bremner and Mulvaney, 1982), P content in plants was estimated through molybdate vanadate method (Chapman and Pratt, 1961), K content was calculated by flame photometry (Kudsen and Peterson, 1982), and Zn content was measured by an atomic absorption device.

Before the experiment, a sample of the soil used in the present study was sent to the soil laboratory to determine its physical and chemical properties. The soil analysis results are listed in Table 1.

Table 1. The physical and chemical properties of the soil used in the study.

Soil texture	pH	EC (dS/m)	N (%)	P (ppm)	K (ppm)	Organic matter (%)
Sandy-loamy-clay	8.03	1.4	0.15	63.2	464.6	1.3

Data analysis

The study was carried out as a factorial experiment based on a Randomized Complete Block Design with five treatments of phosphate biofertilizer (a combination of phosphate solubilizing bacteria, including *Pantoea agglomerans* strain P5 and *Pseudomonas putida* strain P13) and foliar application of $ZnSO_4$ at four rates in three replications. Data were analyzed using SAS 9.1 Software Package and means were compared by Duncan's test at $P<0.05$. The graphs and tables were prepared in MS-Excel Software.

RESULTS

Plant height

According to the analysis of variance, plant height was significantly ($P<0.01$) influenced by biofertilizer, $ZnSO_4$, and their interaction (Table 2).

Table 2. Analysis of variance of morphological traits of the marigold plant exposed to different periods of biofertilizer application and foliar application $ZnSO_4$.

S.o.V	df	MS		
		Plant height	Flower fresh weight	Flower dry weight
Replication	2	0.38^{ns}	0.80^{ns}	0.05^{ns}
Barvar 2 (B)	4	21.34^{**}	28.26^{**}	0.43^{**}
$ZnSO_4$ (Z)	3	34.66^{**}	15.39^{**}	0.53^{**}
B×Z	12	9.55^{**}	7.86^{**}	0.52^{**}
Error	38	1.46	1.14	0.02
CV (%)		7.23	20.20	14.56

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant respectively.

Means comparison of data showed that the highest amount of plant height (20.50 cm) was observed in both treatments of seed inoculation with biofertilizer and foliar application of 1 g L^{-1} $ZnSO_4$ concentration under the application of biofertilizer 2 weeks after plant emergence.

On the other hand, the lowest amount of plant height (11.67 cm) was obtained from the foliar application of 3 g L^{-1} of $ZnSO_4$ under the application of biofertilizer 6 weeks after emergence of plants, which had no significant difference with plant height in biofertilizer application 6 weeks after plant emergence × control treatment (12.83 cm), and biofertilizer application 6 weeks after plant emergence × 3 g L^{-1} $ZnSO_4$ (13.67 cm).

Table 3. Means comparison of plant height and flower fresh and dry weight under the application of biofertilizer and foliar application of $ZnSO_4$ at different periods.

Biofertilizer treatments	$ZnSO_4$ concentrations (g L^{-1})	Plant height (cm)	Flower fresh weight (g)	Flower dry weight (g)
No inoculation	Control	14 efg	3.87 fgh	0.53 gh
	1	17.67 bcd	6.26 b-e	1.19 cd
	2	16.17 b-e	5.21 ef	0.81 ef
	3	16.00 cde	5.05 ef	0.52gh
Seed inoculation by biofertilizer	Control	16.17 b-e	5.38 def	1.20 cd
	1	18.00 bc	6.70 a-e	1.28 bc
	2	20.50 a	8.21 ab	1.22 cd
	3	18.17 bc	6.42 b-e	0.57 fgh
Biofertilizer application 2 weeks after plant emergence	Control	16.67 bcd	5.67 c-f	1.01 de
	1	20.50 a	8.64 a	1.37 bc
	2	18.50 ab	7.51 abc	1.63 a
	3	15.50 def	4.24 fg	1.16 cd
Biofertilizer application 4 weeks after plant emergence	Control	16.67 bcd	4.23 fg	0.35 h
	1	17.50 bcd	7.34 a-d	1.31 bc
	2	18.17 bc	7.82 ab	1.48 ab
	3	13.67 fghg	4.16 fg	0.43 h
Biofertilizer application 6 weeks after plant emergence	Control	12.83 gh	1.09 i	0.41 h
	1	17.50 bcd	3.68 fgh	0.75 fg
	2	18.00 bc	2.37 ghi	0.59 fgh
	3	11.67 h	2.06 hi	0.47 h

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using the LSD test.

Flower fresh weight

In Table 2, it was found that the effects of biofertilizer, $ZnSO_4$, and biofertilizer × $ZnSO_4$ interaction were significant ($P<0.01$) on fresh weight and dry weight of the flowers (Table 2). The interaction of biofertilizer application 2 weeks after plant emergence × 1 g L^{-1} of $ZnSO_4$ resulted in the highest amount of fresh weight of flower (8.64 g). On the other hand, the next two highest fresh weights (8.21 and 7.82 g) were obtained from the seed inoculation treatment by biofertilizer × 2 g L^{-1} $ZnSO_4$ and the application of biofertilizer 4 weeks after plant emergence × 2 g L^{-1} $ZnSO_4$, respectively. In contrast, the lowest fresh weight of the flowers was obtained from the interaction

of biofertilizer application 6 weeks after plant emergence × 1, 3, and 2 g L^{-1} $ZnSO_4$, in which it was 1.09, 2.06, and 2.37 g, respectively (Table 3).

Flower dry weight

The results of analysis of variance for the dry weight of flowers indicated that this index was affected by the treatments of biofertilizer application, $ZnSO_4$ concentrations, and their interaction at P<0.01 (Table 2). Means comparison of the dry weight of the flowers Table 3 showed that the highest flower dry weight (1.63 g) was observed from the application of biofertilizer 2 weeks after plant emergence × 2 g L^{-1} $ZnSO_4$, but it had no significant difference with amount of flower dry weight in the application of biofertilizer 4 weeks after plant emergence × 2 g L^{-1} $ZnSO_4$ (equal to 1.48 g). On the other hand, the lowest flower dry weight (equal to 0.35 g) was seen in plants treated with biofertilizer 4 weeks after plant emergence × control treatment of $ZnSO_4$ concentrations (Table 3).

Plant pigments

Analysis of variance in Table 4 showed that chlorophyll a, b, total chlorophyll, and anthocyanin contents were significantly influenced by biofertilizer applications, $ZnSO_4$ concentrations, and interaction of biofertilizer × $ZnSO_4$ at P<0.01 (Table 4).

Table 4. Analysis of variance of chlorophyll and anthocyanin contents of marigold under the application of biofertilizer and ZnSO4 treatments.

S.o.V	df	MS			
		Chl. a	Chl. b	Total Chl.	Anthocyanin (AC)
Replication	2	0.0006^{ns}	0.0001^{ns}	0.002^{ns}	0.00004^{ns}
Barvar 2 (B)	4	0.133^{**}	0.006^{**}	0.149^{**}	0.0003^{**}
$ZnSO_4$ (Z)	3	0.111^{**}	0.023^{**}	0.212^{**}	0.0002^{**}
B × Z	12	0.025^{**}	0.011^{**}	0.040^{**}	0.0001^{**}
Error	38	0.001	0.0004	0.0016	0.00002
CV (%)		6.34	11.38	5.55	10.85

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant respectively.

Means comparison of chlorophyll *a* in Fig.1 demonstrated that the highest content of chlorophyll *a* (0.84 mg g^{-1}FW) was observed in the interaction of seed inoculation with biofertilizer × foliar application of 2 g L^{-1} $ZnSO_4$. On the other hand, the lowest content of chlorophyll *a* was obtained from the control treatment (no biofertilizer inoculation and no foliar application of $ZnSO_4$), the plants treated with biofertilizer 4 weeks after plant emergence × no application of $ZnSO_4$, and the plants treated with biofertilizer 4 weeks after plant emergence × 3 g L^{-1} $ZnSO_4$. The content of chlorophyll a for these treatments were equal to 0.33, 0.34, and 0.36 mg g^{-1} FW, respectively (Fig. 1).

Also, based on the results of Fig. 2, it was observed that the highest content of chlorophyll b (0.34 mg g^{-1} FW) was achieved in the seeds inoculated with biofertilizer × 2 g L^{-1} $ZnSO_4$. Furthermore, the lowest content of chlorophyll b (0.08 mg g^{-1} FW) was found in the plants inoculated with biofertilizer 4 weeks after plant emergence × no application of $ZnSO_4$. The next lowest chloro-

phyll b content (0.12 mg g^{-1} FW) was observed in two treatments including the control treatment and the interaction of seed inoculation with biofertilizer × no application of $ZnSO_4$ (Fig. 2).

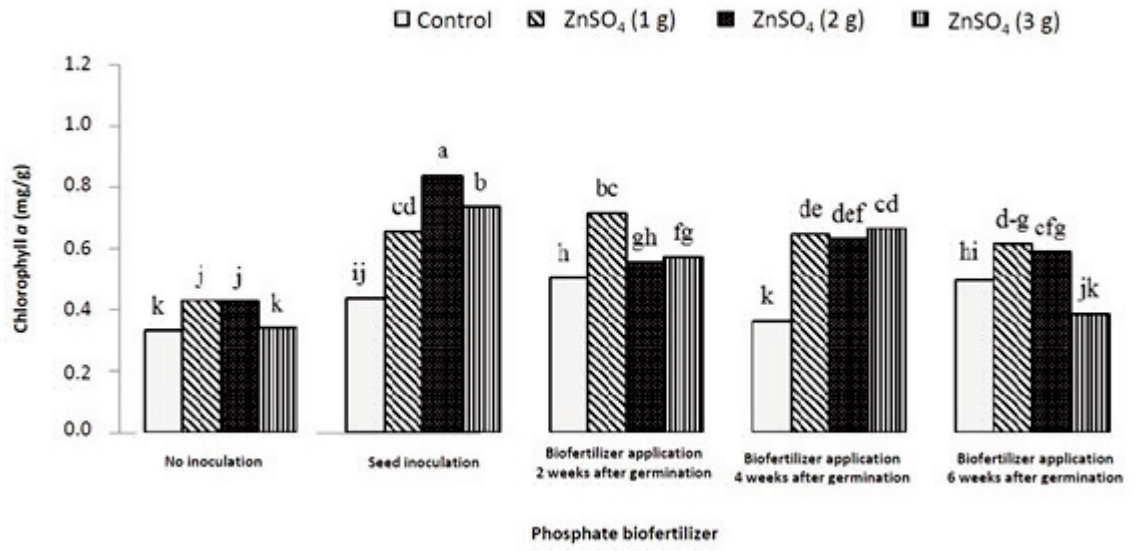

Fig. 1. Mean comparison of the effect of biofertilizer × $ZnSO_4$ interaction on content of chlorophyll a. In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

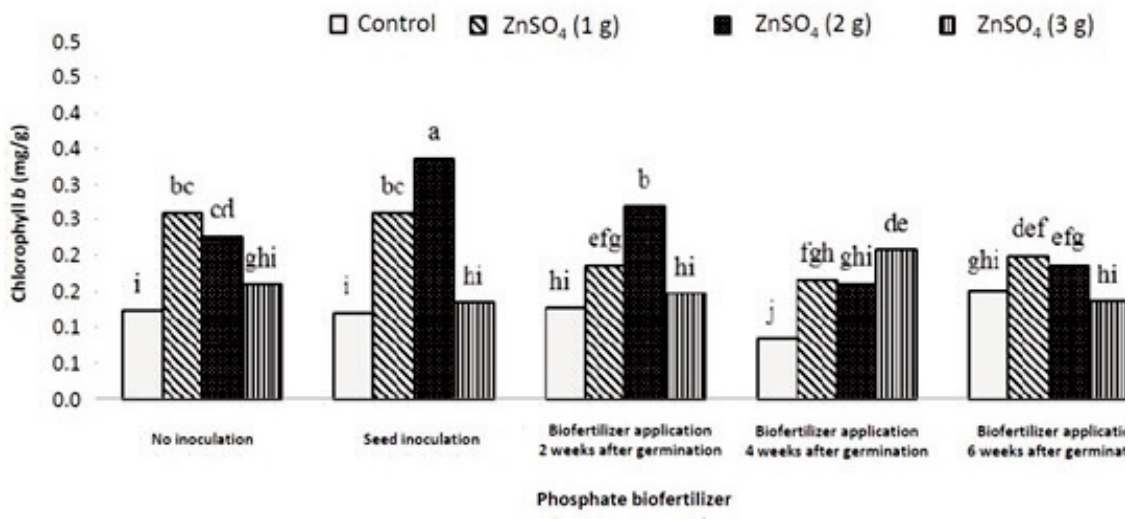

Fig. 2. Means comparison of the effect of biofertilizer × ZnSO4 interaction on chlorophyll b content. In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

On the other hand, seed inoculation with biofertilizer supplemented with 2 mg L^{-1} $ZnSO_4$ resulted in the highest total chlorophyll content (1.17 mg g^{-1}) whereas the lowest ones were 0.45, 0.46 and 0.50 mg g^{-1} FW obtained from the plants treated with biofertilizer 4 weeks after plant emergence × no application of $ZnSO_4$, control (no biofertilizer inoculation and no $ZnSO_4$ application), and 3 g L^{-1} $ZnSO_4$ without inoculation with biofertilizer, respectively (Fig. 3).

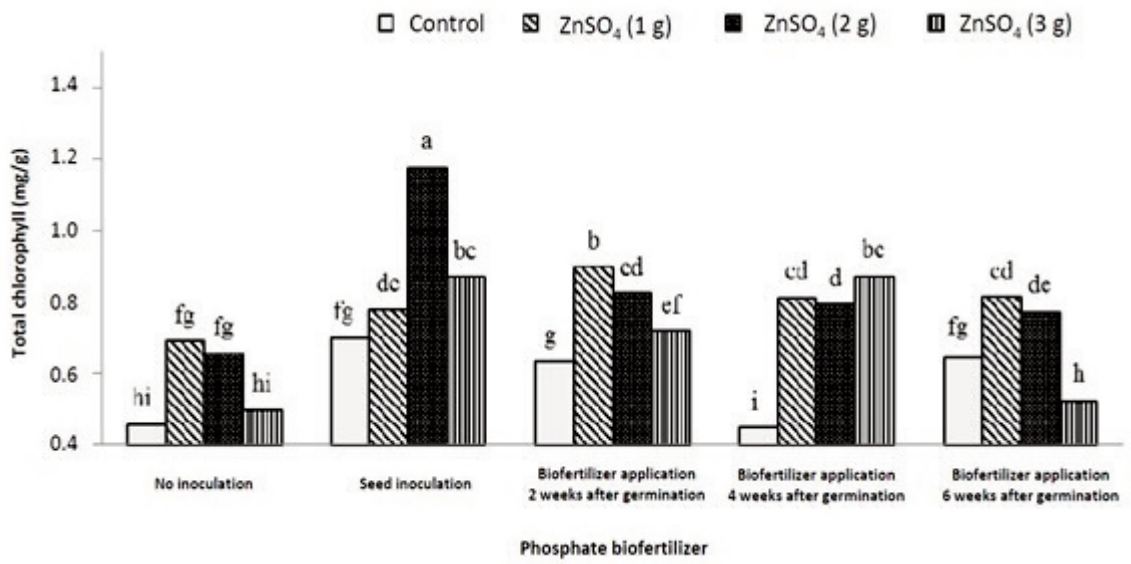

Fig. 3. Means comparison of the effect of biofertilizer × $ZnSO_4$ interaction on total chlorophyll content. In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

The highest content of anthocyanin (0.05 mM g^{-1}) was observed in the treatments of biofertilizer application 2 weeks after plant emergence × 1 g L^{-1} $ZnSO_4$, biofertilizer application 4 weeks after plant emergence × 1 g L^{-1} $ZnSO_4$, seed inoculation with biofertilizer × 2 g L^{-1} $ZnSO_4$, and 1 g L^{-1} $ZnSO_4$ without seed inoculation. Furthermore, the lowest anthocyanin content (0.03 mM g^{-1}) was observed in the plants treated with biofertilizer 6 weeks after plant emergence × no application of $ZnSO_4$, as well as in the plants treated with biofertilizer 6 weeks after plant emergence × foliar application of 3 g L^{-1} $ZnSO_4$. However, some treatments did not differ significantly in Duncan's test at $P < 0.05$ (Fig. 4).

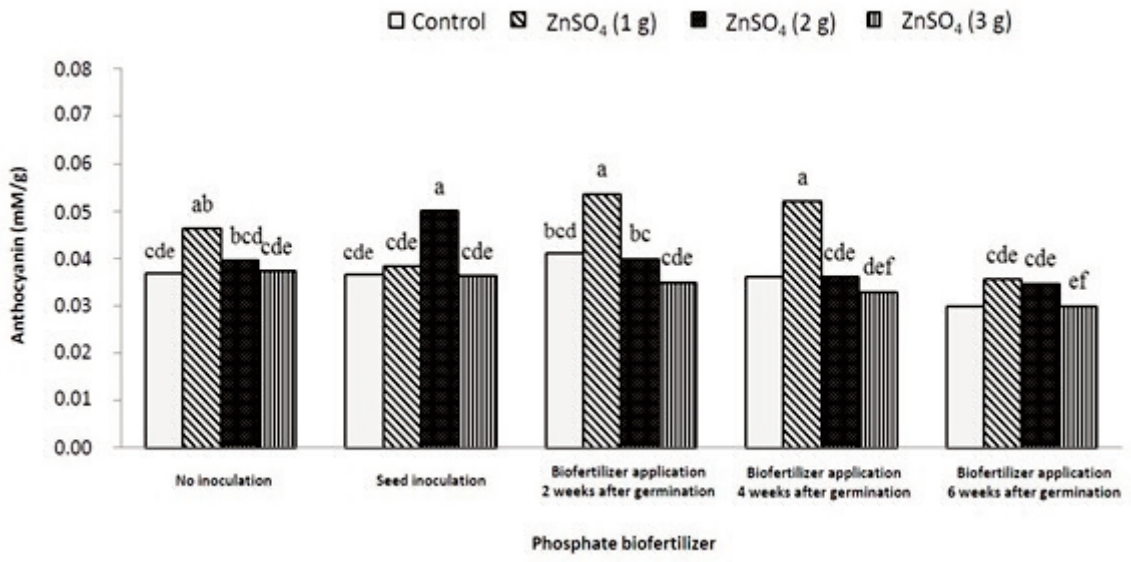

Fig. 4. Means comparison of the effect of biofertilizer × $ZnSO_4$ interaction on anthocyanin content. In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Content of minerals

The results of Table 5 showed that the uptake of N, P, K, and Zn elements was significantly ($P<0.01$) affected by the application of biofertilizer, $ZnSO_4$, and their interaction.

Table 5. Analysis of variance of nutrients content of marigolds as influenced by different treatments.

S.o.V	df	MS			
		Nitrogen	Phosphorous	Potassium	Zinc
Replication	2	0.0003^{ns}	0.0002^{ns}	0.0001^{ns}	0.003^{ns}
Barvar 2 (B)	4	0.10^{**}	0.007^{**}	0.005^{**}	0.240^{**}
$ZnSO_4$ (Z)	3	0.26^{**}	0.001^{**}	0.006^{**}	0.472^{**}
B × Z	12	0.53^{**}	0.001^{**}	0.012^{**}	0.002^{**}
Error	38	0.01	0.0002	0.0002	0.0004
CV (%)		6.67	9.16	6.23	3.12

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant respectively.

The results showed that the highest amount of N uptake (2.51%) was obtained in the plants treated with the application of biofertilizer 2 weeks after plant emergence × 1 g l^{-1} $ZnSO_4$. Besides, The lowest amount of N uptake (0.97%) was achieved in plants treated with the application of biofertilizer 6 weeks after plant emergence × control treatment of $ZnSO_4$ (Fig. 5).

The application of biofertilizer 4 and 6 weeks after plant emergence supplemented with 2 and 1 g L^{-1} $ZnSO_4$ resulted in the highest P contents of 0.21, 0.21, 0.21, and 0.20%, respectively, whilst the lowest P contents, ranged in 0.12-0.15%, was observed in the control plants and the plants treated with different levels of biofertilizer supplemented with 3 g L^{-1} $ZnSO_4$ (Fig. 6).

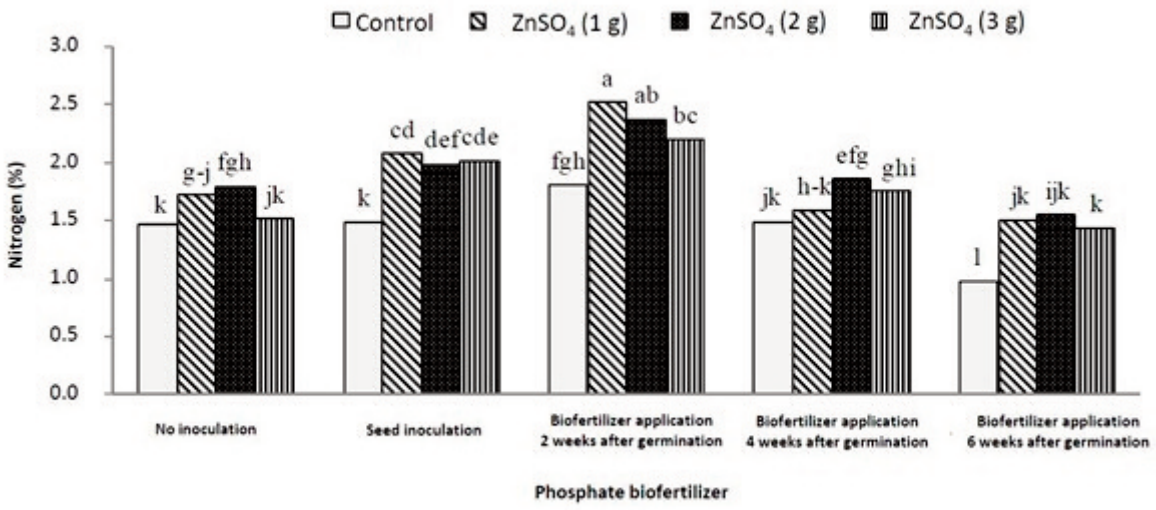

Fig. 5. Means comparison of the effect of biofertilizer × $ZnSO_4$ interaction on uptake of N%. In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

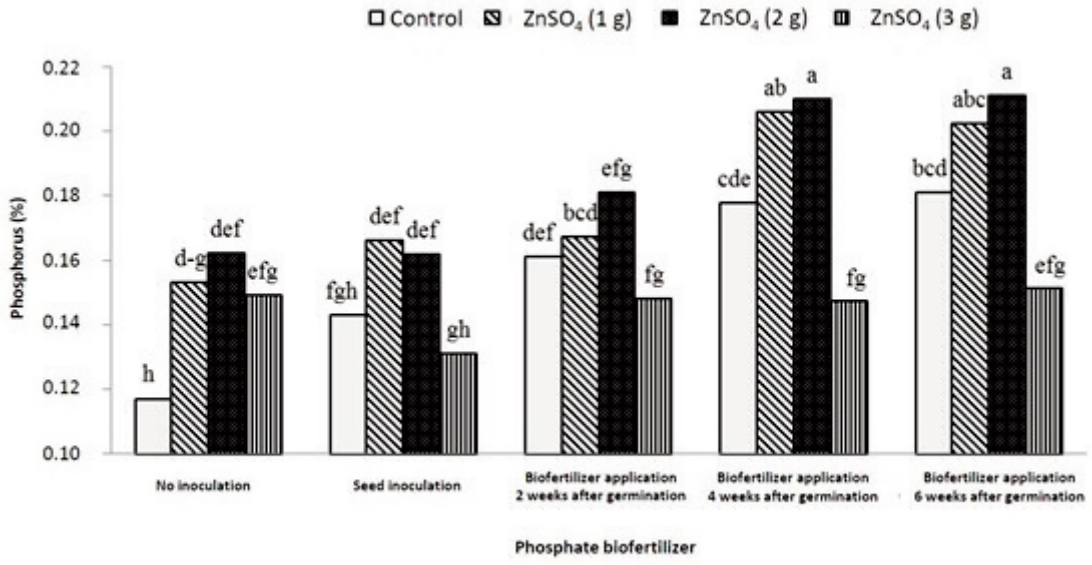

Fig. 6. Means comparison of the effect of biofertilizer × $ZnSO_4$ interaction on uptake of P content. In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Seed inoculation with biofertilizer × 2 mg L^{-1} $ZnSO_4$ and biofertilizer application 2 weeks after plant emergence × 2 g L^{-1} $ZnSO_4$ resulted in the highest K content of 0.33%. But, the lowest one (0.13%) was observed in the plants treated with biofertilizer 6 weeks after plant emergence × 3 g L^{-1} $ZnSO_4$ (Fig. 7).

On the other hand, the highest amount of Zn (97 g per 100 g) was recorded by the plants treated with biofertilizer 6 weeks after plant emergence × 3 g L^{-1} $ZnSO_4$ and the lowest one (0.19 g per 100 g) in the control plants (no biofertilizer inoculation and no $ZnSO_4$ application). However, some treatments did not differ significantly ($P < 0.05$) in Duncan's test (Fig. 8).

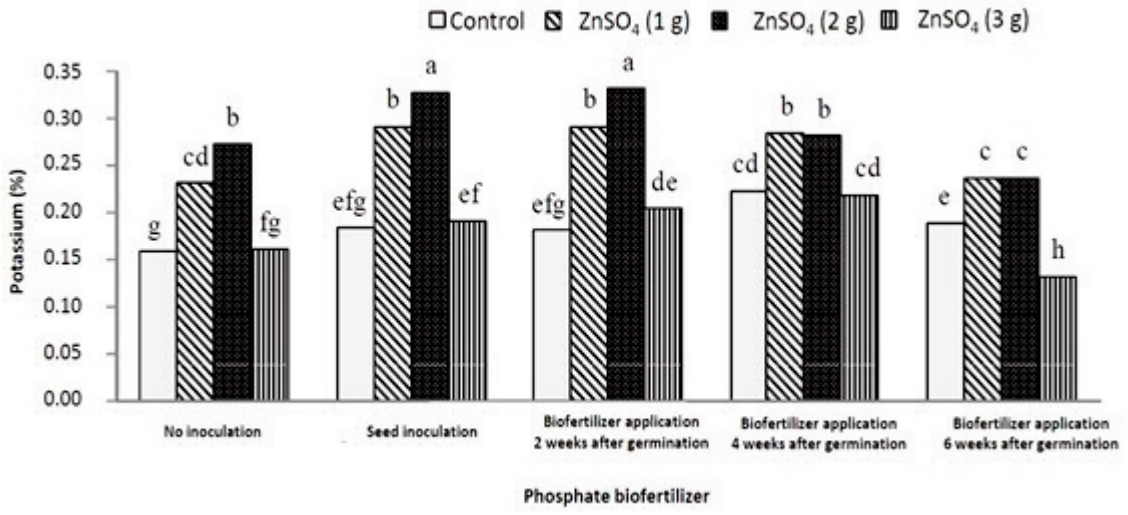

Fig. 7. Means comparison of the effect of biofertilizer × $ZnSO_4$ interaction on uptake of K. In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

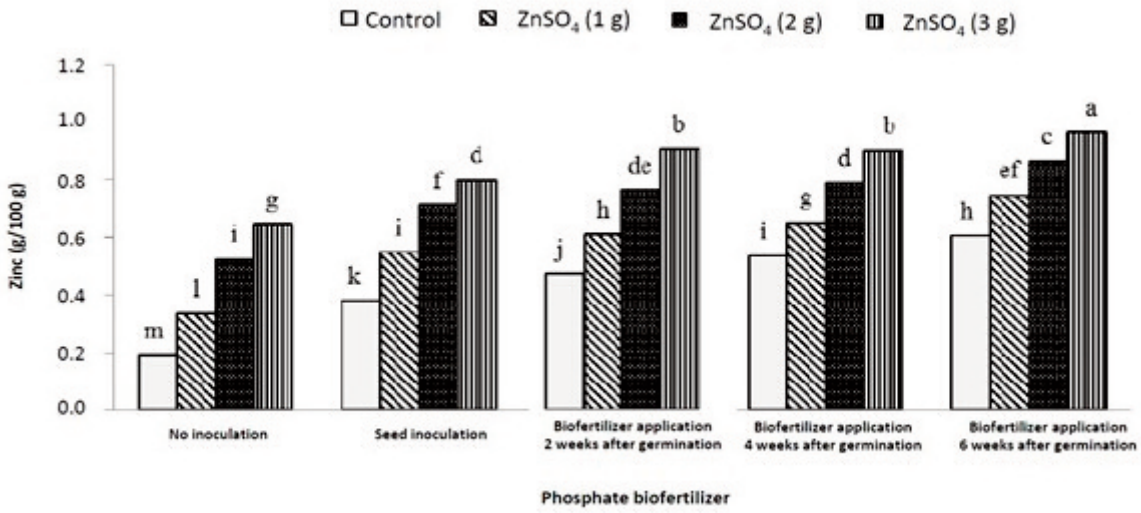

Fig. 8. Means comparison of the effect of biofertilizer × $ZnSO_4$ interaction on uptake of Zn. In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

DISCUSSION

In the studied treatments, an interesting response was obtained in the interaction of biofertilizer (phosphate solubilizing bacteria) × foliar application of $ZnSO_4$. Our findings showed that the utilization of 1 and 2 g L^{-1} of $ZnSO_4$, followed by the application of biofertilizer through seed inoculation and the application of biofertilizer 2 and 4 weeks after plant emergence, significantly increased plant height, fresh weight of flower, fresh weight of flower, nitrogen, phosphor, zinc, and potassium content in marigold plants (Table 3 and Figs. 5-8). Aboutalebian and Khodabandehloo (2017) investigated the effects of application methods of phosphor and $ZnSO_4$ on corn plant and showed that both zinc and phosphor contents were increased in corn grains.

The relationship between Zn application and the increase in plant growth indices such as internode length and plant height can be attributed to the role of Zn in the biosynthesis of the plant growth regulators, energy production (Mousavi, 2011), nitrogen metabolism, and nitrogen uptake (Potarzycki and Grzebisz, 2009). Also, Zn is involved in the conversion of the amino acid tryptophan to indole-3-acetic acid. Therefore, an increase in Zn enhances auxin in plants, thereby enhancing plant height through stimulating plant growth and shoot elongation (Zare Dehabadi *et al*., 2008). Our results about the effect of Zn on plant height improvement is consistent with Zare Dehabadi *et al*. (2008)'s results for spearmint.

The effectiveness of phosphate biofertilizers on the improvement of growth parameters, such as plant height, may be attributed to the synthesis and release of growth stimulators like plant growth regulators including auxins, cytokinins, gibberellins, amino acids, antibiotics, hydrogen cyanide, and siderophores. Gibberellins induce cell elongation, especially stem internode elongation, and auxins stimulate cell division. By this explanation, we can account for the increase in internode length and plant height (Desbrosses and Stougaard, 2011; Hadi *et al*., 2007).

On the one hand, indole compounds, e.g. indole-3-acetic acid, indole-3-pyruvic acid, and indole-3-acetamide, are ramped up in the soil when inoculated with growth-stimulating bacteria. Baha and Bekki (2015) stated that indole-3-lactic acid produced in anaerobic conditions, its synthesis was increased from tryptophan metabolism, and thus this could affect the growth indices of the plants (Baha and Bekki, 2015). Our results as to the effect of biofertilizers and $ZnSO_4$ on the increase in plant height are in agreement with Raesee *et al*. (2015)'s study on cumin.

On the other hand, the improved yield of flower fresh and dry weight induced by P-fixing biological fertilizers may be related to the increased activity of biofertilizers and the production of growth-stimulating hormones by bacteria, which enhances net photosynthesis rate and so, flower fresh and dry weight (Ahmed *et al*., 2012). Also, the increase in the uptake of ions like NO^{3-}, NH^{4+}, PO_4^{3-} and K^+ due to the presence of P supplying bacteria can be the main cause of shoot dry weight increase (Jay *et al*., 2013; Anna *et al*., 2013). P is used in the structure of NADP that acts as an electron carrier and supplies the energy required for the reduction of carbonic gas. Consequently, these reactions increase the synthesis of such nutrients as carbohydrates, proteins, and fats, resulting in a higher dry matter percent in plants (Li *et* al., 1998; Carstensen *et al*., 2018; Xiao *et al*., 2018).

It was found that chlorophyll pigments were bolstered when the seeds were inoculated with the biofertilizer and were fertilized with 2 g L^{-1} $ZnSO_4$. Chlorophyll and photosynthesizing pigments are important factors underpinning a plant's photosynthesis capacity because they influence photosynthesis rate indirectly which, in turn, affects biomass production (Mudgal *et al*., 2009). The buildup of chlorophyll pigments in plants treated with the biofertilizer might be related to the increased uptake of minerals by the plants and the release of plant growth regulators by microorganisms occurring in the biofertilizer that change the biochemical compositions of the plants (Jenschke *et al*., 2000). Sawan *et al*. (2008) stated that phosphorus and zinc are also necessary nutrients for the biosynthesis of pigments and cell division. Therefore, the increase in chlorophyll content and anthocyanin will be likely to be affected by the application of zinc and phosphorus due to the activity of these elements in pigment biosynthesis and cell division.

Also, there is a relationship between Zn availability and the generation of the enzyme carbonic anhydrase. This enzyme plays a key role in photosynthesis and increases the production of carbohydrates, thereby affecting plant pigments. On the other hand, Zn is essential for triggering antioxidant enzymes like ascorbate peroxidase and glutathione reductase to protect chlorophyll from degradation by active oxygen radicals (Yassen *et al*., 2010). Our results regarding the effect of biofertilizer on improving pigments support Han and Lee (2005)'s results about lettuce.

On the other hand, we observed that 3 g L^{-1} $ZnSO_4$ resulted in the loss of plant pigments for which the reduction of chlorophyll synthesis and its degradation can be implicated. It has been documented that high concentrations of Zn impair photosynthesis and photosynthesizing pigments considerably (Subba *et al*., 2014). This can be attributed to the fact that heavy metals directly destruct cell structure and entail oxidative stress, resulting in the loss of growth, the reduction of chlorophyll contents and photosynthesis, the inhibition of enzyme activities, and damage to biomolecules like lipids, proteins, and nucleic acids, especially DNA (Ahmed *et al*., 2012; Nagajyoti *et al*., 2010).

According to the results, the application of phosphate biofertilizer and $ZnSO_4$ influenced anthocyanin content of marigold plants. The improvement in $ZnSO_4$-treated marigold plants' phytochemical properties can be ascribed to more photosynthesis due to the increase in chlorophyll content, higher activity of phosphoenolpyruvate carboxykinase and ribulose bisphosphate, and higher P and N content that positively influence Fe and Mn contents and improve other metabolic activities of the plant (Mudgal *et al*., 2009). Also, biofertilizers provide an appropriate amount of P, thereby contributing to the enhancement of vegetative growth and plant chlorophyll content and the increase in the secondary metabolites as the byproducts of photosynthesis. Therefore, marigold plants inoculated with biofertilizer exhibited a higher level of the generation of secondary metabolites, including pigments. In fact, it can be said that bacteria provide a higher amount of water and nutrients for plant in an optimal manner, increase the generation of pigments, and facilitate the mobilization of water and photosynthates within the plants (Ahmed *et al*., 2012).

In addition, the nutrient content of marigolds responded to the method of biofertilizer application and different rates of $ZnSO_4$. The highest N, P, and K contents were observed in the

plants treated with 1 or 2 g L^{-1} $ZnSO_4$ and biofertilizer 2 weeks after plant emergence and seed inoculation with biofertilizer. The plants that were treated with 3 g L^{-1} $ZnSO_4$ as well as biofertilizer 6 weeks after plant emergence had the highest amount of Zn. With respect to the effect of phosphate-solubilizing bacteria, it can said that by exuding organic acids, e.g. oxalic and citric acids, reducing soil acidity, and releasing phosphatase, these bacteria help the development of plant root systems and thereby improve organic P availability, P uptake efficiency in soil, and its uptake in plant parts. On the other hand, by synthesizing and releasing organic acids like formic acid and some other organic acids in soils, these bacteria dissolve insoluble P resulting in a higher rate of P uptake by plants inoculated with phosphate-solubilizing bacteria (Eftekhari *et al*., 2012).

Furthermore, the application of biofertilizers increases the uptake of micronutrients through root development and access to more volume of soil. In fact, bacteria produce plant hormones and thereby stimulate root branching, increase stem and root biomass, and induce the reproductive cycle of the plant. Thus, the increased penetrability of root and the increased uptake of minerals allow the increase in nutrient concentration in plants (Bashan and de-Bashan, 2010; Violante *et al*., 2002).

The mobilization of cations can be essentially facilitated by carboxylic amino acids and/or organic acids. Biofertilizers are capable of building vitamins B_1, B_2, B_6, and B_{12}, pantothenic acid, nicotinic acid, and organic acids like malic acid and citric acid (Talaat *et al*., 2015). The increased level of nutrients mobilization to the shoot of the plants inoculated with the biofertilizers can be attributed to the likely effect of hormones like cytokinin and also, the effect of siderophore as plant growth stimulator that, in addition to stimulating root growth, facilitates the mobilization of essential ions to the shoot (Kang *et al*., 2014; Rakshapal *et al*., 2013). Furthermore, phosphate-solubilizing bacteria can build chelate and form complexes with metal cations, thereby reducing their concentration in soil solution and increasing their release from minerals (Yadar *et al*., 2011). Our results regarding the increased level of minerals under the effect of biofertilizer are in agreement with Eftekhari *et al*. (2012) and Kang *et al*. (2014).

CONCLUSION

Since soils in most parts of Iran are calcareous with high pH and low organic matter, they are likely to suffer from Zn deficiency. In this type of soils, the solubility of micronutrient is limited and causes the decline in the uptake of these elements. Hence, the demand of plants for these elements is increasing. The results of the present study show that optimal management of plant nutrients can considerably influence their morphological and biochemical traits. Therefore, given the importance of curbing chemical fertilizer use and finding an appropriate approach to replacing them with biological sources, it can be recommended to consider the supply of nutrients by phosphate solubilizing biofertilizer.

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How to cite this article:
Jalali, F. and Naderi, D. 2019. Study of morphological and biochemical traits of marigold as influenced by Phosphorous biofertilizer and Zinc. *Journal of Ornamental Plants,* 9(4), 275-290.
URL: http://jornamental.iaurasht.ac.ir/article_669732_f53b094ecf9af9b0e1d64b1f947ca098.pdf