Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2322-3227 (Online)
Vol. 1, No. 2, 2015
http://JCNS.iauahvaz.ac.ir
OPEN ACCESS

Effect of Biological and Chemical Nitrogen Fertilizers and Plant Density on Quantitative and Qualitative yield of Silage Corn

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RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *29 Mar. 2015*
Received in revised form: *24 Apr. 2015*
Accepted Date: *31 May. 2015*
Available online: *1 Jul. 2015*

To Cite This Article:
Asoodeh Makvandi. Effect of Biological and Chemical Nitrogen Fertilizers and Plant Density on Quantitative and Qualitative yield of Silage Corn. ***J. Crop. Nut. Sci., 1(2):*** *36-48, 2015.*

ABSTRACT

In order to study the effect of chemical and biological fertilizers on silage corn under different plant densities, a split plot experiment was carried out in a randomized completely block design with three replicates in south western Iran. The main plot included nitrogen fertilizer treatment at three levels (N_1: with out chemical and organic fertilizers, N_2: 75% chemical fertilizer + 100% organic fertilizer, N_3: 100% chemical fertilizer+ 100% organic fertilizer) and the sub plot was density in three levels (D_1: 70000 plant.ha^{-1}, D_2: 90000 plant.ha^{-1}, D_3: 110000 plant.ha^{-1}). The results showed that the highest fresh and dry weight of forage, stem, and leaf of the corn was related to the treatment with 75% urea and 100% Nitroxin. D_1 (70000 plant.ha^{-1}) and D_2 (90000 plant.ha^{-1}) had higher yields than the other densities in terms of stem, leaf and forage dry weights, but it was not possible to identify appropriate density in terms of fresh weight of stem, leaf, corn and forage because the effects of different densities on these traits were not significant. N_2 treatment had the highest rate of plant height, number of leaves per plant, and corn/plant ratio. N_1 treatment without chemical or organic fertilizer had the lowest rate of mentioned features. Moreover, N_3 treatment had highest rate of protein and phosphor. Interaction effect of fertilizer and density was not significant on all studied traits. Generally, combined fertilizer treatment with 75% urea and 100% Nitroxin had a significant effect on the increase of forage yield in comparison to control treatment (without chemical and organic fertilizer). The optimum plant density (90000 plant.ha^{-1}) increased yield and more importantly due to competition and shadowing reduced the dry matter and yield in this experiment.

Keywords: *Bio-Fertilizer, Density, Maize, Nitroxin.*

INTRODUCTION

Due to the increasing importance of corn (*Zea Mays* L.) in feeding humans and animals and its wide compatibility with temperate and tropical climate zones, it is considered as one of the strategic crops (Yazdani *et al*, 2009). The increase of corn acreage over the past few decades, intensive planting systems of the corn and its high nutrient demand have not only increased the excessive use of chemical inputs but also have increased costs of production and have

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caused some environmental risks (Biari *et al*., 2008). That is why in the last two decades, due to these outcomes, the use of soil bacteria in feeding the soil and crops has increased within the sustainable agriculture systems throughout the world. These bacteria which have actively occupied plants crops and increase the plants growth and yield (Wu *et al*., 2005). Include Azospirillum, Azotobacter, Bacillus and the Pseudomonas (Stancheva and Dinew, 2003). The use of biological fertilizers for maintaining biological balance and soil fertility in order to maximize the desired biological relationships of the system and to minimize the use of materials or operations which disturb such relations, particularly the use of the chemical fertilizers is particularly important. Even though the use of biological fertilizers has a long history in agriculture and not long ago all the food for human consumption was produces using such valuable resources, the scientific use of such resources does not have a long history (Stancheva et al., 1999). The application of bio-fertilizers especially growth enhancers is the most important strategy for the integrated management of plant nutrition at sustainable agriculture system with adequate input as the integration of chemical fertilizers and aforementioned bacteria (Sharma, 2003). Vadivel *et al.* (1999) concluded that the application of Nitroxin biological fertilizer had a significant effect on all measured parameters except 1000-grian weight and grain-straw ratio. Grain yield, straw yield, plant height, spike length, number of grains per spike and number of spikes per square meter increased in Nitroxin treatment in comparison to the control treatment. The results indicate that the soil of this area due to continuous cultivation, poverty and unavailability of nutrients has positively responded to Nitroxin biological fertilizer and its effect on the plant growth and yield is observed. The increase of dry matter production in corn as a result of nitrogen application has been reported in several studies. For instance, in studying the effect of nitrogen on quantitative and qualitative properties of forage corn, Zahir *et al.* (2004) found that the increase of nitrogen consumption up to 300 $kg.ha^{-1}$ increased the dry matter and mentioned that its main reason was the improvement of corn physiological parameters due to the use of nitrogen fertilizers. Uhart and Andrade (1995) showed that as the soil nitrogen increased, the corn dry weight increased, too which could be due to its effect on the crop growth rate, leaf area index and leaf area duration. Azotobacter and the Azospirillum are the most important growth regulators bacteria which in addition to biological nitrogen fixation, affect the crops growth and yield by producing remarkable amount of growth enhancer hormones particularly different kinds of auxin, gibberellin, and cytokinin. Kapulnik *et al.* (1982) showed that the plant height, dry and fresh weight of corn leaves increase due to the seeds inoculation with azospirillum bacteria. Khan and Joergensen (2009) reported that the fresh weight of plan shoots, number of leaves and the height of corn increased due to the seeds inoculation with the bacteria. In a field experiment, Nanda *et al.* (1995) observed that the corn seeds inoculation with the Azotobacter and Azospirillum increased the yield of green fodder in treatments with different levels of nitrogen fertilizer. Jilani *et al*. (2007) observed that the corn grain yield increased due to the corn seed inoculation with Azotobacter Choorcoccun and Azospirillum Brasilenese and the use of nitrogen fertilizer in comparison to the use of nitrogen fertilizer alone or the seed inoculation with any of these bacteria without use of nitrogen fertilizer. Albayrak and Yuksel (2010) reported the increase of dry matter inoculation due to high effect of nitrogen on leaf area and its better continuity. Pandi *et al*. (1998) observed that due to the seed inoculation with Azospirillum Brasilenese, nitrogen and phosphorus of different parts of plant and the corn yield considerably increased. Corn is very sensitive to plant density and in lower densities, the production factors are not optimally utilized. On the other hand, excessive plant density causes flower sterility and decrease of grain yield (Baser Kouchebagh *et al*., 2012). Turk *et al*. (2009) showed that the effect of the kind of fertilizer on protein percentage and ash percentage was significant, so that the highest ash percentage was observed in manure fertilizer and the highest protein percentage was observed in chemical fertilizer. Chen (2006) reported that the decrease of the rate of accessible assimilates at high levels of plant density due to the reduced light resulted in the decrease of the number of seeds per row and the abortion of seeds at the end of the corn. The results of the research conducted by Ramazan *et al*. (2007) showed that the space between the rows only affected the yield of fresh forage. The corn diameter and the dry weight of single plant was more in the density of 70000 plants per hectare. Rees *et al*. (2009) showed that the use of biofertilizers particularly Azospirillum could considerably improve the rate of dry matter, nutrients uptake, plant height, leaf size, and root length in cereal crops. Koliai *et al*. (2012) reported that even though the density of forage corn was more than that of silage corn, the quality of forage at higher densities decreased. Muchow and Wilson (1999) believed that as the plant density increased, the plant height also increased to some extent. They thought that the only reason was the plants competition to receive more light. Besides, they found out that if the plant density increased too much, plants would compete to receive not only more light but also more resources and environmental factors and through excessive increase of density, the plant height would decrease (Tarang *et al*., 2013). With regard to the conducted researches, this research aimed to study effect of bacterial companions with in corn as a cereal crop family and nitrogen fertilizer (as to decrease exclusive use of nitrogen fertilizer) on quantitative and qualitative yield and features of forage yield for sustainable management of ecological farming structures through plant integrative nutrition.

MATERIALS AND METHODS

Field and Treatment Information

This experiment was carried out in the summer of 2011 in a research field in south western of Iran. The experiment was carried out as split plots based of randomized completely block design with three replicates. Main factor included: integrated fertilizer treatment three levels (N_1: without chemical fertilizer and Nitroxin, N_2: 75% chemical fertilizer + 100% Nitroxin, N_3: 100% chemical fertilizer + 100% Nitroxin). The biological fertilizer used in experiment included Nitroxin (Azotobacter Choorcoccun and Azospirillum Brasilenese) and urea fertilizer source as the sources of nitrogen supply. Sub factors included: D_1= 70000, D_2= 90000 and D_3= 110000 plant.ha^{-1}, respectively.

Crop Management

In order to apply the above treatment, urea fertilizer from ammonium phosphate source with 46% nitrogen was used in the region as recommended that is 400 kg.ha^{-1} (100%) and Nitroxin was used by 2 lit.ha^{-1} and the seeds were treated by Nitroxin before planting. After seed bed preparation, the seeds of each treatment were sowed.

Traits measure

Quantitative traits included measuring the rate fresh and dry forage yield, dry and fresh weight of leaf, dry and fresh weight of stem and dry and fresh weight of corn in area unit during the harvest. In order to measure morphological traits, five plants from each plot were randomly selected and their mean was used. In order to measure the dry weight the samples were placed in an oven in 75°C for 48 hours and weighed. Other qualitative traits such as the rate of phosphorus in the leaves and stem and corn were measured by spectrophotometric method and phosphomolybdate. Nitrate was measured by Kjeldahl method (Sharma, 2003).

Statistical analysis

After collecting data, the variance of all studied traits was analyzed by using Minitab software (Ver. 14). The means were compared through Duncan's multi range test at 5% probability level.

RESULTS AND DISCUSSION

Forage dry and fresh weight

According to the ANOVA results, the interaction effect of chemical and biologic fertilizers on the dry and fresh forage yield was significant at 1% probability level (Table 1). The highest dry and fresh weight belonged to N_2 treatment with 75% urea and 100% nitroxin and the lowest weight occurred in N_1 treatment without any chemical fertilizer and nitroxin (Table 2). It seems that nitrogen is the main component of the structure of ribonucleic acid and proteins in plants. The excessive increase or decrease of urea fertilizer will decrease the effect of Nitroxin and thus will reduce the yield of dry and fresh forage. Density had a significant effect on the yield of dry forage at 1% probability level, but it didn't have a significant effect on the fresh weight of the forage (Table 1). The highest yield of dry and fresh matter belonged to the treatment with the density of the 70000 plants.ha^{-1} by 164.16 and 798.16 g.plant^{-1} respectively and the lowest yield belonged to the density of 110000 plants.ha^{-1} by 152.83 and 758.58 g.plant^{-1} (Table 2). The Maximum weight of dry forage on the interactive effect of fertilizer and density belonged to N_3D_1 which indicated that density of 70000 plants.ha^{-1} was desirable and the lowest weight belonged to N_1D_3 (Table 3). The highest interaction effect of fertilizer and density on fresh forage belonged to N_3D_2, that is the ratio of 75% urea, 100% Nitroxin at the density of 90000 plants.ha^{-1}. The lowest belonged to N_1D_3 in which the plant competition for absorbing the nutrients and receiving more light decreased the yield of fresh forage (Table 3). With regard to correlation coefficient there was a positive significant correlation between the weight of fresh forage and dry forage at 1% probability level, i.e. the increase of the weight of fresh forage increased the weight of dry forage (Table 4).

Stem dry and fresh weight

Effect of chemical and biological fertilizers interaction on dry weight of stem was significant at 1% probability level. Its effect on fresh weight of stem was not significant (Table 1). The highest dry and fresh weight of stem belonged to N_2 treatment (75% urea, 100% Nitroxin) and lowest dry weight was related to N_1 treatment and lowest fresh weight belonged to N_3 treatment (Table 2).

Table 1. The ANOVA of fertilizer and density on measured traits

S.O.V	df.	Stem fresh weight	Stem dry weight	Leaf fresh weight	Leaf dry weight	Corn fresh weight	Corn dry weight	Forage fresh weight	Forage dry weight	Phosphorus	Protein	Plant height	Number of leaves per plant	Ratio of corn/plant
Replication	2	447.4 ^{ns}	180.19*	1534.15**	152.31**	998.7*	418.52 **	427 $^{n.s}$	331.31*	119.3 $^{n.s}$	31.77 n.s	43.94	1.39 $^{n.s}$	0.05 $^{n.s}$
Fertilizer	2	32354.2 ^{ns}	222.06**	332.58 **	40.38**	4620.3**	395.06 **	68023 **	1386.91**	31520.4 **	456.53 **	5234.47**	3.21**	1.4 **
Error a	4	822.8	234.47	54.37	7.11	65.2	35.33	1526	195.03	432.2	46.28	57.3	0.22	0.01
Density	2	4387.5**	195.39 **	65.58 ^{ns}	29.55**	462.9 $^{n.s}$	213.67 **	9461**	855.97**	27.7 $^{n.s}$	39.41**	52.91 $^{n.s}$	0.02 $^{n.s}$	0.15 $^{n.s}$
Density* Fertilizer	4	473.1 ^{ns}	13.48 ^{ns}	16.65 $^{n.s}$	4.83 $^{n.s}$	167.6 $^{n.s}$	14.17 $^{n.s}$	380 $^{n.s}$	23.17 $^{n.s}$	227.9 $^{n.s}$	3.29 n.s	115.97 $^{n.s}$	0.27 $^{n.s}$	0.2 $^{n.s}$
Error b	12	560.1	26.77	30.59	4.05	128.7	17.21	894	44.66	128.4	6.07	57.76	0.29	0.12
CV (%)	26	4.77	7.61	8.73	10.06	5.7	6.06	3.88	4.26	5.06	16.54	4.09	3.6	17.76

,* and ^{ns} respectively mean significant at 5% probability level and 1% probability level and non-significant

Table 2. Mean comparison of fertilizer and plant density on quantitative traits.

Treatment	Stem fresh weight (g.plant^{-1})	Stem dry weight (g.plant^{-1})	Fresh leaf weight (g.plant^{-1})	Dry leaf weight (g.plant^{-1})	Forage fresh weight (g.plant^{-1})	Forage dry weight (g.plant^{-1})	Corn wet weight (g.plant^{-1})	Corn dry weight (g.plant^{-1})	Phosphorus (mg.kg^{-1} dry weight)	Protein (%)	Plant height (cm)	Number of leaves per plant	Ratio of corn/plant
Fertilizer													
N_1= Urea 0%, Nitroxin 0%	424.58^{c*}	63.41^{b}	56.50^{b}	18.50^{b}	665.37^{c}	143.16^{c}	173.33^{c}	61.25^{b}	154.66 ^{c}	8.79 ^{c}	158.91 ^{c}	14.43^{b}	1.50 ^{c}
N_2= Urea 75%, Nitroxin 100%	536.25^{a}	73.75^{a}	69.16^{a}	22.66^{a}	835.16^{a}	169.08^{a}	217.08^{a}	72.66^{a}	244.16 ^{b}	15.91 ^{b}	209.83^{a}	15.46 ^{a}	2.21 ^{a}
N_3= Urea 100%, Nitroxin 100%	532.50^{a}	67.00^{ab}	62.91^{ab}	19.25^{b}	812.58^{a}	159.25^{b}	212.33^{ab}	73.41^{a}	221.66 ^{a}	23.00 ^{a}	184.91 ^{b}	15.28 ^{a}	2.21^{a}
Density (Plant.ha^{-1})													
D_1= 70000	517.08^{a}	71.33^{a}	63.66^{a}	19.16^{ab}	798.16^{a}	164.16^{a}	206.83^{a}	73.66^{a}	222.83 ^{a}	12.91 ^{a}	187.41 ^{a}	14.90 ^{a}	1.83 ^{a}
D_2= 90000	505.41^{a}	70.50^{a}	66.50^{a}	22.33^{a}	788.66^{a}	162.83^{ab}	206.08^{a}	70.00^{ab}	222.58 ^{a}	14.08 ^{a}	185.75 ^{a}	14.93 ^{a}	2.08 ^{a}
D_3= 110000	486.66^{a}	68.75^{ab}	62.08^{a}	19.50^{ab}	758.58^{a}	152.83^{bc}	198.75^{a}	65.00^{b}	225.33 ^{a}	15.25 ^{a}	186.50 ^{a}	14.90 ^{a}	1.83 ^{a}

*Similar letters in each column show non-significant difference according to 5% Level in Duncan Multiple Rang Test.

Table 3. Interactive effect of fertilizer and density on measured traits

Treatment	Stem fresh weight (g.plant^{-1})	stem dry weight (g.plant^{-1})	Leaf fresh weight (g.plant^{-1})	Leaf dry weight (g.plant^{-1})	Corn fresh weight (g.plant^{-1})	Corn dry weight (g.plant^{-1})	Phosphorus (mg.kg^{-1} dry weight)	Protein (%)	Plant height (cm)	Number of leaves per plant	Ratio of corn/plant (g.plant^{-1})	Fresh forage weight (g.plant^{-1})	Dry forage weight (g.plant^{-1})
N1D1	460.00^{fg*}	68.66^{abc}	58.33^{a}	16.66^{b}	169.66^{f}	68.33^{abc}	148.33^{g}	8.00^{f}	150.00^{f}	14.50^{abc}	1.33^{cd}	699.00fg	153.66^{cde}
N1D2	445.00^{gh}	63.33^{abc}	58.33^{a}	21.66^{ab}	173.66^{ef}	65.00^{abcd}	156.66^{g}	8.66^{f}	162.00^{f}	14.73^{abc}	1.66 ^{bc}	688.66^{fg}	150.00^{cde}
N1D3	406.66^{hi}	65.00^{abc}	56.33^{a}	18.66^{ab}	181.66^{def}	57.33^{cd}	156.66^{g}	8.50^{f}	161.66^{f}	14.16^{c}	1.00^{d}	656.50^{gh}	141.00^{ef}
N2D1	560.00 ^{a}	68.33^{abc}	65.33^{a}	20.33^{ab}	223.33^{ab}	77.33^{a}	283.00^{a}	19.33^{abc}	169.00^{bcd}	15.16^{abc}	2.10^{ab}	855.33^{a}	166.00^{abc}
N2D2	530.00^{abc}	72.00^{abc}	62.66^{a}	19.33^{ab}	217.33^{abc}	73.33^{ab}	278.66^{a}	22.66^{ab}	186.33^{de}	14.90^{abc}	2.21^{ab}	815.66^{abcd}	164.66^{abcd}
N2D3	523.33^{abcd}	67.00^{abc}	61.33^{a}	18.66^{ab}	203.33^{bcd}	69.33^{abc}	271.33^{ab}	24.00^{ab}	179.33^{e}	15.63^{a}	2.44^{a}	794.00^{bcde}	153.33^{cde}
N3D1	538.33^{abc}	78.33^{a}	67.66^{a}	21.66^{ab}	231.66^{a}	77.33^{a}	245.00^{cd}	14.33^{cdef}	214.66^{a}	15.30^{abc}	2.10^{ab}	850.66^{ab}	177.33^{a}
N3D2	550.00^{a}	76.66^{ab}	73.33^{a}	25.00^{a}	223.33^{ab}	73.33^{ab}	243.33^{cd}	14.00^{cdef}	206.66^{abc}	15.60^{a}	2.44^{a}	859.66^{a}	175.00^{ab}
N3D3	536.66^{abc}	73.33^{abc}	67.66^{a}	22.33^{ab}	206.66^{bcd}	70.00^{abc}	251.66^{bc}	17.00^{bcde}	213.33^{a}	15.63^{a}	2.22^{ab}	825.00^{abc}	165.66^{abc}

*Similar Letters in each column show non-significant difference according to 5% Level in Duncan Multiple Rang Test.

Table 4. Correlation relation between traits.

Treatment	Stem fresh weight	Stem dry weight	Leaf fresh weight	Leaf dry weight	Corn fresh weight	Corn dry weight	Phosphorus	Protein	Plant height	Number of leaves per plant	Number of leaves per corn	Ratio of Pod/plant	Forage fresh weight
Stem dry weight	0.673*												
Leaf dry weight	0.795**	0.723*											
Leaf dry weight	0.514*	0.565*	0.822**										
Corn fresh weight	0.753**	0.522*	0.717*	0.57*									
Corn dry weight	0.922**	0.657*	0.674*	0.423^{ns}	0.604*								
Phosphorus	0.866**	0.356^{ns}	0.572*	0.289^{ns}	0.819**	0.708*							
Protein	0.629	0.02^{ns}	0.245^{ns}	0.061^{ns}	0.534*	0.539*	0.855**						
Plant height	0.782**	0.663*	0.825**	0.644*	0.855**	0.599*	0.706*	0.305^{ns}					
Number of leaves per plant	0.771**	0.353^{ns}	0.547	0.494^{ns}	0.538*	0.676*	0.726**	0.713*	0.611*				
Ratio of corn/plant	0.716**	0.306^{ns}	0.568^{ns}	0.44^{ns}	0.667*	0.571*	0.756**	0.656*	0.644*	0.771**	0.482		
Fresh forage weight	0.986**	0.731**	0.895**	0.589*	0.825**	0.893**	0.850**	0.563*	0.850**	0.724*	0.614*	0.702*	
Dry forage weight	0.886**	0.893**	0.847**	0.670*	0.668*	0.892**	0.597	0.291^{ns}	0.755*	0.603*	0.459^{ns}	0.510*	0.914**

,* and ns respectively mean significant at 5% probability level and 1% probability level and non-significant

The higher dry weight of stem in N_2 treatment could be due to positive effect of the increase of nitrogen fertilizer which increased dry matter accumulation and ultimately increased the stem weight. In fact, nitroxin increased nitrogen accessibility. The effect of density on dry weight was significant at 1% probability level (Table 1). There was no significant difference between the density of 70000 and 90000 plants.ha^{-1} in terms of dry and fresh weight of stem (Table 2). The highest rate of dry and fresh weight of stem was related to the density of 70000 plants.ha^{-1} by 71.33 and 517.08 g.plan^{-1} respectively and the lowest rate of dry and fresh weight of stem belonged to the density of 110000 plants.ha^{-1} by 68.75 and 486.66 g.plant^{-1} respectively (Table 2). The reason of decrease stem weight as the density increased could be due to the plants competition in absorbing water and nutrition. Moreover, in high densities, due to serious competition to receive light, the stem height increases, but the stem diameter and consequently the stem yield decrease. The highest interaction effect of fertilizer and density on dry and fresh weight of stem was related to N_3D_1 and N_2D_1 respectively (Table 3). This was due to low density and less competition, more leaf area and photosynthesis, so assimilation increased. The lowest effect on dry and fresh weight of stem was related to N_1D_3 and N_1D_2, respectively (Table 3).

Leaf dry and fresh weight

The results showed that the effect of the interaction of chemical and biological fertilizers on dry and fresh weight of leaf was significant at 1% probability level (Table 1). The highest dry and fresh weight of leaf belonged to N_2 treatment with 75% urea and 100% Nitroxin and the lowest weight belonged to N_1 treatment without any chemical fertilizer and Nitroxin (Table 2). Nitrogen is an important element in the structure of organic compounds and proteins and nucleic acids and by absorbing water it increased the leaf weight. As the soil nitrogen increases the yield of corn dry weight increases, which could be due to its effect on the growth rate of crop, leaf area index, and leaf area continuity. The studies of Khaksar *et al.* (2009) reported that the seed inoculation with Azospirillum bacteria increased the plant height and dry and fresh weight of corn leaves. The effect of plant density on dry weight of leaf was significant at 1% probability level but its effect on fresh weight of leaf was not significant (Table 1). The highest dry weight was related to the density of 90000 plants.ha^{-1} by 22.23 g.plant^{-1} (Table 2). The lowest dry weight was related to other densities. There was no significant difference between densities in terms of fresh weight of leaf (Table 2). The decrease of efficiency in low density shows more competition with in the plants. There was no significant difference between the interaction effects of the treatments on dry and fresh weight of the leaf (Table 3). There was a positive significant correlation between the fresh weight of leaf and dry and fresh weight of corn at 5% probability level (Table 4), because leaf area, as a photosynthetic organ, increased the weight of corn. This is because of photosynthesis and assimilates mobilization to the corn.

Corn dry and fresh weight

The effect of density on dry weight of corn was significant at 1% probability level but its effect on the fresh weight of corn was not significant (Table 1). The highest yield of corn dry weight belonged to N_3 treatment with 100% urea, and 100% Nitroxin which was not significantly different from N_2

treatment. The highest yield of fresh weight of corn belonged to N_2 treatment with 75% urea, and 100% Nitroxin. The lowest belonged to N_1 treatment (Table 2). It seems that the increase of corn yield is due to the fact that nitrogen in compounds particularly in proteins and enzymes has a main role and is a component of molecular structure of chlorophyll and chloroplast. Hence by producing more assimilates and mobilizing them to the grains it has increased the corn dry weight. The highest dry weight of corn belonged to the density of 7000 plants.ha^{-1} by 73.66 g.plant^{-1}, and the lowest dry weight occurred the density of 110000 plants.ha^{-1} by 65 g.plant^{-1}, but there was no significant difference between different densities in terms of fresh weight of the corn (Table 2). Considering the dry weight of the corn, as the density increased, (due to high competition in absorbing environmental resources and leaves shadowing) the photosynthesis decreased which led to the decrease of dry matter and the yield of corn dry weight. Koliai *et al*. (2012) reported that the density of 95000 plants.ha^{-1} had the highest fresh weight of the corn by 410.7 g.m^{-2} and the densities of 83000 and 102000 plants.ha^{-1} had the lowest fresh weight of the corn by 388.8 and 350.1 g.m^{-2} respectively (Table 2). The highest rate of the interaction effect of fertilizer and density on dry weight of the corn belonged to N_2D_1 and N_3D_1 and the lowest belonged to N_1D_3 as well (Table 3). However, the highest effect on the fresh weight of corn belonged to N_3D_1 and the lowest belonged to N_1D_1 indicating the positive effect of low density that might decrease the yield of single plant, but in general it increased the yield (Table 3). The table of correlation coefficient shows that there is a positive significant correlation between the fresh weight of corn and fresh forage weight at 1% level. Also, there is a positive significant correlation between the fresh weight of corn and dry forage weight at 5% level (Table 4).

Percentage of forage phosphorus and protein

The effect of different levels of fertilizer on the rate of phosphorus and protein was significant at 1% probability level (Table 1). The highest rate of phosphorus belonged to N_3 and the lowest rate belonged to N_1 and the highest rate of protein was related to N_3 and the lowest rate was related to the treatment without any fertilizers (N_1) (Table 2). As the percentage of urea in interaction with nitroxin decreased, the rate of phosphorus decreased. The increase of phosphorus uptake might be due to higher concentration of phosphorus in the environment or the increase of the root development or both of them. Azospirrilum bacteria considerably increased phosphorus. The increase of urea fertilizer has increased the percentage of nitrogen because it contains nitrogen which is an important element in the structure of organic compound and protein and nucleic acids. Rohitashav *et al*. (1993) inoculated the corn seeds and Azotobacter Choorcoccun by planting them in the farm together with seeds which were not inoculated as the control treatment. They observed that rate of stem phosphate had increased. Generally, it is said that overuse of nitrogen has increased protein percentage of plant (Rees *et al*. 2009). The results related to the combination of fertilizers in this experiment were consistent with the results obtained by Torbati Nejad *et al*. (2001). Increase of density had no significant effect on rate of phosphorus and protein. Highest rate of phosphorus and protein was related to density of 110000 plants.ha^{-1} by 225.33 (mg.kg^{-1} of dry weight) and 15.25 g.kg^{-1} of dry weight

(Table 2). Lowest rate of phosphorus was related to the density of 90000 plants.ha^{-1} by 225.58 mg.kg^{-1} of dry weight. Lowest rate of protein was related to density of 70000 plant.ha^{-1} by 12.91 g.kg^{-1} of dry weight (Table 2). In high densities, due to increase of Auxin hormone, growth of internodes is high and deposition of structural carbohydrates which develop the cellulose tissues decreases and so indirectly increases percentage of protein substances. ANOVA results indicated interactive effect of density and fertilizer on phosphorus and protein was not significant (Table 1), but it was significant in Duncan's mean comparison test (Table 3). The highest interaction effect of fertilizer and density on phosphorus was observed in N_2D_1 and the lowest interaction effect was observed in N_1D_1 (Table 3). The rate of nitrogen in the combination of 100% urea and 100% Nitroxin increased as the density increased which indicates high activity of Nitroxin bacteria and urea in high densities. Regarding the table of coefficients correlation phosphorus had a positive significant correlation with protein, number of leaves per plant, and the ratio of corn/plant at 1% probability level (Table 4). It had a positive significant correlation with plant height at 5% probability level. There was a positive significant correlation between protein and the number of leaves per plant at 5% probability level (Table 4), because protein has leading role in plant structure and as number of plant leaves increased, photosynthesis and consequently protein increased too.

Plant height and number of leaves per plant

The ANOVA results showed that the effect of fertilizer on plant height and number of leaves per plant was significant at 1% probability level (Table 1). The highest rate of plant height and number of leaves per plant was related to N_2 treatment with the combination of 75% urea and 100% Nitroxin (Table 2). The lowest plant height belonged to the control treatment (N_1) which indicates positive coexistence of Nitroxin bacteria (100%) and urea fertilizer (75%) that has increased material production and plant height. The increase of dry matter accumulation was due to the great effect of nitrogen on the leaf area and its better continuity (Table 2). The findings of Ahmadi and Gholami (2010) showed that the corn seeds inoculation with different microorganisms significantly increased the plant height and dry matter accumulation in comparison to the control treatment. Plant density didn't have a significant effect on plant height and the number of leaves per plant. The ANOVA results showed that he interactive effect of density and fertilizer on plant height and number of leaves per plant was not significant (Table 1) but Duncan's test showed that the difference was significant (Table 3). The highest rate of interaction effect of density and fertilizer on plant height was related to N_3D_1 and the lowest rate was related to N_1D_1. The highest rate of interaction effect of density and fertilizer on the number of leaves per plant was related to N_3D_3 and the lowest rate was related to N_1D_3 (Table 3).

The ratio of corn to plant

Different fertilizer levels had a significant effect on the ratio of corn to plant at 1% probability level (Table 1). Corn as a more digestible part than any other organs and as supplier of a large amount of forage protein is particularly important and thus it could be said that the ratio of corn to biomass is determining the nutrition value and more digestible capability of the forage (Wu *et al*., 2005).

The highest ratio of corn.plant^{-1} belonged to the treatment with the combination of 75% urea and 100% Nitoxin and also the treatment with 100% urea and 100% Nitroxin and the lowest ratio of corn/plant was related to N_1 with 0% urea and 0% Nitroxin (Table 2). The highest ratio of corn to plant was related to the density of 90000 plants.ha^{-1} by 2.08 and the lowest ratio was related to 70000 and 110000 plants.ha^{-1} by 1.83 (Table 2). Therefore, as the rate of urea increased, the ratio of corn/plant relatively increased and there was a good coexistence between urea fertilizer and bacteria. Further activity of bacteria produced nitrogen fixation nodes and thus transferred more dry matter to grains and consequently increased the ratio of corn/plant. Density had no significant effect on the ratio of corn/plant (Table 4). In high densities the flowers and fruits which could potentially be produced are not formed or are infertile and this reduces the yield because the total amount of photosynthetic materials which could be reserved in the grain will decrease. The increase of density, due to excessive competition among plants for absorbing light and moisture, will decrease the production of seeds in corn and finally will cause the decrease of corn weight (Tarang *et al*., 2013). Moreover, in high densities growth and production of corn in each plant will decrease and biological yield contribution will increase. Rafiei and Asgharipoor (2009) reported that as the plant intensity increased, the biological yield increased too, however, the grain yield decreased. In other word, the ratio of corn to biomass decreased. The results indicate that the interaction effect of density and fertilizer on the ratio of corn to plant was not significant. The highest rate of interaction effect of fertilizer and density was related to N_2D_3 and N_3D_2, i.e. in high levels of urea and mediocre densities the ratio of corn to plant increased and the lowest ratio was related to N_1D_3 (Table 5). With regard to the table of coefficients correlation (Table 6), the ratio of corn to plant had a positive significant correlation with the weight of fresh forage at 5% probability level. Increase of fresh forage weight increased the ratio of corn to plant.

CONCLUSION

The experiment revealed that highest yield of morphological characteristics was related to the combination of 75% urea, 100% Nitroxin. Highest yield of qualitative traits such as phosphorus was related to treatment with 75% urea, 100% Nitroxin and for protein it was related to treatment with 100% urea and 100% Nitroxin. Lowest yield for both traits was related to N1 (0% urea, 0% Nitroxin). So, it became clear that it was not possible to accurately identify a suitable density for qualitative traits and morphological characteristics in this experiment because effect of different densities on these traits was not significant. Interaction effects of fertilizer and density in table of ANOVA were not significant, but according to mean comparison of Duncan’s tests effects were significant. It should be pointed out that many researchers have stated that biological fertilizers are not able to supply total nitrogen that plant needs by themselves and positive effects of biological fertilizers mostly increases solubility of elements such as phosphorus and also increases absorption per area unit which is due to production of different growth enhancer hormones (Vessy, 2003). Growth enhancer hormones, particularly auxin, increase rate of uptake per area unit via stimulating root expanding system by presence of appropriate amounts of chemical fertilizers, they enhance such effects which ultimately lead to increase of yield.

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