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Original Article

Bio-Inoculants and Organics Influence on Mineral Nutrition and Productivity in *Calendula officinalis* L.

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Abstract

It is important to investigate and develop the strategies and methodologies to maintain the sustainability of plant production. A two-year field study was performed to evaluate calendula response to individual form and also mixtures of selected bio organic fertilizers. The effects of co-inoculation of two plant growth promoting rhizobacteria (PGPR), humic acid (HA), vermicompost (VC), and combinations of these bio organics were investigated in two calendula cultivars, Isfahan multi petalled (MP) and single petalled (SP). The experimental design was a factorial randomized complete block with three replicates. Based on the combined analysis of two years, mean flower yield was highest for the MP cultivar treated by VC + PGPR. Flower essential oil was higher in the MP cultivar than the SP cultivar and did not vary with fertilizers. The highest harvest index of flowers obtained from the MP cultivar treated by HA + PGPR. Furthermore, nutrient contents of leaves increased by fertilizers and combined application of bio organics gave the highest values of nitrogen, phosphorus and potassium contents in calendula leaves. The present results indicate that the selected bio organic fertilizers can be used to obtain higher levels of quality and yield in sustainable agricultural practices.

Keywords: Biofertilizers, Humic acid, Leaf nutrients, Vermicompost, Yield.

Introduction

Sustainable strategies have to be based on methods that conserve natural resources. A post-modern agriculture system uses ecology principles more effectively to improve plants production while reducing negative environmental impacts. Many organic products have advantages as both fertilizers and soil modifying agents.

Calendula or pot marigold (*Calendula officinalis* L., Asteraceae) has various usages as a medicinal plant. It is compatible to both field based and greenhouse floriculture as a model crop. In a sustainable agricultural production, soil microbial structure and function has to be considered. In this regard biological amendments are considered as an agents that contribute to integrated solutions for agro-environmental problems. Plant growth promoting rhizobacteria (PGPR) are a group of soil bacteria which have rhizosphere colonization ability to enhance the production of plants [1]. On the other hand using Organic products such as vermicomposts and humic acids, drived from different sources have a major role in a sustainable nutrient management. Vermicomposts are natural products produced through the accelerated biodegradation process of organic materials by earthworms and microorganisms [2]. Humic acid contains nutrients for microbial communities which can increase their growth and population [3]. The effectiveness of humic matter depends on its concentration, chemical characteristics and molecular dimensions and weight [4].

Comparatively there are few studies about responses of calendula cultivars due to application of fertilizers in organic production systems. Therefore, the objectives of present study were to

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investigate the effects of the application of organics and biofertilizers on vegetative and flowering parameters of two calendula cultivars.

Material and Methods

The present research was performed in 2015 and 2016 at the field research station of the Faculty of Agriculture, University of Tabriz (38° 05′N, 46° 17′E, and 1360 m above free sea level), Tabriz, Iran. Soil type was a sandy loam and climate is characterized by mean annual precipitation of 245.75 mm per year and mean annual temperature of 10°C. The experimental design was a factorial randomized complete block with three replicates.

Bio organic fertilizers and calendula cultivars were two factors of this study. Fertilizer treatments included: plant growth promoting rhizobacteria (*Azotobacter* sp.145 PI and *Azospirillum* sp. AC49I), humic acid (Mobicel-H) (10 kg ha^{-1}), vermicompost (Tak Vermicompost *Azerbaijan*) (7 t ha^{-1}), HA + PGPR, VC + PGPR, and control. The two calendula cultivars used in this research were Isfahan multi petalled (MP) and single petalled (SP). The vermicompost was applied into the top 10 cm of the rows by hand uniformly one week before planting and incorporated to the soil. Nitrogen fixing bacteria were inoculated with the seeds before planting. Used bacteria were cultured in the laboratory of soil biology. Humic acid was applied with first irrigation immediately after planting. Individual plot size was 3 m × 2 m and consisted of six rows. The seeds were obtained from Pakan Bazr Company (Isfahan, Iran) and planted by hand on 18 may 2015 and 8 may 2016. Because of calendula's indeterminate growth, half of the plots were allocated for flower yield and the other half for seed yield measurements. Flower yield were assessed by sequential hand harvesting of one m^2 of the plots. Flowers were dried in the shadows and then weighted to obtain values of the yield. Biological yield determined by collected plant samples from one m^2 of the plots and oven dried at 65°c for 72 h. The percent ratio of flower and biological yields were calculated as harvest index. At the end of season, biometric measurements were taken by 7 randomly selected plants from each plot to collect data on number of flower heads per plant and plant height. Flower heads of calendula used for essential oil content determination. Samples of 25 g were subjected to conventional hydro distillation for 3 hours using a Clevenger-type apparatus and the percentage of essential oil extracted was determined. Nitrogen (N), phosphorus (P) and potassium (K) content of the leaves was measured at the end of the experiment. Kjeldhal method used for total N measurement [5]. P content was determined using vanadomolybdate yellow color method (Olsen and Sommers 1982) [6] and K content obtained photometrically according to Emami [7].

Analysis of variance (ANOVA) and mean separations conducted using the general linear model (GLM) procedure of SAS 9.1 program. Duncan Multiple Range Test was used to determine statistically significant differences among treatment means, at a probability level of 0.05.

Result and Discussion

According to combined analyses of variance significant effects of fertilizers and cultivars on plant growth and quality parameters were detected. The plant height showed significant differences under application of different fertilizers (Table 1). Plants treated with VC + PGPR recorded the maximum value with 40.3% followed by VC with 32.4% increase in calendula plant height compared to the control. The plants grown under HA+PGPR were slightly taller than HA but no significant difference was observed among these treatments. The MP cultivar was significantly taller than the SP cultivar.

The interactions between factors indicated the highest flower yield for the MP treated by VC + PGPR (1241.87 kg ha^{-1}) and the lowest value for the SP in control plots (932.55 kg ha^{-1}) which was not significantly different from the SP treated by PGPR (974.13 kg ha^{-1}) (Fig. 1a). Flower yield of both cultivars in the second year was higher than that in the first year and VC + PGPR and HA + PGPR recorded the highest increases of 28.4% and 26.9% respectively. The minimum yield was recorded from control plots in both years, which was not significantly different from PGPR in the first year (Fig. 1b). The enhanced flower yield as a result of applied fertilizers could be due to the uptake of nutrients, but the hormone like activity of the HAs should also be considered.

Table 1 Calendula cultivars plant characteristics as affected by fertilizers in 2015 and 2016. Within columns values followed by the same letter are not significantly different at the $P \leq 0.05$ level (mean ± standard error).

Treatments	Plant Height (mm)	Flower yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index%	Essential oil%	Essential oil yield (kg ha^{-1})	N%	P%	K%
Control	372.6±21.56 e	964.41±41.49d	6763.50±69.24 f	14.26±0.56 d	0.13±0.057 a	1.26±0.59 b	1.78±0.36 c	0.19±0.062 d	1.86±0.176 c
PGPR	426.3±38.04 d	1050.78±89.11c	7032.64±136.29 e	14.92±0.98 c	0.13±0.094 a	1.48±1.05 b	2.10±0.28 b	0.25±0.095 cd	2.24±0.314 bc
HA	450.0±21.45 cd	1070.90±80.90 c	7214.00±120.24 d	14.83±0.91 c	0.16±0.085 a	1.82±1.02 ab	2.22±0.44 b	0.24±0.112 cd	2.04±0.218 b
VC	493.3±22.49 b	1178.11±19.80 b	7719.76±144.04 b	15.26±0.23 b	0.24±0.091 a	2.91±1.10 a	2.31±0.45 b	0.31±0.067 bc	2.20±0.206 b
VC+PGPR	522.9±22.56 a	1228.75±30.56 a	7812.95±85.59 a	15.72±0.31 a	0.18±0.067 a	2.24±0.84 ab	2.76±0.19 a	0.37±0.017 ab	2.52±0.200 a
HA+PGPR	473.6±35.74 c	1185.7±24.87 b	7441.52±78.97 c	15.92±0.25 a	0.21±0.104 a	2.54±1.28 ab	2.82±0.15 a	0.39±0.070 a	2.64±0.290 a
MP	470.2 a	1150.73 a	7395.39 a	15.54 a	0.22 a	2.56 a	2.45 a	0.31 a	2.33 a
SP	442.6 b	1075.50 b	7266.06 b	14.76 b	0.13 b	1.52 b	2.21 b	0.28 a	2.17 b

Trinh *et al*. [8] reported promoting effects of used PGPR on growth and yield of *Arabidopsis thaliana* plants. In another study, Ansari and Mahmood [9] found that the bio organic fertilizers produced more crop growth and yield than control in pigeon pea (*Cajanus cajan* L.) plants. Also according to Gong *et al*.[10] growth and flowering of Geranium and calendula were enhanced in vermicompost-based media.

The biological yield of the SP cultivar was less than that of the MP cultivar and both cultivars showed significant differences under application of different amendments (Table 1). The highest biological yield was found in VC + PGPR with the increase of 15.5%, followed by VC treatment, but the difference between HA+PGPR and HA treatments was significant which showed 10% and 6.7% increase respectively.

Abdelaziz *et al*. [11] similarly reported the effect of VC and PGPR application on rosemary (*Rosmarinus officinalis* L.). Gholami *et al*. [12] also stated that the humic acid and vermicompost enhanced the chicory (*Cichorium intybus* L.) yield and some phytochemical properties. According to Salehi Sardoei [13], the highest yield and growth of marigold were obtained in 60% vermicompost treatment. Furthermore, the effect of biofertilizers was significant on dry matter of flower and fresh yield of calendula flowers as stated by Hoseini Mazinani [14].

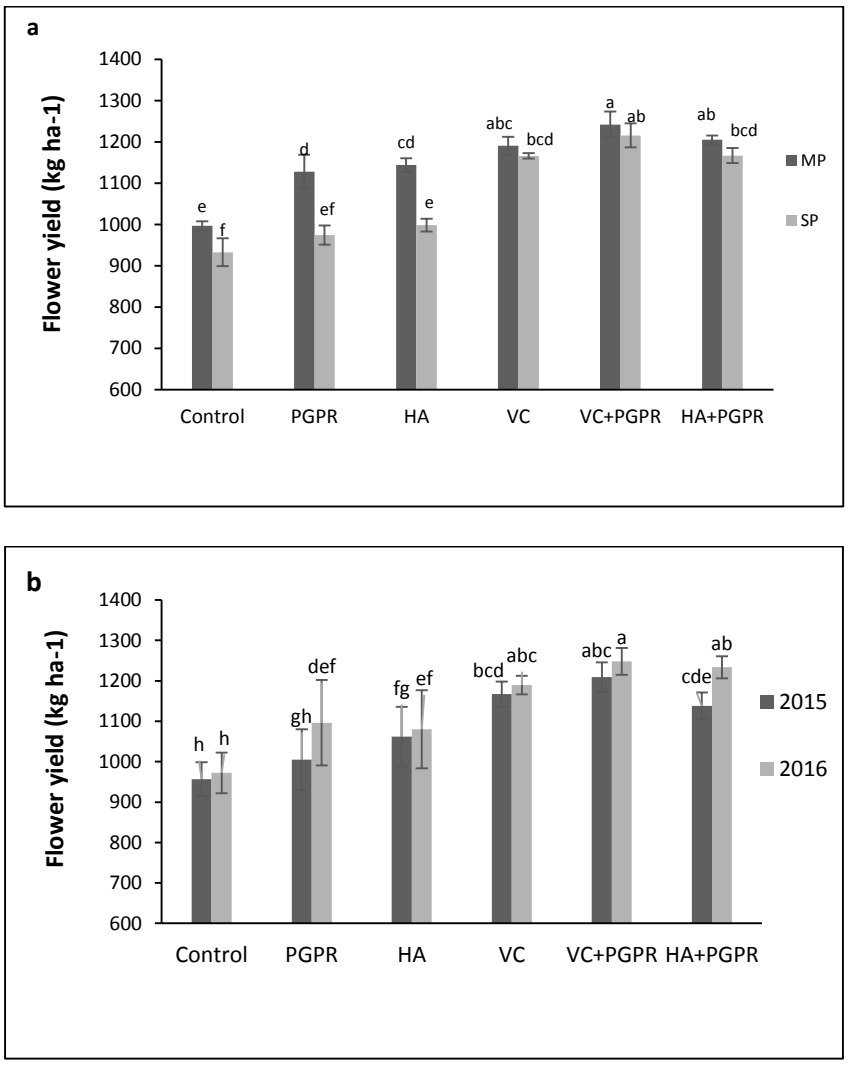

Fig. 1 Calendula flower yield as affected by fertilizers and cultivars (a) and compared by years (b). Values followed by the same letter are not significantly different at the $P \leq 0.05$ level.

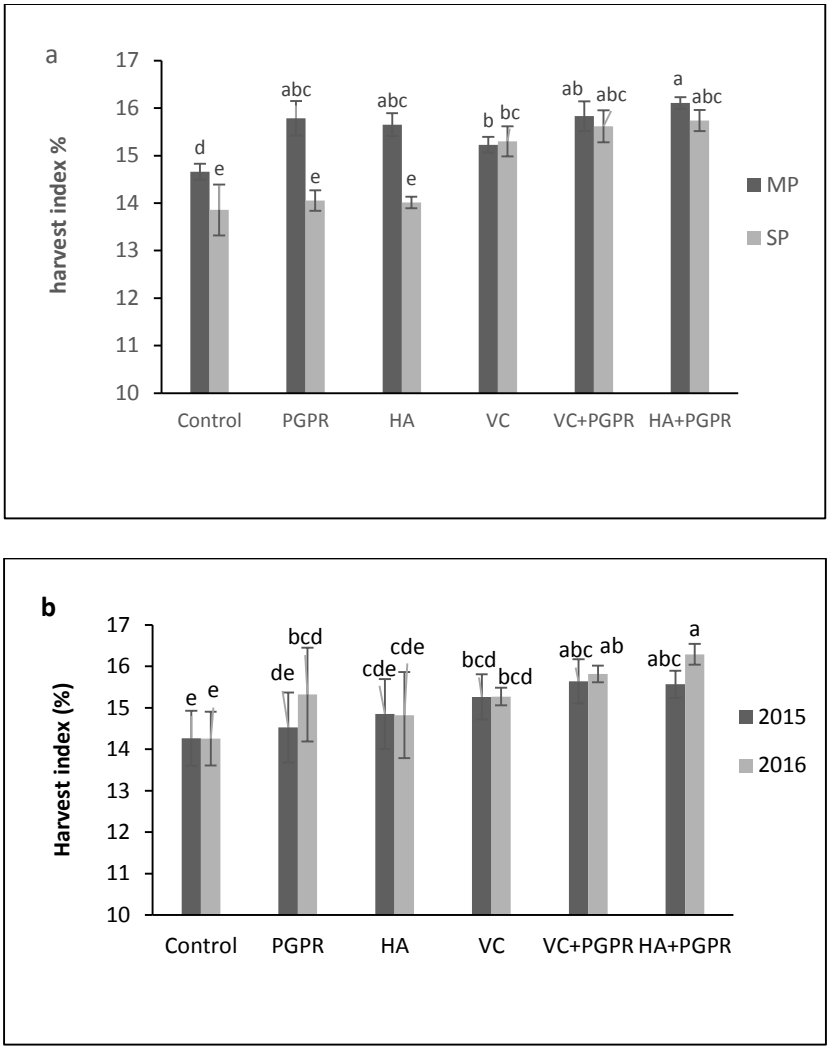

Fig. 2 Calendula flower harvest index as affected by fertilizers and cultivars (a) and compared by years (b). Values followed by the same letter are not significantly different at the $P \leq 0.05$ level.

In this study the increase in yield and yield traits of calendula could be attributed to direct or indirect effects of organics and bio fertilizers on plant growth and development. It is possible that organics increase microbial biomass and activity in soils which may have been responsible for the increased calendula growth. Nevertheless, there appear to be noticeable differences between the effects of VC and HA that were used in this study, in terms of their influence on plant growth, depending upon the source of the parent material used in their production.

In the current investigation there was a variation in the percent ratio of flower and biological yield of the crop (Table 1). The interaction between cultivars and fertilizers revealed that there were not significant differences between the MP cultivar treated by HA + PGPR, VC + PGPR and VC (16.11, 15.83 and 15.23% respectively) and the SP cultivar when treated by the same fertilizers. The lowest harvest index obtained from the SP cultivar treated by control, HA and PGPR (13.85, 14.01 and 14.05% respectively) which was significantly lower than the MP cultivar treated by the same fertilizers (Fig. 2a). The harvest index had the highest value in response to HA + PGPR treatment (16.29%), followed by VC + PGPR (15.81%) in the second year which were not significantly different from the same treatments in the first year (Fig. 2b). According to Akladious and Mohamed [15] humic acid increased fruit length, fruit diameter and fresh and dry weights of fruit in pepper (*Capsicum annuum* L.) plants. Another study by Sharma *et al.* [16] revealed the efficiency of applied organics to improve improving soil enzyme activities which leads to soil biological health. They also reported increases in the flower number and pigment content of Calendula and Marigold flowers. Hosseinzadeh *et al.* [17] also reported positive effects of vermicompost on physiological, biochemical, and

photosynthetic responses of chickpea (*Cicer arietinum* L.).Furthermore, results from a research by Azizi [18] showed positive effects of the bio fertilizers on the seed number per plant, harvest index, total dry matter and seed yield of Fennel (*Foeniculum vulgare* Mill.).

Organic and bio fertilizers gave better results in essential oil content while no significant deference were observed among these treatments (Table 1) but the difference between cultivars was significant. The results showed a higher amount of essential oil content in the MP cultivar (0.222%) than the SP cultivar (0.137%). A similar trend was found for essential oil yield. A report by Gopichand *et al*. [19] indicates that essential oil content of *Curcuma aromatic* Salisb. did not significantly affect by levels of different organic fertilizer. Although increases in the essential oil content following the application of VC and/or PGPR were observed in fennel [20] and Lemon Balm (*Melissa officinalis* L.) [21]. Rostaei *et al*.[22] also reported positive effects of applied organic manure on the quality of the essential oil and antioxidant activity of dill (*Anethum graveolens* L.).

Leaf contents of N, P and K found for each cultivar is given in Table 1. The results showed greater variation within than between cultivars. Combined application of fertilizers gave the highest values of N, P and K content of calendula leaves. Leaf N content varied from 1.87 to 2.82% and was minimum for control plants. Similarly minimum leaf P 0.19% and K 1.86% contents were found in untreated plants. Although there was not significant increase in leaf P content with the application of PGPR or HA individually and in leaf K content with the application of PGPR. The MP cultivar showed the higher contents of N and K than the SP cultivar but there was no significant difference of P content between cultivars. The results indicated the advantages of organics and bio fertilizers due to their effects on nutrient contents of calendula plants. Suitable source of nutrients in bio organic fertilizers and high availability of them, can promote the overall plant growth and development. Positive influences of the combination of organics and bio fertilizers on plant nutrients such as N, P and K contents in calendula reported by Hussein *et al*. [19] and, Maie Mohsen and Ismail [23]. Akladious and Mohamed [15] found that humic acid enhanced N, P and K levels in stressed plants compared to the plants treated with salt stress alone. Ansari and Mahmood [9] also reported similar results by combined application of biological and organic fertilizers. It was determined that humic acid improved the nutritional element contents of different tagates species [24].

An important role of VC is that, during the degradation of the different organic materials by earthworms, many of the nutrients contents are change to more suitable forms that are easily used by plants [25]. The amount of VC had a significant effect on growth and flowering parameters of the Marigold (*Tagetes erecta* L.) plants, as stated by Pritam *et al*. [26].

There are several reports of HAs application on plant growth. According to Ciarkowskaa *et al.* [27] humic materials increased different soil parameters and tested plants growth. Also as stated by Pizzeghello *et al*. [28] HS products seems to influence positively the signaling pathways and metabolism in plants, by stimulating physiological responses. Another study by Aslam *et al*. [29] revealed that the humic acid levels significantly affected most of the growth and flowering parameters of marigold.

In this study we also used bio fertilizers individually and in combination with organic fertilizers. Results from some other studies indicated the ineffectiveness of the single-strain inoculations of PGPR [30], but mixed PGPR inoculations showed much more beneficial effects [31]. Singh *et al*. [32] found that the use of PGPRs improved yield and yield-related indexes in calendula. The individual effects from VC, HA or PGPR on increasing growth parameters, flower and biological yields were clear, and effects were stronger when all inputs were combined suggesting synergy. Yilmaz and Sonmez [33] indicated that PGPRs in combination with VC mostly had stronger effects on stability and organic carbon content of aggregates in comparison with control and PGPR treatments alone.

Many researches have focused on individual effects of VC, HA or PGPR on soil and plant performance or on a comparison of organics with inorganic fertilization. In the absence of VC or HA, PGPR had only slightly stimulatory effects on plant growth. Unfavorable condition in the soil, could have led to poor survival of the microorganisms. Sing *et al*. [34] indicated the survival of introduced bacteria depends on different factors such as the resource availability, soil environment and endemic microbial diversity.

Conclusions

Findings in this study explain that the combined application of PGPR and organic fertilizers could be effective in improving nutritional and reproductive parameters of both calendula cultivars more than individual uses of these fertilizers, although in some cases differences were not significant. In addition, the increase in growth and yield by these treatments might be due to the effect of balanced nutrition supplied and Physiological processes through the application of organic and bio fertilizers. Hence, using these amendments as environmentally friendly products could be considered.

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