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Evaluation of Seed Yield, Its Component and Nitrogen Use Efficiency of Sorghum in Response to Nitrogen and Nitroxin Fertilizers

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ABSTRACT

BACKGROUND: Fertilizer management plays an important role for obtaining satisfactory yields and to increase crop productivity. Nutrient management may be achieved by the involvement of organic sources, bio fertilizers, and micro-nutrients.
OBJECTIVES: Assessment effect of chemical and biological fertilizer on seed yield, its components and plant height of Sorghum crop.
METHODS: This research was carried out via split plot experiment based on randomized complete blocks design with three replications. Pure nitrogen from urea source was used as the main plot in four levels (a_1 = 0 or control, a_2 = 50, a_3 = 100, a_4 = 150 Kg N ha^{-1}) and Nitroxin bio-fertilizer was used as the sub plot in two levels (b_1 = non-use of bio-fertilizer or control and b_2 = use of 1 $L.ha^{-1}$ Nitroxin per 200 kg seeds).
RESULT: The results of analysis of variance indicated that the effect of different level of nitrogen and Nitroxin on all measured traits was significant but interaction effect of treatment was significant only on seed yield, number of seed per spike and nitrogen use efficiency traits at 1% probability level. The maximum number of spikelet per spike, seed per spikelet, seed per spike, 1000-seed weight, seed yield and nitrogen use efficiency belonged to the treatment with consumption of 150 Kg N ha^{-1} and also to the treatment with consumption of Nitroxin bio-fertilizer. Crop under 150 kg nitrogen treatments and not using fertilizers with the mean of 116 and 108 cm had the highest and lowest plant height.
CONCLUSION: Finally according result of current research consumption 150 Kg N ha^{-1} with 1 $L.ha^{-1}$ Nitroxin led to achieve maximum seed yield (349 $gr.m^{-2}$) and it can be advice to producers.

KEYWORDS: *Biological fertilizer, Plant height, Sorghum bicolor.*

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1. BACKGROUND

Nowadays, world's attention (permanent agriculture) to the stable agriculture and applying new technology and science in farms to minimize damage to resources and maximum utilization of it is considered. (Gholami *et al.*, 2013). With the growth population increase, the request for food has been increasing while land availability has been declining. Thus, the only way to increase production is to increase yield per unit area (Hirpa, 2014). More recently, attention is focused on the global environmental problems. The world elite society is giving emphasize on utilization of organic wastes, farm yard manure, compost, vermin compost and poultry manures as the most effective measure to save the environment to some extent. Organic materials are the safer sources of plant nutrient which have no detrimental effect to crops and soil. Cow dung, farm yard manure, poultry manure and also green manure are excellent sources of organic matter as well as primary plant nutrients (Pieters, 2005). Crop management is important to attain higher stalk yield in sweet sorghum. Among the various inputs that improve the efficiency of a cultivar in realizing its potential, fertilizers (nitrogen in particular) play a crucial role. Nitrogen is generally a limiting nutrient in crop production, and especially in sorghum, as it has been said to be the most responsive nutrient for its production (Singh *et al.*, 1972). To achieve economically viable returns, efficient use of available resources, like nitrogen, is necessary to maximize yields in all seasons. Variable responses to the application of nitrogen fertilizer have been observed in sorghum owing to differences in climatic, soil and genotypic factors across seasons and locations (Muchow, 1988). After the industrial revolution widespread introduction of inorganic fertilizers led to a decline in the use of organic material in the cropping systems (Hasan uzzaman *et al.*, 2010). Fertilizer management plays an important role for obtaining satisfactory yields and to increase crop productivity. Nutrient management may be achieved by the involvement of organic sources, bio fertilizers, and micro-nutrients (Singh *et al.*, 2002). Among the macro nutrients essential for crop growth, nitrogen (N) is a very mobile element in the soil, due to its susceptibility to leaching, de nitrification, and volatilization losses. Excessive use of N fertilizer can lead to pollution of water bodies and may lead to soil acidification. Balanced and efficient use of applied N is of paramount importance in the overall nutrient management system than any other plant nutrient in order to reduce its negative impact on the environment. Besides, even under the best management practices, 30%-50% of the applied nitrogen is lost through different routes and hence more fertilizer needs to be applied than actually needed by the crop to compensate for the loss. The transitory loss of N not only causes loss to the farmer but also causes irreversible damage to the environment. High rates of chemical fertilizer cause environmental pollution (Shamme *et al.,* 2016). Nitrogen is essential for plants growth and it is still one of major factors limiting crop yield (Zhao *et al.*, 2005). To achieve economically viable returns, efficient use of available resources, like nitrogen, is necessary to maximize yields in all seasons. There is a need to use the minimum amount of nitrogen required for the maximum growth rate at any time during the growing season (Sheehy *et al.*, 1998). The nitrogen requirement for crop production has traditionally been determined from field experimentation involving different rates of application of nitrogen fertilizer (Muchow, 1998). Variable responses to the application of nitrogen fertilizer have been observed in maize and in sorghum owing to differences in climatic, soil and genotypic factors across seasons and locations (Muchow, 1990). Who also mentioned that nitrogen requirement is dependent on the yield expectation in a given environment as determined by climate, management and cultivar. Depending on soil nitrogen fertility, farmers apply anywhere between 45 and 224 kg N ha in sorghum production (Zhao *et al*., 2005). Although adequate supply of nitrogen to crops is fundamental to optimize crop yields, mismanagement of nitrogen, such as excessive nitrogen application, can result in contamination of the groundwater (Jaynes *et al*., 2001). Mengel and Kirkby (2001) mentioned that corn and sorghum yield would have dropped by 41% and 19%, respectively, without nitrogen fertilizer application. Application of nitrogen fertilizer increase sweet sorghum stem yield and corn fodder. Nitrogen fertilizer is a key nutrient in the production of non-legume crops. It is a component in many biological compounds that plays a major role in photosynthetic activity and crop yield capacity (Cathcart and Swanton, 2003) and its deficiency constitutes one of the major yield limiting factors for cereal production (Shah *et al*., 2003). Nitrogen is the most limiting essential nutrient for maize production (Aftab *et al*., 2007). Nitrogen has positive effect on storage of protein in Maize seed and hence, the rates of this element are effective in its distribution in plant (Souza *et al*., 1998). A low nitrogen content in the soil leads to poor absorption of micronutrients by plants, which may be insufficient for the complete development of the plant tissue (Szulc, 2013). On the other hand, an excessive accumulation of mineral nitrogen in the soil poses a risk of water pollution as a result of nitrate leaching by precipitation (Ladha *et al*., 2005). Use of inorganic fertilizers has increased considerably to meet the higher nutrient requirements of the present day improved varieties. However, ecological and environment concerns over the increased and indiscriminate use of inorganic fertilizers have made research on use of organic materials as a source of nutrients very necessary (Upadhyaya *et al*., 2003). Organic manure is cheap and could be used as a substitute for chemical fertilizers (Delate and Camberdella, 2004). Studies have shown that long-term use of fertilizers reduces crop yields. This decrease is due to the acidification of the soil, the reduction of biological activity of the soil and the inappropriate physical properties of the soil (Alexandratos, 2003). Including ways to increase the yield per unit area can be mentioned to appropriate management in crops nutrition and intercropping systems plants. Increasing nitrogen fertilizer applications has been a major management strategy to obtain high yield (Guo *et al*., 2010). The reduction of nitrogen fertilizer inputs and improved nitrogen use efficiency are crucial for sustainable production. 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 crop production, reduce the overuse of chemical fertilizers, and decrease greenhouse emissions (Kennedy *et al*., 2004). Chemical fertilizers have several negative impacts on environment and sustainable agriculture. Therefore, bio fertilizers are recommended in these conditions and growth prompting bacteria uses as a replacement of chemical fertilizers (Wu *et al*., 2005). To alleviate the problem, integrated plant nutrient management is an option as it utilizes available organic and inorganic nutrients to build ecologically sound and economically viable farming system. Research has suggested that integrated nutrient management strategies involving chemical fertilizers and bio-fertilizers enhance the sustainability of crop production. Integrated plant nutrient management is the combined use of mineral fertilizers with organic resources such as cattle manures, crop residues, urban/rural wastes, composts, green manures and bio-fertilizers (Kemal and Abera, 2015). Combined application of organic fertilizer and urea fertilizer or combination urea fertilizer and polyamines significantly increased yield, vegetative growth and chlorophyll index (Zeid, 2008). For gave to highest seed yield in agriculture addition to both nitrogen and phosphate fertilizer is very important (Shaban, 2013). Biological fertilizers cause the economic sustainability of soil resources, production, long-term maintenance and prevent of environmental pollution. On the other hand, the quality of food is a product of biological fertilizers not only consumer satisfaction but also supply and guarantee their physical health (Shoaei *et al*., 2012). The term of biological fertilizer is not particularly for organic matters from manure, crop residue, green manure, etc., but also includes bacterial and fungus micro organisms, specially plant growth regulators and compounds from their activity (Manaffee and Kloepper, 1994). These types of bacteria, in addition to increasing mineral elements of soil through biological N fixation, phosphate and potassium solubilizing and inhibition of pathogens, also by growth regulator hormones produce affect crop yield (Sturz and Chrisite, 2003). Overall, biological fertilizers term refers to fertile materials that involve one or more beneficial soil organism within a suitable preservative. In fact, this fertilizers include different types of micro organisms (Chen, 2006), that could converse nutrients from unavailable form to available form during a biological process (Rajendran and Devaraj, 2004), and resulted in develop root system and increase seed germination rate (Chen, 2006). Biological fertilizers have special significance in increasing crop production and reserve soil sustainable fertility (Sharma, 2003). The application of bio fertilizers has become of great necessity to get a yield of sufficient high quality and to avoid environmental pollution (Shevananda, 2008). Biological fertilizers are obviously an important part of a sustainable agricultural system and have an important role in crop production by maintaining soil fertility (Chen, 2006). Biological fertilizers are produced from a variety of microorganisms that have the ability to convert nutrients from non-absorbable to absorbable forms (Yu *et al*., 2005). The highest values of vegetative growth, oil yield, chlorophyll content and NPK percentages were recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer (Ahmed *et al*., 2013). Nouraki *et al*. (2016) reported bacteria have positive role in the production of bio-fertilizers and hormones which play a significant role in regulating plant growth while mixing them with chemical fertilizers as a supplement the level and depth of the roots. This combination also increases the rate of water and nutrient absorbance which raise the rate of growth and photosynthesis. These combination also increase the grain yield, yield components, and biological function, it has been found that bio-fertilizers can be combined with chemical fertilizers in a complementary way to reduce the excessive amount of chemical fertilizers used to grow corn. It was shown that the mixing of biological fertilizers with chemical fertilizers could reduce the needs of chemical fertilizers up to 25% and these results are comparable to the application of 100% chemical fertilizers. Therefore, the best hybrid maze is the single cross 704 that has good yield potential when the chemical fertilizer is used at either 25% or 50% of the current application when mixed with the bio-fertilizer. Among microorganisms, The *Azotobacter* has attracted more attention because of their ability to communicate with important crop plants such as wheat, corn, and sorghum (Hegde *et al*., 1999). *Azotobacter* is a free-living bacteria that stabilizing the molecular nitrogen for stimulating and enhancing plant growth through nitrogen fixation, increasing the production of hormones, B vitamins, the development of the root system and the release of organic acids in the rhizosphere (Gaind and Gaur, 1989). Rai and Caur (1998) studied *Azotobacter* and *Azospirillum* and double-inoculation and alone inoculation effects on wheat growth and yield. Double-inoculation of *Azotobacter* and *Azospirillum* had positive effects on plant height, spike length, grain yield, biological yield and harvest index in various wheat genotypes. It is proved that hormones such as oxine, giberline and cytokenine are synthesized by many *Azotobacter* spp (Singh *et al*., 2004). Nitroxin is an Azotobacter biological fertilizer which leads to higher quality yields (Kholdi *et al*., 2015). The Nitroxin biological fertilizer also contains nitrogen stabilizing bacteria, which is produced and supplied with the approval of the country's research institutes (Asadi Kupaland and Isa Zadeh Laserjan, 2009). The bacteria in the Nitroxin biological fertilizer, in addition to stabilizing nitrogen of the air and balancing the absorption of macro and micronutrient elements, stimulate growth of the hormones by synthesizing and securing growth promoters such as hormones (Fulchirri and Frioni, 1994). Rahi (2013) reported that increase in Nitroxin also increased fresh and dry weights of leaf, stem, chlorophylls a, b, total carotenoids, and anthocyanin content of the plants linearly. Sorghum it is the fifth most important cereal crop in the world and it is the dietary staple of more than 500 million people in more than 30 countries, and it ranking the fourth food grains of the world (El Naim *et al*., 2012). Grain sorghum has so many local names among these: Durra, Feterita, Daza, Sorgo, Gonia corn, Egyptian millet, Jowar, Kaffir corn, Milo, Shallo and Sudan grass (Ahmed *et al*., 2016). Most of people in Africa and Asia depend on sorghum as the staple food. In addition, the fodder and Stover is feed to millions of animals providing milk and meat for people, over 55% of grain produced globally is used for human consumption and about 33% of grain used in feeding livestock. Globally sorghum is cultivated over an area of about 42.7 million ha with a production of about 58.7 million tons in many parts of the world (Alikhani *et al*., 2012). To manage the sorghum crop for achievement of maximum forage production, the farmer should be concerned about nitrogen requirement (Vanderlip, 2012). Although sorghum is a C4 crop and uses nitrogen in a more efficient way compared to most C3 crops, nitrogen is the most essential nutrient for sorghum growth, which is still one of the major factors limiting its yield (Young and Long, 2000). On the other hand, while nitrogen fertilization increases growth of sorghum and its yield, inappropriate amount of fertilizer during cultivation leads to lower plant performance and reduction in efficiency of the applied fertilizer compared to the actual potential of fertilizer use efficiency (Zhao *et al.*, 2005). The rate of nitrogen fertilization to optimize sorghum growth and yield varies with cultivars. Genetic diversity of nitrogen use has been demonstrated in sorghum with some of the most efficient types being cultivars that evolved from low-fertility environments (Gardner *et al.*, 1994). Bebawi (1981) showed that sorghum cultivars are different in their ability to respond to different fertilization. Mansouri-Far *et al.* (2011) showed that increase in nitrogen fertilizer level improves maize growth and yield. They also found that the responses of different hybrids to nitrogen supply were different. Mahmud *et al.* (2003) reported that N fertilization increases crude protein, fodder and dry matter yield in forage sorghum. Under nitrogen deficit conditions, photosynthetic are not used fully in the synthesis of organic nitrogen compounds and hence sugars are accumulated (Karic *et al.*, 2005).

2. OBJECTIVES

The goals of this study were to find out the effect of different level of chemical and bio-fertilizer on productivity of sorghum, yield components, plant height and nitrogen use efficiency.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

Current research was carried out to assessment effect of chemical and biological fertilizer on seed yield, its components and plant height of Sorghum via split plot experiment based on randomized complete blocks design with three replications along 2015 year. Place of research was located in research farm of Islamic Azad University of Ahvaz Branch at longitude 48°40'E and latitude 31°20'N in Khuzestan province (South west of Iran). Pure nitrogen from urea source was used as the main plot in four levels (a_1 = 0 or control, a_2 = 50, a_3 = 100, a_4 = 150 Kg N ha^{-1}) and Nitroxin bio-fertilizer was used as the sub plot in two levels (b_1 = nonuse of bio-fertilizer or control and b_2 = use of 1 L.ha^{-1} Nitroxin per 200 kg seeds). Urea and triple super phosphate were used from the sources of chemical fertilizers of nitrogen and phosphorus used in the experiment.

3.2. Farm Management

The required nitrogen was provided by the urea source. In order to prevent horizontal movement of urea fertilizer during the fertilization, some furrows were made in irrigation streams and the fertilizer was evenly placed in the furrows. Then they were covered by soil and immediately irrigated. While planting at the first stage, urea fertilizer was distributed to the experiment land as the basic fertilizer. Potassium fertilizer was not used due to high level of absorbable potassium. One liter of Nitroxin bio-fertilizer was used before planting as mixed with the seeds. The required amounts of nitrogen fertilizers were identified after the soil analysis and the needed fertilizer for each plot was calculated with regard to the plot size and the levels of studied treatments and 25% of pure nitrogen as the base fertilizer was added to the land before planting and 75% was added at 8-leaf stage. There were 8 plots in each block. The space between each sub plot from the other one was as one non-planting line and the space between every two main plots was as two non-planting lines. There were 6 planting rows in each plot and the space between the rows was 75 cm and over the rows was 12 cm. Cultivar seeds were used. The seeds were planted at the end of July as ridge and furrows at the depth of 3-4 cm. in seed mixing method, after blending the seeds they were dried in shadow and immediately planted. After sowing the seeds, the field was irrigated. During the growth stage, growing operations such as irrigation, thinning and controlling the weeds (at 4-leaf stage) were done.

3.3. Measured Traits

In order to determine the yield and its components two planting lines from each plot and after removal of marginal effect were carried to laboratory and were placed in the oven at 75°C for 48 hours and after ensuring that samples were completely dry, they were weighed and finally total yield was measured. Also to determine the yield components such as the number of spikelet per spike, number of seeds per spikelet, number of seeds per spike, and 1000-seed weight, after selecting 10 plants from each plot their means were considered as the yield components. 1000-seed weight was measured after accurate sifting and cleaning of seeds and drying them in open air. Nitrogen use efficiency (NUE) was calculated by flowing equation (Craswell and Godwin, 1984): **Equ. 1.** NUE = (Seed $yield_F$ – Grain $yield_C$) / Fertilizer N applied $gr.\ gr^{-1}$

F: fertilized crop; C: unfertilized.

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS (Ver.8) software and Duncan multiple range test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. Seed Yield

Yield is complex trait resulting from interaction of morphological, physiological and environmental parameters on the growth of plants. Identification of the variations of morphological and physiological traits influencing the yield of a plant in a certain environment is an essential tool for selecting and breeding of yield (Azarpour *et al.*, 2014). Several reports showed that sorghum had severed reaction to nitrogen fertility. Beyart *et al.* (2005) studied nitrogen fertility on sorghum Sudan grass and reported that highest yield was produced by application 125 kg nitrogen per hectare. The results of analysis of variance showed that the effect of different level of nitrogen, Nitroxin and interaction effect of treatments on seed yield was significant at 1% probability level (Table 1). Mean comparison result of different level of nitrogen indicated that maximum seed yield (341 $gr.m^{-2}$) was noted for 150 Kg N ha^{-1} and minimum of that (200 $gr.m^{-2}$) belonged to control treatment (Table 2). It seems like that the increase of seed yield is due to the positive effect of nitrogen and receiving light and the increase of photosynthesis, crop growth rate, leaf area index, and leaf area duration. The results are consistent with the findings of (Nawas *et al.,* 2005).

Table 1. The ANOVA results of studied traits affected different level of nitrogen and Nitroxin

S.O.V	df	Seed yield	No. spikelet per spike	No. seed per spikelet	No. seed per spike	1000-seed weight	Plant height	Nitrogen use efficiency
Replication	2	51.73^{ns}	20.41^{ns}	19.67^{ns}	14.54^{ns}	17.02^{ns}	14.89^{ns}	11.22^{ns}
Nitrogen	3	248.27**	41.27*	35.27*	82.56**	35.23*	33.27*	47.43**
Error I	6	48.46	16.61	14.94	11.13	14.19	12.61	7.13
Nitroxin	1	230.58**	44.25*	34.59*	83.87**	30.27*	36.32*	43.17**
Nitrogen × Nitroxin	3	245.29**	22.44^{ns}	17.45^{ns}	88.44**	19.88^{ns}	19.3^{ns}	13.11**
Error II	8	54.68	16.54	13.86	17.77	13.93	14.9	9.44
CV (%)	-	9.38	11.93	10.93	10.2	6.53	12.44	6.34

^{ns}, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

In another study conducted by Garg *et al.* (2005) increasing nitrogen to soil increased the plant photosynthetic efficiency and ultimately increased the seed yield and growth rate. On the other hand, since the rate of light absorption by leaves and converting it into photosynthetic materials are the other factors affecting the plant growth and production, the increase of leaf area in the farm leads to the increase of light absorption and ultimately leads to the increase of seed yield. The highest rate of seed yield by 324 gr.m^{-2} belonged to consumption 1 L.ha^{-1} Nitroxin and the lowest one by 214 gr.m^{-2} belonged to control treatment (Table 3). Useful soil bacteria, by facilitating elements uptake, atmospheric nitrogen fixation, plant hormone production such as auxins and gibberellins, increase the yield components and the seed yield, ultimately (Gary *et al.,* 2005). Hamidi *et al.* (2007) examined the effect of growth enhancer bacteria on post-mature hybrids of maize in an experiment and reported that application of such bacteria increased the seed yield. Mean comparison interaction effects of treatments revealed that the highest and the lowest rate of seed yield by 349 and 207 gr.m^{-2} respectively belonged to consumption of 150 Kg N ha^{-1} along 1 L.ha^{-1} Nitroxin and control (Table 4). Majidian (2006) reported that the integration of bio-fertilizers and chemical fertilizers significantly increased the number of seed rows per corn in comparison to their single application which confirms the present result. Moosavi *et al.* (2013) recommended to apply 225 kg N ha^{-1} with the minimum density of 50 plants m^{-2} to obtain economical yield of grain sorghum had the positive effect to increase grain yield. In a study on the effect of different N fertilization rates on different cultivars of grain sorghum, Asghari *et al*. (2006) reported that the increase in fertilization rate from 0 to 150 kg ha^{-1} led to increase grain yield (8.56 kg.ha^{-1}) significantly. Almodares *et al.* (2009) suggested applying 200 kg.ha^{-1} urea because the highest biomass and protein content and the lowest fiber content will be achieved with at this amount of nitrogen fertilizer. Although, this amount of nitrogen will decreased soluble carbohydrates content but it seems this reduction dose not effect on forage palatability and digestibility considerably.

Table 2. Mean comparison of studied traits affected different level of nitrogen

Treatment	Seed yield (gr.m^{-2})	No. spikelet per spike	No. seeds per spikelet	No. seeds per spike	1000 seed weight (g)	Plant height (cm)
Nitrogen						
Control	200^{*d}	40^{b}	20.7^{c}	798^{d}	20.35^{c}	108^{c}
50 Kg.h^{-1}	231^{c}	41.5^{b}	22.2^{b}	910^{c}	21.07^{b}	110^{b}
100 Kg.h^{-1}	304^{b}	45.19^{a}	24.6^{ab}	1109^{b}	23.71^{ab}	114^{ab}
150 Kg.h^{-1}	341^{a}	45.31^{a}	26.9^{a}	1135^{a}	25.11^{a}	116^{a}

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test.

Joorabi *et al*. (2015) reported that nitrogen fertilizer (150 kg.ha^{-1}) could increase qualitative and quantitative traits such as seed yield (9.82 t.ha^{-1}) of sorghum forage in Speed feed variety. Tarang *et al*. (2013) reported applications of Nitroxin bio-fertilizer and chemical fertilizer (400 kg.ha^{-1} urea with 300 kg.ha^{-1} ammonium phosphate) had a significant effect on traits such as root dry weight, number of seed per row (36.5), number of seed per ear (458.56), 1000 grain weight, seed (13.23 t.ha^{-1}) and biological yield (26.4 t.ha^{-1}), and harvest index (53.88%). Moghimi and Emam (2015) in order to evaluate the impact of different amounts of nitrogen fertilizer on yield of sorghum cultivars reported Pegah cultivar and application of 205 kg N ha^{-1} might be offered for producers (in similar climate). Nitrogen is an important nutrient for optimum crop growth and yield performance. Although its effect on growth and yield of sweet sorghum has been demonstrated to be dependent on the factors of climate, soil type and genotype which also vary across seasons and locations, the application nitrogen generally results in increase in the biomass and yield of sweet sorghum until an optimum rate is reached. This optimum rate varies from one location to another and from one season to another. However, from this matter, the optimum rate can be said to lie within the range of 60 and 120 kg N ha^{-1} depending on the location, the soil type and the native N of the soil determined through the soil test (Olugbemi, 2017).

4.2. Number of Spikelet per Spike

Result of analysis of variance revealed effect of different level of nitrogen and Nitroxin on number of spikelet per spike was significant at 5% probability level, but interaction effect of treatments was not significant (Table 1). Mean comparison of different levels of nitrogen in terms of the number of spikelet per spike indicated that the highest number of spikelet per spike by 45.31 belonged to the treatment with consumption of 150 Kg N ha^{-1} and the lowest one (40) belonged to control (Table 2). Fallahi *et al.* (2008) founded that Nitroxin bio-fertilizer had significant effects on main yield components, seed yield; essential oil .They concluded that this bio-fertilizer can be considered as a replacement for chemical fertilizers the absorbed nitrogen during this time leads to the increase of the number of spikelet. In different experiments it was observed that the yield and yield components increased in the crop inoculated with Azospirillum. Furthermore, the comparison different level of Nitroxin matter indicated the highest and the lowest number of spikelet per spike trait by 44.7 and 41.3 belonged to one $L.ha^{-1}$ Nitroxin and control treatment (Table 3).

Table 3. Mean comparison of studied traits affected different level of Nitroxin

Treatment	Seed yield ($gr.m^{-2}$)	No. spikelet per spike	No. seeds per spikelet	No. seeds per spike	1000 seed weight (g)	Plant height (cm)
Nitroxin						
Control	214^{*b}	41.3^{b}	21.38^{b}	901^{b}	21.37^{b}	110^{b}
1 $Lit.ha^{-1}$	324^{a}	44.7^{a}	25.82^{a}	1075^{a}	23.75^{a}	114^{a}

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test.

4.3. Number of Seeds per Spikelet

According result of analysis of variance effect of different level of nitrogen and Nitroxin on number of seeds per spikelet was significant at 5% probability level, but interaction effect of treatments was not significant (Table 1). Mean comparison of different levels of nitrogen indicated that the highest number of seeds per spikelet by 26.9 belonged to 150 Kg N ha^{-1} and the lowest one by 20.7 belonged to control treatment (Table 2). Hamidi and Dabbagh Mohammadi Nasab (2000) reported that nutrients availability particularly nitrogen at critical stage of seed formation affects the number of seeds through increase of plant growth rate. Comparison different level of Nitroxin revealed the highest and the lowest number of seeds per spikelet by 25.82 and 21.38 belonged to 1 $L.ha^{-1}$ Nitroxin and control (Table 3). Application of biological fertilizers significantly increased the number of seeds per spikelet and these results were already reported by (Kumar *et al.,* 2009). Results of Hammad *et al.* (2011) revealed that maximum plant growth, number of kernels per ear and grain yield of maize was found in 250 kg N ha^{-1} treatment and the highest days to maturity and biological yield were recorded from 300 Kg N ha^{-1} application. It has been clearly shown in the literature that applying optimum rate of N at proper time is crucial in improving crop productivity. Farmers usually apply high rates of nitrogen fertilizer to ensure the fulfillment of crop needs, while they are using both water and nitrogen in an inefficient way by increasing leaching potential of nutrients into the ground water (Ramos *et al*., 2012). Depending on soil nitrogen fertility, farmers apply between 45 and 224 kg N ha^{-1} in sorghum production (Zhao *et al*., 2005).

4.4. Number of Seeds per Spike

The results of analysis of variance showed that the effect of different level of nitrogen, Nitroxin and interaction effect of treatments on number of seeds per spike was significant at 1% probability level (Table 1). The mean comparison of different levels of nitrogen indicated that the highest number of seeds per spike by 1135 belonged to 150 Kg N ha^{-1} and the lowest one by 798 belonged to control (Table 2). It sees that in this experiment nitrogen caused the availability of assimilates for spike through the photosynthesis continuity, and the number of seeds per spike increase because of the decrease of seeds competition for nutrients. The positive effect of nitrogen on the number of seeds per spike trait was reported by Sepehri (2002). Mean comparison of different level of Nitroxin showed the highest and the lowest number of seeds per spike by 1075 and 901 belonged to 1 $L.ha^{-1}$ Nitroxin and control (Table 3). Sarige *et al.* (1988) reported that sorghum seed inoculation with Azospirillum increased yield by 25-28% which was due to greater number of seeds in each panicle. Mean comparison of the interaction effects of treatment showed that the highest and the lowest number of seeds per spike by 1200 and 810 respectively belonged to the treatments with consumption of 150 $KgNha^{-1}$ along with 1 $L.ha^{-1}$ Nitroxin and control, respectively (Table 4).

Table 4. Mean comparison of studied traits affected interaction effect of nitrogen and Nitroxin

Treatment		Seed yield ($gr.m^{-2}$)	No. seeds per spike	Nitrogen use efficiency ($gr.gr^{-1}$)
Control	**Control**	207^{*g}	810^{f}	-
	1 $Lit.ha^{-1}$	217^{f}	870^{e}	-
50 $Kg.h^{-1}$	**Control**	233^{e}	976^{d}	46.6^{b}
	1 $Lit.ha^{-1}$	258^{cd}	996^{c}	51.4^{a}
100 $Kg.h^{-1}$	**Control**	275^{c}	971^{d}	27.2^{cd}
	1 $Lit.ha^{-1}$	296^{ab}	1016^{b}	29.3^{c}
150 $Kg.h^{-1}$	**Control**	317^{b}	1063^{b}	21.09^{e}
	1 $Lit.ha^{-1}$	349^{a}	1200^{a}	23.07^{d}

*Similar letters in each column show non-significant difference at 5% probability level via Duncan test.

Kupulnik *et al.* (1982) reported that the effect of Azospirillum inoculation at various levels of nitrogen fertilizer on wheat spike was positive. The obtained results indicate that inoculation with Azospirillum improves the stem growth which results in faster emergence of spikes and therefore more time is provided for seed filling in plant and consequently the number of seeds per spike will increase.

4.5. 1000-Seed Weight

According result of analysis of variance effect of different level of nitrogen and Nitroxin on 1000-seed weight was significant at 5% probability level, but interaction effect of treatments was not significant (Table 1). Mean comparison of different levels of nitrogen indicated that the highest rate of 1000-seed weight by 25.11 g belonged to 150 Kg N ha^{-1} and the lowest one by 20.35 g belonged to control (Table 2). The results show that nitrogen increases 1000-seed weight by increasing the mobilization of assimilates made by plant. Mean comparison different level of Nitroxin revealed that the highest and the lowest rate of 1000-seed weight by 23.75 and 21.37 g belonged to 1 $L.ha^{-1}$ Nitroxin and control (Table 3). Biari *et al.* (2008) reported that in case of consumption of chemical and bio-fertilizers the weight of 1000-seed would increase which is consistent with the present results.

4.6. Plant height

Result of analysis of variance revealed effect of different level of nitrogen and Nitroxin on plant height was significant at 5% probability level, but interaction effect of treatments was not significant (Table 1). Mean comparison of different levels of nitrogen indicated that the highest plant height by 116 cm belonged to 150 Kg N ha^{-1} and the lowest one by 108 cm belonged to control (Table 2). An important reason that can increase the impact of bio-fertilizers for Plant height this is that use of fertilizers leading to increased internode length and it can stimulate the production of plant hormones produced by these fertilizers (Hassanpour *et al*., 2011). Increasing nitrogen consumption seems to increase the amount of protein and, as a result, increases the amount of protoplasm in cell. Mean comparison different level of Nitroxin revealed that the highest and the lowest rate of plant height by 114 and 110 cm belonged to 1 $L.ha^{-1}$ Nitroxin and control (Table 3). Biari *et al.* (2008) also examined the effects of Azospirillum and Azotobacter on corn and stated that inoculation of corn seeds increases with bacteria that stimulate plant height growth. They attributed this to increasing the absorption of plant nutrients and the secretion of growth stimulants through the use of bio fertilizers.

4.7. Nitrogen use efficiency (NEU)

The nitrogen use efficiency in the world for cereals is about 30 percent. Low levels of nitrogen efficiency are mainly due to the leaching, nitrogen outflow from the plant and ammonium emissions. This waste will not only reduce the efficiency of nitrogen use, but also has long-term harmful effects on the environment and human health. From an economic point of view it is necessary to increase this efficiency, in which case the environmental protection will be achieved (Pourmirza and Tajbakhsh, 2003). Evaluation of nitrogen use efficiency (NUE) is one of the current methods for measuring the N efficiency. Nitrogen use efficiency has been defined N recovered in yield as a proportion of N applied, allowing for initial soil N conditions (Raun *et al*., 2002). Severe yield limitations where there is a lack of N supply. Moreover, this might increase environmental pollution where high N fertilizer doses are applied to attain maximum yields (Hirel *et al*., 2007). The results of analysis of variance showed that the effect of different level of nitrogen, Nitroxin and interaction effect of treatments on nitrogen use efficiency was significant at 1% probability level (Table 1). Mean comparison of the interaction effects of treatment showed that the highest and the lowest nitrogen use efficiency by 51.4 and 21.09 $gr.gr^{-1}$ respectively belonged to the treatments with consumption of 150 Kg N ha^{-1} along with 1 $L.ha^{-1}$ Nitroxin and 150 Kg N ha^{-1} along with control, respectively (Table 4). Wu *et al.* (2005) also reported that corn seed insemination with the growth promoting bacteria increases the efficiency of fertilizer use, which, as result of present experiment, is consistent with the results of these investigators and can be due to this reason. They use ability of Azotobacter and Azospirillum to grow and conserve plant through biological processes and absorption of nutrients and nutrients required by the plant, consequently, development of root cause a better growth of the plant and maintain its health (Kizilkaya, 2008). The efficiency of nitrogen consumption for grain products, maize, and rice in developed countries is 15 kg grains per 1 Kg N consumption. Global efficiency of N fertilizers recycling in grains has been reported to be 33% which reaches to 29 and 42% respectively in developing and developed countries (Raun and Johnson, 1999). As level of nitrogen increased to 140-230 $kg.ha^{-1}$, grain yield in sorghum increased, too. The increase of nitrogen fertilizer consumption up to 200 $kg.ha^{-1}$ has increased yield of millet (Mirlohi *et al.,* 2010). The highest efficiency of nitrogen consumption belonged to the cultivar with minimum absorption during grain filling stage (Amiri *et al*., 2014). Increase of the nitrogen consumption increased relative content of nitrogen and reduced the efficiency of this element in oat. Collins *et al.,* (1990) reported that nitrogen efficiency decreased as its consumption increased from 90 to 180 $kg.ha^{-1}$ in millet. One of the reasons of decrease of crop yield and nitrogen use efficiency is the increasing speed of loss of nitrogen through leaching and sublimation or lack of efficient use of it (Amiri *et al*., 2014). Collins *et al.* (1990) found that the increase of nitrogen consumption enhanced nitrogen relative content but reduced nitrogen use efficiency in oats. Stally *et al.* (1991) stated that as the consumption of nitrogen increased from 90 to 180 kg/ha, nitrogen use efficiency decreased in millet. Malakouti and Nafisi (2003) stated that when a large percentage of nitrogen is used during cultivation, the loss of nitrate due to leaching in early irrigations increases and nitrogen use efficiency decreases. Therefore, several application of nitrogen fertilizer at different growth stages is highly important for increasing nitrogen use efficiency. Bowadr and Waskom (2003) reported that due to further loss of nitrogen in first irrigation, the application of a high percentage of total nitrogen at planting stage should be avoided. Huggins and Pan (1993) found that at high levels of nitrogen fertilizer consumption the absorption of the available nitrogen increased gradually which led to decrease of nitrogen use efficiency. Also Amiri *et al*. (2014) reported by increasing nitrogen consumption led to decrease the NUE significantly and the highest one is related to 80 kg N ha^{-1}. Split application of nitrogen has also had significant effects on nitrogen use efficiency so that the highest rate for all above cases has been related to use N in 50% at planting stage with 50% at stem elongation. The nitrogen management protocols require recognizing cultivar differences and critical stages of crop growth that fertilization is necessary to avoid potential yield loss (Senanayake *et al*., 1994). Nitrogen use efficiency of crops can be improved by adopting adequate management practices. Use of nitrogen fertilizers in adequate amount, form and methods of application are important management strategies of this element. NUE has been defined in various ways, but these definitions generally take into account quantity of N accumulated in the plant, known as uptake efficiency and quantity of N utilized in grain production known as utilization efficiency (Tayefe *et al*., 2011).

5. CONCLUSION

Result of current research showed that the highest seed yield belonged to the integrated treatment with application of 150 Kg N ha^{-1} together with Nitroxin inoculation by 349 $gr.m^{-2}$ and the lowest one by 207 $gr.m^{-2}$ belonged to the integrated treatment without use of nitrogen and lack of inoculation with bio-fertilizer. The highest nitrogen use efficiency was related to the treatment of 50 Kg N ha^{-1} pure fertilizer with Nitroxin fertilizer, which increased by 43% compared with 150 Kg N ha^{-1} without inoculation. It can be stated that in a plant such as seed sorghum which has high nutrient needs the available bio-fertilizers cannot replace chemical fertilizers by themselves, but they can be used as complements beside chemical fertilizers. This matter has improved the stability of seed sorghum production systems in the Khuzestan Province (south west of Iran) and chemical fertilizers, of the course with optimized application, will still be considered as the necessary component of the sustainable agriculture.

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