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Growth and chlorogenic acid content of artichoke (*Cynara scolymus* L.) affected by bio and chemical fertilizer

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Key words: Artichoke, Bio-fertilizers, Chlorogenic acid, Integrated plant nutrition system

Abstract

Considering the importance of artichoke plant in the pharmaceutical industry and due to the negative effect of excessive consumption of chemical fertilizers on human health, this study was conducted to assess the effect of bio and chemical fertilizer on morphological traits and the leaves chlorogenic acid content of artichoke in Isfahan, Iran during 2014 -2016. A factorial experiment in the base of randomized complete block design with three replications was used. The factors were chemical fertilizers in three levels of control, 50% chemical fertilizer (100 and 50 kg ha^{-1} NP), 100% chemical fertilizer (200 and100 kg ha^{-1} NP) and bio-fertilizer in four levels of non-inoculated, inoculated seeds with Nitroxin (include *Azotobacter, Azospirillium* and *Pseudomonas*), inoculated seeds with Barvar 2 (*Pantoea agglomerans*-p5 and *Pseudomonas putida*-p13) and co-inoculation seeds with Nitroxin and Barvar 2. The traits were measured at the flowering stage. The highest chlorogenic acid amount was obtained from the residual effect of 50% chemical fertilizer+ 50 kg ha^{-1} supplementary nitrogentreatment. Integrated treatments showed the maximum plant height and leaf length in artichoke plant. Among chemical fertilizer levels, the highest number of leaf per plant, plant fresh weight, plant dry weight and number of capitol per plant were recorded by residual effect of 100% chemical fertilizer+ 100 kg ha^{-1} supplementary nitrogen. Co-inoculation of Nitroxin and Barvar 2 produced the highest plant height, number of leaf per plant, leaf length, plant fresh weight, plant dry weight and number of capitol per plant among bio-fertilizer levels. Totally, the application of 50 % chemical fertilizer along with Nitroxin+ Barvar 2 is suggested for improving the chlorogenic acid content and morphological traits of artichoke.

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Introduction

Artichoke (*Cynara scolymus* L.) is a perennial plant from the composite family that possesses both food value and medicinal properties. Nearly 85% of artichokes cultivated in the world are grown in countries bordering the Mediterranean basin (Bianco, 2005). This plant has been used since Roman times, for its health benefits due to the high content of polyphenols and inulin (Lattanzio *et al.,* 2009; Pandino *et al.,* 2011).Fresh leaves gathered in the first year are the most suitable material for the formulation of medicinal preparations (Dermarderosian, 2001). The main pharmacological properties of the artichoke are liver and gallbadder bile stimulation, hepatoprotective, antihepatotoxic and hypocholestrolemic (Aksu and Altinterim, 2011).Moreover, it has antioxidant, anti-proliferative, anti-migratory, anti-angiogenic and antimicrobial activities (Ramos *et al.,* 2014). According to the world wide spread demand to use of organic products due to the harmful effects of chemical products, such as chemical fertilizers on environmental and human health as well as medicinal plants, it is necessary to the serious attention of organic cultivation of plants(Raei and Alami-Milani, 2014). Nowadays, attention to sustainable agricultural systems and application of bio-fertilizers has been increased (Ehteshami *et al.,* 2007).Bio-fertilizers containing beneficial microorganism improve soil chemical and biological characteristics, phosphate solutions and agricultural production (Yosefi *et al.,* 2011). The application of bio-fertilizers can reduce the use of chemical fertilizers and lead to the production of high qualitative products of free of harmful materials for human safety (Mahfouz and Sharaf-Eldin, 2007).

Free-living nitrogen fixing bacteria such as; *Azotobacterchr oococcum* and *Azospirillum lipoferum*, were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (El-Ghandour *et al.,* 2009).

Some studies showed that the improvement in growth characters of medicinal plants might be due to the fact that the inoculated plants with phosphate solubilizing bacteria 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 (Premsekhar and Rajashree, 2009; Castagno *et al.,* 2011; Saharan and Nehra, 2011). Rahimi *et al.,* (2013) reported that bio-stimulators and bio-fertilizers had a significant positive effect on the growth parameters of basil (*Ocimum bacilicum* L.). Application of bio-fertilizers plays a remarkable role in improving quantity and quality of ajowan (*Carum copticum*) and can be considered a suitable replacement for chemical fertilizers (Habibi and Talaei, 2014). The real value is given to the quality in the cultivation of medicinal plants. Furthermore, application of chemical fertilizers can threat the human health, because of increasing the soil and water pollution and accumulation of some heavy metals in plants (Singh *et al.,* 2008). Therefore, the real aim of this study was to evaluate the effect of bio and chemical fertilizer on morphological traits and chlorogenic acid content of artichoke inorder to reduce chemical fertilizers applications with the replacement of bio-fertilizers in a part of chemical fertilizer as well as improvement of the quality of artichoke production.

Materials and methods

Study area

This research was conducted in Isfahan (32° 37’ N, 51° 28’E and an altitude of 1612 m), Iran during 2014-2016. This site was characterized by a semi-arid climate with a mean annual precipitation of 140 mm and an average temperature of 16 °C (Yaghmaei *et al.,* 2009). The Soil of the field was also analyzed before applying fertilizers in the field. The soil was a clay loam with 31% clay, 24 % silt and 45 % sand. Soil pH was 7.7. Organic matter in soil was 0.065% while Nitrogen level was 0.04%, Phosphorous was 12 ppm and Potassium was 250 ppm.

Experimental design and treatments

The study was conducted in Randomized Complete Block Design (RCBD) using factorial arrangement with three replications. The experiment, consisting of two factors: chemical fertilizers at levels of C_1: 100% chemical fertilizer (200 and 100 kg ha^{-1} NP), C_2: 50% chemical fertilizer (100 and 50 kg ha^{-1} NP), C_3: control and bio-fertilizer at levels of B_1: 1 liter ha^{-1}Nitroxin (include *Azotobacter, Azospirillium* and *Pseudomonas*), B_2: 100 gr ha^{-1}Barvar 2 (*Pantoea agglomerans*-p5 and *Pseudomonas putida*-p13), B_3: 1 liter ha^{-1} Nitroxin+100 gr ha^{-1}Barvar 2 and B_4: control. Chemical fertilizers were urea and triple super phosphate. The seeds were sown on 26 April 2014. The first half of the nitrogen and total phosphorus fertilizers were utilized as strip method before sowing and the rest at 7-8 leaf stage. Bio-fertilizer treatment was performed by inoculating seeds with Nitroxin and Barvar 2. All other cultural practices were kept normal and uniform for all treatments. Each plot size was 5×3.5 m which included 5 rows of plants spaced 60 cm apart. Each row was planted with plants at 30 cm intervals. Irrigation was applied immediately after sowing seeds. Weeds were hand controlled during crop growth and development. Artichoke is a perennial plant and therefore, according to soil test, half of the recommended nitrogen fertilizer was added to the chemical fertilizer plots in the second year. Plants were harvested at flowering stage (July 2015).

Evaluated traits

Some morphological characteristics such as plant height, number of leaf per plant, number of capitol per plant, leaf length, plant fresh weight, plant dry weight and leaves chlorogenic acid content were determined.

Methods

Five plants were selected from each plot and morphological characteristics were evaluated. After the harvest, samples were air dried, then dried leaves were ground into powder and chlorogenic acid amount of plant extracts was determined by using reflux balloon method with HPLC (Adzet and Puigmacia, 1985). The used HPLC equipment and conditions: Gradient pump: Maxi- Star K-1000, Column: Erospher 100 C-18, 250 x 4 mm (5 µm), Detection: Spectrophotometer K-2500, Wavelength: 325 nm, Flow rate: 1.0 ml/min, Column temperature: 25 °C, Injection volume: 20 µl, Analysis time: 20 min.

Statistical analysis

Data analysis was performed using SAS and mean of the treatments were compared using Duncan's Multiple Range Test ($p \leq 0.05$).

Results and discussion

Leaves chlorogenic acid content

The main effects and interaction effect of chemical and biological fertilizers were statistically significant ($p<0.01$) for chlorogenic acid content of leaves (Table 1). Chlorogenic acid content improved as chemical fertilizers increased from 0 to 50 %, whereas it was decreased aschemical fertilizer enhanced from 50 to 100%. For the integrated system, maximum chlorogenic acid value (4.17 mg/g) was obtained by residual effect of 50% chemical fertilizer+ 50 $kgha^{-1}$ supplementary nitrogen treatment (Fig. 1). Bio-fertilizer increased the chlorogenic acid content compared to control, but there was no significant effect (Fig. 1).Lux-Endrich *et al.* (2000)mentioned that the relationship between nitrogen and chlorogenic acid was dependent on cultivar and chlorogenic acid accumulation in artichoke leaves was happened by nitrogen reducing. Bahreininejad *et al.* (2004) mentioned that increasing nitrogen fertilizer up to 200 kg urea ha^{-1} reduce the amount of chlorogenic acid content of artichoke leaves. Nitrogen caused competition between the primary precursor of L-Phenylalanine for protein production via phenolic compounds and manufacturing phenols were reduced with the arrival of phenylalanine for protein production. Hejazi *et al.* (2013) showed that there was a decrease inchlorogenic acid percentage of artichoke leaves due to the increase of nitrogen manure. Fateh *et al.* (2013) stated that organic and integrated fertilizing systems produced the most chlorogenic acid content in leaves and bracts of artichoke plant.

Table 1. Analysis of variance for morphological traitsand chlorogenic acid content of Artichoke under different nutritional treatments.

SOV	df	Mean squares (MS)						
		Plant height	Number of leaf per plant	Leaf length	Number of capitol per plant	Plant fresh weight	Plant dry weight	Leaf chlorogenic acid content
Replication	2	29.361 ^{ns}	2.583**	9.521**	0.778 ^{ns}	3114850.38**	48669.53 **	0.019 ^{ns}
Chemical fertilizer	2	3007.528**	91**	478.083**	94.694 **	2.740×10^{-7**}	428198.6 **	2.242**
Bio-fertilizer	3	157.259**	8.546**	43.525**	5.435 **	607039.01*	9484.98 *	0.074**
Chemical fertilizer* Bio-fertilizer	6	7.454**	0.407 ^{ns}	3.907**	0.435 ^{ns}	44432.64 ^{ns}	694.26 ^{ns}	0.046**
Error	22	2.301	0.432	0.134	0.202	156483.4	2445.05	0.008

ns: Means no significant at level (p≤0.05), *Means significant at level (p≤0.05) and **Means significant at level (p≤0.01).

Morphological traits

Plant height

Plant height was significantly affected by bio and chemical fertilizers and interaction effect of them (Table 1). The highest and the lowest plant height (89.66, 49 cm) were achieved from residual effect of 100% chemical fertilizer+ 100 kgha^{-1} supplementary nitrogen + Nitroxin+ Barvar 2 and control treatment, respectively (Fig. 2). There was an increasing trend in plant height with the application of all fertilizers compared to control. Plant height was increased as NP fertilizers consumption increased. This effect could be attributed to intensifying effect of N on vegetative growth and cell division in plant`s stem. Application of bio-fertilizers enhanced plant height with promoting plant growth regulators (Rajput and Singh, 1996).

Table 2. Mean comparison of morphological traitsand chlorogenic acid content of artichokeas affected by soil fertilizing systems.

Chemical fertilizer	Plantheight (cm)	Number of leaf per plant	Leaf length (cm)	Number of capitol per plant	Plantfreshweight(g)	Plant dryweight(g)	Leaf chlorogenicacid content (mg/g)
Control	53.25 c	9.91 c	59.62 c	2.66 c	3591.22 c	448.90 c	3.05 c
Residual effect of 50% chemical fertilizer+ 50 kg ha^{-1} supplementary nitrogen	73.66 b	12.91 b	66.79 b	6 b	4904.56 b	613.07 b	3.92 a
Residual effect of 100% chemical fertilizer+ 100 kg ha^{-1} supplementary nitrogen	84.41 a	15.41 a	72.2 a	8.25 a	6605.33 a	825.67 a	3.53 b

In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan's test.

This may be attributed due to more atmospheric nitrogen fixed in the soil, which was probably due to mobilization of bacteria, providing favorable conditions (Senthil-Kumar *et al.,* 2009). Furthermore, bio-fertilizers enlarged root uptake through root development (Yosefi *et al.,* 2011). Subb-Rao (1984) stated that the favorable effect of bio-fertilizers on growth parameters might be ascribed to its important role in fixing atmospheric N as well as increasing the secretion of natural hormones namely IAA, GA_3 and cytokinins, antibiotics and possibly raising the availability of various nutrients.

The highest plant height of black cumin (*Nigella sativa* L.) was obtained with using of Nitroxinbio-fertilizer (Akbarian *et al.,* 2013).

Kariminejad and Pazoki (2015) stated that increasing nitrogen use led to a significant enlargement in plant height of German chamomile (*Matricaria chamomilla* L.). The maximum height of dill (*Anethum graveolens* L.) plant was obtained froma bio-fertilizer application (Nejatzadeh-Barandozi and Gholami-Borujeni, 2014). Said-Al Ahl*et al.* (2015) reported that the combined treatment between bio-fertilizer and 80 kg N/feddan gave the largest values

of dill (*Anethum graveolens* L.) plant height. They stated that the exudate of some hormonal substances like cytokinins and auxins enhanced plant height. Meanwhile, this may be due to the increase of N in the root zone and the synergistic effect of microorganisms on the physiological and metabolic activities of the plant.

Table 3. Mean comparison of morphological traitsand chlorogenic acid content of artichoke as affected by soil fertilizing systems.

Bio-fertilizer	Plantheight (cm)	Number of leaf per plant	Leaf length (cm)	Number of capitol per plant	Plantfreshweight(g)	Plant dryweight(g)	Leaf chlorogenicacid content (mg/g)
Control	65.33 d	11.66 c	64 d	4.88 c	4792.74 b	599.09 b	3.59 a
Nitroxin	72.55 b	12.88 b	66.55 c	5.77 b	5121.19 ab	640.15 ab	3.45 b
Barvar 2	69 c	12.44 b	65.16 b	5.22 c	4860.59 b	607.57 b	3.56 a
Nitroxin+ Barvar 2	74.88 a	14 a	69.11 a	6.66 a	5360.30 a	670.04 a	3.4 b

In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan's test.

Number of leaf per plant

The maximum number of leaf per plant (15.41) was recorded by residual effect of 100% chemical fertilizer+ 100 kg ha^{-1} supplementary nitrogen andthe minimum number of leaf per plant (9.91) was obtained in control plants (Table 2). Increasing levels of chemical fertilizer significantly improved the number of leaf per plant. This could be attributed to the high values of available nutrients for plant growth. Among bio-fertilizer levels, the maximum number of leaf per plant (14) was achieved via seeds inoculation with Nitroxin+ Barvar 2. So, no significant difference was observed between alone application of Nitroxin and Barvar 2 (Table 3). The number of artichoke leaves significantly increased when N-fertilization increased from 95 to 285 kg N ha^{-1} (Salamah *et al.* 1997). Darzi *et al.* (2012) reported that use a 142 kg N, 114 kg P_2O_5 and 238 kg K_2O ha^{-1} had the best results for the number of leaves in Coriander (*Coriandrum sativum* L.).

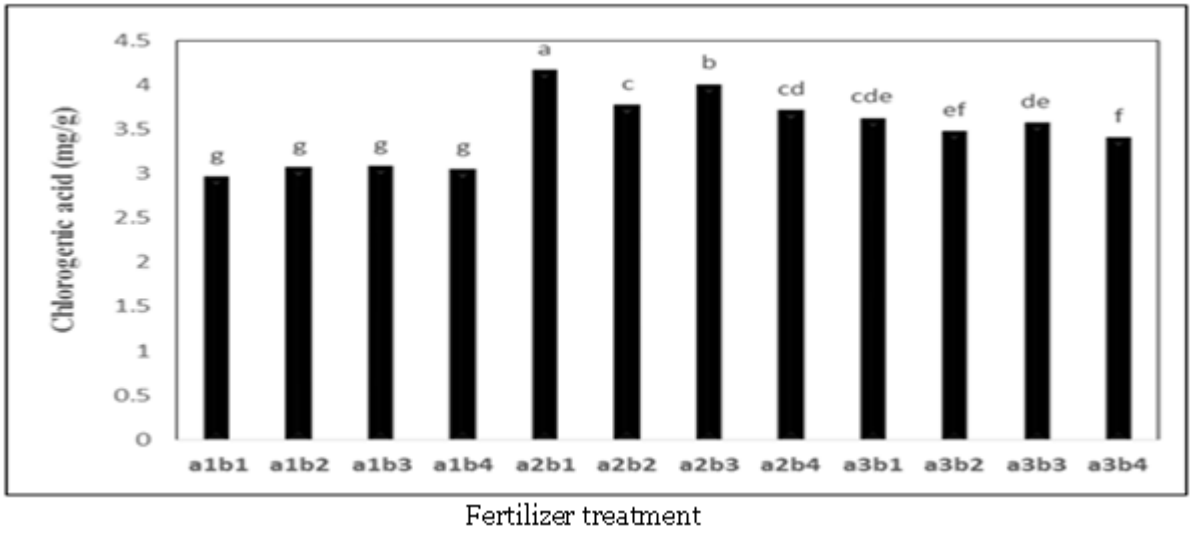

Fig. 1. Interaction effect of chemical and biological fertilizers on leaves chlorogenic acid content of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.05 level of probability. (Control (a1), residual effect of 50% chemical fertilizer + 50 kg ha^{-1} supplementary nitrogen (a2), residual effect of 100% chemical fertilizer 100 kg ha^{-1} supplementary nitrogen (a3), control (b1), Nitroxin (b2), Barvar 2 (b3), Nitroxin + Barvar 2 (b4)).

The maximum number of leaf per plant in *Calendula officinalis* L. was observed under application of bio-fertilizer along with 75% the recommended of chemical fertilizer (Singh *et al.,* 2008). The inoculation of marigold (*Tagetes erecta* L.) seeds and roots with bio-fertilizer × 400 mg L^{-1} phosphorus produced the maximum number of leaf per plant (Hashemabadi *et al.,* 2012). The highest number of leaves in saffron (*Crocus sativus* L.) was observed in the treatment of 5 kg Nitroxin and 150 kg Urea treatment (Omidi *et al.,* 2009).

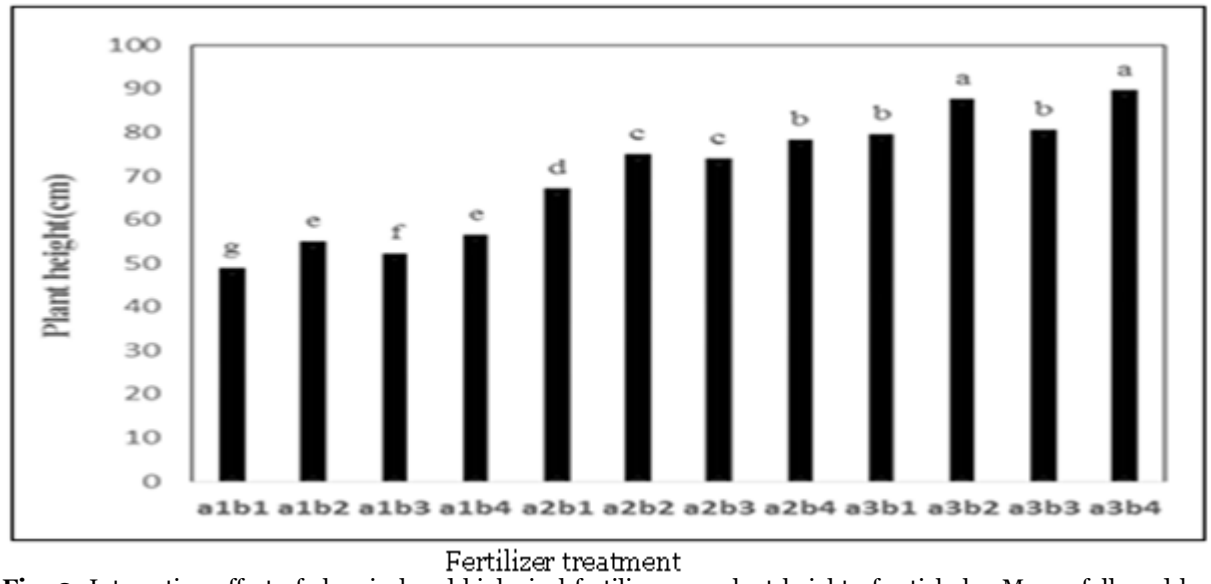

Fig. 2. Interaction effect of chemical and biological fertilizers on plant height of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.05 level of probability. (Control (a1), residual effect of 50% chemical fertilizer + 50 kg ha^{-1} supplementary nitrogen (a2), residual effect of 100% chemical fertilizer 100 kg ha^{-1} supplementary nitrogen (a3), control (b1), Nitroxin (b2), Barvar 2 (b3), Nitroxin + Barvar 2 (b4)).

Leaf length

According to significant main and interaction effects of all treatments on leaf length (Table 1),this trait was increased with increasing levels of chemical fertilizer at all levels of bio-fertilizers. The highest and the lowest artichoke leaf length were observed in the combination (residual effect of 100% chemical fertilizer+ 100 $kgha^{-1}$ supplementary nitrogen + Nitroxin+ Barvar 2) and control treatments with values of 76.16 and 56.33 cm, respectively (Fig. 3). Application of bio-fertilizer containing PGPR, increased the leaf length of saffron (Rasouli *et al.,* 2013).The leaf length of onion (*Allium cepa* L.) has been increased via inoculation seed with Barvar 2 bio-fertilizer (Bolandnazar *et al.,* 2014). The maximum length of peppermint (*Mentha piperita* L.) leaves obtained in the integrated and chemical systems (Kodori *et al.,* 2015).

Number of capitol per plant

The presented results in Table 1 have demonstrated that only the main effects of chemical and biological fertilizers on the number of capitol per plant were significant. Among the bio-fertilizer levels, the highest (6.66) and the lowest (4.88) capitol per plant were obtained by application of Nitroxin+ Barvar 2 bio-fertilizers and control levels, respectively (Table 3). Also, among chemical levels, the highest number of capitol (8.25) was recorded in residual effect of 100% chemical fertilizer+ 100 kg ha^{-1} supplementary nitrogen (Table 2). Darzi *et al.* (2012)indicated that the maximum umbel number per plant were obtained with two treatments of inoculation with *Azotobacter* and inoculation with *Azospirillum* in coriander (*Coriandrum sativum*). Kariminejad and Pazoki (2015) showed that the highest number of capitols per

plant in German chamomile (*Matricaria chamomilla* L.) belonged to a treatment with 130 Kg N ha^{-1}. Moghimi *et al.* (2011) reported that application of Nitroxin had significant and positive effects on the number of bush heads of safflower (*Carthamus tinctorius*).

Plant fresh weight

Based on Table 1 results, among bio-fertilizer levels, the highest (5360.30 g) and the lowest plant fresh weight (4792.74 g) were obtained with application of Nitroxin + Barvar 2 bio-fertilizers and control, respectively (Table 3). Increasing chemical fertilizer levels caused a significant increase in weight of artichoke plant. Among chemical levels, the highest weight (6605.33 g) was dedicated to the application of residual effect of 100% chemical fertilizer+ 100 kg ha^{1} supplementary nitrogen also increasing chemical fertilizer levels cause a significant improvement in weight of artichoke plant (Table 2). The increase in plant fresh weight may be due to the increase of N in the root zone as a result of chemical fertilizer application and fixed N by bacteria. Also, the solubilization of mineral nutrients, synthesis of vitamins, amino acids and gibberellins, which stimulate growth and yield (Sprenat, 1990).

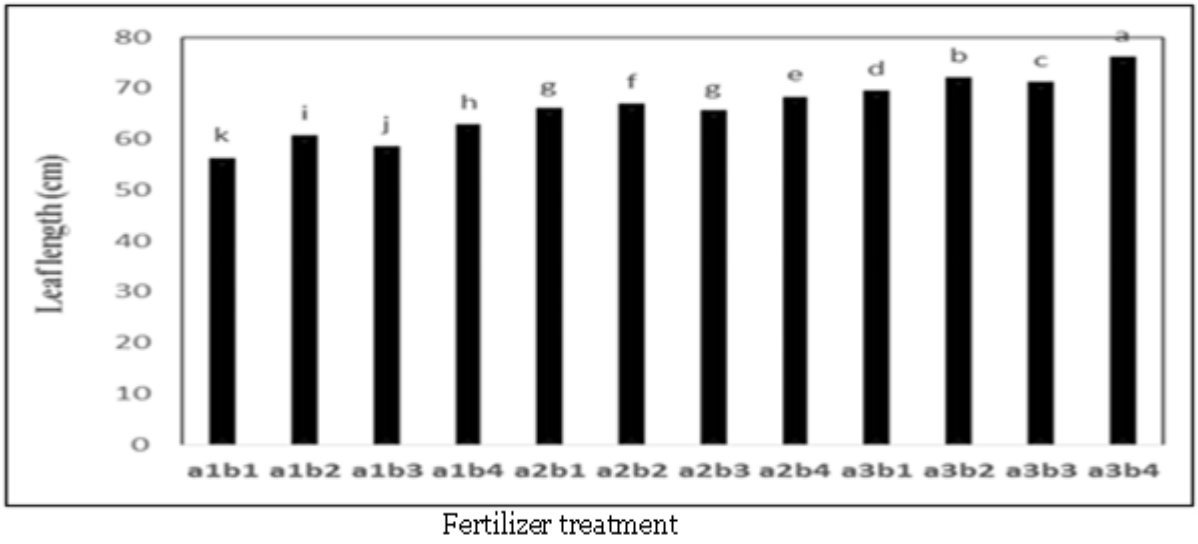

Fig. 3. Interaction effect of chemical and biological fertilizers on leaf length of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.05 level of probability. (Control (a1), residual effect of 50% chemical fertilizer + 50 kg ha^{-1} supplementary nitrogen (a2), residual effect of 100% chemical fertilizer 100 kg ha^{-1} supplementary nitrogen (a3), control (b1), Nitroxin (b2), Barvar 2 (b3), Nitroxin + Barvar 2 (b4)).

Some hormone substances, such as gibberellins, auxins and cytokinins with stimulate the cell elongation and division increase plant growth (Paleg *et al.,* 1985; Cacciari *et al.,* 1989).A nutrient solution containing at least 130 mg N L^{-1} and rates of 100 and 250 mg L^{-1} of P and K resulted in maximum shoot fresh and dry weight in artichoke plant (Elia and Santamaria, 1994). In a study artichoke leaf fresh weight significantly increased when N-fertilization increased from 95 to 285 kg N ha^{-1} (Salamah, 1997).

The reduction of nitrogen application from 500 to 300 kg N ha^{-1} resulted in a reduction of the total biomass of artichoke (Pedreno *et al.,* 1996). The inoculation of *Hibiscus sabdariffa* with the mixture of phosphate dissolving bacteria in combination to 50 or 100% NPK increased shoot fresh weight (Abo-Baker and Mostafa, 2011).

The fresh weight of *Majorana hortensis* L. plant was positively affected by bacterial inoculation under faba bean straw or sheep manure compared to control plant (El Ghadban *et al.,* 2006).

Plant dry weight

According to Table 1, the main effects of bio and chemical fertilizers on plant dry weight were significant. Among chemical levels, the highest (825.67g) and the lowest (448.90 g) plant dry weight achieved in residual effect of 100% chemical fertilizer+ 100 kg ha^{-1} supplementary nitrogen and control, respectively (Table 2). Bio-fertilizer showed a significant effect on the dry weight of plant (Table 3), as the maximum plant dry weight (670.04g) was obtained in Nitroxin + Barvar 2 and had no significant difference with the use of Nitroxin alone. As same as shoot fresh weight. Effect of Nitroxin on the dry weight of plant was due to enhancing nitrogen uptake and growth rate improvement. The favorable effect of chemical fertilizer and bio-fertilizers on plant growth attributes might be due to the improved nutrition and production of growth-promoting substances by microorganisms (Dhillo *et al.,* 1980). Salem (1986) stated that the application of nitrogen + bio-fertilizers increase in available amounts of nutrients, also, microbiological processes can change unavailable forms of nutrients into available ones that can be easily assimilated by plants (Alaa El-Din, 1982). Artichoke leaf dry weight increased with increasing nitrogen application from 95 to 285 kg N ha^{-1} (Salamah, 1997).

The dry weight of *Majorana hortensis* L. plants was positively affected by bacterial inoculation with sheep manure compared to control plant (El Ghadban*et al.,* 2006). The highest dry weight of black cumin (*Nigella sativa* L.) plant was obtained in Nitroxinusage (Akbarian *et al.,* 2013). Said-Al Ahl*et al.* (2015) indicated thatthe effect of nitrogen or bio-fertilizers was significantly increased fresh and dry weights of dill (*Anethum graveolens* L.) plant.

Conclusion

This study indicated that the highest amount of chlorogenic acid content was obtained by residual effect of 50% chemical fertilizer+ 50 kg/ha supplementary nitrogen treatment. The use of chemical fertilizers up to 50% increased the acid content, but further consumption reduced it. Bio-fertilizers had significant and positive effects on all the measured traits compared to control through improving the availability of nutrients. Maximum of plant height, number of leaf per plant, leaf length, plant fresh weight, plant dry weight and number of capitol per plant were achieved in inoculation of artichoke seeds with Nitroxin and Barvar 2. Application of combined chemical and bio-fertilizers resulted in maximum plant height and leaf length in artichoke plant. Finally, it was concluded that using the integrated plant nutrition system significantly improved the chlorogenic acid content and morphological traitsof artichoke.

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