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Evaluation Effect of Chemical and Biological Fertilizer on Nitrogen Efficiency Indexes of Safflower Genotypes under Rain fed Condition

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ABSTRACT

BACKGROUND: Management of nutrients, especially nitrogen, in order to economic production, maintains sustainable agriculture, provide food security and prevent environmental pollution is considered to have an important priority. Also select and propose responsible genotype to environmental conditions is a great factor.

OBJECTIVES: The current research was conducted to evaluate effect of Azotobacter and nitrogen fertilizer on Nitrogen consumption efficiency indices of some safflower genotypes under dry land conditions.

METHODS: This research was conducted via combined analysis factorial experiment based on randomized complete blocks design with three replications along 2015-16 and 2016-17 year. The treatments included different level of Nitrogen (N_0: nonuse of fertilizer or biofertilizer as control, N_1: Seed inoculation with Azotobacter + nonuse of urea chemical fertilizer, N_2: Seed inoculation with Azotobacter + 50% nitrogen from urea source, N_3: 100% nitrogen from urea source according soil test) and 6 genotypes (G_1: 312-S6-697, G_2: PI-401478, G_3: PI-253895, G_4: PI-306974, G_5: Padideh and G_6: Sina).

RESULT: Result of analysis of variance revealed effect of nitrogen factor and genotypes on all studied traits was significant. In this study combined application of 50% nitrogen chemical fertilizer with Azotobacter in terms of seed yield was not significantly different from the treatment of 100% nitrogen fertilizer application and the nitrogen use efficiency in this treatment was significantly higher than the application of 100% chemical fertilizer.

CONCLUSION: Sina genotype and combined use of 50% nitrogen fertilizer with Azotobacter to produce maximum yield and reduce the use of nitrogen fertilizer in order to achieve sustainable agriculture and environmental protection in the dry land conditions and can be recommended to producers in studied region.

KEYWORDS: *Azotobacter, Nitrogen harvest index, Nitrogen uptake, Nutrition, Oilseed.*

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1. BACKGROUND

Environmental factors (such as consumption of macro and micro fertilizers) and use of agronomic bio-fortification strategy can exert larger influences on seed yield (GY), agro-morphological traits and grain micronutrients concentration of food crops (Mishra *et al.*, 2015; Esfandiari *et al*., 2016). Soil fertility is an important factor, which determines the growth of plant. Soil fertility is determined by the presence or absence of nutrients i.e. macro and micronutrients, which are required in minute quantities for plant growth (Zayed *et al*., 2011). Increasing price of fertilizers worldwide, necessary for production economy, ground water pollution, and soil structure degradation due to uncontrolled and unwise consumption of chemical fertilizers are the problems that have to be solved by proper methods (Cakmack, 2002). 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). 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). Availability of nitrogen is important for growing plants. It is a main constituent of protein and nucleic acid molecules. It is also a part of chlorophyll molecules. It is well known that the use of fertilizer helps in production and is a quick method resulted in the best yields (Farooqui *et al*., 2009). Nitrogen deficiency in the wheat plant may be due to: decrease in fertilizer usage, using organic methods of crop management (David, 1997) and nitrogen consumption in an inappropriate time (Mainard *et al*., 2001). In these conditions number of seeds per area unit (Modhej *et al*., 2009) will be decreased because of decrease in number of spikes per area unit, number of spikelet per spike, number of fertile florets in spikelet, decrease of survival and decrease in fertilization of florets (Peltonen and Peltonen, 1995). Some researches realized nitrogen fertilizer can increase vegetative growth and seed yield. A desirable increase of nitrogen can expand the most important factor of seed yield, number of seeds per spike (Fang *et al*., 2010; Khalilzadeh *et al*., 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 microorganisms, 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). Nouraki *et al*. (2016) reported bacteria have positive role in the production of biofertilizers 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 biofertilizers 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). 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).

2. OBJECTIVES

Due to the improper use of nitrogen fertilizer and the problems caused by it, also due to the importance of safflower as an oil plant adapted to rainfed conditions, the need for this research was felt. Also, lack of documented and comprehensive information on nitrogen application efficiency indices to nitrogen and Azotobacter chemical fertilizers in rainfed conditions the current research was conducted to evaluate effect of Azotobacter and nitrogen fertilizer on Nitrogen consumption efficiency indices of some safflower genotypes under dry land conditions.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was conducted via combined analysis factorial experiment based on randomized complete blocks design with three replications along 2015-16 and 2016-17 year. Place of research was located in Sarabeleh Agriculture Research station at longitude 46°36'E and latitude 33°47'N in Ilam province (Southwest of Iran). The treatments included different level of Nitrogen (N_0: nonuse of fertilizer or biofertilizer as control, N_1: Seed inoculation with Azotobacter + nonuse of urea chemical fertilizer, N_2: Seed inoculation with Azotobacter + 50% nitrogen from urea source, N_3: 100% nitrogen from urea source according soil test) and 6 genotypes (G_1: 312-S6-697, G_2: PI-401478, G_3: PI-253895, G_4: PI-306974, G_5: Padideh and G_6: Sina). The study region has an average annual rainfall of 402 mm, average annual temperature of 17.9 ° C, maximum and minimum absolute annual temperature of 42.2 and 8-6 ° C, respectively. Rising temperatures at the end of the safflower growing season are usually accompanied by cessation of rainfall and rain-fed cultivation of this crop in Ilam region is facing drought stress. The average monthly temperature, rainfall and relative humidity in the crop years 2015-16 and 2016-17 are presented in table 1. The physical and chemical properties of the soil at the test site during the two years of the experiment are presented in table 2.

Table 1. Monthly mean value of temperature, precipitation and relative air humidity Research Station of Agriculture Ilam at 2015-16 and 2016-17 cropping seasons

Year 2015-16

Month	Precipitation (mm)	Mean monthly temperature (C^0)	Mean Relative Humidity (%)
Oct.	0.5	24.2	29
Nov.	319.8	13.9	69
Dec.	67.7	8.1	64
Jan.	75.9	6.7	70
Feb.	56.9	7.0	62
Mar.	53.0	12.0	58
Apr.	138.6	12.9	61
May.	18.6	20.4	51
Jun.	0	25.0	28
Jul.	0	32.0	19
Aug.	0	32.2	18
Sep.	0	29.1	20

Continue table 1.

Year 2016-17

Month	Precipitation (mm)	Mean monthly temperature (C^0)	Mean Relative Humidity (%)
Oct.	2.7	22.0	25
Nov.	19.1	17.3	34
Dec.	34.3	8.1	45
Jan.	87.5	7.3	60
Feb.	102.3	4.9	65
Mar.	74.7	10.2	54
Apr.	80.0	14.0	60
May.	9.7	21.1	43
Jun.	0	26.2	22
Jul.	0	31.7	17
Aug.	0	32.7	15
Sep.	0	30.0	16

3.2. Farm Management

According to the results of soil analysis in both years of the experiment, 25 $kg.ha^{-1}$ of phosphorus from the superphosphate source was added to the soil at the time of cultivation and there was no need to use potassium fertilizer. Also, nitrogen fertilizer was supplied from urea source in both years of experiment. In relation to nitrogen (urea fertilizer) for consumption of 50 and 100% nitrogen consumption, based on soil test, 50 and 100 $kg.ha^{-1}$ of urea fertilizer source (46% of pure nitrogen), respectively, in two stages, one-half of which is basic at the time of planting, the other half was added to the soil at the beginning of the stemming stage. The land of the test site was plowed deeply in October every two years, and in early November, additional land preparation operations were carried out, including plowing, discing and plotting. Each experimental plot consisted of six planting lines with 30 cm line spacing, 10 cm plant spacing and 4 m planting line length. For inoculation of seeds, the amount of seven grams of inoculum, each gram of which contained 108 live and active bacteria, was moistened with sugar water at a concentration of 20% and in the ratio of 2 kg of inoculum per 100 kg of seeds was used. The treated seeds were placed in the shade on a clean surface for ten minutes to dry and ready for planting. According to the meteorological forecast, one day before the first effective rainfall in both years was considered as the planting date. No irrigation was done and only rainfall was used.

Table2. Physical and chemical properties of soil in the experimental site (0-30 cm depth)

Year	Soil Texture	pH	EC ($ds.m^{-1}$)	OC (%)	N (mg Kg^{-1})	P_2O_5 ($mg.kg^{-1}$)	K_2O ($mg.kg^{-1}$)
2015-16	Clay Loam	7.31	0.45	1.40	13	6.2	282
2016-17	Clay Loam	7.34	0.43	1.38	13	6.1	270

3.3. Measured Traits

In order to measure nitrogen in the grain ripening stage, seed and straw were sampled separately and the percentage of seed nitrogen content (SNC) and straw nitrogen content (StNC) was measured by Kjeldahl method (Svecnjak and Rengel, 2006). The amount of nitrogen absorbed by the plant was obtained from the total seed nitrogen (percentage of seed nitrogen content × seed yield) and straw nitrogen content (percentage of nitrogen straw content straw × straw weight). To calculate the nitrogen use efficiency, in addition to fertilizer application, nitrogen storage in the soil before planting was also considered. For this purpose, the depth of fertile soil for safflower was 30 cm and also according to the percentage of soil nitrogen and soil Bulk density (Table 2), the amount of nitrogen in the soil was obtained using Equation (1), the amount of which is 50.7 kg in Hectares.

Equ. 1. Available soil nitrogen ($mg.kg^{-1}$) × Bulk density ($gr.cm^{-3}$) × soil depth (m) × 10000/ 10000 (Kilogram per hectare conversion factor)

Equ. 2. NUE (Nitrogen use efficiency) = (Gw/Ns) (Huggins and Pan, 1993)
Ns = Nf + Nr + Nm + Nx + Nd, Gw= Seed yield (kg.ha^{-1}), Ns= Nitrogen storage available to the plant (kg.ha^{-1}), Nf= Nitrogen content of Nitrogen Fertilizer, Nr= Mineral nitrogen residues in the soil before planting, Nm= Mineralized nitrogen during the soil season, Nd= Nitrogen added to soil through atmosphere, irrigation water and running water. The amounts of other sources of mineral nitrogen (Nx, Nd) are very small and are ignored.

Equ. 3. Nitrogen harvest index= Gn/Tn × 100 (Delogu *et al.*, 1998)
Gn= Seed nitrogen content
Tn= Total nitrogen of plant

Equ. 4. Nitrogen utilization efficiency (NUte) = SDM/N (Plant) (Moll *et al.*, 1982)
SDM= shoot dry mass
N (plant): total nitrogen content in the plant

Equ. 5. NAE (nitrogen absorption or uptake) efficiency)= N (plant)/N (applied) (Moll *et al.*, 1982)
N (plant): total nitrogen content in the plant, N (applied): amount of nitrogen supplied to the plant.

3.4. Statistical Analysis

Before combined analysis, Bartlett uniformity test was performed on variances. The results of Bartlett test showed that in all cases the calculated Chi-Score was smaller than the Chi-Score of the table, so, ensuring that the variances were uniformity, combined analysis was done on the data. In the combined analysis, the F test was performed for the significance of the sources of variation using the mathematical expectation of the mean squares, assuming that the effect of the experimental treatments was constant and the effect of the year was random. 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

The results of combined analysis of variance of experimental data in rainfed conditions showed that the effect of year on straw nitrogen content and nitrogen harvest index was significant at the level of 5% probability (Table 3). This result indicates that environmental conditions in different years have different effects on straw nitrogen content and nitrogen harvest index. Nitrogen factor had significant effect on Seed nitrogen content, Straw nitrogen content, Total nitrogen uptake, Nitrogen utilization efficiency and Nitrogen use efficiency at 1% probability level (Table 3). Effect of genotype factor on all studied traits was significant at 1% probability level (Table 3). This matter indicates the existence of considerable genetic diversity in terms of the studied traits and the possibility of selection for these traits among the studied genotypes. Interaction effect of year × nitrogen factor had a significant effect on all studied traits (Table 3). Therefore, it can be seen that the nitrogen factor for each year has heterogeneous changes on SNC, StNC, Total nitrogen uptake, seed yield, Nitrogen harvest index, Nitrogen uptake efficiency, Nitrogen utilization efficiency and Nitrogen use efficiency.

Table 3. Combine analysis of variance for nitrogen and genotype effect for studied traits

S.O.V	df	Straw nitrogen content	Seed nitrogen content	Total nitrogen uptake	Seed yield
Year (Y)	1	0.323*	0.13^{ns}	132.21^{ns}	51900^{ns}
Rep. /Y	4	0.040	1.65	390.18	6298988
Nitrogen (N)	3	0.086**	10.01**	7378.66**	906950*
Y × N	3	0.014**	0.14**	133.84**	35541*
Genotype (G)	5	0.092**	1.57**	1585.75**	311251**
Y × G	5	0.002^{ns}	0.01^{ns}	9.63^{ns}	8367^{ns}
N × G	15	0.007**	0.09**	116.55**	10661^{ns}
Y × N × G	15	0.004^{ns}	0.02^{ns}	11.58^{ns}	6655^{ns}
Error	92	0.003	0.06	15.23	4970
CV (%)	-	12.73	8.71	9.50	7.29

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

Continue table 3.

S.O.V	df	Nitrogen harvest index	Nitrogen uptake efficiency	Nitrogen utilization efficiency	Nitrogen use efficiency
Year (Y)	1	806.32*	0.008^{ns}	254.32^{ns}	96.10^{ns}
Rep. /Y	4	106.24	0.078	127.35	15.99
Nitrogen (N)	3	383.42*	0.182*	1174.42**	478.94**
Y × N	3	17.58*	0.016**	21.89**	5.86*
Genotype (G)	5	25.68**	0.268**	109.21**	60.61**
Y × G	5	7.58^{ns}	0.001^{ns}	1.05^{ns}	1.08^{ns}
N × G	15	2.96^{ns}	0.003^{ns}	3.32*	0.95^{ns}
Y × N × G	15	4.09^{ns}	0.001^{ns}	1.11^{ns}	1.47^{ns}
Error	92	9.54	0.002	2.43	1.03
CV (%)	-	4.97	8.75	5.64	6.75

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

Interaction effect of nitrogen × genotypes factor had a significant effect on SNC, StNC, Total nitrogen uptake and Nitrogen utilization efficiency (Table 3). That matter indicates a different response of genotypes in terms of mentioned traits to nitrogen factor. Interaction effect of year × genotype and year × Nitrogen × genotype was not significant on studied traits (Table 3). This matter indicates a similar response of genotypes to nitrogen factor in two years of experiment. According to the experimental results, it seems that the application of 100% of nitrogen fertilizer application under dry land conditions in both years compared to other treatments has led to a significant reduction in nitrogen harvest index. The reason for this trend can be stated that with increasing nitrogen application, safflower vegetative growth increased, and with increasing vegetative growth, remobilization from vegetative organ to reproductive organ limited. A decrease in nitrogen harvest index in wheat was also observed with increasing fertilizer application (Hosseini *et al.*, 2013). The highest nitrogen harvest index among the studied genotypes was related to PI-253895 genotype (Table 4). Mean comparison interaction effect of year × nitrogen for straw nitrogen content showed that all nitrogen source treatments in the second year compared to the first year of the experiment had significantly higher percentage of straw nitrogen content (Table 5). However, the amount of total nitrogen uptake of the crop only in the first year of the experiment in the treatment of 100% application of nitrogen fertilizer was significantly higher than the treatment of 100% application of nitrogen fertilizer in the second year. Other treatments in both years were not significantly different in terms of total nitrogen uptake (Table 5). This matter indicates that in the first year of the experiment, due to higher rainfall and soil moisture, the application of 100% nitrogen fertilizer causes the transfer of more nitrogen from the shoot to the seed and ultimately increases the total nitrogen uptake of the plant. This result was consistent with a report by Ehdaie *et al*. (1988) who stated that the uptake of nitrogen by wheat after flowering and its transfer from vegetative tissues to seeds is highly dependent on growth conditions. The highest amount of seed nitrogen content, straw nitrogen content and total nitrogen uptake of the plant affected by the interaction effect of nitrogen × genotype achieved by 100% nitrogen fertilizer application treatment with Sina genotype and the lowest ones belonged to 312-S6-692 and PI-401478I genotypes under non consumption of fertilizer (Table 4). This indicates that the full application of nitrogen chemical fertilizer, by increasing the availability of more nitrogen for the plant, causes more uptake of soil nitrogen by the plant and thus increases more storage of nitrogen in the seeds and straw of safflower. Tufenkci *et al*. (2006) also reported that with more use of nitrogen, grain nitrogen increased. Moslehi *et al*. (2016) also reported that the amount of straw nitrogen in the treatments in which nitrogen fertilizer was used was much higher than other treatments, which was consistent with the results of this experiment. Azotobacter inoculation treatment in all studied genotypes significantly increased the percentage of nitrogen in safflower seeds and straw compared to the control treatment in all genotypes (Table 6). Among the reasons for the superiority of the treatment inoculated with Azotobacter compared to the control can be mentioned of various hormones that increase root volume and nitrogen uptake from the soil, the effect on NO_3^- uptake by reducing this compound by bacteria in the root zone, improve N_2 stabilization in soil to increase crop nitrogen content due to bacterial activity.

Table 4. Mean comparison of main effects on studied characteristics in safflower

Nitrogen Source	Straw nitrogen content (%)	Seed nitrogen content (%)	Total nitrogen uptake (kg.ha^{-1})	Seed yield (kg.ha^{-1})
N_0	0.31*c	2.21c	25.62b	869b
N_1	0.36c	2.69b	32.92b	968b
N_2	0.46b	3.28a	48.67a	1172a
N_3	0.65a	3.44a	56.94a	1196a
Genotypes				
G_1	0.39e	2.68d	32.27e	914d
G_2	0.41d	2.64d	33.15e	938d
G_3	0.39e	2.82e	37.01d	1016c
G_4	0.46c	3.02b	44.54c	1120b
G_5	0.49b	3.17a	46.91b	1117b
G_6	0.54a	3.25a	52.37a	1203a

N_0: nonuse of fertilizer or biofertilizer as control, N_1: Seed inoculation with Azotobacter + nonuse of urea chemical fertilizer, N_2: Seed inoculation with Azotobacter + 50% nitrogen from urea source, N_3: 100% nitrogen from urea source according soil test

G_1: 312-S6-697, G_2: PI-401478, G_3: PI-253895, G_4: PI-306974, G_5: Padideh and G_6: Sina

*Means with similar letters in each column are not significantly differentt by Duncan test at 5% probability level.

Continue table 4.

Nitrogen Source	Nitrogen harvest index (%)	Nitrogen uptake efficiency (kg.kg^{-1})	Nitrogen utilization efficiency (kg.kg^{-1})	Nitrogen use efficiency (kg.kg^{-1})
N_0	76.95*ab	0.50b	34.51a	17.15b
N_1	79.55a	0.64a	29.80b	19.10a
N_2	79.65a	0.60ab	24.69c	14.45c
N_3	72.89b	0.50b	21.55c	10.68d
Genotypes				
G_1	77.57ab	0.46e	29.81a	13.50d
G_2	76.34b	0.45e	29.57c	13.78d
G_3	79.50a	0.51d	29.12a	14.72c
G_4	78.07ab	0.61c	26.74b	16.27b
G_5	77.75ab	0.64b	25.63c	16.32b
G_6	77.33ab	0.71a	24.94c	17.49a

N_0: nonuse of fertilizer or biofertilizer as control, N_1: Seed inoculation with Azotobacter + nonuse of urea chemical fertilizer, N_2: Seed inoculation with Azotobacter + 50% nitrogen from urea source, N_3: 100% nitrogen from urea source according soil test.

G_1: 312-S6-697, G_2: PI-401478, G_3: PI-253895, G_4: PI-306974, G_5: Padideh and G_6: Sina.

*Means with similar letters in each column are not significantly differentt by Duncan test at 5% probability level.

Panahi *et al.* (2015) reported that the application of biofertilizer containing Azotobacter crococcum increased the grain nitrogen content in rice, which was consistent with the findings of this experiment. However, it contradicted the report of Canbolat *et al.* (2006), who stated in their study of spring barley that growth-promoting bacteria did not alter the grain nitrogen content. In the present study, Sina genotype had the highest percentage of seed and straw nitrogen content affected in all nitrogen factor (Table 6).

Table 5. Mean comparison of interaction effects of Year × Nitrogen Source for studied characteristics in safflower

Treatments	Straw nitrogen content (%)	Seed nitrogen content (%)	Total nitrogen uptake ($kg.ha^{-1}$)	Seed yield ($Kg.h^{-1}$)
$Y_1 \times N_0$	0.28*h	2.24f	24.6de	885d
$Y_1 \times N_1$	0.31g	2.64d	33.29d	1029c
$Y_1 \times N_2$	0.39e	3.21c	49.50c	1259a
$Y_1 \times N_3$	0.63b	3.51a	60.55a	1272a
$Y_2 \times N_0$	0.35f	2.37e	26.59e	754d
$Y_2 \times N_1$	0.42d	2.74d	32.55d	907d
$Y_2 \times N_2$	0.53c	3.35b	47.85e	1085bc
$Y_2 \times N_3$	0.68a	3.38b	53.33b	1120b

Y_1 and Y_2: 2015 -16 and 2016 – 17 growing seasons, respectively.
N_0: nonuse of fertilizer or biofertilizer as control, N_1: Seed inoculation with Azotobacter + nonuse of urea chemical fertilizer, N_2: Seed inoculation with Azotobacter + 50% nitrogen from urea source, N_3: 100% nitrogen from urea source according soil test.
*Means with similar letters in each column are not significantly differentt by Duncan test at 5% probability level.

Continue table 5.

Treatments	Nitrogen harvest index (%)	Nitrogen uptake efficiency ($kg.kg^{-1}$)	Nitrogen utilization efficiency ($kg.kg^{-1}$)	Nitrogen use efficiency ($kg.kg^{-1}$)
$Y_1 \times N_0$	81.09b	0.48e	36.39a	17.46bc
$Y_1 \times N_1$	82.24ab	0.69a	31.50a	20.3a
$Y_1 \times N_2$	82.78a	0.61bc	26.25e	15.53d
$Y_1 \times N_3$	74.39d	0.54d	21.71g	11.36f
$Y_2 \times N_0$	76.80c	0.52d	32.63b	16.84c
$Y_2 \times N_1$	76.86c	0.64ab	28.10d	17.90b
$Y_2 \times N_2$	76.53c	0.58c	23.14f	13.38e
$Y_2 \times N_3$	71.38e	0.47e	21.36g	10.00g

Y_1 and Y_2: 2015 -16 and 2016 – 17 growing seasons, respectively.
N_0: nonuse of fertilizer or biofertilizer as control, N_1: Seed inoculation with Azotobacter + nonuse of urea chemical fertilizer, N_2: Seed inoculation with Azotobacter + 50% nitrogen from urea source, N_3: 100% nitrogen from urea source according soil test.
*Means with similar letters in each column are not significantly differentt by Duncan test at 5% probability level.

Nowadays, the ability of different plant genotypes to absorb and consume nutrients has been considered by many researchers. Differences in their effectiveness in nutrient utilization are affected by root spread, or by plant consumption, or both, and the relative importance of these strategies can vary depending on the type of element and the type of plant species. The reason for increasing the total nitrogen uptake in the treatment of 100% application of nitrogen fertilizer and Sina genotype in dryland conditions is related to high nitrogen concentration and dry matter weight of this treatment due to the availability of nitrogen available to the crop. Considering that the amount of nitrogen uptake of safflower genotypes is obtained by multiplying the percentage of nitrogen by the safflower genotype and the dry matter produced per unit area. As a result, increasing the nitrogen uptake of safflower in Sina genotype and fertilizer treatment is not far from expectation, because the use of 100% nitrogen chemical fertilizer in this experiment increased the percentage of seed and straw nitrogen content of Sina genotype under dryland conditions per unit area. On the other hand, increasing the nitrogen uptake of the whole plant as a result of the application of 100% nitrogen chemical fertilizer can be related to stimulating the photosynthetic surface and safflower vegetative growth and thus nitrogen uptake. So that this stimulation leads to an increase in dry weight and ultimately increase the total nitrogen uptake of the plant per unit area. This result was similar to finding of Yadavi and Yuosefpur (2015), they are stated that high consumption of nitrogen fertilizer led to increase the availability of nitrogen for the plant, causes more uptake of soil nitrogen by the plant and thus increases the storage of nitrogen in the shoots of sunflower. Safflower seed yield was affected by year × nitrogen fertilizer so that the highest seed yield (1272 $kg.ha^{-1}$) was obtained from 100% nitrogen fertilizer application in the first year of the experiment and with combined treatment of Azotobacter + 50% nitrogen fertilizer had no significant difference (Table 5). High seed yield due to use of these treatments can be attributed to the availability and greater uptake of nitrogen in dryland. The combined Azotobacter + 50% of nitrogen fertilizer application has provided the nitrogen required by the plant for plant growth and achieving potential yield under dryland conditions. However, adding more chemical fertilizer does not have a significant effect on increasing seed yield. Because in rainfed conditions, it causes wastage of nitrogen fertilizer and environmental pollution. Increasing plant's accessibility to nitrogen with the combined application of biological and chemical nitrogen fertilizers and its greater uptake by the plant, thus increasing growth and photosynthesis by increasing grain yield components are factors in increasing grain yield in integrated treatment in rainfed conditions. On the other hand, the results confirmed that a combination of Azotobacter and nitrogen fertilizer is possible. Because Azotobacter are free living and their activity increases with use of nitrogen fertilizer. Based on the results of mean comparison interaction effect of year × nitrogen (Table 5), it was found that inoculation treatment with Azotobacter + 50% nitrogen fertilizer application in first year had the highest nitrogen harvest index and lowest one belonged to application 100% nitrogen fertilizer in the second year. Mean comparison interaction effect of year × nitrogen for nitrogen uptake efficiency showed the highest amount in both years belonged to Aoztobacter (Table 5).

Table 6. Mean comparisons of interaction effect of nitrogen ×genotype for studied characteristics in safflower.

Treatment	Nitrogen content straw (%)	Nitrogen content grain (%)	Nitrogen uptake plant total (kg.ha^{-1})	Nitrogen utilization efficiency (kg.kg^{-1})
$N_1 \times G_1$	0.305l	2.07n	21.37h	37.55a
$N_1 \times G_2$	0.310kl	2,14n	22.41h	35.94b
$N_1 \times G_3$	0.256m	2.22n	27.77h	36.91ab
$N_1 \times G_4$	0.333j-l	2.41m	27.77g	33.07c
$N_1 \times G_5$	0.345i-k	2.47m	28.87g	32.03cd
$N_1 \times G_6$	0.356ij	2.52lm	31.1g	31.58d
$N_2 \times G_1$	0.320j-l	2.59lm	28.08g	31.01de
$N_2 \times G_2$	0.331j-l	2.47m	27.08g	31.93cd
$N_2 \times G_3$	0.346i-k	2.68kl	30.63g	30.13fe
$N_2 \times G_4$	0.38hi	2.78jk	35.47f	28.96fe
$N_2 \times G_5$	0.395gh	2.79jk	36.55f	28.75gh
$N_2 \times G_6$	0.436f	2.82jk	39.77ef	28.04g-i
$N_3 \times G_1$	0.381hi	2.91h-j	36.18f	27.54h-j
$N_3 \times G_2$	0.428fg	2.88i-k	39.62ef	26.92ij
$N_3 \times G_3$	0.410f-h	3.09f-h	43.85e	26.25j
$N_3 \times G_4$	0.486e	3.38de	52.77d	23.95k
$N_3 \times G_5$	0.518e	3.67bc	57.4c	22.01lm
$N_3 \times G_6$	0.57d	3.74ab	62.25b	21.49m
$N_4 \times G_1$	0.575d	3.14fg	43.46e	23.14kl
$N_4 \times G_2$	0.573d	3.07g-i	43.52e	23.49k
$N_4 \times G_3$	0.570d	3.27ef	51.35d	23.18kl
$N_4 \times G_4$	0.675c	3.52cd	62.19b	20.98mn
$N_4 \times G_5$	0.725b	3.75ab	64.8b	19.73no
$N_4 \times G_6$	0.823a	3.92a	76.26a	18.67o

N_0: nonuse of fertilizer or biofertilizer as control, N_1: Seed inoculation with Azotobacter + nonuse of urea chemical fertilizer, N_2: Seed inoculation with Azotobacter + 50% nitrogen from urea source, N_3: 100% nitrogen from urea source according soil test.

G_1: 312-S6-697, G_2: PI-401478, G_3: PI-253895, G_4: PI-306974, G_5: Padideh and G_6: Sina.

*Means with similar letters in each column are not significantly differentt by Duncan test at 5% probability level.

The reason for this matter can be attributed to the biological stabilization of nitrogen by Azotobacter and the gradual and simultaneous uptake of safflower according crop situation in dryland conditions. Kumar and Ahlawat (2006) also positively evaluated the role of Azotobacter in significantly increasing nitrogen uptake by wheat. The lowest nitrogen uptake efficiency was allocated to the treatment of non-fertilizer application in the first year and the treatment of 100% nitrogen fertilizer application in the second year, which were statistically in the same class (Table 5). It seems that the reason for the low efficiency of nitrogen uptake in the conditions of full application of nitrogen chemical fertilizer is largely due to the difference in the yield of safflower seed produced under the influence of nitrogen application (which directly affects the uptake of nitrogen from the soil). As a result, although with increasing the full application of nitrogen fertilizer, yield increased in rainfed conditions, but the plant's ability to absorb nitrogen is not in line with increasing fertilizer application. In fact, the full application of nitrogen fertilizer under rainfed conditions in safflower is not in line with its use. A decrease in nitrogen uptake efficiency with increasing application of nitrogen fertilizer has been reported in some studies (Haile *et al*., 2012). The mean comparison of between the studied safflower genotypes in rainfed conditions showed that the highest nitrogen uptake efficiency with an average of 0.710 $kg.kg^{-1}$ belonged to the Sina genotype (Table 5). Considering that Sina genotype has a higher percentage of nitrogen and total nitrogen uptake compared to other genotypes in rainfed conditions, the high efficiency of nitrogen uptake in this genotype can be justified. The means comparison interaction effect of nitrogen × genotype revealed the highest and lowest nitrogen utilization efficiency was assigned to non-fertilizer and 312-S6-692 genotype treatments. The lowest nitrogen utilization efficiency belonged to the treatment application of 100% of nitrogen fertilizer application in Sina genotype (Table 6). According to the results obtained in dryland conditions, it seems that the nitrogen utilization efficiency is a direct function of the grain yield of safflower genotypes. In this experiment, the amount of this index decreased with increasing application of nitrogen fertilizer. In other words, with increasing nitrogen consumption, the ratio of seed production to the amount of nitrogen content in plant tissues decreased. This decrease can be attributed to the non-linear relationship between increased nitrogen consumption and improved seed yield. Ahmadi *et al*. (2018) reported that with increasing nitrogen consumption, nitrogen use efficiency decreased. It should be noted that the nitrogen use efficiency is the product of the nitrogen uptake efficiency and nitrogen utilization efficiency. These components are in balance with each other, so an increase in one of these components will be accompanied by a decrease in the other component (Moll *et al*., 1982). Therefore, in dryland conditions (drought stress), 312-S6-692 genotype test had the highest nitrogen utilization efficiency and the lowest nitrogen uptake efficiency. The highest nitrogen use efficiency was assigned to Azotobacter inoculation treatment in the first year and the lowest one (10.00 $kg.kg^{-1}$) belonged to the treatment of 100% of nitrogen fertilizer in the second year (Table 5). The results of this study in rainfed conditions indicate that the application of 100% nitrogen chemical fertilizer in the second year of safflower plant failed to produce seeds in proportion to the nitrogen fertilizer received. Shahrasbi *et al*. (2016) reported that in dryland areas with reduced rainfall at the end of the growing season, excessive application of nitrogen fertilizer significantly reduces nitrogen use efficiency. Because increasing nitrogen consumption has little effect on increasing seed yield. Liu *et al*. (2018) also stated that with increasing fertilizer application, the amount of grain yield increased less according to the law of diminishing returns, which reduced the nitrogen use efficiency. In general, the efficiency of nutrient consumption decreases with the gradual elimination of plant needs and usually the highest efficiency of fertilizer consumption is achieved in the first units of its consumption. The higher nitrogen use efficiency in Azotobacter inoculation treatment may be related to the ability of Azotobacter to stabilize nitrogen and increase root growth and nutrient uptake by safflower plants. Seyed Sharifi *et al*. (2016) reported same result. Reports indicate that Azotobacter as a plant growth stimulant, in addition to molecular nitrogen fixation, produces hormones and plant growth stimulants that increase fertilizer efficiency by increasing root fiber production, root volume and nutrient uptake from soil. The results of this study also showed that regardless of the amount of nitrogen fertilizer application in rainfed conditions, the highest nitrogen use efficiency with an average of 17.49 belonged to Sina genotype (Table 5). Ahmadi *et al*. (2018) also reported genetic differences in nitrogen use efficiency in maize genotypes.

5. CONCLUSION

In this study, considering that the combined application of 50% nitrogen chemical fertilizer with Azotobacter in terms of seed yield was not significantly different from the treatment of 100% nitrogen fertilizer application and the nitrogen use efficiency in this treatment was significantly higher than the application of 100% chemical fertilizer. Also in this experiment, Sina genotype showed its superiority over other genotypes in terms of seed yield and nitrogen use efficiency. Therefore, Sina genotype and combined use of 50% nitrogen fertilizer with Azotobacter to produce maximum yield and reduce the use of nitrogen fertilizer in order to achieve sustainable agriculture and environmental protection in the dry land conditions and can be recommended to producers in studied region.

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