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Influence of phosphate bio-fertilizer on quantity and quality features of marigold (*Tagetes erecta* L.)

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Abstract

The present investigation was conducted to study the effect of bio-fertilizer (Barvar-2) and phosphorus on quantity and quality characteristics of marigold (*Tagetes erecta* L.). A factorial experiment based on randomized complete blocks design (RCBD) with four replications was carried out with two factors including methods of bio-fertilizer (Barvar-2) application (without bio-fertilizer inoculation, seed inoculation, root inoculation and seed plus root inoculation), and different levels of chemical phosphorus (100, 200, 300 and 400 mg l^{-1}). In the present study, plant height, the number of leaf per plant, shoot fresh weight, shoot dry matter percentage, the content of total phosphorus of cultivation media and shoot and flower diameter were measured. Results revealed that the application of bio-fertilizer and phosphorus was significant on most characters in 1% probability level. The interaction effect of bio-fertilizer and phosphorus was not significant on studied characters except for total shoot phosphorus and the number of leaf per plant. The best plant height (26.87 cm), the number of leaf per plant (56.27), flower diameter (84.420 mm), shoot fresh weight (19.94 g) and total shoot phosphorus (0.353%) was obtained under inoculation of seeds and roots with bio-fertilizer × 400 mg l^{-1} phosphorus. Maximum of shoot dry matter (19.86%) and total medium phosphorus (0.235%) was calculated in treatments of 200 mg l^{-1} phosphorus without bio-fertilizer inoculation and 200 mg l^{-1} phosphorus with seed inoculation, respectively.

Keywords: Marigold, bio-phosphate, growth response, ornamental plants.

Introduction

Marigold (*Tagetes erecta* L.) (Asteraceae) is a medicinal and ornamental plant. It is used for its nematocide, cosmetic and medicinal properties. The essential oil of the flower contains antioxidants (Pérez Gutiérrez et al., 2006). Growth of marigold (*Tagetes erecta* L.) (Asteraceae) is influenced by chemical fertilizers, particularly phosphate fertilizers. Since, application of these fertilizers increase the soil and water pollution and accumulation of some heavy metals such as cadmium, they can threat the human health. Moreover, the long-term use of chemical fertilizers tends to the soil structure degradation (Singh et al., 2008). Nowadays, attention to biological fertilizer has been increased due to price of chemical fertilizers and attention to sustainable agricultural systems (Ehteshami et al., 2007). Bio-fertilizers containing beneficial bacteria and fungi improve soil chemical and biological characteristics, phosphate solutions and agricultural production (El-Habbasha et al., 2007; Yosefi et al., 2011). Some bacteria provide plants with growth promoting substances and play major role in phosphate solubilizing (Abou-Aly et al., 2006). Bio-fertilizers have improved quantity and quality features of some plants (Ratti et al., 2001; Ojaghloo et al., 2007; Mubassara et al., 2008; Yosefi et al., 2011). Bio-fertilizers comprised of nitrogen fixers, phosphate dissolvers and available potassium (Ezz et al., 2011). It is necessary to use phosphate solubilizing microorganisms to change insoluble phosphorus into soluble form. An advantageous of phosphate solubilizing microorganisms is related to their propagation rate that can relatively remove the plant requirements to phosphorus at the root region (Sharma, 2002). Belimov et al. (1995) demonstrated that, inoculation of soil with bacterial mixtures caused a more balance nutrition for plants and improvement in root uptake of nitrogen and phosphorus in a main mechanism of interaction between phosphate solubilizing and bacteria nitrogen fixing. Ratti et al. (2001) investigated effect of some varieties of phosphate solubilizing bacteria on the yield of Lemon Grass and concluded that the plant height and biomass increased compared to the control condition. Hazarika et al. (2000) reported that the use of phosphate solubilizing bacteria significantly increased the height of tea plant. Gupta et al. (2002) found that inoculation of mint root by mycorrhyza fungi has considerably increased the height and yield of plant. In another research, Kapoor et al. (2004) showed that fennel root symbiosis with two species of VAM fungi significantly increased the flowering, 1000 seeds weight, dry matter and seed yield. Kapoor et al. (2002) reported that the inoculation of root with a kind of phosphate solubilizing bacteria enhanced the shoot dry matter. The present investigation was done in order to evaluate the effect of different treatments methods of phosphate biological fertilizer (Barvar-2) and phosphate chemical fertilizer on some quantity and quality features of marigold (*Tagetes erecta* L.).

Results and Discussion

Effect of phosphate bio-fertilizer on final yield

Results demonstrated that the different methods of bio-fertilizer application had positive effect on most characters except for dry matter percentage of aerial parts. Inoulation of seeds and transplant roots with bio-fertilizer had maximum effect on most characters. Moreover, the effect of different levels of pure chemical phosphorus on most characters was significant (Tables 2 and 3). Overall results showed that bio-fertilizer application is effective on quality and quantity traits of *Tagetes erecta* L., whether alone or with chemical fertilizer.

Effect of phosphate bio-fertilizer on plant height

Results showed that plant height was significantly ($p \leq 0.01$) affected by biological phosphate fertilizer (Barvar-2) and pure chemical phosphorus (Table 2). The mean comparison of data in different treatments (Table 3) showed that the highest plant height (25.59 cm) was achieved by application of bio-fertilizer on seeds and transplant roots along with 400 mg l^{-1} chemical phosphate. The lowest plant height (21.68 cm) was obtained in control treatment (100 mg l^{-1} chemical phosphate without application of bio-fertilizer) (Table 3). Among the bio-fertilizer treatments, the highest (25.59 cm) and lowest (24.00 cm) plant height were obtained by application of bio-fertilizer on seeds and transplant roots, and control, respectively (Fig. 1). Also, among chemical phosphate treatments, the highest (25.55 cm) and lowest (23.06 cm) plant height were recorded by application of 400 and 100 mg l^{-1} phosphate, respectively (Fig. 2).

The obtained data are in general agreement with those reported by Shaalan (2005), Singh et al. (2008) and Ezz et al. (2011). Bio-fertilizers increase root uptake through root development (Yosefi et al., 2011). Furthermore, application of bio-fertilizers increases the plant height by promoting the plant growth regulators (Senthil-Kumar et al., 2009). Studies of Ezz et al. (2011) on banana (*Musa* spp.) showed that the using of phosphorus fertilization and/or effective microorganisms as a bio-fertilizer increased all studied vegetative growth characters including plant height. Singh et al. (2008) revealed the positive effect of bio-fertilizer on the plant height of *Calendula officinalis* L. Similar observations have been reported by Chandrikapure et al. (1999) in marigold. Abou El-Yazeid and Abou-Aly (2011) showed the positive effect of phosphate solubilizing microorganisms on the most plant growth parameters of tomato. The effect of phosphate solubilizing bacteria on growth may be due to the activity of phosphate solubilization caused by the strain and increased further mineral availability uptake.

Moreover, some researchers showed that the increase in growth characters might be due to the fact that phosphate solubilizing bacteria inoculated plants were able to absorb nutrients from solution at faster rates than un-inoculated plants resulting in accumulation of more N, P and K in the leaves (Rai, 2006; Premsekhar and Rajashree, 2009; El-Tantawy and Mohamed, 2009; Castagno et al., 2011; Saharan and Nehra, 2011). Studies of El-Ghandour et al. (2009) demonstrated that growth parameters of *Majorana hortensis* L. were positively affected by bacterial inoculation as well as organic phosphorus sources. Abo-Baker and Mostafa (2011) showed that the inoculation of *Hibiscus sabdariffa* with the mixture of bio-fertilizers combined with 50 or 100% chemical fertilizer improved the growth characters. Similar results were observed on some plants such as *Nigella sativa*, *Ammi visnaga* and *Salvia officinalis* (Yuonis et al., 2004; Shaalan, 2005; Abd El-Latif, 2006).

Effect of phosphate bio-fertilizer on the number of leaf per plant

Table 2 shows that the number of leaf per plant was significantly ($p \leq 0.01$) affected by biological phosphate fertilizer and pure chemical phosphorus. The mean comparison of data in different treatments (Table 3) showed that maximum number of leaf per plant (56.27) was determined by application of bio-fertilizer on seeds and transplant roots along with 400 mg l^{-1} chemical phosphate (Fig. 3). Minimum number of leaf per plant (25.70) was obtained in control plants (100 mg l^{-1} chemical phosphate without application of bio-fertilizer) (Table 3). Among bio-fertilizer treatments, maximum (39.06) and minimum (29.32) number of leaf per plant were obtained by application of bio-fertilizer on seeds and transplant roots and control, respectively (Fig. 4). Also, among chemical phosphate treatments, maximum (44.58) and minimum (27.52) number of leaf per plant were recorded by application of 400 mg l^{-1} phosphate and 100 mg l^{-1} phosphate, respectively (Fig. 5). Our findings are in agreement with those reported by some other researchers. Singh et al. (2008) showed that maximum number of leaf per plant in *Calendula officinalis* L. was obtained under application of bio-fertilizer along with 75% of chemical fertilizer. Studies of Abou El-Yazeid and Abou-Aly (2011) demonstrated that the number of leaves had significantly higher value under application of phosphate solubilizing microorganisms combined with rock phosphate treatments compared to control. This could be attributed to the highest values of available P compared to other treatments.

Effect of phosphate bio-fertilizer on flower diameter

Our findings revealed that flower diameter was significantly ($p \leq 0.01$) affected by biological phosphate fertilizer and pure chemical phosphorus (Table 2). The mean comparison of data in different treatments (Table 3) showed that the highest flower diameter (84.420 mm) was determined by application of bio-fertilizer on seeds and transplant roots along with 400 mg l^{-1} chemical phosphate. The lowest flower diameter (39.033 mm) was obtained in treatment of 100 mg l^{-1} chemical phosphate when seeds inoculated by bio-fertilizer (Table 3).

Among bio-fertilizer treatments, the highest (68.871 mm) and lowest (57.118 mm) flower diameter were obtained by application of bio-fertilizer on seeds and transplant roots and control, respectively (Fig. 6). Also, among chemical phosphate treatments, the highest (76.229 mm) and lowest (48.761 mm) flower diameter were determined by application of 400 and 100 mg l^{-1} phosphate, respectively (Fig. 7). The

Table 1. Physicochemical properties of the media used for seeds and roots culture of marigold (*Tagetes erecta* L.).

Properties	Medium (cocopeat plus sand) for plant seeds	Medium (cocopeat, sand and mold) for plant roots
pH	7.02	6.7
Available K (mg/kg)	51.2	340.0
Available P (mg/kg)	25.1	59.0
Total N (%)	2.7	4.3
Organic Carbon (%)	1.0	7.8

Table 2. Analysis of variance (ANOVA) of the effect of different application methods of bio-fertilizer and different levels of chemical phosphorus on plant height, the number of leaf per plant, shoot fresh weight, shoot dry matter percentage, the content of total phosphorus of cultivation media and shoot and flower diameter of marigold (*Tagetes erecta* L.).

Treatments	Plant height	Number of leaf in plant	Flower diameter	Shoot fresh weight	Shoot dry matter	Total shoot phosphorus	Total medium phosphorus
Application Methods	6.115^{**}	309.789^{**}	508.551^{**}	109.276^{**}	3.202^{ns}	0.120^{*}	4435.44^{**}
Phosphorus Levels	14.283^{**}	688.879^{**}	1667.259^{**}	187.480^{**}	32.946^{**}	0.220^{**}	372.917^{ns}
Methods × Phosphorus	1.033^{ns}	63.122^{**}	24.771^{ns}	2.39^{ns}	1.772^{ns}	0.230^{**}	667.361^{ns}
Error	1.384	5.157	20.122	1.328	6.274	0.003	348.958
Total	22.815	1066.947	2220.703	300.482	44.197	0.573	5824.706
CV (%)	6.36	26.3	19.95	38.08	14.99	2.770	13.45

**: Significant at $\alpha = 1\%$, *: Significant at $\alpha = 5\%$, ns= Not significant

Table 3. Mean comparison of the effect of different application methods of bio-fertilizer and different levels of chemical phosphorus on plant height, the number of leaf per plant, shoot fresh weight, shoot dry matter percentage, the content of total phosphorus of cultivation media and shoot and flower diameter of marigold (*Tagetes erecta* L.).

Treatments	Plant height (cm)	Number of leaf in plant	Flower diameter(mm)	Shoot fresh weight(g)	Shoot dry matter (%)	Total shoot phosphorus (%)	Total medium phosphorus (%)
M_1	24.16b	29.71b	57.378b	9.33b	18.12a	0.343a	0.187bc
M_2	24.60ab	37.40a	68.150a	14.10a	17.64a	0.344a	0.212a
M_3	25.59a	39.06a	68.871a	14.78a	17.52a	0.340ab	0.202ab
M_4	24.00b	29.32b	57.118b	9.13b	16.87a	0.337b	0.168bc
P_1	23.06b	27.52c	48.761d	7.37d	15.25b	0.339b	0.190a
P_2	24.58a	29.73c	58.849c	10.12c	19.12a	0.341ab	0.196a
P_3	25.15a	33.64b	67.678b	13.35b	17.55ab	0.337b	0.198a
P_4	25.55a	44.58a	76.229a	16.49a	18.23a	0.347a	0.186a
M_1P_1	22.77a	26.53e	39.033a	6.05a	14.82a	0.344bcd	0.180a
M_1P_2	24.73a	28.20de	52.603a	7.83a	19.86a	0.332de	0.180a
M_1P_3	24.40a	28.93de	65.130a	10.16a	18.64a	0.336cde	0.190a
M_1P_4	24.73a	33.60bcd	71.703a	12.50a	19.16a	0.352a	0.200a
M_2P_1	23.27a	29.13de	55.780a	8.65a	15.61a	0.336cde	0.220a
M_2P_2	24.47a	30.67cde	62.537a	11.79a	18.93a	0.348abc	0.235a
M_2P_3	25.47a	36.93b	72.373a	16.40a	18.19a	0.348abc	0.210a
M_2P_4	25.20a	52.87a	81.910a	19.53a	17.85a	0.344bcd	0.185a
M_3P_1	24.53a	28.73de	55.777a	9.81a	16.54a	0.340bcde	0.200a
M_3P_2	25.47a	33.47bcd	65.417a	13.34a	18.96a	0.340bcde	0.205a
M_3P_3	25.48a	37.77b	69.870a	16.02a	16.68a	0.328e	0.220a
M_3P_4	26.87a	56.27a	84.420a	19.94a	17.88a	0.353a	0.185a
M_4P_1	21.68a	25.70e	44.453a	4.96a	14.01a	0.336cde	0.160a
M_4P_2	23.65a	26.60e	54.840a	7.53a	18.72a	0.344bcd	0.165a
M_4P_3	25.27a	30.93cde	63.337a	10.83a	16.70a	0.336cde	0.173a
M_4P_4	25.40a	35.60bc	66.883a	14.01a	18.04a	0.332de	0.175a

*In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan's test. M_1: Seeds inoculation to bio-fertilizer, M_2: Transplant roots inoculation to bio-fertilizer, M_3: Seeds and transplant roots inoculation to bio-fertilizer, and M_4: Control (without bio-fertilizer). P_1-P_4: Different levels of phosphorus; P_1: 100 mg l^{-1}, P_2: 200 mg l^{-1}, P_3: 300 mg l^{-1}, and P_4: 400 mg l^{-1}.

obtained data are in general agreement with those reported by Fallahi et al. (2009) in *Matricaria chamomilla*, Khalaj et al. (2009) in *Polianthes tuberose* and Dehghani Meshkani et al. (2011) in *Matricaria recutita*.

Effect of phosphate bio-fertilizer on shoot fresh weight

The effect of biological phosphate fertilizer and pure chemical phosphorus on shoot fresh weight was significant ($p \leq 0.01$) (Table 2). The mean comparison of data in different treatments (Table 3) showed that the highest shoot fresh weight (19.94 g) was achieved by application of bio-fertilizer on seeds and transplant roots along with 400 mg l^{-1} chemical phosphate. The lowest shoot fresh weight (4.96 g) was obtained in control plants (100 mg l^{-1} chemical phosphate without application of bio-fertilizer) (Table 3). Among bio-fertilizer treatments, the highest (14.78 g) and lowest (9.13 g) shoot fresh weight were obtained by application of bio-fertilizer on seeds and transplant roots and control, respectively (Fig. 8).

Among chemical phosphate treatments, the highest (16.49 g) and lowest (7.37 g) shoot fresh weight were scored by application of 400 and 100 mg l^{-1} phosphate, respectively (Fig. 9). Investigations of Sanchez Govin et al. (2005) on *Calendula officinalis* and *Matricaria recutata*, Mahfuz and Sharafeldin (2007) in *Foeniculum vulgare* Mill., Abou El-Yazeid et al. (2007) in *Cucurbita*, and Dehghani Meshkani et al. (2011) in *Matricaria recutita* confirm our findings. Study of Ezz et al. (2011) on banana (*Musa* spp.) showed that the using of phosphorus fertilization and/or effective microorganisms as a bio-fertilizer increased all studied vegetative growth characters. Abo-Baker and Mostafa (2011) showed that the inoculation of *Hibiscus sabdariffa* with the mixture of phosphate dissolving bacteria combined with 50 or 100% NPK increased shoot fresh weight in the first season. All bio-fertilizer treatments without NPK reduced the fresh weight of the vegetative growth (Abo-Baker and Mostafa, 2011). Similar results were observed in some plants such as *Nigella sativa*, *Ammi visnaga* and *Salvia officinalis* (Yuonis et al., 2004; Shaalan, 2005; Abd El-Latif, 2006). Study of El-Ghandour et al. (2009) demonstrated that fresh weight of *Majorana hortensis* L. plants were positively affected by bacterial inoculation under faba bean straw or sheep manure compared to control plant. Gewaily et al. (2006) reported that, inoculation of *Majorana hortensis* L. with bio-fertilizer and using organic residues enhanced the vegetative growth.

Effect of phosphate bio-fertilizer on shoot dry matter percentage

The results of ANOVA showed that the effect of bio-fertilizer on shoot dry matter percentage was not significant (Table 2). However, the effect of pure chemical phosphorus on this character was significant ($p \leq 0.01$) (Table 2). The mean comparison of data in different treatments (Table 3) showed that the highest shoot dry matter percentage (19.86%) was determined by application of bio-fertilizer on seeds along with 200 mg l^{-1} chemical phosphate. The lowest shoot dry matter percentage (14.01%) was obtained in control plants (100 mg l^{-1} chemical phosphate without application of bio-fertilizer) (Table 3). This result showed the positive effect of bio-fertilizer on shoot dry matter percentage. Among chemical phosphate treatments, the highest (19.12%) and lowest (15.25%) shoot dry matter percentage were determined by application of 200 and 100 mg l^{-1} phosphate,

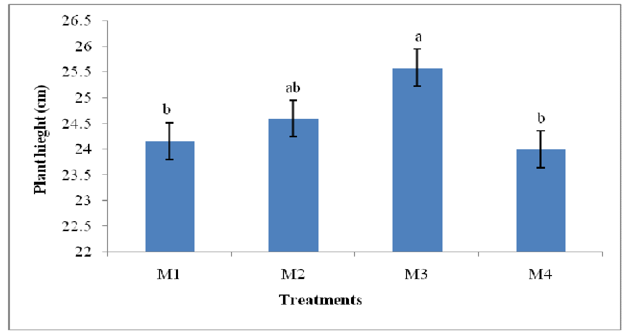

Fig 1. Effect of different methods for application of bio-fertilizer (Barvar-2) on plant height of marigold (*Tagetes erecta* L.).

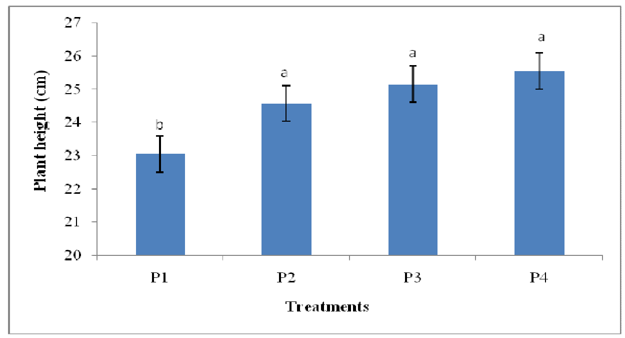

Fig 2. Effect of different levels of chemical phosphorus on plant height of marigold (*Tagetes erecta* L.).

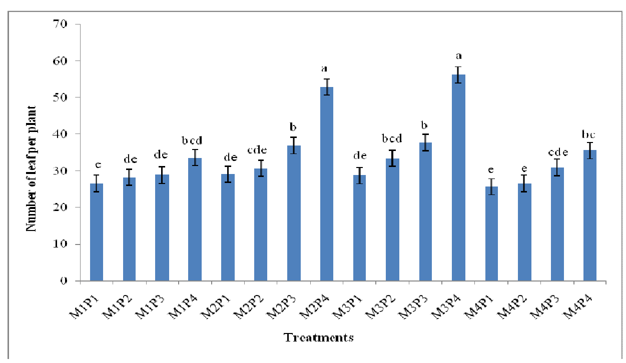

Fig 3. Effect of different methods for application of bio-fertilizer (Barvar-2) and different levels of chemical phosphorus on the number of leaf in marigold (*Tagetes erecta* L.).

respectively (Fig. 10). Investigation of Bagheri et al. (2009) in tea confirms our findings. Abo-Baker and Mostafa (2011) showed that the inoculation of *Hibiscus sabdariffa* with the mixture of phosphate dissolving bacteria, the mixture of nitrogen fixing bacteria and phosphate dissolving bacteria with 100% chemical fertilizer increased the shoot dry weight in the second season. All bio-fertilizer treatments without NPK reduced the dry weight of the vegetative growth (Abo-Baker and Mostafa, 2011). Similar results were observed in some plants such as *Nigella sativa*, *Ammi visnaga* and *Salvia officinalis* (Yuonis et al., 2004; Shaalan, 2005; Abd El-Latif, 2006). Phosphate solubilizing bacteria releases the organic and inorganic acids which reduces soil pH leading to change of available phosphorus and other nutrients and their uptake by plants (Singh and Kapoor, 1999). Study of El-Ghandour et al. (2009) demonstrated that dry weight of *Majorana hortensis* L. plants were positively affected by bacterial inoculation with sheep manure compared to control plant. Similar result was obtained by Gewaily et al. (2006).

Effect of phosphate bio-fertilizer on total shoot phosphorus

Analysis of variance (ANOVA) of data showed that the effect of various procedures of bio-fertilizer application, different concentrations of phosphorus, and their interaction effect on the content of total shoot phosphorus were significant at 5%, 1% and 1% probability level, respectively (Table 2). The results of total shoot phosphorus content revealed that all application methods of bio-fertilizer were superior to the control (Table 3, Fig. 11). Among chemical phosphate treatments, the highest (0.347%) and lowest (0.337%) shoot dry matter percentage were determined by application of 400 and 300 mg l^{-1} phosphate, respectively (Fig. 12).

Highest content of shoot phosphorus was obtained in treatments of bio-fertilizer on seeds and transplant roots along with 400 mg l^{-1} chemical phosphate and bio-fertilizer on seeds along with 400 mg l^{-1} chemical phosphate with 0.353% and 0.352%, respectively, and 400 mg l^{-1} chemical phosphate with 0.347% (Table 3, Fig. 13). It seems the increase in phosphorus uptake at the treated root to biological fertilizer is due to the increase in phosphorus availability and the improvement of plant uptake capacity. Elhami et al. (2007) believed that the phosphorus deficiency is compensated by phosphate solubilizing bacteria and mycorrhiza fungi in the absent of chemical phosphate fertilizers. Thus, the use of biological phosphate fertilizers containing phosphate solubilizing bacteria and mycorrhiza fungi not only causes to decrease the application of chemical phosphate fertilizer, but also it increases plant growth. Khalaj et al. (2009) investigated impact of bio-fertilizer Barvar-2 on phosphorus uptake of *Polianthes tuberosa* and concluded that the best result obtained by using biological fertilizer and chemical fertilizer at the 50:50% ratio. Study of El-Ghandour et al. (2009) showed that nutrient uptake of *Majorana hortensis* L. plants positively responded to the bacterial inoculation and the concerned organic phosphorus sources. Many researchers explained the role of phosphate solubilizing bacteria on the bases of increases in the availability of phosphorus in soil through the secretion of phosphatase enzyme which leads to transfer organic phosphorus to their available forms (Melero et al., 2006; El-Ghandour et al., 2009). Consequently, it enhances phosphorus absorption and accumulation in plant tissues.

Effect of phosphate bio-fertilizer on total medium phosphorus

Analysis of variance (ANOVA) showed that the effect of various procedures of bio-fertilizer application on the content of total medium phosphorus was significant at 1% probability level (Table 2). The effect of different concentrations of phosphorus, and its interaction with various procedures of bio-fertilizer application on the content of total medium phosphorus was not significant (Table 2). The results related

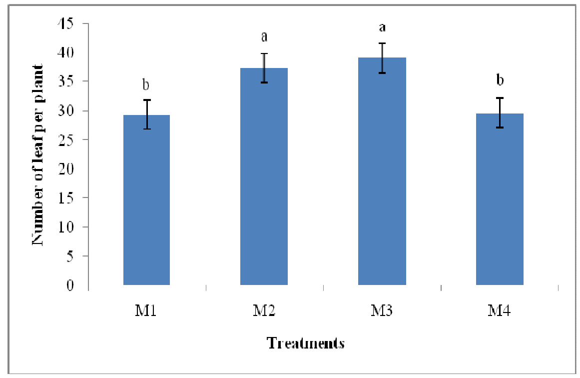

Fig 4. Effect of different methods for application of bio-fertilizer (Barvar-2) on the number of leaf in marigold (*Tagetes erecta* L.).

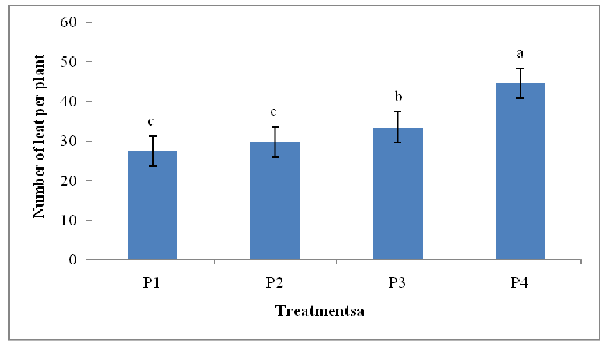

Fig 5. Effect of different levels of chemical phosphorus on the number of leaf in marigold (*Tagetes erecta* L.).

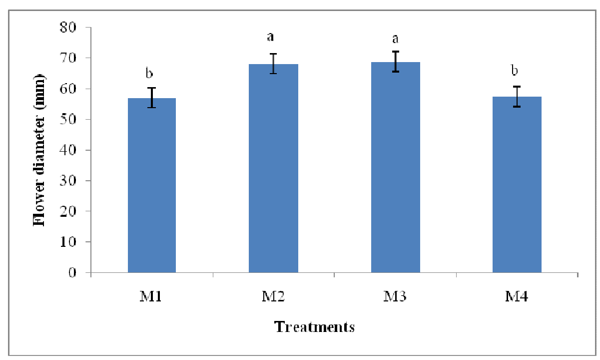

Fig 6. Effect of different methods for application of bio-fertilizer (Barvar-2) on flower diameter of marigold (*Tagetes erecta* L.).

to the content of total medium phosphorus revealed that highest (0.212%) and lowest (0.168%) content were obtained in transplant roots inoculated with bio-fertilizer and the control, respectively (Fig. 14). Among the chemical phosphate treatments, there was no significant difference between the highest and lowest total medium phosphorus. Highest (0.235%) and lowest (0.160%) content of total medium phosphorus were obtained in treatments of bio-fertilizer on transplant roots along with 200 and 100 mg l^{-1} chemical phosphate without bio-fertilizer, respectively (Table 3). One of the abilities of phosphate dissolving bacteria is that they increase the absorbable phosphorus around the plants roots (Peix et al., 2001). Some researchers believe that phosphate bio-fertilizer increases the soil phosphorus, more uptake and improvement of plant growth and development due to increase of solubility and realizing of phosphorus from insoluble phosphate compounds (Toro et al., 1997; Sharma, 2002).

Materials and methods

Plant materials

The present investigation was carried out in order to study the response of marigold (*Tagetes erecta* L.) cv. Tiashan to phosphate bio-fertilizer and chemical fertilizer during 2010/2011 experimental season (since April until August) at a greenhouse (photoperiod of 16 h per day), located in Rasht city, Guilan province, the northern part of Iran.

Experimental design and treatments

Experiment was conducted in factorial arrangement based on randomized completely block design with two factors consisting the methods of bio-fertilizer application, and different levels of chemical phosphorus. The first factor was seed inoculation with bio-fertilizer, transplanted roots inoculated with bio-fertilizer, seeds and transplant roots inoculated with bio-fertilizer and control (without bio-fertilizer). For preparation of bio-fertilizer solution, 100 g Barvar-2™ was added to 2000 ml water and was sprayed on seeds. This solution was applied at the stage of transplanting of seedlings to bigger pots. The second factor included chemical phosphorus treatments (from Crystalon source with 12-12-12 ratio of NPK) at four levels as 100, 200, 300 and 400 mg l^{-1} phosphorus. Plantlets were treated with chemical phosphorus at two stages: 15 days after planting the seeds (first stage), and 10 days after transplant transition (second stage). At first stage, the contents of 0.42, 0.84, 1.26 and 1.68 g Crystalon fertilizer were added to 500 ml water for preparation of 100, 200, 300 and 400 mg l^{-1} chemical phosphorus, respectively. At second stage, the contents of 2.40, 4.80, 7.20 and 9.60 g of Crystalon fertilizer were added to 3000 ml water for preparation of 100, 200, 300 and 400 mg l^{-1} chemical phosphorus, respectively. The study was investigated at a temperature 20±2 and relative humidity of 70%. The phosphate bio-fertilizer was purchased from Green Biotechnology Company, Tehran, Iran.

Soil characteristics

The seeds were planted in a medium contains cocopeat and sand (50:50 v/v) (Table 1). After 45 days of seeding, plantlets in four leaves stage were transplanted to the larger pots containing cocopeat, sand and mold (1:1:1 ratio) (Table 1).

Measurements

Measurements were done at the end of June until the end of July. In current study, plant height, the number of leaf per plant, flower diameter, shoot fresh weight, shoot dry matter, and the content of phosphorus in cultivation media and shoots were measured. Plant height was determined via a ruler at the end of the experiment by cutting aboveground parts at soil surface. Also, the number of leaf per plant was counted at the same time. Flower diameter was calculated by a digital caliper (Guanglu model) from complete flowers. For determination of shoots fresh weight, plant was cut from aboveground parts and then weighted by a digital balance. Following obtaining of fresh weight, shoots was dried in Oven at 75°C for 24 h, and their dry weight was recorded by a digital balance. Dry matter percentage was calculated by the following formula:

$$Dry\ matter\ (\%) = \frac{dry\ weight}{fresh\ weight} \times 100$$

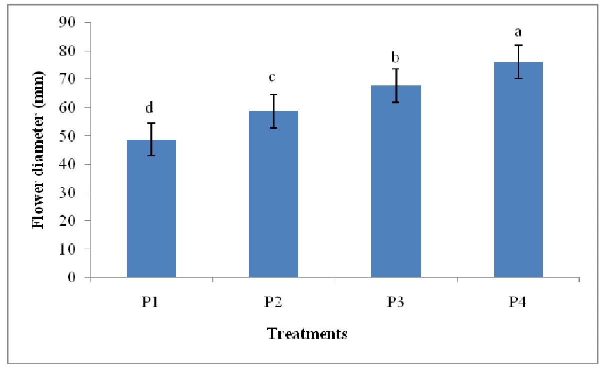

Fig 7. Effect of different levels of chemical phosphorus on flower diameter of marigold (*Tagetes erecta* L.).

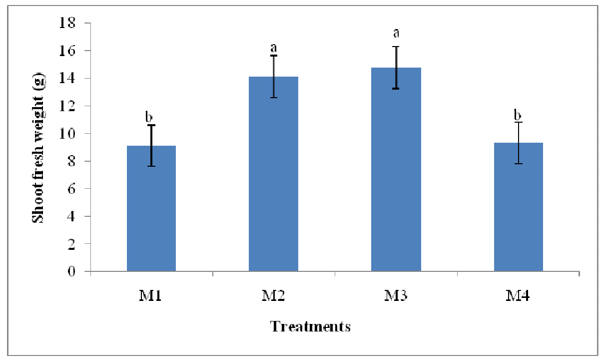

Fig 8. Effect of different methods for application of bio-fertilizer (Barvar-2) on shoot fresh weight of marigold (*Tagetes erecta* L.).

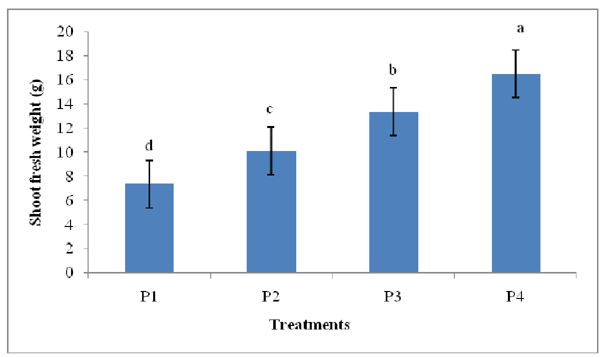

Fig 9. Effect of different levels of chemical phosphorus on shoot fresh weight of marigold (*Tagetes erecta* L.).

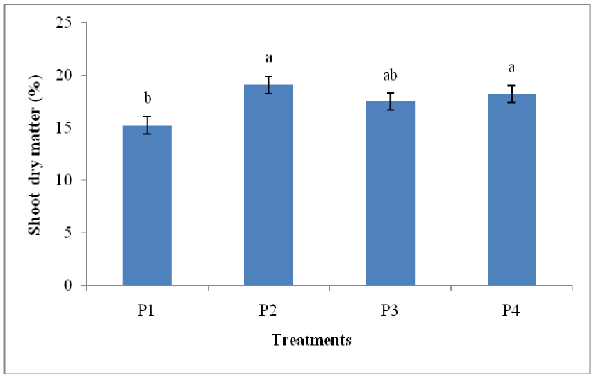

Fig 10. Effect of different levels of chemical phosphorus on shoot dry matter of marigold (*Tagetes erecta* L.).

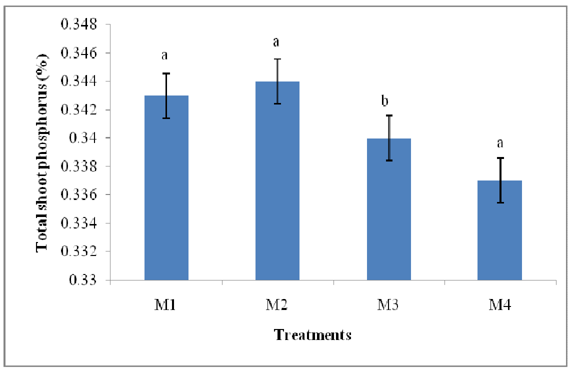

Fig 11. Effect of different methods for application of bio-fertilizer (Barvar-2) on total shoot phosphorus in marigold (*Tagetes erecta* L.).

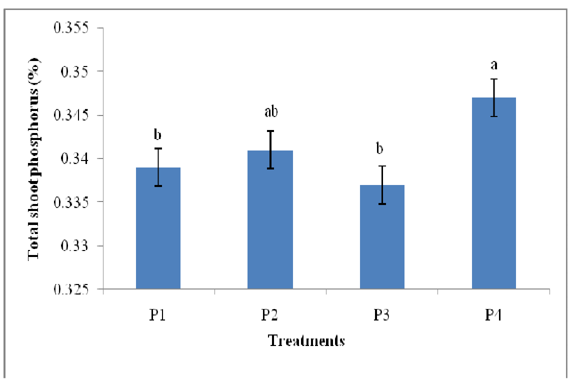

Fig 12. Effect of different levels of chemical phosphorus on total shoot phosphorus in marigold (*Tagetes erecta* L.).

Total phosphorus content of cultivation media and shoots were determined by method of extraction using AB-DTPA and colorimetric in wavelength of 470 nm, respectively, through a spectrophotometer (PD-303 uv model, Japan).

Statistical analysis

The statistical setup in this study was Randomized Complete Block Design with sixteen treatments and four replications. The statistical analysis was carried out using analysis of variance (ANOVA) and compared the means between treatments using the Duncan's test at 1% and 5% probability

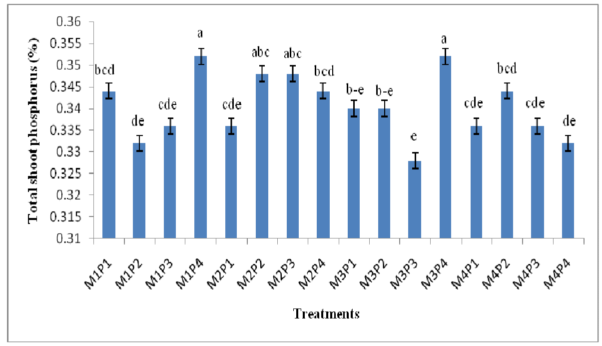

Fig 13. Effect of different methods for application of bio-fertilizer (Barvar-2) and different levels of chemical phosphorus on total shoot phosphorus in marigold (*Tagetes erecta* L.).

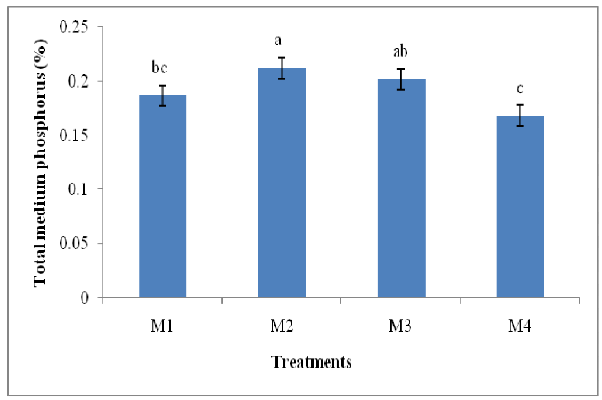

Fig 14. Effect of different methods for application of bio-fertilizer (Barvar-2) on total medium phosphorus in marigold (*Tagetes erecta* L.).

levels by SPSS software package. Data processing of the results was carried out by an EXCEL.

Conclusion

Bio-fertilizers are widely applied in crop production and they are proper substitutions for chemical fertilizers. Application of bio-fertilizer significantly improved quality and quantity features in marigold. Maximum of plant height, the number of leaf, flower diameter, shoot fresh weight and total shoot phosphorus was obtained in treatment of seeds and transplant roots inoculated with bio-fertilizer along with 400 mg l^{-1} phosphorus. Totally, the obtained results revealed that using bio-fertilizer combined with chemical fertilizer significantly improved the quantity and quality characters compared to control.

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