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EVALUATION OF YIELD, YIELD COMPONENTS AND SOME PHYSIOLOGICAL AND QUALITATIVE TRAITS OF CORN AFFECTED BY CHEMICAL AND BIOLOGICAL NITROGEN FERTILIZERS

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ABSTRACT

In order to evaluate the yield, yield components and some physiological and qualitative traits of corn treated with nitrogen fertilizers (biological, chemical and integrated), a field experiment was conducted at the Agricultural Research Station of Khorramabad during 2016 growing season. Treatments were arranged in a complete randomized block design with four replications. Experimental treatments consisted of 100% chemical fertilizer (urea), bio-fertilizer (nitroxin), integration of bio-fertilizer + 25% chemical fertilizer, integration of bio-fertilizer + 50% chemical fertilizer, integration of bio-fertilizer + 75% chemical fertilizer and the control. Results showed that the effect of different treatments of fertilizers on all measured traits, except for number of rows in each ear and carotenoids, was significant. The results indicated that integration of bio-fertilizer + 75% chemical fertilizer affected the highest grain yield (9.31 t ha^{-1}), dry biological yield (20.96 t ha^{-1}), number of kernels in each row (41.67), plant height (201.02 cm), hectoliter weight (0.82 g cm^{-3}), chlorophyll *b* (0.43 mg g^{-1} FW), total chlorophyll (1.1 mg g^{-1} FW) and leaf area index (LAI) (4) and there was no significant difference among this treatment and 100% chemical fertilizer (N) and integration of bio-fertilizer + 50% chemical fertilizer treatments in all measured traits. The greatest harvest index (45.8) and grain protein-content (9.1%) resulted from the integration of bio-fertilizer + 50% chemical fertilizer treatment. Also the highest 1000-grains weight (281.13 g) and chlorophyll *a* (0.66 mg g^{-1} FW) were caused by 100% chemical fertilizer (urea) treatments. Results showed that integration of bio-fertilizer + chemical fertilizer could be considered as a means to reduce the consumption of chemical fertilizers for sustainable agriculture.

Key words: corn, grain yield, integrated system, nitroxin, photosynthetic pigments

INTRODUCTION

Maize is an important cereal crop that provides food to many people all over the world. In developing countries, maize is a major source of income for many farmers [Tagne et al. 2008]. Maize is capable of utilizing inputs more efficiently and is potentially capable of producing large quantity of food grains per unit area [Chaudhary 1993]. The crop can be used as food for people, livestock feed, oultry feed and is also used in many branches of industry [Bibi et al. 2010]. With the growth in population, the demand for food has been increasing while land availability has been decreasing. Thus, the only way to increase the production is to increase the yield per unit area [Hirpa 2014]. Boosting crop

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yields and closing the gap between actual and attainable yield can be achieved by the implementation and advancement of numerous practices and technologies, including nutrient management practices and fertilizer technologies. Growers are continually striving to overcome nutrient deficiencies and adopt improved management practices in order to increase the yields for more profit. Great progress in fertilizer technology and the use of plant nutrients has been made in recent years, which led to improved fertilization and farming practices that have improved crop yields worldwide [Tisdale et al. 1985]. In recent years, the real challenge for agricultural researchers has been to stop using high rates of agrochemicals, which negatively affects the human health and the environment. Large quantities of chemical fertilizers are used to replenish the soil N, resulting in high costs and severe environmental contamination [Dai et al. 2004].

Nitrogen is one of the most important nutrients for plant production that plays a major role in photosynthetic activity and crop yield capacity and its availability influences the plants morphological, physiological and biochemical parameters [Werner and Newton 2005, Caliskan et al. 2008]. There have been many attempts to replace those harmful fertilizers with a biofertilizer [El Kholy et al. 2005]. In this regard, microbial inoculants have paramount significance in integrated nutrient management systems to sustain agricultural productivity and healthy environment [Adesemoye and Kloepper 2009]. Using biological fertilizers is one of effective managerial methods to maintain soil quality [Kokalis et al. 2006]. One alternative to decrease the application of nitrogen fertilizer could be the use of free-living N-fixing bacteria in agricultural systems [Cocking 2003], which could improve the crop production, reduce the overuse of chemical fertilizers, and decrease the greenhouse emissions [Kennedy et al. 2004]. Inoculation of maize with free-living N-fixing bacteria has been shown to raise crop yields through supplemental nitrogen input to the soil [Hungria et al. 2010]. Bacteria from *Azotobacter* and *Azospirillum* strains have the ability to create and leak some biological activity compounds such as vitamin B, nicotinic acid, biotin, oxins, gibbrellins, etc. to plant root environment, which has a beneficial role in incrementing the root absorbance [Kader 2002]. Little information is available on the effect of chemical and biological nitrogen fertilizers on various traits of corn in Iran conditions, therefore this study was carried out in order to evaluate the yield, yield components and some physiological and qualitative traits of maize treated with different nitrogen fertilizers – biological, chemical and integrated (biological + chemical).

MATERIAL AND METHODS

Location and plant materials

This study was conducted at the Agricultural Research Station of Khorramabad, Iran (33°27'N, 48°17'E, and altitude 1,162 m) during 2016 growing season. Physical soil analysis and chemical characteristics of soil at the depth of 0–40 cm have been shown in Table 1. Seed of crop was planted in plots, the area of which was 7 m^2, in five 2-meter rows in early May, 2016. Row spacing was 70 cm and intra-row spacing was 20 cm. Three seeds per hill were planted. One plant per hill was maintained at 4–6 leaf stage of the crop. All plots were irrigated immediately after sowing. Subsequent irrigations were carried out every 6 days. Manual weeding of the experimental area was performed as required.

Table 1. Physical and chemical analysis of soil before the experiment

Soil texture	Clay (%)	Silt (%)	Sand (%)	pH	EC (ds m^{-1})	Total N (%)	Available P (ppm)	Available K (ppm)
Clay loam	31.52	41.5	26.98	7.36	0.536	0.285	6	356

Fertilizer and microbial inocula

Before cultivation, all P (150 kg ha^{-1} triple superphosphate) and K (50 kg ha^{-1} potassium sulfate) fertilizers were added to plots according to the soil test. Nitrogen in 100% chemical fertilizer treatment was applied at 375 kg N ha^{-1} as urea. With last plowing before planting, half the amount of nitrogen (187.5, 140.6, 93.7 and 46.9 kg ha^{-1}) was added to 100% chemical fertilizer, integrated of biofertilizer + 75% chemical fertilizer, integrated of biofertilizer + 50% chemical fertilizer and integrated of biofertilizer + 25% chemical fertilizer plots, respectively. The rest of nitrogen was distributed in the following two phases: eight-leaf stage of maize and before the start of crown flower as head in the plots. Before cultivation, biofertilizer was mixed completely with seeds and kept for half an hour in shade and finally dried seeds were planted in early May. The liquid biofertilizers (nitroxin) were applied at the dose of 2 l ha^{-1}. The biofertilizers were *Azospirillum* and *Azotobacter* strains. Both *Azospirillum* and *Azotobacter* strains consisted of 10^8 CFU ml^{-1} inoculant. The inocula of the two rhizobacteria genera, *Azotobacter* and *Azospirillum* were purchased from Mehr Asia Biotechnology Company, Tehran, Iran.

Plot experiment

The experimental design was a complete randomized block design with four replications. Experimental treatments included F1: control (without biofertilizer and chemical nitrogen fertilizer), F2: 100% chemical fertilizer (urea), F3: bio-fertilizer (nitroxin), F4: integration of bio-fertilizer + 25% chemical fertilizer, F5: integration of bio-fertilizer + 50% chemical fertilizer and F6: integration of bio-fertilizer + 75% chemical fertilizer.

Measurement of yield, yield components and qualitative traits

Standard procedures were followed to collect data for yield, yield components and some qualitative properties. Five plants from each plot were selected at random and their height was measured with the help of a measuring tape and the average was calculated from those five measured values. The number of rows in each ear and the number of kernels in each row were counted and averaged. From each plot, five samples, each of 1000 grains, were randomly collected and their weight was recorded. In order to measure the hectoliter weight, a container with known mass and volume was filled with the corn seeds to the top. The seeds were poured to the container in excess and with a constant rate from a height of about 150 mm [Singh and Goswami 1996]. Razavi et al. [2007] reported that dropping the seeds from the height of 150 mm produces a tapping effect in the container, which reproduces the settling effect during the storage. After filling the container, excess seeds were removed by passing a flat stick across the top surface, using 2 zigzag motions. The seeds were not compacted in any way. The container was weighed on a digital balance (Model GT2100, Germany) reading with an accuracy to 0.01 g. Hectoliter weight (ρ_b) was calculated from the ratio of seeds mass in the container (m_b) to its volume (v_b):

$$\rho_b \frac{m_b}{v_b}$$

The hectoliter weight was measured from 10 replications for each treatment.

In order to measure the grain yield after physiological maturity, when grain moisture content was between 23–25%, the harvest was performed. Grain yield of each plot was recorded using a portable balance and based on 14% seed moisture was calculated. In order to measure the dry biological yield, the samples were oven dried at 75°C for 72 h and weighed. The harvest index was accounted as follows:

HI = (Economical yield / Biological yield) × 100.

Measurement of chlorophyll *a*, chlorophyll *b* and carotenoids

Leaf samples were taken to measure the photosynthetic pigments at the flowering stage. Chlorophyll *a*, chlorophyll *b* and carotenoids were extracted using pure acetone from fresh leaf samples (0.1 g). Absorbance was measured at 663 nm, 645 nm and 470 nm for chlorophyll *a*, chlorophyll *b* and carotenoids, respectively, using a UV spectrophotometer (UNICO, Model SUV–S2100, USA). The mentioned traits were calculated according to the procedure described by Lichtenthaler [1987].

Chlorophyll *a* = 11.24 × A 662 – 2.04 × A 645

Chlorophyll *b* = 20.13 × A 645 – 4.19 × A 662

Total chlorophyll = 7.05 × A 662 + 18.09 × A 645

Carotenoids = (1000 × A 470 – 1.90 chlorophyll *a* – 63.14 chlorophyll *b*) /214

Leaf area index (LAI) was measured at the flowering stage. LAI was computed as the ratio between leaf area and the corresponding ground surface area. Leaf area was measured using a leaf area meter (LI 3100C Area Meter, LI-COR Inc., USA).

Data analysis

SAS (version 9.1) and MSTATC programs were used to conduct an analysis of variance (ANOVA) and means comparison, respectively. Treatment mean differences were separated by Duncan test at the 5% probability level. Figures were drawn using Microsoft Excel and figures display the standard deviation (SD) of data using error bars.

RESULTS AND DISCUSSION

Physiological traits of maize. The effects of different fertilizer treatments were significant in the case of chlorophyll *a*, chlorophyll *b*, total chlorophyll and LAI (Tab. 2). Result of mean comparisons showed that among different fertilizer treatments, the highest chlorophyll *a*, chlorophyll *b*, total chlorophyll and LAI were due to integration of bio-fertilizer + 75% chemical fertilizer, integration of bio-fertilizer + 50% chemical fertilizer and 100% chemical fertilizer treatments and there was no significant difference among these treatments (Figs. 1, 2, 3 and 4). These results indicate the effective role of nitrogen-fixing bacteria in meeting the plants' nutrient demands in terms of nitrogen. Nitroxin biofertilizer treatment had better effect on majority of traits compared to the control group. Application of nitroxin biofertilizers (F3) resulted in 11, 55, 25 and 8% extra chlorophyll *a*, chlorophyll *b*, total chlorophyll and LAI of maize, respectively, as compared to the control (F1). High nitrogen fertilization can increase photosynthesis and plant growth in several crops [Evans 1989, Cechin and Fumis 2004].

Up to 75% of leaf N is found in chloroplasts and most of it is part of ribulose bisphosphate carboxylase (Rubisco) alone. At low nitrogen level, the lower photosynthesis is often attributed to the reduction in chlorophyll content and Rubisco activity [Evans and Terashima 1987, Fredeen et al. 1991]. Chandrasekhar et al. [2005] reported that useful effects of bacteria inoculation on increased chlorophyll content are due to higher availability of nitrogen to the growing tissue and organs supplied by free-living N-fixing bacteria. Another study showed that bio-fertilizer (nitroxin) increased chlorophylls *a*, *b*, total and carotenoids content of the plants linearly [Rahi 2013]. No significant

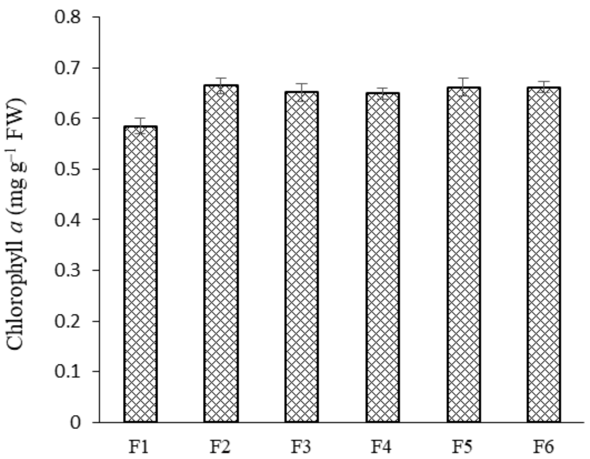

Fig. 1. Effects of different fertilizer treatments on chlorophyll *a*
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

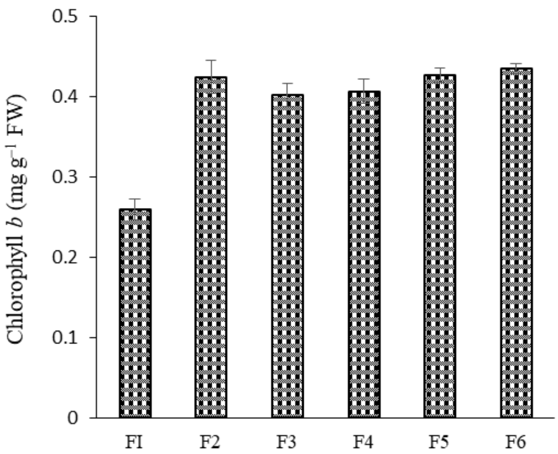

Fig. 2. Effects of different fertilizer treatments on chlorophyll *b*
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

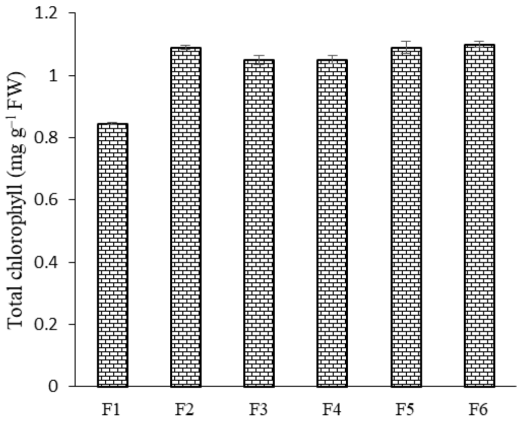

Fig. 3. Effects of different fertilizer treatments on total chlorophyll
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

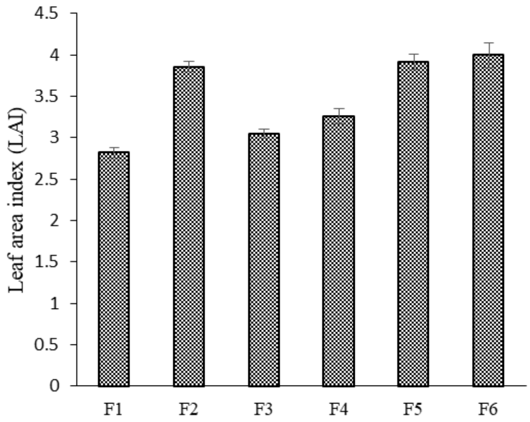

Fig. 4. Effects of different fertilizer treatments on leaf area index (LAI)
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

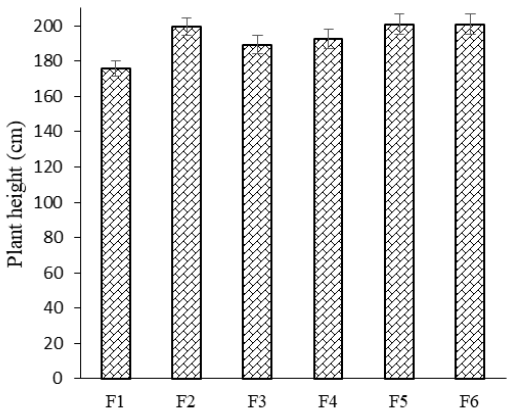

Fig. 5. Effects of different fertilizer treatments on plant height
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

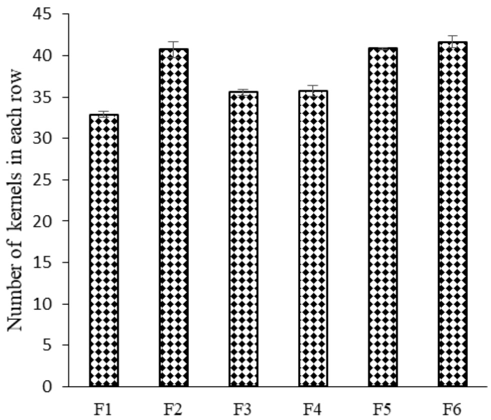

Fig. 6. Effects of different fertilizer treatments on number of kernels in each row
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

difference, as regards to carotenoids, was observed between the control and other fertilizer treatments (Tab. 2). Smolen and Sady [2009] showed that nitrogen fertilization did not cause any significant changes in carotenoid compounds in carrot.

Yield and yield components of maize. Fertilizer treatments had significant effects on the yield and yield components of maize except from the number of rows in each ear (Tab. 3). The highest plant height (201.02 cm), number of kernels in each row (41.67), grain yield (9.31 t ha^{-1}) and dry biological yield (20.96 t ha^{-1}) were achieved from F6 treatment (Figs. 5, 6, 8 and 9). Also the greatest 1000-grains weight (281.13 g) and harvest index (45.8) resulted from F2 and F5 treatmens, respectively (Figs. 7 and 10). Comparison of mean values of the plant height showed that among different fertilizer treatments, the lowest plant height (175.73 cm) was obtained from

F1 (control) treatment (Fig. 5). As the nutrient deficit plays a major role in the height of plants, it seems that, due to the deficit of nutritional matters, the control plants grew less than those nourished properly with chemical and integrated fertilizers. Zahir et al. [1998] also reported an 8.5% increase in the height of corn, that was infected with *Azotobacter* and *Pseudomonas*. According to the results, F6 and F1 (control) treatments had the highest and the lowest number of kernels in each row, respectively. Thus it can be said that inoculation of *Azospirillum* and *Azotobacter* caused an increase in sink portion, increasing the maize yield by increasing the number of kernels in row.

The effect of fertilizer treatments was not significant as regarding the number of rows in each ear, indicating that it is a genetic trait that is less affected by environmental factors. The same results have been already reported by Moscheler [1988]. The comparison of mean values of the 1000-grains weight showed that among different fertilizer treatments, the highest (281.13 g) and lowest (220.79 g) 1000-grains weight values appeared in treatments F2 and F1, respectively (Fig. 7). However, F2 treatment in terms of 1000-grains weight, showed no significant difference with F5 and F6 treatments. Increasing 1000-grain weight under inoculation treatment can be due to the improved attributes such as leaf area, photosynthetic pigments and height, which finally caused an increase in assimilates production. Hey and Walker [1989] reported that grain weight can be increased, reduced or maintained constant by some factors determining the length of grain filling after flowering stage, such as nitrogen fertilizer, plant density and environmental stresses.

The comparison of mean values showed that inoculation of corn grains with *Azotobacter* and *Azospirillum* strains as compared to the control group, led to increased grain yield and dry biological yield equal to 23 and 12%, respectively (Figs. 8 and 9). Malakouti [1998] reported that an increase in nitrogen leads to an increase in the leaf area and duration of leaf area and this increase stems from a higher rate of assimilation and yield. Regarding the role of nitrogen deficit in increasing time span between releasing pollen and the emergence of corn silk, this agent is one of the main factors causing a sever reduction in ear weight and grain yield of the control group (without applying fertilizers) owing to sterilized ears of maize. It seems that application of nitrogen fertilizer plays a significant role in increasing the plant vegetative growth and, consequently, paves the way for increasing the yield of corn. Higher dry mass of nitrogen treated plants could be connected with the positive effect of nitrogen in some important physiological processes. In other words, due to nitrogen-fixing and the effects of nitrogen-fixing bacteria on secreting the growth regulator

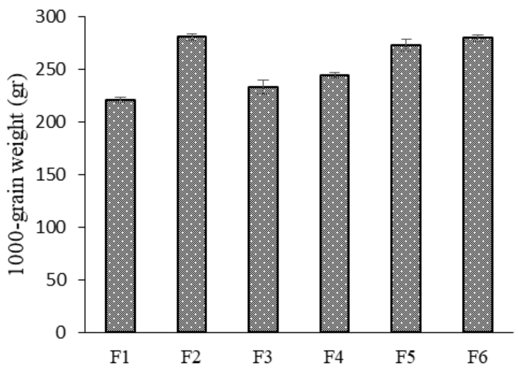

Fig. 7. Effects of different fertilizer treatments on 1000-grain weight
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

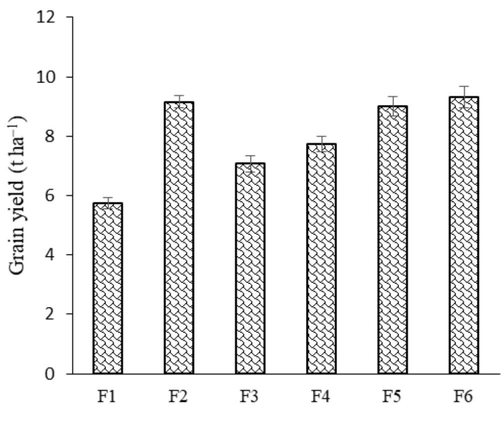

Fig. 8. Effects of different fertilizer treatments on grain yield
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

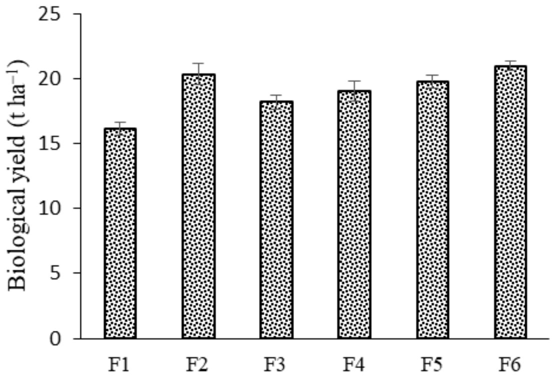

Fig. 9. Effects of different fertilizer treatments on biological yield
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

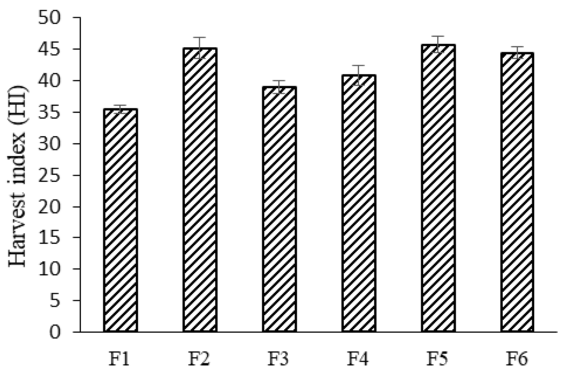

Fig. 10. Effects of different fertilizer treatments on harvest index
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer;
F4: integration of bio-fertilizer + 25% chemical fertilizer
F5: integration of bio-fertilizer + 50% chemical fertilizer
F6: integration of bio-fertilizer + 75% chemical fertilizer

as well as stimulating the plant-growth, the maize yield was increased due to the application of nitroxin biofertilizer as compared to the control group. It was also proved the positive role of nitrogen in qualitative and quantitative traits of corn, such as dry weight [Cox et al. 1993]. In this respect, Nanda et al. [1995] also showed that inoculating the corn seeds with nitrogen-fixing bacteria caused an increase in complete soiling yield through treating plants with different levels of nitrogen fertilizer.

Mean comparisons indicated that the highest and the lowest harvest indices were achieved from F5 and F1 treatments, respectively (Fig. 10). Increasing the harvest index value is affected by biofertilizer application due to its increasing effect on vegetative and reproductive growth. Therefore, it can be argued that bacteria increased the harvest index by increasing the dry weight of the plant and allocating more dry matter to the seed. Biofertilizer (nitroxin), compared to control group, led to increased harvest index equal to 9.6%. As reported by Vessey et al. [2003], biofertilizers not only contributed to N fixation, but they were also involved in the biological control of plant pathogens, solubilization of nutrients and phytohormone synthesis. Moreover, they can also bind soil particles into stable aggregates, which improves the soil structure and reduces erosion potential [Kohler et al. 2006]. Nitrogen deficiency leads to reduced leaf area and the rate of photosynthesis and it decreases dry matter production and grain yield.

Qualitative traits of maize

The effects of different fertilizer treatments were significant for grain protein and hectoliter weight (Tab. 3). Result of mean comparisons showed that among different fertilizer treatments, the highest (9.1%) and lowest (7.2%) grain protein values appeared in treatments F5 and F1 (control), respectively (Fig. 11). Biofertilizer (nitroxin) compared to the control group, led to increased grain protein equal to 8.9%. Rahmani et al. [2008] reported that nitrogen is the most important element in protein synthesis and its increase in optimum conditions increases the amount of protein. In addition, Jalilian et al. [2012] showed that application of biological fertilizer on sunflower increased the seed protein. They stated that nitrogen-fixing bacteria activity increased the nitrogen fertilizer recovery by providing a part of the required nitrogen during the growing season and reducing losses of nitrogen from the soil.

Mean comparisons showed that the highest (0.82 g cm^{-3}) and the lowest (0.72 g cm^{-3}) hectoliter weight appeared in F6 and F1 treatments, respectively (Fig. 12). Kordi et al. [2013] reported that urea application methods influenced the amount of nitrogen available to the plant, resulting in changes in the hectoliter weight of maize and the foliar urea application increased the hectoliter weight significantly as compared to the soil fertilization. Campillo et al. [2010] reported that an increase in the level of applied nitrogen increased hectoliter weight of the wheat grain.

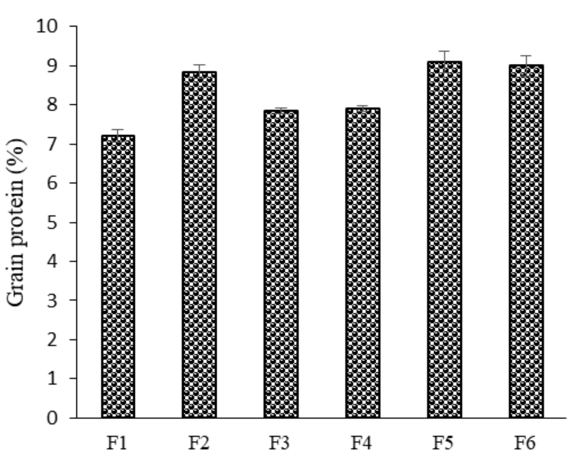

Fig. 11. Effects of different fertilizer treatments on grain protein
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer; F4: integration of bio-fertilizer + 25% chemical fertilizer F5: integration of bio-fertilizer + 50% chemical fertilizer F6: integration of bio-fertilizer + 75% chemical fertilizer

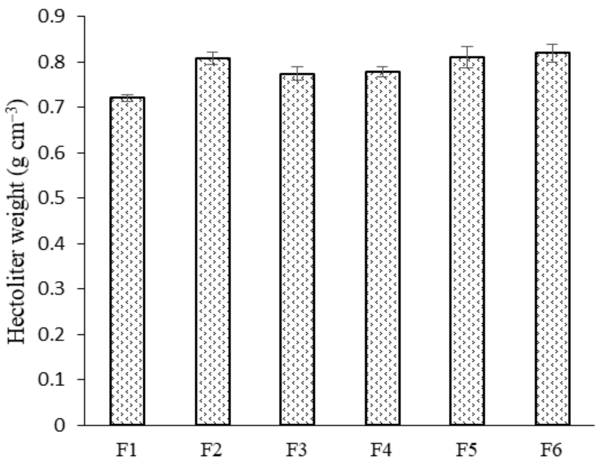

Fig. 12. Effects of different fertilizer treatments on hectoliter weight
F1: control; F2: 100% chemical fertilizer; F3: bio-fertilizer; F4: integration of bio-fertilizer + 25% chemical fertilizer F5: integration of bio-fertilizer + 50% chemical fertilizer F6: integration of bio-fertilizer + 75% chemical fertilizer

Table 2. Variance analysis for some of physiological properties of maize plant

Source of variation	Df	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids	Leaf area index (LAI)
Replication	3	0.0004 ^{ns}	0.002 ^{ns}	0.0008 ^{ns}	0.00003 ^{ns}	0.10 ^{ns}
Treatment	5	0.004*	0.02 **	0.04 **	0.0002 ^{ns}	1.02 **
Error	15	0.0009	0.0005	0.0006	0.00009	0.02
CV (%)	–	4.87	5.64	2.43	7.45	4.04

*,** and ns show significant difference at probability of 5%, 1% and no significant difference, respectively

Table 3. Variance analysis for yield, yield components and some qualitative properties of maize plant

Source of variation	Df	Biological yield	Grain yield	Harvest index (HI)	1000-grain weight	Number of rows in each ear	Number of kernels in each row	Plant height	Grain protein-content	Hectoliter weight
Replication	3	0.20 ^{ns}	303 671.89^{ns}	0.42 ^{ns}	32.81^{ns}	1.11^{ns}	1.03^{ns}	166.87^{ns}	0.11^{ns}	0.0007^{ns}
Treatment	5	11.92**	8 143 721.77**	66.87**	2730.23**	0.19^{ns}	52.71**	383.48*	2.38**	0.005**
Error	15	1.76	300170.19	7.78	79.15	0.52	1.6	101.58	0.15	0.001
CV (%)	–	6.96	6.84	6.67	3.48	4.98	3.34	5.22	4.68	4.23

*,** and ns show significant difference at probability of 5%, 1% and no significant difference, respectively

CONCLUSIONS

The highest values in this research, obtained from a majority of traits and grain yield, were due to the integrated treatment of biofertilizer + 75% chemical fertilizer. As a remarkable difference between the mentioned treatment with that of biofertilizer + 50% chemical fertilizer was not observed in a majority of traits, the integrated treatment of biofertlizer + 50% chemical fertilizer can be used as the most appropriate treatment for reducing the extensive employment of chemical fertilizers in agriculture and paving the way for sustainable agricultures. Results of this research clearly suggested that nitroxin biofertilizer and its integration with chemical fertilizer had a useful effect on enhancing the yield and growth parameters of maize under Khorramabad conditions.

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