Influence of chemical fertilizers and bioinoculants on growth and yield of sunflower (*Helianthus annuus* L.)

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ABSTRACT

The present study was conducted to investigate and compare the effect of applying various levels of chemical fertilizers and bioinoculants on the growth and yield of sunflower during the 2017-2018 crop year in Hamedan, Iran. This study was executed as two factorial experiments, as a randomized complete block design in three repetitions. Chemical fertilizers containing nitrogen (N) and phosphorus (P) were used in the first experiment, and nitrogen-fixing (NI) and phosphate-solubilizing (BI) bioinoculants were used in the second experiment. The experimental treatments included applying a urea fertilizer (N_0=0, N_1=45, N_2=90 kg pure nitrogen/ha) and a triple superphosphate (P_0=0, P_1=40, P_2=80 kg pure phosphorus/ha), as well as a nitroxin biofertilizer (NI_0=0, NI_1=0.5, NI_2=1 L/ha) and a biophosphorus (BI_0=0, BI_1=0.5, BI_2=1 L/ha). The results indicated that the highest levels of leaf dry weight, number of seeds per head, head diameter, head weight, seed yield, and the plant's biological yield were obtained for the chemical treatment (N_2P_2) and biological treatment of (NI_2BI_2). Group comparisons between the chemical and biological treatments did not show a significant difference for any of the studied characteristics, therefore, the results of this study conclude that the investigated levels of bioinoculants could be appropriate alternatives to chemical fertilizers.

Keywords: chemical fertilizer, nitrogen-fixing bacteria, phosphate-solubilizing bacteria, biological nitrogen fixation, oil yield, sustainable agriculture

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is considered the fourth oil-producing plant in the world in the amount of seed production after soybeans, rapeseed, and safflower (Khandekar et al., 2018). Sunflower is tolerant of environmental conditions such as drought, cold, and heat. This plant is extremely efficient in extracting water from the soil through the development of its root system. This attribute is of great importance for Iran which is located in an arid and semi-arid region (Khajepour, 2012). According to the report published by FAO, in 2014 the area under cultivation and seed production of sunflowers in Iran was 24000 hectares and 15517 tonnes, respectively (FAO, 2014). Despite the existence of many vast, cultivable lands in Iran, suitable for the production of oilseeds, sunflower oil is among the twenty food items imported from outside of Iran (FAO, 2014). Therefore, the optimal utilization of chemical fertilizers and bioinoculants could lead to increased production per unit of area, preventing the importation of this product.

Nutritional elements play a significant role in the growth and development of plants. Nitrogen is among the elements with high demand in sunflower (Fallah-Toosi and Azizi, 2014). This element is the most important nutrient that improves the quality of sunflower seeds (Ullah et al., 2010) and also increases the plant's metabolic process, eventually leading to increased vegetative growth, reproductive growth, and plant yield (Nasim et al., 2012).

Phosphorus is in second place after nitrogen regarding the plant's growth and development, and the plant requires optimal levels of phosphorus during the initial stages of growth up to crop production (Grant et al., 2005). Phosphorus leads to increased root development, seed size, and, eventually, the yield of sunflower seeds (Arif et al., 2003). Phosphorus deficiency has immense negative impacts on the plant's growth (Chojnacka, 2015).

The excessive consumption of chemical fertilizers is one of the main reasons for the pollution of surface and ground waters which eventually leads to poisoning in humans, livestock, and aquatic animals. In addition to its negative environmental consequences, leads to increased production expenses (Chandrasekar et al., 2005). The disruption of biological activities, soil structure, and salt accumulation in the soil have also been identified as the adverse effects of the excessive usage of chemical fertilizers (Omidi et al., 2009). Excessive consumption of nitrogen chemical fertilizers in sunflower cultivation has drastic, negative effects on seed quality and decreases the amount of seed oil (Gok et al., 2006; Azeez and van Averbeke, 2010). Therefore, to decrease these risks, sources should be used that, in addition to supplying the plant's needs, lead to the sustainability of agricultural systems in the long-term as well (Wu and Ma, 2015). A solution to this problem is to implement sustainable agricultural principles in cultivation systems, an element of which is the application of bioinoculants to eliminate or reduce the usage of chemical inputs and increasing soil fertility (Floody et al., 2018).

Bioinoculants include adequate levels of one or several species of beneficial soil microorganisms that are supplied with appropriate preservatives and have a positive role in providing the plant's nutritional requirements and improving the growth conditions. These microorganisms include *Azospirillum*, *Azotobacter*, *Bacillus*, and *Pseudomonas bacteria* (Bashan et al., 2004) and *Rhizobium* had been classified into four genera, *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Sinorhizobium* (Zahran, 2001).

In addition to the biological fixation of nitrogen, the solubilization of phosphorus and potassium, and the inhibition of pathogenic factors, biofertilizers also produce growth-stimulating hormones to increase the yield of field crops (Sturz and Christie, 2003; Herman et al., 2008).

Bioinoculants are currently being considered as alternatives to chemical fertilizers to increase soil fertility and crop production in sustainable agriculture systems (Wu et al., 2005). Shaukat et al. (2006) have studied the sunflower plant and reported that inoculating its seeds with growth-stimulating bacteria increased the plant height, head diameter, and seed weight, as compared to the control (non-inoculated) plants. It appears that a combination of various types of growth-stimulating bacteria can enable a synergistic and intensifying relationship that increases the plant growth and eventually the plant yield (Nezarat and Gholami, 2009). Maleki and Balouchi (2012) have reported that the *Azotobacter*, *Azospirillum*, and *Pseudomonas* bacteria lead to the development of the aerial organs of maize and, causing major changes in the plant physiology, result in the drastic increase of plant yield and quality. Bashan et al. (2004) have reported that the application of bioinoculants, especially *Azospirillum*, could significantly improve dry matter, nutrient absorption, plant height, leaf size, and root length in cereals. Ehteshami et al. (2007) have also stated that inoculating maize seeds with phosphate-solubilizing bacteria has had a positive effect on the absorption of nutrients and yield. A study conducted by Mirzakhani and Sajedi (2015), observed that applying the nitroxin biofertilizer and nitrogen and phosphorus chemical fertilizers increased the head diameter, grain yield, number of seeds per head, and the 1000-grain weight of sunflower. Ghaderi et al. (2012) have stated that the application of biofertilizers has a significant role in the improvement of yield and the yield components in wheat.

A research study conducted by Soleimanzadeh and Gooshchi (2013) has reported that plant height, number of seeds per panicle, and the biological yield of wheat were significantly higher than that of the non-inoculated plants. Moreover, applying a nitrogen chemical fertilizer has had a significant impact on the studied characteristics such as plant height, panicle length, the number of grains per panicle, grain yield, and biological yield.

Evaluating the results of the previous studies has shown that the application of biofertilizers had positive impacts, both quantitatively and qualitatively, on the plants. The objective of the present experiment is to compare the effect of chemical fertilizers and bioinoculants, separately, on the growth and yield of sunflower. This study will also proceed to investigate the possibility of replacing chemical fertilizers with bioinoculants to achieve the goals of sustainable agriculture.

MATERIALS AND METHODS

This research study was conducted in the 2017 crop year in the Agriculture and Natural Resources Research Center of Hamedan, Iran, with loam soil and a pH of 8 (Table 1). The studied region (1758 meters above sea level and with the geographic coordinates of 34° 52´ latitude and 48° 52´ E longitude) is considered a semi-arid region of the country with an annual mean precipitation rate of 302 mm and a mean temperature of 24°C during the warmest month of the year, according to meteorological statistics.

Table 1. Chemical and physical characteristics of the soil at the beginning of the experiment

Property	Result
pH	8
EC ($ds.m^{-1}$)	1.4
T.N.V (%)	0.81
Total N (%)	0.08
P (AV.1) (ppm)	8.2
K (Au) (ppm)	39.4
Clay (%)	21.8
Silt (%)	29.2
Sand (%)	49
Soil Texture	Loam

T.N.V: Total neutralizing value

The research was conducted as two separate factorial experiments that were performed in the same block. The first experiment was executed using a nitrogen-containing chemical fertilizer (N) from a urea source and phosphorus-containing chemical fertilizer (P) from a triple superphosphate source (as a 3×3 design with nine treatments) and the second experiment was conducted using a nitrogen-fixing bioinoculant (NI) from a nitroxin source and a phosphorus-solubilizing bioinoculant (BI) from a biophosphorus source (as a 3×3 design with nine treatments), designed as a randomized complete block design with three repetitions.

The chemical fertilizers factorial treatments are:
N_0: not using a urea fertilizer
N_1: 45 kg/ha pure nitrogen from a urea source
N_2: 90 kg/ha pure nitrogen from a urea source
P_0: not using a triple superphosphate fertilizer
P_1: 40 kg/ha pure phosphorus from a triple superphosphate source
P_2: 80 kg/ha pure phosphorus from a triple superphosphate source

The bioinoculants factorial treatments are:
NI_0: not using a nitroxin fertilizer
NI_1: 0.5 L/ha nitroxin fertilizer
NI_2: 1 L/ha nitroxin fertilizer
BI_0: not using a biophosphorus fertilizer
BI_1: 0.5 L/ha biophosphorus fertilizer
BI_2: 1 L/ha biophosphorus fertilizer

Each experimental plot consisted of four, 6 m long, cultivated rows with 60-cm spacing between rows. The cultivation spacing on each row was 20 cm. Plots were spaced 120 cm from each other and the distance between the blocks was 2 m. Plant density was considered 83300 plants per hectare (5.5 kg seeds per hectare). Land preparation including surface tillage, disking, and land leveling was carried out before cultivation.

The sunflower seed used for experimentation was of the Eroflor Hybrid type, which was planted manually and via the ridge and furrow method (plough with an iron plow twice and then form ridges and furrows, using 3 ridges) on the fourth of June. Three to four seeds were planted in each spot. Thinning was carried out at the 3-4 leaf stage. Irrigation was performed according to the region's weather conditions and the requirements of the sunflower. All experimental plots were free of diseases and pests, and weed control was performed manually and according to the land's requirement.

The nitroxin fertilizer, as the inoculant, is a combination of nitrogen-fixing bacteria, including the *Azotobacter* and *Azospirillum* genera, with 10^8 live and active bacteria per milligram of inoculant for each of the bacteria genera. The Biophosphorus fertilizer, as the inoculant, is a combination of phosphate-solubilizing bacteria, including the *Pseudomonas* and *Bacillus* genera, with 10^7 live and active bacteria per milligram of inoculant for each of the bacteria genera. The required amount of bioinoculant was calculated for all the combined treatments based on the levels of bioinoculants application (0, 0.5, and 1.0 L/ha) and added to seeds. According to the manufacturer's recommendation, inoculation was performed by soaking 5.5 kg of sunflower seeds per hectare in bacterial suspension for 4 hours at 28 °C before sowing. Inoculation of the seeds was carried out an hour before cultivation and drying in the shade. The inoculants were purchased from Mehr Asia Biotechnology Company, Tehran, Iran.

To supply the levels of 45 and 90 kg of pure nitrogen per hectare, the urea chemical fertilizer (46% N) was added to the soil at 98 and 196 kg urea per hectare, respectively, in two rounds, one pre-cultivation and the other at the 6-8 leaf stage. Also, to provide the levels of 40 and 80 kg of pure phosphorus per hectare, fertilization was carried out pre-cultivation and in one round using the triple superphosphate chemical fertilizer (46% P_2O_5) at 87 and 174 kg per hectare, respectively. At the time of complete ripening, harvesting was carried out from the two middle rows of each plot and in a 2-meter length.

For the dry weight of plants, plants were cut above the ground and separated into stems, leaves, and heads. To estimate yield and yield components, samples were oven-dried at 70 °C for 72 hours. The dried weight was measured by using a digital scale. Furthermore, dry weight of the plant, number of seeds per head, head weight (head weight represents the dry weight of head including seeds), and seed yield, as well as biological yield, were determined by measuring the weight of 3 random samples. Head diameter was measured at the maturity stage and the number of seeds per head counted by using a laboratory seed counter. The biological yield included the total above-ground dry weight. The percentage of oil in seed was determined via a Soxhlet device and an ether solvent. The oil yield was calculated by multiplying the oil percentage by the grain yield.

Data were analyzed with the analysis of variance (ANOVA) procedure of the statistical analysis system, SAS (Ver8.2). The significance of differences among main treatment effects and their interaction were determined Duncan's multiple range test (DMRT) with the level of significance established at $p \leq 0.05$.

RESULTS AND DISCUSSION

The analysis of variance results for the measured characteristics is depicted in Table 2, indicating that none of the group comparisons between the chemical fertilizers and bioinoculants on the studied characteristic were significant.

The leaf and stem ,dry weight

The results provided in Table 2 indicate that the effect of various levels of the chemical nitrogen and bioinoculant containing nitrogen-fixing bacteria on the dry weight of leaf and stem was significant. Also, the effect of various levels of phosphorus chemical fertilizer on the stem dry weight was significant at a probability level of 5%. However, the group comparisons of the chemical fertilizers versus the bioinoculants did not show a significant difference. The comparison of means of the nitrogen and phosphorus levels showed that increasing the amount of chemical nitrogen and phosphorus fertilizer led to the increase of the leaf and stem dry weight (Table 4). The maximum leaf and stem dry weight among the chemical treatments was that of the N2P2 treatment at the average values of 23.98 and 35.67 grams per plant, respectively. According to the comparison of means (Table 4), the maximum leaf and stem dry weight among the bioinoculants treatments

Table 2. Analysis of variance of mean squares for the measured characteristics

S.O.V	d.f	Dry Weight of Plant		No. Seed per Head	Head Diameter	Head Weight	Oil Yield	Biologi-cal Yield	Seed yield
		Leaves	Stems						
rep	2	34.805	5.8928	6655.32	1.5288	54.9099	0.004	1.7952*	0.128
Chemical Fertilizer									
N	2	57.5866*	31.1577*	30880.6959*	2.1377*	336.1658**	0.0446	2.2358*	0.7524**
P	2	14.4492	21.1477*	33329.5959*	4.0411**	100.495*	0.039	1.6389*	0.3606*
N×P	4	9.2054	0.8399	7638.59	0.3805	43.3274	0.0023	0.5744	0.0136
Bioinoculant									
NI	2	97.0584**	53.5852**	3168.55	2.4677*	328.9306**	0.072	3.1913*	0.4126*
BI	2	13.8122	12.4581	3920.82	1.6033	113.3017*	0.019	0.1477	0.6536**
NI×BI	4	6.1671	3.24	2817.49	0.9077	13.9689	0.02	0.6399	0.1374
Chemical vs Bioinoculant	1	0.2373	9.0815	1587.63	0.0816	2.8566	0.029	0.8231	0.135
Error	34	14.1092	6.952	6822.18	0.7206	41.5008	0.036	0.6321	0.1137
CV (%)		20.77	8.05	8.42	4.99	6.06	9.26	6.02	6.14

*: Significant at 0.05 level, **: Significant at 0.01 level

Table 3. The comparison of means for the measured characteristics affected by various treatments of chemical fertilizers and bioinoculants

Treatments		Dry Weight of Plant (g/plant)		No. Seed per Head	Head Diameter (cm)	Head Weight (g/plant)	Oil Yield (ton/ha)	Biologi-cal Yield (ton/ha)	Seed yield (ton/ha)
		Leaves	Stems						
Chemical Fertilizer	Level								
	0	16.04 b	30.65 b	939 b	16.41 b	103.39 b	2.34	12.74 a	5.12 b
	1	17.43 b	31.96 ab	966 ab	17.05 ab	101.39 b	2.42	12.81 a	5.39 ab
	2	20.95 a	34.32 a	1051 a	17.36 a	112.86 a	2.48	13.64 b	5.8 a
	0	16.8 a	30.57 a	941 b	16.42 b	102.76 b	2.35	12.62 b	5.21 b
	1	18.28 a	32.93 ab	961 b	16.71 b	105.53 ab	2.41	13.1 a	5.39 ab
	2	19.33 a	33.44 a	1055 a	17.7 a	109.41 a	2.48	13.47 a	5.61 a
Bioinoculant									
	0	14.35 b	31.21 b	944	16.52 b	100.52 b	2.36	12.67 b	5.19 b
	1	18.42 ab	32.31 b	955	16.97 ab	105.97 ab	2.48	13.43 ab	5.63 a
	2	20.14 a	35.88 a	985	17.56 a	112.59 a	2.54	13.84 a	5.88 a
	0	16.67 a	32.34 a	913	16.54 a	102.63 a	2.41	13.24 a	5.26 b
	1	17.67 a	34.48 a	958	17.17 a	106.77 a	2.49	13.46 a	5.54 ab
	2	18.56 a	32.57 a	1007	17.34 a	109.69 ab	2.48	13.24 a	5.8 a

Mean which have at least once common letter are not significantly different at the 5%level using (DMRT)
N_0, N_1 and N_2= 0, 45 and 90 kg/ha, respectively — BI_0, BI_1 and BI_2= 0, 0.5 , 1 litr/ha, respectively
P_0, P_1 and P_2= 0, 40, 80 kg/ha, respectively — NI_0, NI_1 and NI_2= 0, 0.5 , 1 litr/ha, respectively

were that of the treatments of NI_2BI_2 at 22.41 and NI_2BI_1 at 37.13 grams per plant, respectively.

Stancheva et al. (1992) observed that inoculating maize with *Azospirillum* increased the dry weight of the plant. Tialk et al. (1982) also reported that inoculating maize seeds with *Azotobacter chroococcum* and *Azospirillum brasilense* increased the dry weight of the plant, which is consistent with the results of the present study.

The study of Moradi et al. (2011) on wheat showed that the effective utilization of microorganisms as bioinoculants leads to the increase of all vegetative characteristics. Talebzadeh and Marashi (2019) reported that the effect of various levels of chemical and biological nitrogen on total dry weight was significant which is consistent with the results of the present study.

Biological yield

The analysis of variance results shows that the effect of applying chemical nitrogen and bioinoculant containing nitrogen-fixing bacteria on the biological yield is significant at a 5% probability level. Also, the effect of applying chemical phosphorus is significant at a 5% level. The interaction effects of applying nitrogen and phosphorus for the chemical fertilizer and bioinoculant treatments did not reveal a significant difference. The maximum and minimum levels of biological yield among the chemical treatments were obtained for the N_2P_2 treatment at 14.52 t/ha and the N_0P_0 control treatment at 12.54 t/ha (Table 4). The study of Namvar et al. (2012) on sunflower revealed that using 100 kg/ha chemical nitrogen fertilizer increased biological yield (8837 kg/ha), compared to the no fertilizer treatment (8094 kg/ha), which is consistent with the results of the present study.

The comparison of means table shows that the highest level of biological yield among the inoculant treatments is that of the NI_2BI_0 treatment with an average yield of 14.21 t/ha, followed by the NI_2BI_2 treatment with an average value of 13.76 t/ha, whereas the lowest value is that of the NI_0BI_0 control treatment with an average value of 12.4 t/ha (Table 4). Ghaderi et al. (2012) have stated that the application of biofertilizers leads to a significant difference in the biological yield of wheat. Naserirad et al. (2011) reported that the highest level of biological yield in maize was achieved from the treatments inoculated with *Azotobacter* and *Azospirillum* (21320 kg/ha) while the lowest level was that of the control treatment (18400 kg/ha). It appears that the growth-stimulating bacteria increase the biological yield by allocating more dry matter to the vegetative parts of the plant (Naserirad et al., 2011). In conditions where nitrogen is available to the plant, the photosynthetic activities increase and the biological yield increases through vegetative and reproductive growth. The increased application of nitrogen fertilizers increases the plant's total dry weight (Maleki et al., 2011). Mirparsa et al. (2016) investigated the effect of nitrogenous and phosphate growth-stimulating bacteria on the yield of sunflower and reported that the biofertilizer has significantly increased the biological yield of sunflower compared to the control treatment (no fertilizer), which is consistent with the results of the present study.

Seed yield

The analysis of variance results showed that the effect of various levels of chemical nitrogen and phosphorus on seed yield were significant at a 1% and 5% probability level, respectively, whereas the interaction effects of these two were non-significant (Table 2). The comparison of means for nitrogen and phosphorus levels showed that increasing the level of chemical nitrogen and phosphorus fertilizers from level zero to two increased seed yield (Table 3). The maximum and minimum levels of seed yield among the chemical treatments were obtained for the N_2P_2 treatment at 5.9 t/ha and the N_0P_0 control treatment at 4.84 t/ha (Table 4).

The analysis of variance results showed that the effect of various levels of bioinoculants on seed yield was significant at a 5% and 1% probability level for the levels of Nitroxin and biophosphorus, respectively, whereas the interaction effects of these two were non-significant (Table 2). Despite the quantitative increase of seed yield, the group comparison of chemical and biological treatment didn't show a significant difference which indicates the lack of difference between the chemical treatments and the biological treatments, in terms of seed yield (Table 2). The maximum and minimum levels of seed yield among the bioinoculant treatments were obtained for the NI_2BI_2 treatment at 5.9 t/ha and the NI_0BI_0 control treatment at 4.81 t/ha (Table 4).

The results of the present study are consistent with those of previous studies conducted in this field. Choobforoosh et al. (2014) studied the application of biofertilizers containing nitrogen-fixing bacteria in sunflower and reported the significant increase of seed yield, resultant of biofertilizer application. Also, the application of *Azotobacter*, *Azospirillum*, and *Bacillus* biofertilizers increased seed yield in the medicinal plant, fennel (Melero et al. 2008). Abbadi and Gerendas (2009) also reported that compared to lower levels of nitrogen application, the application of nitrogen fertilizers in high levels leads to increased seed yield. Hashemi and Mojaddam (2015) investigated the effect of triple superphosphate (0, 45, and 90 kg/ha) and Barvar2 phosphate (0, 100, and 200 g/ha) biofertilizers on yield and yield components of sesame and reported that triple superphosphate fertilizer and Barvar2 biofertilizer both significantly increased the seed yield. Martinez et al. (2017) reported that the application of 100 kg/ha chemical nitrogen increased seed yield at 11.1 t/ha and the lowest maize yield was determined in the control treatment (6.8 t/ha), the application of chemical nitrogen on maize yield increased seed yield more than sunflower seed yield at the present study.

Number of seeds per head

The effect of various chemical nitrogen and phosphorus levels on the number of seeds per head is significant at a 5% probability level (Table 2). However, the effect of various levels of nitroxin and biophosphorus on the number of seeds per head is not significant. The comparison of means for the chemical fertilizers and bioinoculants levels revealed that by increasing their level of application, the number of seeds per head showed an increasing trend (Table 3). Zaongo et al. (1997) have reported the significant effect of nitrogen on increasing the number of seeds per panicle in sorghum. The maximum number of seeds per head, obtained for the chemical and biological treatments, was that of the N_2P_2 and NI_2BI_2 combination treatments with the average values of 1186 and 1058, respectively (Table 4), and the minimum number of seeds per head was that of the N_0P_0 and NI_0BI_0 control treatments with the average values of 897 and 897, respectively. The group comparisons between the chemical and bioinoculant treatments didn't show a significant difference (Table 2).

Applying phosphorus-solubilizing microorganisms in sunflower cultivations can reduce the path of phosphorus distribution and absorption and facilitate phosphorus availability to the plant, and also increase the number of seeds per head (Moradi et al., 2008). The experimental results of Mirzakhani and Sajedi (2015) showed that the effect of chemical nitrogen and phosphorus fertilizer treatments on the number of seeds per head was significant at a 1% probability level. Increasing the amount of phosphorus in the plant generally increases the ability for successful pollination and flower fertility in the head. Therefore, a higher number of seeds will form in each head. On the other hand, the higher level of nitrogen will increase the green area and photosynthesis of the plant, positively impacting the transfer of photosynthetic material and grain filling. The study results of Omidi and Bahrani (2010) have revealed that the effect of a nitrogen application treatment on the number of seeds per head is significant at a 1% probability level and the maximum and minimum number of seeds were obtained for the 140 kg/ha application treatment and the treatment with no fertilizer application, respectively.

Head diameter

The effect of chemical nitrogen and bioinoculant containing nitrogen-fixing bacteria application on the head diameter was significant at a 5% probability level. The effect of various levels of chemical phosphorus on this characteristic was also significant at a 1% level (Table 2).

Table 4. The mean comparison of the interaction effect of chemical fertilizer and bioinoculant treatments on the measured characteristics

Treatments	Dry Weight of Plant (g/plant)		No. Seed per Head	Head Diameter (cm)	Head Weight (g/plant)	Oil Yield (ton/ha)	Biological Yield (ton/ha)	Seed yield (ton/ha)
	Leaves	Stems						
Chemical Fertilizer								
N_0P_0	16.27	28.33	897	15.83	101.88	2.24	12.54b	4.84
N_0P_1	15.80	31.64	938	16.53	102.92	2.34	12.82b	5.15
N_0P_2	16.05	31.99	980	16.86	105.59	2.44	12.88b	5.38
N_1P_0	16.56	30.57	946	16.60	101.19	2.39	12.66b	5.26
N_1P_1	17.76	32.66	955	16.83	100.80	2.42	12.76b	5.36
N_1P_2	17.95	32.65	997	17.73	102.18	2.46	13.02b	5.55
N_2P_0	17.58	32.80	978	16.83	105.22	2.43	12.67b	5.53
N_2P_1	21.28	34.50	989	16.76	112.88	2.46	13.73ab	5.68
N_2P_2	23.98	35.67	1186	18.50	120.48	2.55	14.52a	5.90
Bioinoculant								
NI_0BI_0	14.23	29.11	897	15.66	95.32	2.22	12.40	4.81
NI_0BI_1	15.01	33.07	960	17.40	103.74	2.48	13.29	5.53
NI_0BI_2	13.80	31.62	976	16.50	102.50	2.39	12.32	5.44
NI_1BI_0	16.32	32.01	917	16.76	103.05	2.41	13.11	5.25
NI_1BI_1	19.46	33.43	961	16.80	104.50	2.55	13.54	5.68
NI_1BI_2	21.15	31.49	986	17.36	110.37	2.50	13.64	5.70
NI_2BI_0	19.46	35.90	943	17.20	109.51	2.59	14.21	5.81
NI_2BI_1	20.21	37.13	953	17.30	112.06	2.46	13.56	5.70
NI_2BI_2	22.41	34.60	1058	18.16	116.20	2.57	13.76	5.90

N_0, N_1 and N_2= 0, 45 and 90 kg/ha, respectively
P_0, P_1 and P_2= 0, 40, 80 kg/ha, respectively
BI_0, BI_1 and BI_2= 0, 0.5 , 1 Litr/ha, respectively
NI_0, NI_1 and NI_2= 0, 0.5 , 1 Litr/ha, respectively

The comparison of means showed that increasing the application of chemical fertilizers and bioinoculants application increases the head diameter (Table 3). The maximum head diameter was that of the N_2P_2 treatment with an average value of 18.5 cm and the NI_2BI_2 treatment with an average value of 18.16 cm, whereas the minimum value was that of the control treatments (Table 4). The head diameter is considered the most important parameter of yield and has a significant impact on the number of seeds per head and oil yield (Cosge and Bayraktar, 2004). The study results of Skiner et al. (1981) have shown that using chemical fertilizers with nitrogen and phosphorus nutritional elements increased the head diameter in sunflower.

Choobforoosh et al. (2014) have stated that applying Nitragin biofertilizer significantly increased the head diameter in sunflower which is consistent with the results of the present study. Salehi and Bohrani (2000) have also shown that increasing the amount of nitrogen fertilizer significantly increased the head diameter of sunflower. Mirzakhani and Sajedi (2015) reported that the head diameter was significantly affected by the application of chemical nitrogen and phosphorus fertilizers. Supplying a higher amount of the plant's required nutritional elements is followed by the increased vegetative and reproductive growth of the plant. Followed by the increased reproductive growth stage of the plant via the increased number of reproductive units (fertile flowers per sunflower head), the head diameter will increase as well.

Head weight

According to Table 2, the effect of applying chemical nitrogen and bioinoculant containing nitrogen-fixing bacteria levels on the head weight shows a very significant difference at a 1% probability level, and a significant difference at a 5% probability level is also observed for the levels of chemical phosphorus and bioinoculant containing phosphorus-solubilizing bacteria. The maximum head weight is that of the N_2P_2 treatment at 120.48 g/plant (Table 4).

The comparison of means has shown that increasing the levels of nitroxin and biophosphorus from level zero to one liter per hectare increases the head weight (Table 3). The maximum and minimum head weights obtained for the biofertilizer treatments were that of the NI_2BI_2 combination treatment at 116.2 g/plant and the NI_0BI_0 control treatment at 95.32 g/plant, respectively (Table 5).

Mirshekari et al. (2009) concluded that Nitragin biofertilizer increased the maize cob weight, when compared to the non-inoculated treatment. Inoculating sunflower seeds with four strains of plant growth-promoting bacteria - two strains of which were of the *Azotobacter* genus and two strains of the *Pseudomonas* genus - Zahir et al. (1998) found that the head weight increased in seed inoculation treatments using both bacteria, compared to the control treatment, while inoculating with each bacteria also showed a significant promoting effect, which is consistent with the results of the present study.

Seed oil yield

According to the analysis of variance table, it is evident that applying chemical fertilizers and bioinoculants at various levels does not significantly affect the seed oil yield (Table 2). The maximum oil yield among the chemical treatments was that of the N_2P_2 treatment with an average value of 2.55 t/ha and the minimum value was that of the N_0P_0 treatment at 2.24 t/ha (Table 4). Also, among the biological treatments, the maximum and minimum oil yield were obtained for the NI_2BI_0 treatment (2.59 t/ha) and the NI_0BI_0 control treatment (2.22 t/ha). The group comparison of the chemical treatments versus the biological treatments did not reveal a statistically significant difference. The experimental results of Gul and Kara (2015) indicated the non-significant effect of various levels of nitrogen fertilizer on sunflower oil yield, which is consistent with the results of the present study.

Namvar et al. (2012) reported that the sunflower seed oil yield increased with the increase of nitrogen application and the biofertilizers increased the seed oil yield by 10.84% compared to the control treatment. Scheiner et al. (2002) investigated the sunflower nitrogen requirement and found that the oil yield of the inoculated treatments was higher than that of the control treatment, which is consistent with the results of the present research. The increased oil yield of the inoculated treatments could be due to the secretion of plant growth regulators (PGRs) such as Auxin, Gibberellin, and Cytokinin by *Azospirillum* and *Azotobacter* (Mehran et al., 2011; Vessy, 2003).

Simple correlation analysis of characteristics

Investigating the simple correlation between the measured characteristics showed a positive correlation between oil yield and all studied characteristics. The results also revealed the significant correlation of oil yield with stem dry weight, head diameter, head weight, and seed yield. As depicted in Table 5, the highest significant correlation was that of the seed yield and oil yield (0.73) which is significant at a 1% probability level.

Table 5. Simple correlation analysis of characteristics for the chemical fertilizer and bioinoculant treatments

	Dry Weight of Plant		No. seed per head	Head Diameter	Head weigh	Biological Yield	Oil Yield	Seed yield
	leaves	Stems						
	1	2	3	4	5	6	7	8
1	1	0.29*	0.34*	0.39**	0.39**	0.42**	0.18	0.39**
2		1	0.23	0.36**	0.34*	0.19	0.34*	0.34*
3			1	0.39**	0.47**	0.42**	0.24	0.41**
4				1	0.39**	0.3*	0.35**	0.55**
5					1	0.4**	0.38**	0.67**
6						1	0.21	0.4**
7							1	0.73**
8								1

*: Significant at 0.05 level, **: Significant at 0.01 level significant

CONCLUSION

Fertilizers can influence the quantitative and qualitative yield indices of plants, therefore, fertilizer and nutrition management is one of the most important factors in the successful cultivation of field crops. The results were based on a one-year study, revealed that nitrogen-fixing bioinoculants significantly increased the leaf and stem dry weight, head diameter, head weight, and biological yield, and the effect of applying phosphorus-solubilizing bioinoculants had a significant effect only on head weight (Table 2). Meanwhile, the effect of various levels of urea fertilizer application significantly affected all studied characteristics except for oil yield. Moreover, the effect of various levels of triple superphosphate fertilizer application on stem dry weight and oil yield was not significant.

The results of this study reveal that 90 kg of pure nitrogen per hectare were achieved the maximum leaf and stem dry weight, biological yield, and head weight. Also, 80 kg of pure phosphorus per hectare was achieved the maximum head diameter and the number of seeds per head. Applying one liter per hectare of nitroxin fertilizer showed the optimal amount for leaf and stem dry weight, head weight, head diameter, and biological yield, whereas using 1 L/ha of biophosphorus fertilizer was increased the number of seeds per head.

Nowadays, considering the pollution caused by the application of chemical fertilizers, the effect of bioinoculants and native sources of nutrients in absorbing nutritional elements has become of interest, and using these types of fertilizers could be an effective step towards reaching sustainable agriculture and reducing the environmental pollution.

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