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Effect of Chemical and Biological Fertilizers on Quantitative and Qualitative Yield of Artichoke (*Cynara scolymus* L.)

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ABSTRACT: In order to evaluate the effect of different nutritional treatments on forage yield and some qualitative traits of artichoke (*Cynara scolymus* L.), an experiment was conducted as factorial experiment on the basis of RCBD design with three replications in Isfahan Agriculture and Natural Resources Research Station, Iran during 2014. The first factor consisted of chemical fertilizers at levels of 100% chemical fertilizer (200-100 kgha^{-1} NP), 50% chemical fertilizer (100-50 kg ha^{-1} NP), and control. The second factor comprised biofertilizer at levels of 1 liter ha^{-1} Nitroxin (include *Azotobacter, Azospirillium* and *Pseudomonas*), 100 gha^{-1} Barvar 2 (include *Pseudomonas potida* and *Bacillus lenthus*), 1 liter ha^{-1} Nitroxin +100 gha^{-1} Barvar 2 and control. The results showed that soil fertilization treatments significantly affected forage quantity and qualitative traits of artichoke. The effects of chemical fertilizers on the majority of traits in artichoke were statically significant, however, there was no significant differences between treatments in about ash%. The interaction between biological and chemical fertilizer was significant on all traits. The best quality and forage yield were obtained in integrated plant nutrition. The highest dry matter yield (12.1 t ha^{-1}), crude protein (19.53%), phosphorous content (622.2 mg/kg) organic matter digestibility (86.56%)were obtained by Nitroxin + Barvar 2+ 100% chemical fertilizers (urea + triple super phosphate).The control treatment had the highest acid detergent fiber % (ADF) and neutral detergent fiber % (NDF). In general, results showed that seed inoculation with biofertilizer in the integration with chemical fertilizers improved forage yield and quality of artichoke. The application of bio fertilizers alone could not fulfill the nutritional requirements of artichoke. It seems that it could be used as a complementary fertilizer with chemical fertilizer in sustainable agricultural practices.

Key Words: Artichoke; biofertilizers; forage quality; gas production; integrated plant nutrition system

INTRODUCTION

Artichoke (*Cynara scolymus* L.) (Asteraceae) is an herbaceous perennial plant of Mediterranean origin, North Africa, Canary isles and Southern Europe. Nowadays, artichoke is cultivated in many parts of the world, such as the United States - mainly in California, in South America (Argentina, Chile, Peru), North Africa, Near East (Turkey and Iran) and China (Ierna & Mauromicale 2010; Lattanzio *et al.* 2009; Pandino *et al.* 2011a,b). Several uses such as (i) human food (ii) lignocellulosic biomass for energy (Ierna & Mauromicale 2010; Gominho *et al.* 2011) and paper pulp (Gominho *et al.* 2009), (iii) seed oil for biodiesel fuel production (Raccuia & Melilli 2004), (iv) roots for insulin (Raccuia & Melilli 2010), (v) leaves and heads for pharmaceutical compounds (Pandino *et al.* 2011; Rondanelli *et al.* 2011; Aksu & Altinterim 2013), (vi) green forage for ruminant feeding (Meneses *et al.* 2005; Sallam *et al.* 2008; Fatma *et al.* 2011) are considered for the species. There are limited research on the types and amounts of chemical and biological fertilizers effects on performance of artichoke. Bahreininejad *et al.* (2004) with the survey of appropriate densities and nitrogen fertilizer in *Cynara scolymus* L. reported that fresh weight, dry matter and plant height of artichoke significantly increased with fertilizer utility in the levels up to 200 kg urea /ha.

Fateh *et al.* (2009) with the survey of various systems of soil fertility (chemical, organic and chemical × organic) on the quantity and quality of artichoke reported that except for ash percentage, soil fertility system had significant effect on quantitative and qualitative traits of artichoke and the combined fertilization of chemical × manure treatments produced the highest dry and crud protein. Elia *et al.* (1996) investigated 4 different ratios 100:0, 70:30, 30:70 and 0:100 of ammonium: nitrate (NH_4^+:NO_3^-) for artichoke growth. Their results showed that NO_3^- was the preferred N form, with 70-100% NO_3^- resulting in the best vegetative growth, largest leaf area and root volume and the greatest dry weight. Increasing NO_3^--N to 100% increased water use efficiency by 2.5-fold compared with 100% NH_4^+. Pedreno *et al.* (1996) reported that the reduction of nitrogen application from 500 to 300 kg N ha^{-1} resulted in reduction of total biomass of artichoke. Positive effects of biofertilizers and combination of biofertilizers with organic or chemical fertilizers on growth and yield of crops were reported in several studies. Tarang *et al.* (2013) with the survey of grain yield and quality of corn (Maxima Cv) in responses to Nitroxin bioferilizer and chemical fertilizers indicated that Nitroxin bioferilizer and chemical fertilizers had significant impact on leaf chlorophyll at anthesis stage, 1000 grain weights grain and biological yield, harvest index, protein, soluble carbohydrate, K and Na content of seed. Azarpour *et al.* (2012) reported that application of Nitroxin enhanced the growth parameter and yield in soybean cultivar. Moghimi *et al.* (2012) showed that Nitroxin biologic fertilizer had positive effect on increase of grain yield of safflower through nitrogen fixation and production growth stimulant hormones. Banari *et al.* (2015) with the survey of effect of bio-fertilizer (Barvar2) on the yield and yield components of bread wheat and durum showed that the use of phosphorus biological fertilizer can be used as a substitute for a portion of the phosphorus fertilizer, without a decrease in yield occurs. Shabani *et al.* (2015) stated that application of integrated fertilizing treatments not only decreased the chemical fertilizer application, but it also enhanced forage quality of Annual Medic (*Medicago scutellata* cv. Robinson) in terms of higher macro- and micro-nutrients concentrations. Verlinden *et al.* (2010) found that application of biofertilizers increased vegetative organs of the grassland plants. Since there is no enough evidence on the effects of bio-fertilizer application on artichoke yield, the aim of the present study was to investigate the effects of biofertilizers and chemical fertilizers on artichoke yield and, consequently, reducing the application of chemical fertilizers.

MATERIALS AND METHODS

This experiment was conducted at the Research Station of Isfahan Agricultural and Natural Resources (18 km west Isfahan, 32° 37' N, 51° 28'E and an altitude of 1612 m), Iran during 2014. This site was characterized by a semi-arid climate with a mean annual precipitation of 140 mm (mainly during the fall and winter) and an average temperature of 16°C) Karimi, 1992; Yaghmaei *et al.* 2009). Soil samples were taken before sowing of crop at a depth of 30 cm for physio-chemical analysis. The soil characteristics were composed as the following: sand (45%), silt (24 %), clay (31%), pH (7.7), EC (2.8 dS m^{-1}), organic matter (0.45%), total nitrogen (0.04%), P_2O_5 (12ppm) and K_2O (250 ppm). The factorial set of treatment was arranged within RCBD with three replications. The experiment, consisted of two factors chemical fertilizers as the first factor with levels 100% chemical fertilizer (200-100 $kgha^{-1}$ NP), 50% chemical fertilizer (100-50 $kgha^{-1}$ NP), control and the second factor at biofertilizer levels of 1 $literha^{-1}$ Nitroxin (include *Azotobacter, Azospirillium* and *Pseudomonas*), 100 gha^{-1} Barvar 2 (include *Pseudomonas potida* and *Bacillus lenthus*), 1 liter ha^{-1} Nitroxin+100 gha^{-1} Barvar 2 and control. Chemical fertilizers were included urea (46%N) and triple super phosphate (46%P_2O_5). The first half of the nitrogen and total phosphorus fertilizers were utilized as strip takes under seed before sowing (26 April, 2014) and the rest at 7-8 leaf stage of crop on 26 May, 2014. Artichoke seeds were inoculated with Nitroxin and Barvar 2, according to the manufacturer's instructions. All other cultural practices were kept normal and uniform for all treatments. Plot size was 5×3.5 m. Each plot consisted of 5 rows of 5 m length, spaced 60 cm. The desired plant population was obtained by over seeding and hand thinning. Intra row plant intervals was 30 cm. Plant density was 4 plants/m^2. Artichoke seeds of the local ecotypes were provided from Isfahan Agriculture and Natural Resources Research Station. Seeds were sown by hand in April 2014 in 5 cm depth of soil. All plots were irrigated after sowing and subsequent irrigations were conducted as required. Weeds were controlled by hand during crop growth and development. The plants were harvested at vegetative rosette stage in from1 m2inthe middle rows of each plots on 6 September, 2014. After harvesting, samples were dried normal air circulation until to reach constant dry weight and then was recorded for each plot. Samples were then ground to pass through a 1 mm sieve and then were used for chemical analysis and in vitro gas production technique. Crude protein (CP, method ID 984.13) total ash (method ID 942.05) and phosphorous content (method ID 965.17) were determined by procedures of AOAC (1999). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured using the methods of Van Soest *et al.* (1991). Fedorak and Hrudey (1983) water displacement technique were used for gas production measurement. In this method, water displacement in a test tube with glasses containing rumen fluid and food samples, indicate the rate of gas production. Foods were ground evenly by mill having sieve pores with a diameter of 2 mm then 300 mg of each grounded foods weighted carefully and transferred into a sterile 50 ml glass serum.

For each food sample three replications were considered. Rumen fluids required in the test gas production were gathered from two fistula sheep two hours after the morning meal feed. The rumen fluid before moving into glass serum, were mixed buffer prepared by Mcdougall method (1948) at 1 to 2 ratios (a section of the rumen fluid and 2 section of the buffer). 20 ml mixture of rumen fluid and buffer were added each test tube and transferred into the shaker incubator at 120 rpm and at 39°C. The amount of gas produced from the fermentation of food was recorded at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h after incubation, respectively. The obtained data from gas production at 24 h after incubation were used according to the following equations adapted from Menke and Steingass (1988) for estimation of OMD.

$$OMD\ (\%\ DM) = 9.00 + 0.9991\ GP + 0.0595\ CP + 0.0181\ CA \quad (n=200,\ r^2 = 0.92) \qquad \ldots(1)$$

The collected data were analyzed using MSTAT-C software and mean values were grouped by Duncan (in significant level of 1 & 5%).

RESULTS AND DISCUSSION

A. Dry matter yield

Results showed that interaction effect of bio and chemical fertilizers on dry matter yield of artichoke was significant (Table 1). The highest and the lowest artichoke yield were observed at the Nitroxin+ Barvar 2+ 100% (urea + triple super phosphate) treatment combination and control treatment with values of 12.1 t ha^{-1} and 5.2t ha^{-1}, respectively (Fig. 1). According to these result, it was showed that application of any fertilizer significantly increased artichoke yield. These priority were cleared that 64.3, 23.8 and 132.7 % for chemical fertilizers, biological fertilizers and integrated system, compared to control, respectively. Also, between biofertilizers, Nitroxin effect in yield important was more than that of Barvar 2 in all treatments. It can be attributed to beneficial effect of PGPRs. Plant-growth promotion by PGPRs appears to be due to the release of factors that either prevent the deleterious effects of pathogenic organisms or facilitate nutrient uptake from the environment (Kloepper 1993). Shabani *et al.* (2011) reported that application of integrated nutrient system in annual medic had a synergetic effect on yield improvement. Chaichi *et al.* (2015) indicated that the highest forage yield of berseem clover was produced in integrated fertilizer application (urea chemical fertilizer + mycorrhiza treatment). Ehteshami *et al.* (2012) in case reported similar results of phosphate fertilizer and phosphate solubilizing bacteria effects on forage quantitative and qualitative of two barley cultivar.

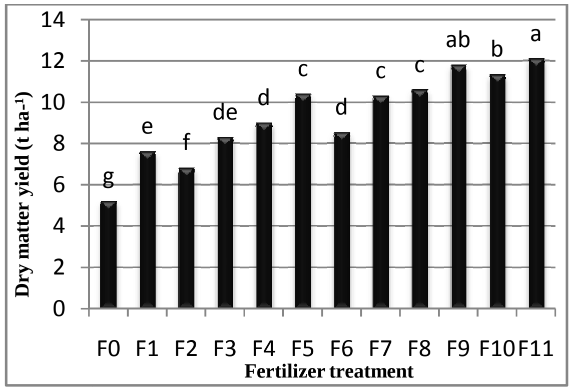
Table 1: Analysis of variance for dry matter yield and quality traits of Artichoke.

SOV	df	Mean squares (MS)						
		Dry matter yield	CP	Ash	NDF	ADF	P	OMD
Replication	2	1.250**	4.059*	0.79^{ns}	2.114^{ns}	1.42^{ns}	188.814*	113.278**
Chemical fertilizer	2	60.768**	54.794**	0.745^{ns}	38.280**	25.72**	48595.290**	980.556**
Biofertilizer	3	7.516**	28.671**	4.252**	54.530**	36.621**	4320.383**	133.026**
Chemical fertilizer* Biofertilizer	6	0.857**	5.840**	3.533**	40.677**	27.319**	957.205**	29.437*
Error	22	0.183	0.930	0.44	1.662	1.115	33.557	7.927
CV%		4.59	6.34	4.18	5.44	5.43	1.15	3.73

Ns: Means no significant at level ($p \le 0.05$), *Means significant at level ($p \le 0.05$) and **Means significant at level ($p \le 0.01$)

B. Crude protein percentage (CP%)

On the basis of results, analysis of forage quality the main effects and interaction effect of chemical and biological fertilizers were statistically significant ($p<0.01$) for CP% (Table 1). For the integrated system, maximum CP% value (19.53%) was obtained at Nitroxin + Barvar 2 + 100% urea + triple super phosphate fertilizer (Fig. 2). Crude protein percentage was enhanced increased in chemical, biological and integrated systems by 32, 26 and 96 % in comparison with control treatment. In study of the effect of fertilizer types on corn forage quality, it was concluded of chemical and manure fertilizer combinations could be decreased chemical fertilizer utilization without any considerable reduction in forage quality (Ebrahim-Ghoshchi *et al.* 2012). Co-inoculation of alfalfa seed with different biological fertilizers in a phosphorus deficient soil resulted in increased forage dry matter, more N_2 fixation and better phosphorus content, compared to inoculation with single bacteria (Stancheva *et al.* 2008). Singh *et al.* (2010) reported a significant improvement in the crude protein content of corn and wheat following inoculation with PGPR.



F0: Control, F1: Nitroxin, F2: Barvar 2, F3: Nitroxin+ Barvar 2, F4: 50% Chemical fertilizer, F5: 50% Chemical fertilizer + Nitroxin, F6: 50% Chemical fertilizer + Barvar 2, F7: 50% Chemical fertilizer+ Nitroxin+ Barvar 2, F8: 100% Chemical fertilizer, F9: 100% Chemical fertilizer + Nitroxin, F10: 100% Chemical fertilizer + Barvar 2, F11: 100% Chemical fertilizer + Nitroxin+ Barvar 2.

Fig. 1. Interaction effect of chemical and biological fertilizers on dry matter yield of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.05 level of probability.

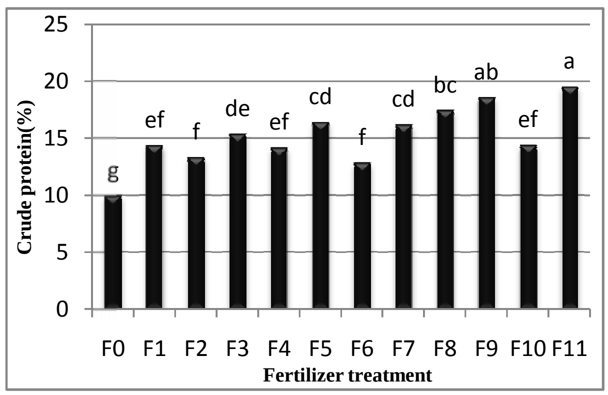


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Fig. 2. Interaction effect of chemical and biological fertilizers on crude protein percentage of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.05 level of probability.

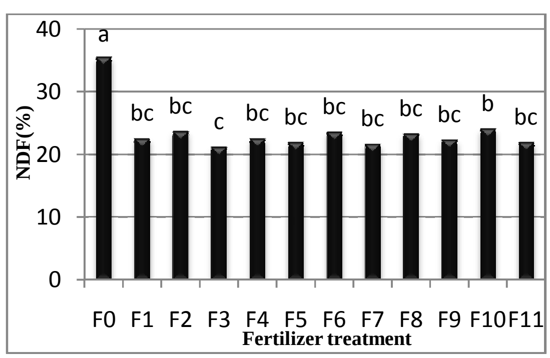

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Fig. 3. Interaction effect of chemical and biological fertilizers on neutral detergent fiber of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at level of 0.05 probability.

C. Neutral detergent fiber (NDF)

Neutral detergent fiber (showing intake potential) is an index indicating forage quality (Hackmann *et al.* 2008). According to the significant effects of biological × chemical fertilizers on NDF %, the maximum and the minimum values of this trait were observed in control and Nitroxin + Barvar 2 treatment combination with 35.53 and 21.20 %, respectively (Fig. 3). Eshaghi Sardrood *et al.* (2013) in study of biofertilizers and chemical fertilizers effects on qualitative and quantitative yield of forage sorghum reported that the highest and the lowest NDF percentage were achieved in control and treatment combination of 50% urea +50% triple super phosphate + barvar 2, respectively. Chaichi *et al.* (2015) in a study noted that different fertilizer types had significant effects on NDF % in berseem clover and the highest NDF with value of 54.9% was observed in nitrogen-fixing bacteria+ triple superphosphate. Application of *Pesudomonas* bacteria decreased the soluble natural detergent fiber (NDF) up to 50.4 %, however, the lowest soluble natural detergent fiber were achieved by using of 60 kg.ha^{-1} triple superphosphate (Mehrvarz & Chaichi 2009).

D. Acid detergent fiber (ADF)

Acid detergent fiber (ADF) is an important factor that affects energy or total digestible nutritious material of forage (Hackmann *et al.* 2008). Interaction effect of chemical and biological fertilizers on ADF% were significant (Table 1). The highest and the lowest ADF %were achieved in the control treatment and Nitroxin + Barvar 2+ with values of 29.12 and 17.38 %, respectively (Fig. 4). According to results it was represented that biofertilizers can reduce ADF%, with both application and non - application chemical fertilizers. Also Nitroxin and barvar 2 had similar effect on this trait, so this effect wasn't affected by the usage of alone or in combination together. Eshaghi Sardrood *et al.* (2013) reported that the maximum and minimum ADF% were observed in the control treatment and 50 % urea +50 % triple super phosphate + barvar 2, respectively in forage sorghum. In study of the effect of *Rhizobium leguminosarum, Pseudomonas* florescence and different levels of chemical fertilizers on forage quantitative and qualitative of Persian clover the lowest ADF (34.25%) was belonged to *Pseudomonas* (Shahverdi *et al.* 2014). Mehrvarz and Chaichi (2009) reported that the co-application of bacteria strain 41 and Mycorrhiza had a positive effect on decreasing the ADF value in barely (*Hordeum vulgare* L.).

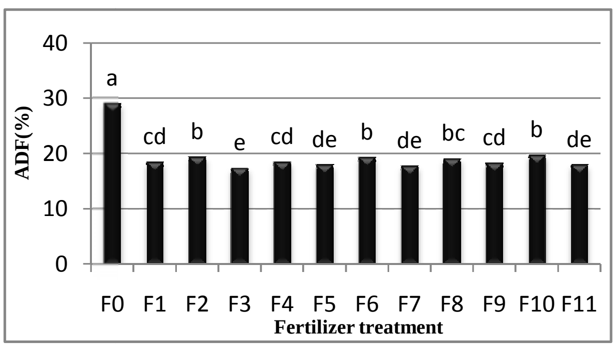

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Fig. 4. Interaction effect of chemical and biological fertilizers on acid detergent fiber of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.01 level of probability.

E. Organic matter digestibility (OMD)

On the finding of this research, co-application of bio and chemical fertilizers enhanced OMD, and among treatment combinations, Nitroxin + Barvar 2+ 100% urea + triple super phosphate values in the highest increasing (Fig. 5). This result was similar to traits of yield, P, CP. Sultana (2003) indicated that different doses of N fertilizer on cowpea forage production, a non-significant effect on in vitro OM digestibility was observed as N-fertilizer increased from 0 to 45 kg N/ ha. Similarly, Khan *et al.* (1992) did not find any significant increasing in the in vitroOM digestibility (70.80 and 71.60%) at N-fertilizer application rate of 20, 40, 60 kg/ha on cowpea forage.

Eshaghi Sardrood *et al.* (2013) stated that the highest digestible organic matter of forage sorghum was obtained from treatment of 50% (urea + triple super phosphate) + biosoper + barvar 2.

F. Ash percentage

The results presented in Table 1 have revealed that the effect of biofertilizers and interaction of biological × chemical fertilizers had significant effects on ash percentage in artichoke plants ($P \leq 0.01$). Nitroxin + Barvar 2 + 50% urea+ triple super phosphate and control treatment with 17.45 and 13.78 % had the highest and the lowest of total ash values, respectively (Fig. 6).

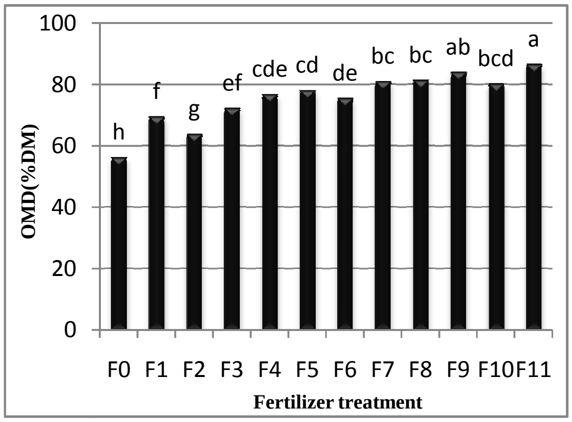

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Fig. 5. Interaction effect of chemical and biological fertilizers on organic matter digestibility of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.05 level of probability.

The effect of Barvar2 in total ash was more than that of nitroxin without application of chemical fertilizer, but there were no significant differences in the integrated treatment. Ehteshami *et al.* (2012) reported that barley seed inoculation with *P. fluorescens* +75% triple super phosphate fertilizer showed the highest percentage of total ash, where as minimum resulted in control. Eshaghi Sardrood *et al.* (2013) noted that the highest ash percentage was achieved in the urea (100%) treatment in forage sorghum. Shahverdi *et al.* (2014) reported that native rhizobium with reduced application of chemical fertilizers treatment as compared to no application of biological fertilizers with recommended chemical fertilizers increased ash percentage (3.25%) in Persian clover (*Trifolium rosapinatum* L.).

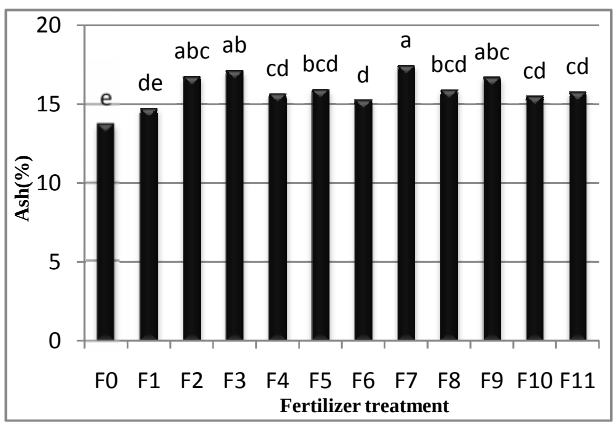

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Fig. 6. Interaction effect of chemical and biological fertilizers on ash percentage of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.05 level of probability.

G. Phosphorous content (P)

Result showed that treatment combination of Nitroxin + Barvar 2+ 100% (urea + triple super phosphate) and control treatment with values of 622.2 and 437.8 mg/kg had maximum and minimum P content, respectively (Fig. 7). Therefore, biofertilizer and chemical fertilizer application had synergetic effect on P content.

Increasing the availability of P in soils with inoculation of PGPR, which may lead to increased P uptake and plant growth, was reported by many researchers. Çakmakçi *et al.* (2007; 2009) reported that phosphate solubilizing and N_2-fixing PGPR increased the uptake of P in spinach and wheat plants.

Mehrvarz and Chaichi (2008) stated that biological fertilizers containing phosphate solubilizing bacteria have the ability to supply the phosphorus needed by plants and stimulate the growth of the plant. Shabani *et al.* (2015) stated that integrated application of P solubilizing bacteria, Mycorrhiza fungi, and N-fixing bacteria had a synergetic effect on the plant P concentration. This result could be explained by PGPR positive contribution in the efficiency of nutrient absorption by plants, which improved uptake of minerals such as P. Zarabi *et al.* (2011) showed that phosphate solubilising microorganisms can positively have effect on the increase of plant growth and phosphorus absorption in maize plant.

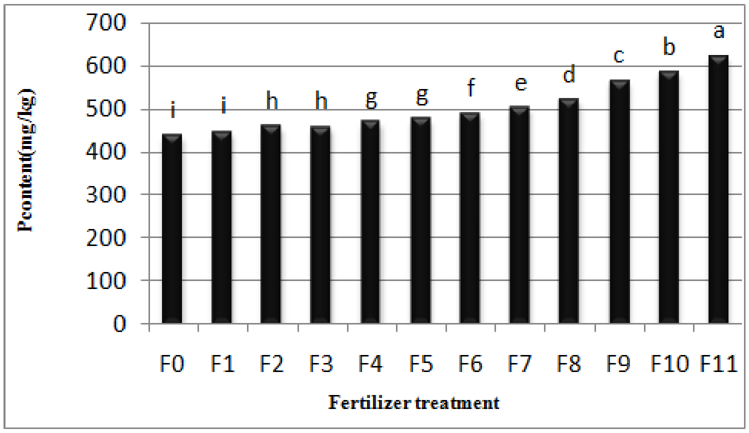

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Fig. 7. Interaction effect of chemical and biological fertilizers on phosphorus content of artichoke. Means followed by dissimilar letters are significantly different based on Duncan test at 0.05 level of probability.

Table 2: Means comparison of dry matter yield and quality traits of artichoke in four levels of chemical fertilizer.

Chemical fertilizer	Dry matter yield (t ha^{-1})	CP (%)	P (mg/kg)	NDF (%)	ADF (%)	OMD (% DM)
Control	6.9750 ^{c}	13.25 ^{c}	450.2 ^{c}	25.75 ^{a}	21.10 ^{a}	65.43 ^{c}
50% Chemical fertilizer	9.5583 ^{b}	14.90 ^{b}	484.5 ^{b}	22.92 ^{b}	18.79 ^{b}	77.82 ^{b}
100% Chemical fertilizer	11.4583 ^{a}	17.49 ^{a}	573.5 ^{a}	22.44^{b}	18.39 ^{b}	83.03 ^{a}

Means in each column, followed by similar letter(s) are not significantly different at 5% probability Level, using Duncan's Multiple Range Test

Table 3: Means comparison of dry matter yield and quality traits of artichoke in three levels of bio-fertilizer.

Bio-fertilizer	Dry matter yield (t ha^{-1})	CP (%)	P (mg/kg)	Ash (%)	NDF (%)	ADF (%)	OMD (% DM)
Control	8.2667 ^{c}	13.51 ^{b}	476.3 ^{d}	15.11 ^{b}	27.13 ^{a}	22.23 ^{a}	71.42 ^{c}
Nitroxin	9.9333 ^{a}	16.45 ^{a}	495.8 ^{c}	15.78 ^{ab}	22.28 ^{b}	18.26 ^{c}	77.17 ^{ab}
Barvar 2	8.8889 ^{b}	13.86 ^{b}	511.0 ^{b}	15.83 ^{ab}	23.80 ^{b}	19.51 ^{b}	73.20 ^{bc}
Nitroxin+ Barvar 2	10.2333 ^{a}	17.03 ^{a}	527.8 ^{a}	16.78 ^{a}	21.60 ^{b}	17.7 ^{c}	79.93 ^{a}

Means in each column, followed by similar letter(s) are not significantly different at 5% probability Level, using Duncan's Multiple Range Test

CONCLUSIONS

In this research soil fertilization had a significant effect on forage yield and quality of artichoke. Obtained findings indicated that the soil on the experimental site was poor and all the fertilization treatments significantly increased forage quality parameters compared to control. The effect of biofertilizers on the majority of traits in artichoke were significant.

The best quality and forage yield were obtained in integrated plant nutrition systems. The highest amount of dry forage yield (12.1 t ha^{-1}), crude protein (19.53%), p content (622.2 mg/kg) and organic matter digestibility (86.56%) obtained in integrated application of biofertilizers and chemical fertilizers, particularly, inoculation with Nitroxin + Barvar 2+100 % chemical fertilizers. This study showed synergistic effects of combined inoculation of PGPRs. 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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