Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 9, No. 1, 2023
https://jcns.ahvaz.iau.ir/ OPEN ACCESS

Investigating the Effect of Nitrogen and Nitroxin Biological Fertilizer on Quantitative and Qualitative Characteristics of Dual-purpose Barley (*Hordeum vulgare* L.) Forage

Najmeh Haghighatzadeh[1] Mani Mojadam[2]

1. *Msc. Graduated, Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.*

2. *Assistant Professor, Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.*

RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *11 Jan. 2023*
Received in revised form: *13 Feb. 2023*
Accepted Date: *15 Mar. 2023*
Available online: *30 Mar. 2023*

To Cite This Article:
Najmeh Haghighatzadeh, Mani Mojadam. Investigating the Effect of Nitrogen and Nitroxin Biological Fertilizer on Quantitative and Qualitative Characteristics of Dual-purpose Barley (*Hordeum vulgare* L.) Forage. ***J. Crop. Nutr. Sci.,*** 9(1): 58-70, 2023.

ABSTRACT

BACKGROUND: Optimum uses of nutrients is the most important factor in agricultural systems due to limitations of nutrients especially nitrogen.

OBJECTIVES: Current study was done to assess the consumption of different amounts of nitrogen and Nitroxin biological fertilizer on crop production of dual-purpose barley forage.

METHODS: This research was conducted in the cropping year of 2013-2014 in Ahvaz city in the form of a split plot using randomized block (RCDB) with three replications. The investigated treatments include the use of pure nitrogen at three levels (including the use of 50, 100 and 150 kg.ha^{-1}) and the use of Nitroxin biofertilizer at three levels (including no use of nitroxin (control), use of 2 and 4 liters per hectare), were placed in the main and sub plots, respectively.

RESULT: The results showed that the effect of different amounts of nitrogen fertilizer and nitroxin biofertilizer on leaf area index, dry forage yield, forage protein yield and seed protein percentage were significant. The highest leaf area index at the flowering stage (4.5), dry forage yield (2450.3 kg.ha^{-1}) and forage protein yield (544.9 kg.ha^{-1}) belonged to the treatment of 150 kg N.ha^{-1}. The consumption of higher doses of nitroxin caused a significant increase in the leaf area index at the flowering stage, dry forage yield and forage protein yield, although there was no significant difference between the consumption values of 2 and 4 liters per hectare in terms of these traits.

CONCLUSION: The interaction effect of the treatments on the studied traits were not significant, according to this, under the consumption of larger amounts of nitrogen (up to 150 kg.ha^{-1}) or the use of biological fertilizer at the rate of 2 liters per hectare (considering the lack of significant difference with the consumption of 4 liters per hectare) can achieve quantitative and qualitative yield of forage in dual-purpose barley cultivation.

KEYWORDS: *Forage yield, Leaf area index, Nitroxin, Nutrition, Protein.*

***Corresponding Author:** Mani Mojadam ✉ manimojaddam@yahoo.com

1. BACKGROUND

Cereals provide 70% of food for people on earth. Among the plants of this region, wheat, rice, corn and barley are the most important sources of food. More than three-fourths of energy and one-half of the protein needed by humans are provided by cereals. It seems that despite the obvious features of these plants, cereals will not be replaced in the future and their importance will increase in the future. Barley is one of the oldest agricultural plants and its cultivation dates back to seven thousand years BC. This plant is the least expected agricultural plant, whose range of adaptation and distribution is more than other agricultural plants. Barley is the fourth most important cereals in the world after wheat, rice and corn (Nourmohamadi *et al*., 2001). Every year, a relatively large area of land in Khuzestan is dedicated to forage barley cultivation. The use of one and sometimes two times of barley before seed production, either by direct grazing or picking, especially if it is not accompanied by correct management and scientific principles, can cause a decrease in seed production, which sometimes reaches 100 percent. This point, together with the low natural quality of forage, causes that the cultivation of such a product is ineffective in many cases (Fatahi Doost, 2018). One of the important factors in increasing the agricultural production in line with the operation according to the breed and according to the crop is the optimal management of the use of chemical fertilizers. One of these important elements is nitrogen, which is widely used as a chemical fertilizer by most plants (Fataii, 2007). One of the main pillars in sustainable agriculture is the usage of biological fertilizers with the aim of eliminating or reducing the consumption of chemical inputs and increasing soil fertility (Koocheki *et al.*, 2008). Among the biological fertilizers, we can mention Nitroxin, which contains the most effective nitrogen-fixing bacteria from the genus Azotobacter, Azospirillium, and phosphate dissolvers from the genus Pseudomonas, which improve the organic matter and biological activity of the soil and the supply of nutrients to the plant increases the yield (Kocabas *et al*., 2010). By using nitroxin biological fertilizer, not only can avoid using nitrogen chemical fertilizers, but also because of the multiple effects of nitroxin biological fertilizer, can produce more products. Nitrogen is one of the main elements required by plants and the need for nitrogen is more than other elements. Cereals need to absorb 22 to 25 kg of nitrogen to produce one ton of seeds. The amount of nitrogen fixation by free air nitrogen-fixing bacteria in suitable conditions is reported to be about 20-40 $kg.ha^{-1}$ per year, which requires a large amount of organic matter for nitrogen fixation (Fataii, 2007). Due to the fact that Azotobacter is a heterotrophic bacterium, it is necessary for the soil to be rich in organic matter to supply its carbon. For this purpose, the combined use of nitroxin and chemical fertilizers is recommended in Iran's soils, which mostly have little organic matter (Sharifi Ashoorabad, 1998).

2. OBJECTIVES

This research was carried out with the aim of investigating the consumption of different amounts of nitrogen and nitroxin biological fertilizer on increasing the quantitative and qualitative yield of dual-purpose barley forage.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out in the cropping year of 2013-2014 in the city of Ahvaz with a longitude of 48 degrees and 40 minutes east and latitude of 31 degrees and 20 minutes north and a height of 22.5 meters above sea level. To determine the physical and chemical characteristics of the soil, before any land preparation operation, samples were randomly collected from 0-30 cm depth of the soil from five points, and after drying in the air and passing through a 2 millimeter sieve, some of its physical and chemical characteristics was determined.

Table 1. Physiochemical characteristics of the soil in the experimental area

Soil texture	K ($mg.kg^{-1}$)	P ($mg.kg^{-1}$)	N (%)	OC (%)	pH	EC ($ds.m^{-1}$)	Depth (cm)
Loam-sand	113	6.4	0.097	0.96	7.18	2.04	0-30

The results of soil analysis are shown in table 1**.** The research was carried out as a split plot using completely randomized block (RCBD) with three replications. The investigated treatments include the usage of pure nitrogen fertilizer at three levels [including the usage of 50 $kg.ha^{-1}$ (N_1), 100 $kg.ha^{-1}$ (N_2) and 150 $kg.ha^{-1}$ (N_3)] and nitroxin biological fertilizer at three levels [including the absence of Nitroxin) (control) (B_0), consumption of 2 liters per hectare (B_1) and 4 liters per hectare (B_2)] were placed in the main and sub plots, respectively. This experiment consisted of 27 plots. Each plot included 7 planting 5 lines meters long with a distance of 20 cm from each other. The distance between the two main plots was 1.5 m and secondary plots were 0.5 m apart.

3.2. Farm Management

Tillage operations included irrigation before planting, semi-deep plowing, disc, troweling and fertilizer spraying. 50% of the nitrogen consumed at the same time as planting and the rest in the middle stage of tillering was consumed from the source of urea. The amount of 80 kg of phosphorus from the triple superphosphate source was mixed with the soil before planting. Nitroxin fertilizer was used as a solution in irrigation water. The first irrigation was done immediately after planting, and subsequent irrigations were done as usual according to needs and rainfall conditions. Weeds were controlled by manual weeding.

3.3. *Measured Traits*

3.3.1. *Plant height*

To calculate the plant height, the height of about 20 plants was randomly measured from soil surface to the tip of stem in the physiological maturity stage and their average was considered as plant height (Yaghoubian *et al*., 2017).

3.3.2. *Leaf area index*

To measure the leaf area index at the flowering stage, the area of the leaves of each plant was determined using the copying method. Leaf area index (LAI) was calculated using the following equation (Gardner *et al*., 1985).

Equ. 1. LAI=LA/SA

LA: leaf area in square meters

SA: land area in square meters

3.3.3. *Forage yield*

Harvesting of green forage was done at the end of tillering at the 30th Zadox stage. Green forage was harvested from the 3 middle lines, after removing 0.5 meters above and below the plots at a level equal to 1.5 square meters and weighed separately. In order to determine the yield of dry forage, 200 gram sample of the product of each plot is separated and dried in an oven at 70 degrees for 48 hours until the yield of dry forage is calculated through the following equation (Mardasi and Mojaddam, 2016). **Equ. 2.** Dry weight of forage on the harvested surface =

Dry weight of the sample in the oven × weight of fresh forage/200

3.3.4. *Forage protein percentage*

To determine forage protein percentage, at first total nitrogen was determined by Kjeldahl method and then multiplied by a factor of 5.7 and the forage protein percentage were calculated (Walton, 1983). To determine forage protein yield per unit area, forage protein percentage of each experimental unit was multiplied by its dry forage yield (Mojaddam, 2009).

3.3.5. *Seed protein content*

The amount of seed nitrogen was calculated using the Kjeldahl method. The amount of seed protein was obtained by multiplying the Kjeldahl nitrogen by 6.25 coefficients (Keeney and Nelson, 1982).

3.4. Statistical Analysis

Variance analysis of data was done in the form of split plots with SAS software (Ver.9), and the averages were compared by Duncan's multi-range test at 5% probability level, and graphs were drawn by Excel software (Ver.2010).

4. RESULT AND DISCUSSION

4.1. *Plant height*

The effect of different amounts of nitrogen consumption and nitroxin biofertilizer was significant at the 1% of level on plant height, but their interaction did not have a significant effect on this trait (Table 2). The highest and lowest plant height with an average of 25.90 and 16.73 cm belonged to the treatments of 150 and 50 kg.ha^{-1} of nitrogen, respectively (Table 3). By creating favorable conditions for the plant and providing elements such as nitrogen, the materialization process becomes favorable and the height of the plant also increases. Nitrogen consumption reduces ratio of abscisic acid/gibberellin and increases the plant growth (Marschner, 2012). These results were consistent with the results of Momen *et al*. (2013) who reported that wheat stem height can be affected by nutritional factors and considered main reason for height increase to be elongation between nodes.

Mosanaei *et al*. (2017) and Mojiri and Arzani (2003) reported that increased nitrogen consumption increased plant height. The highest plant height was obtained from the treatment of 4 liters per hectare of nitroxin with an average of 87.12 cm and the lowest from the absence of nitroxin (control) with an average of 72.31 cm (Table 3). In addition to bio fixing nitrogen and dissolving soil phosphorus (especially in areas with high soil calcium), biological fertilizers produce significant amounts of growth stimulating hormones, mainly auxin, gibberellin and cytokinin, on growth and development, yield crops as well as growth characteristics such as plant height affect and cause its increase (Zahir *et al*., 2004). Ahmadi and Jaafarinia (2015) reported that nitroxin biofertilizer increased plant height.

Table 2. Result of analysis of variance effect of treatment on studied traits

S.O.V	df	Plant height	Leaf area index at flowering stage	Dry forage yield
Replication (R)	2	2.04	0.04	76.31
Nitrogen (N)	2	300.03^{**}	6.54^{**}	1558.01^{**}
E_a	4	28.51	0.54	130.45
Nitroxin (B)	2	357.25^{**}	8.78^{**}	1780.92^{**}
N×B	4	0.81^{ns}	0.023^{ns}	12.63^{ns}
E_b	12	25.06	0.34	125.17
CV (%)	---	6.14	14.4	5.06

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 2.

S.O.V	df	Forage protein percentage	Forage protein yield	Seed protein percentage
Replication (R)	2	4.55	0.032	1.85
Nitrogen (N)	2	56.13^{*}	160.27^{**}	18.11^{*}
E_a	4	7.31	14.05	4.03
Nitroxin (B)	2	33.04^{*}	108.01^{**}	11.68^{*}
N×B	4	1.65^{ns}	1.52ns	0.91^{ns}
E_b	12	4.75	9.67	3.06
CV (%)	---	10.57	6.78	14.6

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively.

On the other hand, Biari *et al*. (2008) also stated that application of biofertilizer containing growth promoting bacteria such as Azotobacter increases plant height. They attributed reason for increase the absorption of required nutrients by plant and secretion of growth-promoting substances due to the use of biofertilizers. As Yosefi *et al*. (2011) reported that the application of biological fertilizer increases plant height by producing regulatory hormones such as auxin and gibberellin acid.

4.2. *Leaf area index at flowering stage*

Leaf area index was affected by nitrogen consumption and nitroxin biofertilizer at the 1% of probability level, but the interaction of treatments on this trait was not significant (Table 2). The highest leaf area index was assigned to the treatment of 150 $kg.ha^{-1}$ of nitrogen fertilizer with an average of 4.5 and the lowest was assigned to the treatment of 50 kg/ha of nitrogen fertilizer with an average of 3.57 (Table 3). Increasing the availability of nitrogen fertilizer for the plant stimulated the growth and increased the leaf surface index. Increasing the leaf area and rapid closing of the canopy can increase the received radiation and photosynthesis and increase yield**.** As the studies of Athernadeem *et al*. (2009) showed that nitrogen fertilizer had a positive and significant effect on the number of leaves, so that the maximum number of leaves was produced with the increase of nitrogen consumption up to 150 $kg.ha^{-1}$. They also stated that the leaf area index showed a significant increase with increasing nitrogen consumption. The results of the research Gharaati (2007) and Ahmad *et al*. (2006) confirmed the results of this research**.**The highest leaf area index was obtained from the treatment of using 4 liters per hectare of nitroxin, with an average of 3.21, and the lowest leaf area index was obtained from the treatment of not using nitroxin (control) with an average of 3.54 (Table 3). Azospirillum and Azotobacter bacteria improve vegetative growth and leaf development by providing nitrogen, and as a result, the leaf area index also increases. In this regard, Bakhshaie *et al*. (2014) stated that nitroxin biological fertilizer with Azospirillium and Azotobacter bacteria increased the leaf area index. As Hamidi *et al*. (2009) showed with the application of biofertilizer, the number of upper leaves of the cob and the number of leaves per plant increased. They explained the reason for this issue to improve access and better absorption of nutrients. The results of the study by Biari *et al*. (2008) showed that growth-promoting bacteria had a positive and significant effect on the leaf area index.

4.3. *Dry forage yield*

Dry forage yield was significantly affected by different amounts of nitrogen and nitroxin biofertilizer, but the interaction of treatments did not have a significant effect on this trait (Table 2). The highest yield of dry forage belonged to the treatment of 150 $kg.ha^{-1}$ of nitrogen fertilizer with an average of 2450.34 $kg.ha^{-1}$ (Table 3). Some researchers have attributed the effect of nitrogen in increasing forage yield to the regulatory role of nitrogen in the production of amino acids and plant hormones related to the division and expansion of the cell wall, and others have attributed the role of nitrogen to the development of developmental stages, which in later stages lead to the reason for receiving more light energy leads to the production of more dry matter (Siam *et al*., 2008).

Table 3. Mean comparison effect of different level of Nitrogen and Nitroxin Biofertilizer on studied traits

Treatments	Plant height (cm)	Leaf area index at flowering stage	Dry forage yield ($kg.ha^{-1}$)
Nitrogen			
50 ($kg.ha^{-1}$)	73.16c	3.57b	1938.47c
100 ($kg.ha^{-1}$)	81.04b	4.08ab	2234.65b
150 ($kg.ha^{-1}$)	90.25a	4.5a	2450.34c
Nitroxin Biofertilizer			
0 (Control)	72.31b	3.21b	1952.13b
2 ($L.ha^{-1}$)	85.03a	4.40a	2300.25a
4 ($L.ha^{-1}$)	87.12a	4.54a	2371.08a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

Continue table 3.

Treatments	Forage protein (%)	Forage protein yield ($kg.ha^{-1}$)	Seed protein (%)
Nitrogen			
50 ($kg.ha^{-1}$)	18.68b	362.1c	10.57b
100 ($kg.ha^{-1}$)	20.91ab	467.26b	11.93ab
150 ($kg.ha^{-1}$)	22.24a	544.95a	13.44a
Nitroxin Biofertilizer			
0 (Control)	18.33b	357.82b	10.25b
2 ($L.ha^{-1}$)	21.72a	499.61a	12.69a
4 ($L.ha^{-1}$)	21.80a	516.89a	13a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

In this regard, Niazkhani *et al*. (2014) announced in triticale plant by increasing nitrogen fertilizer up to 80 $kg.ha^{-1}$, the highest yield of dry forage was obtained, which was consistent with the results of this research. The highest yield of dry forage (2371.08 $kg.ha^{-1}$) was obtained from the treatment of nitroxin biofertilizer at the rate of 4 liters per hectare and the lowest yield of dry forage (1952.13 $kg.ha^{-1}$) was obtained from the treatment of no use of nitroxin (control). It seems that the fixation of nitrogen by the bacteria present in nitroxin and its release and better absorption by the plant had a positive effect on the process of increasing the forage weight. As Mardasi and Mojaddam (2016) reported that the effect of nitroxin fertilizer on forage protein yield and forage yield was significant. In this regard, Ahamdi and Jaafarnia (2015) showed based on their research that nitroxin biofertilizer increased the dry weight of forage compared to control. On the other hand, Romani *et al*. (2015) reported that the highest amount of dry forage was obtained from the biological fertilizer treatment and the lowest from the control treatment. These results prove the positive effect of biofertilizer in improving the nutritional conditions of the plant that as a result of bacterial inoculation in these treatments, the effectiveness of appropriate growth regulation, physiological and metabolic activity in the plant has increased. Other researchers such as Siahmarguee *et al*.

(2022) and Keshavarz *et al*. (2012) have also pointed out the role of biological fertilizers in increasing the yield of dry forage, which was consistent with the results of this study.

4.4. *Forage protein percentage*

The effect of nitrogen consumption and nitroxin on forage protein percentage was significant at the 5% of level, but their interaction on the forage protein percentage was not significant (Table 2). The highest forage protein percentage belonged to the usage of 150 $kg.ha^{-1}$ of nitrogen fertilizer with an average of 22.24% and the lowest belonged to the usage of 50 $kg.ha^{-1}$ of nitrogen fertilizer with an average of 18.68% (Table 3). With the increase of nitrogen, the leaf surface also increases and as a result, the increase in the ratio of leaf to stem increases the amount of protein and decreases the woody and lignin parts in the forage (Vos *et al*., 2005). In this regard, Kiani *et al*. (2014) reported that with the increase of nitrogen levels, the amount of crude protein of forage also increased, so that the highest amount of protein was related to the level of 210 kg of nitrogen with an average of 19.5%. Nitroxin biofertilizer at the rate of 4 liters per hectare with 21.8 forage protein percentage and no application of nitroxin (control) with 18.33 percent of forage protein had the highest and lowest of this trait, respectively (Table 3). These results prove the positive effect of nitroxin biofertilizer in improving the nutritional conditions by increasing the physiological and metabolic activities of the plant (Ram Rao *et al*., 2007). Due to the presence of stimulating bacteria, biofertilizers, in addition to more nitrogen fixation, will increase the quality and forage protein percentage and, as a result, it will be palatable. In this regard, Mardasi and Mojaddam (2016) reported that the effect of nitroxin fertilizer on forage protein yield, seed yield, and forage protein percentage and forage yield was significant. They stated that the effect of nitroxin biofertilizer can achieve the best yield of seed and forage in terms of quality, which was consistent with the results of this research.

4.5. Forage protein yield

The difference between different amounts of nitrogen consumption and nitroxin biofertilizer was significant in terms of forage protein yield, although the interaction of treatments did not have a significant effect on this trait (Table 2). The highest (544.95 $kg.ha^{-1}$) and the lowest (362.1 $kg.ha^{-1}$) forage protein yields were obtained in the treatments of 150 and 50 kg/ha of nitrogen fertilizer, respectively (Table 3). As the levels of nitrogen consumption increased, the yield of protein also increased. Increasing the percentage and yield of forage protein with increasing nitrogen is the result of more nitrogen absorption and increased vegetative growth (Mardasi and Mojaddam, 2016). Nitrogen, in addition to having a small effect on yield, because it is one of the main structures of amino acids, it also increases the percentage of protein, and in general, nitrogen more than the required amount of yield increases the protein content in the plant. Increasing the percentage of dry matter and crude protein makes the plant palatable for livestock and improves the quality of silage (Mirlohi *et al.,* 2000). The highest (516.89 kg.ha^{-1}) and the lowest (357.82 kg.ha^{-1}) yield of forage protein was obtained from the treatments of nitroxin biofertilizer at the rate of 4 liters per hectare and no nitroxin application (control), respectively (Table 3). Due to the increase in nutrient absorption, higher consumption of nitroxin increased the amount of protein, which was consistent with the results of the research of Ardakani *et al*. (2006). The bacteria in nitroxin fertilizer had a higher protein yield with the gradual release of nutrients and their absorption by the plant. In this regard, Rajaee *et al*. (2007) stated that the reason for the increase in protein as a result of the use of nitrogen biofertilizer is the supply of more nitrogen through biological fixation and the release of absorbable nitrogen around plant roots by bacteria. These results were consistent with the reports of Mardasi and Mojaddam (2016) and Moradi *et al*. (2011) who pointed out the positive role of nitroxin biofertilizer in increasing the forage protein yield.

4.6. *Seed protein percentage*

Seed protein percentage was affected by different amounts of nitrogen fertilizer and nitroxin biofertilizer, but the interaction effects on seed protein percentage was not significant (Table 2). The highest percentage of seed protein belonged to 150 kg/ha nitrogen fertilizer (with an average of 13.44%) and the lowest to 50 kg.ha^{-1} of nitrogen fertilizer (with an average of 10.57%) (Table 3). Consumption of high amounts of nitrogen, in addition to more accumulation of nitrogen in vegetative organs, increases the rate of nitrogen transfer to seeds in comparison with carbohydrates, and as a result, seed protein increases. Nitrogen plays an important role in protein biosynthesis and many biological molecules. Therefore, the use of nitrogen causes more productivity of this element in the plant, while nitrogen plays an active role in the photosynthesis of the plant, so that the speed of photosynthesis in the plant increases and finally the production of protein also increases (Litkeh *et al*., 2018). In a study, Alazamani (2014) reported that the effect of nitrogen fertilizer application on seed protein content was significant. The research of Overman *et al*. (1995) showed that with the increase in nitrogen consumption, the concentration of this element in the plant organs increases during the vegetative growth stage, and during the seed filling stage, more substances are transferred to the seeds through the re-transfer of nitrogen, the result of which is an increase in the percentage of seed protein. The above results were consistent with the results of Ebadi *et al*. (2009) and Klikocka and Ebadi *et al*. (2016). The highest percentage of seed protein was obtained from the treatment of 4 liters per hectare of nitroxin, with an average of 13%, and the lowest percentage of seed protein was obtained from the absence of nitroxin (control) with an average of 10.25% (Table 3). Nitroxin fertilizer has probably improved conditions and increased soil enzyme activity and nitrogen supply in the soil, which increases seed protein. By secreting organic acids and phosphatase, biological organisms lead to the release of elements from the complexes in the soil, and plant access to nutrients increases (Tejada *et al*., 2008). Other researchers considered the reason for the increase in seed protein as a result of the use of nitrogen biofertilizer to provide more nitrogen through biological fixation and the release of absorbable nitrogen around plant roots by Azotobacter bacteria. This can be due to the improvement of seed nitrogen supply and the increase of nitrogen consumption efficiency (Egamberdiyeva, 2007). Azadi *et al*. (2013) reported a 4.5% increase in seed protein due to the combined use of Azospirillium and Azotobacter, which was consistent with the results of this research.

5. CONCLUSION

Increasing nitrogen consumption had a positive and significant effect on plant height, forage dry forage, protein and yield of forage protein and seed protein. The use of nitroxin biofertilizer at the rate of 4 liters per hectare (which was not statistically significantly different from the treatment of 2 liters per hectare) in addition to the production of growth stimulating hormones, caused the development of the active level of the root system and increased plant access to nutrients. The highest dry forage yield and forage protein yield were obtained from the treatment of 4 liters per hectare of nitroxin biofertilizer. The use of biofertilizers along with nitrogen chemical fertilizers can reduce the consumption of nitrogen chemical fertilizers in addition to producing enough yields, which significantly contributes to the health of the environment and is an important strategy in moving towards sustainable agriculture.

ACKNOWLEDGMENT

The authors thank all colleagues and other participants, who took part in the study.

FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

FUNDING/SUPPORT: This study was done by support of Department of Agronomy, Islamic Azad University, Ahvaz Branch.

REFRENCES

Ahmad, G., A. Jan, I. Arif. and M. Arif. 2006. Phenology and physiology of canola as affected nitrogen and sulfur fertilization. Agron. J. 5(4): 555-562.

Ahmadi, G. and M. Jaafarinia. 2015. Evaluating the effect of biofertilizers on growth and emergence of barley (*H. vulgare* L.). Indian J. Fundamental and Applied Life Sci. 5(3): 93-99.

Alazmani, A. R. 2014. Effect of nitrogen fertilizer on feed and seed yield of Barley cultivar. Intl. Res. J. Appl. Basic Sci. 8(11): 2013-15.

Ardakani, M. R., A. Farah Bakhsh, B. Sani. and H. Khosiravi. 2006. Investigating the effectiveness of diazotrophic biological fertilizers in sustainable soybean cultivation, The 9th Cong. Agri. Sci. Plant Breed. Iran. Univ. Tehran. Iran.

Ather Nadeem, M., Z. Iqbal, M. Ayoub. and K. Mubeen. 2009. Effect of nitrogen application on forage yield and quality of maize sown alone and in mixture with legumes. Pak. J. Life Social Sci. 7: 161-167.

Azadi, S., S. A. Siadat, R. Naseri, A. Soleimani Fard. and A. Mirzaei. 2013. Effect of integrated application of Azotobacter chroococcum and Azospirillum brasilense and nitrogen chemical fertilizers on qualitative and quantitative of durum Wheat. J. Crop Eco-Physiol. 7(2)26: 129-146.

Bakhshaie, S., P. Rezvani Moghaddam. and M. Goldani. 2014. The effects of nitroxin and nitrogen fertilizer on yield and yield components of winter wheat (*Triticum aestivum* L.). J. Iranian Field Crop Res. 12: 360-368.

Biari, A. A. Gholami. and H. Rahmani. 2008. Growth promotion and enhanced nutrient uptake of maize by application of plant growth promoting Rhizobacteria in arid region of Iran. J. Biol. Sci. 8(6): 1015-1020.

Ebadi, A., H. R. Mohammaddoost-Chamanabad. and H. R. Mustafaei. 2009. Investigating the effect of sulfur and nitrogen on yield and absorption of nutrients in spring safflower. Msc. Thesis. Mohaghegh Ardabili Univ. Faculty Agri. Nat. Res. 118 pages.

Egamberdiyeva, D. 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl. Soil Ecol. 36: 184-189.

Fatahi Doost, R. 2018. Investigating the effect of forage harvesting time and nitrogen levels on the yield of forage and seed of southern barley in Ahvaz weather conditions. Msc. Thesis. Dep. Agron. Ahvaz Islamic Azad University. 105 pages.

Fataii, E. 2007. An introduction to environmental knowledge. Mahd Tamdan Publications.

Gharaati, L. 2007. Effects of nitrogen levels on yield and yield components of safflower. MsC. Thesis. Faculty Agri. Tech. Univ. Isfahan.

Gardner, F. P., R. B. Pearce. and R. L. Mitchell. 1985. Physiology of Crop Plants. Iowa State Univ. Press. Ames 1985. 327 pp. USA.

Hamidi, A., R. Chokan, A. Asghar Zadeh, M. Dehghan shoaar, A. Ghalavand. and M. J. Malakoti. 2009. The effect of use bacterial growth promoting (PGPR) on the phenology of late maturity Hybrid. Iranian J. Crop Sci. 11:270-249.

Keeney, D. R. and D. W. Nelson. 1982. Nitrogen in organic forms. PP. 643-698. *In*: Page, A. L., Miller, R. H., and Keeney, D. R (Eds.), Method of soil analysis. Part II.

Keshavarz Afshar, R., M. R. Chaichi, H. Moghadam. and S. M. R. Ehteshami. 2012. Irrigation, phosphorus fertilizer and Phosphorus solubilizing microorganism effects on yield and forage quality of Turnip (*Brassica rapa* L.) in an arid region of Iran. Agri. Res. 1(4): 370-378.

Kiani, S., S. A. Siadat, M. R. Moradi Telavat, A. R. Abdali Mashhadi. and M. Sare. 2014. Effect of nitrogen fertilizer application on forage yield and quality of Barley (*Hordeum vulgare* L.) and fennel (*F. vulgare* L.) intercropping. Iranian J. Crop Sci. 16(2): 77-90.

Klikocka, H., M. Cybulska, B. Barczak, B. Narolski, B. Szostak, B. Kobiałka, A. Nowak. and E. Wójcik. 2016. The effect of Sulphur and nitrogen fertilization on seed yield and technological quality of spring wheat. Plant Soil Environ. 62(5): 230-236.

Kocabas, I., M. Kaplan, M. Kurkcuoglu. and K. H. C. Base. 2010. Effects of different organic manure applications on the essential oil components of Turkish sage (*Salvia fruticosa* mill). Asian J. Chem. 22(2): 1599-1605.

Koocheki, A. R., M. Hoseini. and A. Dezfoli. 2008. Constant Agriculture. Mashhad Univ. Pub. Iran.

Litke, K., Z. Gaile. and A. Ruza. 2018. Effect of nitrogen fertilization on winter wheat yield and yield quality. Agron. Res. 16(2): 500 509.

Mardasi, Z. and M. Mojaddam. 2016. Study of combination of nitrogen chemical and biological fertilizers and harvesting time on yield quantity and quality of dual purpose barley. Res. Agri. 8(5): 1-13.

Marschner, H. 2012. Mineral nutrition of higher plants. Second Edition. 889pp. London: Academic Press. UK.

Mirlohi, A. F., N. Bozorgvar. and M. Basiri. 2000. Effect different rates nitrogen Fertilizer on growth, yield and forage qualitative three hybrid forage sorghum. Iranian J. Agri. Natural Res. 2: 105-115.

Mojaddam, M. 2009. Investigating the effect of nitrogen management and forage harvesting time on forage yield, seed and amount of retransmission of Jonoob cultivar Barley. Crop Physiol. J. 1(4): 85-99.

Mojiri, A., and A. Arzani. 2003. Effect of nitrogen rate and plant density on yield and yield components of Sunflower J. Water and Soil Sci. 7(2):115-125.

Momen, A., A. R. Pazoki. and M. R. Momayezi. 2013. Effects of granular Sulfur (bentonitic) and compost on quantitative and qualitative characteristics of Bam Wheat in Semnan Region. 3(9): 31-46.

Moradi, M., S. A. Siadat, K. Khavazi, R. Naseri, A. Maleki, and A. Mirzae. 2011. Effect of application of biofertilizers and Phosphorus fertilizers on qualitative and quantitative traits of spring Wheat (*Triticum aestivum* L.). 5(18)2-18: 51-66.

Mosanaei, H., H. Ajamnorozi, M. R. Dadashi, A. Faraji. and M. Pessarakli. 2017. Improvement effect of nitrogen fertilizer and plant density on wheat (*Triticum aestivum* L.) seed deterioration and yield. Emirates J. Food Agri. 29(11): 899-910.

Niazkhani, M., A. Khorshid. and A. Eivazi. 2014. Evaluation forage clipping stages and different levels of nitrogen on seed and forage yields of Triticale. Am. J. Plant Sci. 5: 2199-2206.

Nourmohamadi, Gh., S. A. Siadat. and A. Kashani. 2010. Cereal. Pub. Shahid Chamran Univ. Ahvaz. 446 pages.

Overman, A. R., D. M. Wilson, W. Vidak, M. N. Allhands. and T. C. Perry. 1995. Model for partitioning of dry matter and nutrients in Corn. J. Plant Nutrition, 18(15): 959-968.

Ram Rao, D. M., J. Kodandaramaiah. and M. P. Reddy. 2007. Effect of VAM fungi and bacterial biofertilizers on mulberry leaf quality and silkworn cocoon characters under semi-arid conditions. Caspian J. Environmental Sci. 5(2): 111-117.

Roumani, A., S. M. R. Ehteshami. and M. Rabiei. 2015. Effect of seed inoculation with plant growth promoting Rhizobacteria (PGPR) on forage quality and yield of turnip (*Brassica rapa* L.) at the different values of nitrogen and phosphorus fertilizers. J. Plant Prod. Technol. 14(2): 89-99.

Rajaee, S., H. A. Alikhani. and F. Raiesi. 2007. Effect of plant growth promoting potentials of *Azotobacter chroococcum* native strains on growth, yield and uptake of nutrients in Wheat. J. Water and Soil Sci. 11(41): 85-97.

Sharifi Ashoorabad, E. 1998. Investigating soil fertility in agricultural ecosystems. PhD Thesis. Islamic Azad Univ. Sci. Res. Unit. 115 pages.

Siahmarguee, A., F. Taliei. and M. Yazdandost. 2022. Evaluation of resistance and biochemical responses of different varieties of tomato to Dodder (*Cuscuta campestris* Yunck.). J. Pant Environmental Physiol. 17(65): 19-37.

Siam, H. S., G. M. Abd-El-Kader. and H. I. El-Alia. 2008. Yield and yield components of maize as affected by different sources and application rates of nitrogen fertilizer. Res. J. Agri. Biol. Sci. 4(5): 399-412.

Tejada, M., J. L. Gonzalez, A. M. Garcia-Martinez. and J. Parrado. 2008. Effect of different green manures on soil biological properties and Maize yield. Bio-Res. Technol. 99: 1758-1767.

Vos, J., P. E. L. Vander Putten. and C. J. Birch. 2005. Effect of nitrogen supply on leaf appearance, leaf nitrogen economy and photosynthetic Maize (*Zea mays* L.). Field Crops Res. 93: 64-73.

Walton, P. D. 1983. Production and management of the cultivated forages. Reston Publishing Company. Univ. Minnesota. USA. 336p.

Yaghoubian, I., S. Ghassemi. and Y. Yaghoubian. 2017. Effect of sowing date and urea fertilizer on some morphological traits, yield and yield components of wheat in Hashtroud, Iran climate condition. Agro-Ecol. J. 13(3): 52-46.

Yosefi, K., M. Galavi, M. Ramrodi. and S. R. Mousavi. 2011. Effect of biophosphate and chemical phosphorus fertilizer accompanied with micronutrient foliar application on growth, yield and yield components of maize (Single Cross 704). Aust. J. Crop Sci. 5(2):175-180.

Zahir, A. Z., M. Arshad. and W. F. Frankenberger. 2004. Plant growth promoting rhizobacteria: Application and perspectives in agriculture. Adv. Agron. 81: 97-168.