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THE EFFECTS OF BIOLOGICAL AND CHEMICAL FERTILIZER SOURCES ON THE PRODUCTION AND QUALITY OF SUNFLOWER

Pengaruh Sumber Pupuk Hayati dan Pupuk Kimia Terhadap Produksi dan Kualitas Bunga Matahari

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ABSTRACT

Bio-fertilizers improve nutrient availability and reduce the need to use chemical fertilizers and hold a great promise to increase crop yields. To study the effects of different nutrition systems, this research evaluated the individual and combined effects of bio-fertilizers and compared their efficiency with nitrogen (N) and phosphorus (P) chemical fertilizers on the yield of sunflower (*Helianthus annuus* L.) in the 2017–2018 crop year in Hamedan, Iran. The field experiment included a combination of two factorial experiments, consisting of (a) the application of chemical nitrogen (N_0=0, N_1=45, N_2=90 kg ha^{-1}) and phosphorus (P_0=0, P_1=40, P_2=80 kg ha^{-1}) fertilizers, and (b) the application of N-fixing bacteria *Azospirillum* and *Azotobacter* (BN_0=0, BN_1=0.5, BN_2=1 L ha^{-1}), and P-solubilizing bacteria *Pseudomonas* and *Bacillus* (BP_0=0, BP_1=0.5, BP_2=1 L ha^{-1}) bio-fertilizers. Each experiment used a 3×3 design with nine treatments and arranged in a randomized complete block design with three replicates. Results indicated that the chemical N and P fertilizers significantly increased the grain yield, thousand-grain weight, plant dry weight, protein percentage, and harvest index. Furthermore, bio-fertilizers significantly increased the grain yield and harvest index. The application of bio-fertilizers with N-fixing bacteria and P-solubilizing bacteria improved the growth and yield characteristics of sunflower. Sunflower oil yield was similar among all treatments, ranged from 2.22 to 5.7 t ha^{-1}. Grain yield ranged from 5.12 to 5.88 t ha^{-1}. A similar result between treatments with the chemical and bio-fertilizers suggests that bio-fertilizers are potential alternatives to chemical fertilizers.

[**Keywords:** bio-fertilizer, growth-promoting bacteria, nitrogen fixation, sunflower, sustainable agriculture.]

ABSTRAK

*Pupuk hayati dapat memperbaiki ketersediaan hara sehingga mengurangi kebutuhan pupuk kimia dan meningkatkan hasil panen. Untuk mempelajari pengaruh sistem hara yang berbeda, penelitian ini bertujuan untuk mengevaluasi pengaruh individu maupun kombinasi penggunaan pupuk hayati dan membandingkan efisiensinya dengan pupuk kimia nitrogen (N) dan fosfor (P) terhadap hasil panen bunga matahari (*Helianthus annuus *L.). Penelitian dilaksanakan pada musim tanam 2017–2018 di Hamedan, Iran. Rancangan percobaan lapangan meliputi kombinasi dua percobaan faktorial, yaitu (a) aplikasi pupuk kimia nitrogen (N_0=0, N_1=45, N_2=90 kg ha^{-1}), dan fosfor (P_0=0, P_1=40, P_2=80 kg ha^{-1}), dan (b) aplikasi bakteri penambat N* Azospirillum *dan* Azotobacter *(BN_0=0, BN_1=0,5, BN_2=1 L ha^{-1}) dan bakteri pelarut P* Pseudomonas *serta pupuk hayati Bacillus (BP_0=0, BP_1=0,5, BP_2=1 L ha^{-1}). Masing-masing percobaan menggunakan rancangan 3 x 3 dengan sembilan perlakuan dan disusun dalam rancangan acak kelompok lengkap tiga ulangan. Hasil penelitian menunjukkan bahwa pupuk N dan P kimia secara nyata meningkatkan hasil biji, bobot seribu biji, bobot kering tanaman, persentase protein, dan indeks panen. Selanjutnya, pupuk hayati secara signifikan meningkatkan hasil biji dan indeks panen. Aplikasi pupuk hayati dengan bakteri penambat N dan bakteri pelarut P meningkatkan karakteristik pertumbuhan dan hasil bunga matahari. Rendemen minyak bunga matahari pada semua perlakuan adalah sama, berkisar 2,22–5,7 t ha^{-1}, dan hasil biji 5,12 – 5,88 t ha^{-1}. Hasil yang hampir sama antara perlakuan pupuk kimia dan pupuk hayati menunjukkan bahwa pupuk hayati merupakan alternatif potensial untuk pupuk kimia.*

[**Kata kunci:** pupuk hayati, bakteri pemacu pertumbuhan, penambatan nitrogen, bunga matahari, pertanian berkelanjutan]

INTRODUCTION

In recent decades, the ever-increasing demand for food as an outcome of population growth suggests the optimal application of chemical and biological fertilizers to achieve maximum crop yield (Raei et al. 2013). The occurrence of environmental and economic issues caused by the loss of chemical nitrogen (N) fertilizers because of ammonia sublimation, denitrification, nitrate leaching, volatilization, and other N transformation processes has led chemical fertilizers to be substituted with N-fixing biologic systems as a part of sustainable agriculture programs (Raei et al. 2013). An essential requirement of farm planning is to evaluate various plant-nutrition systems that will affect crop quality by impacting the plant's vegetative and reproductive growth period and the balance between them (Kiani et al. 2016).

Sunflower is a native plant of the central regions of America (Kiani et al. 2016). After soybean (*Glycine max*), groundnut (*Arachis hypogaea*), and rapeseed (*Brassica napus*), sunflower ranks fourth among annual oil-producing plants in terms of oilseed production (Khandekar et al. 2018). Sunflower is a very demanding plant in terms of soil elements, so the balance between nutritional elements is an imperative factor for maximizing the grain yield (Khodaei-Joghan et al. 2018). The area under sunflower cultivation has been increased because of its adaptation to different climatic and soil conditions worldwide (Forleo et al. 2018).

Nutrient deficiency is one of the primary reasons for the low yield of sunflowers. Using the right proportion and optimum quantity of essential macro- and micro-nutrients is the key factor for boosting and sustaining crop productivity. Nitrogen is the most limiting nutrient in sunflower production, which results in the decline of sunflower production. Therefore, fertilizer management is one of the most important factors in the successful cultivation of crops, altering yield quality and quantity (Mehmood et al. 2018). Over application of N fertilizer increases fertilizer costs, N losses to the environment, and negatively affects sunflower seed quality (Ghasem et al. 2018).

The application of bio-fertilizers and bacterial products has advantages over common chemical substances. The bacteria not only increase the availability of soil nutrients, but also enhance plant productivity through the biological N fixation and solubilization of phosphorous (P) and potassium (K), the control of pathogenic agents, and the production of growth-regulating and stimulating hormones (Rajiv K. Sinha et al. 2010). Bio-fertilizers also help plant health and crop yield, intended to proffer long-lasting solutions to the problems associated with the continuous use of chemical fertilizers by enhancing soil fertility to increase crop productivity (Adeniji et al. 2019).

Farnia and Moayedi (2015) reported that the application of N and P bio-fertilizers on sunflower improves morphological characteristics and makes longer phonological stages compared with no bio-fertilizer, and eventually has a better impact on seed yield. Another study shows that the application of Nitroxin bio-fertilizer increases the seed yield of sunflower by 28% compared with no-fertilizer treatment (Bahamin et al. 2014). Moghimi et al. (2012) found that Nitroxin increases the grain yield elevation of safflower (*Carthamus tinctorius*) by fixing N and producing growth-stimulating hormones. Ahmad et al. (2010) have also reported similar results. Azarpour et al. (2012) showed that application of Nitroxin improves the growth characters and yield in soybean cultivars. Ardakani et al. (2011) reported that inoculating wheat seeds with *Azospirillum* increases the NPK absorption rate. Yadav et al. (2011) have also reported that *Azospirillum* inoculation reduces the need to apply chemical N fertilizers significantly.

Banari et al. (2015) investigated the impact of a bio-fertilizer (Fertile 2) on the yield and yield components of durum and bread wheat. They found that P bio-fertilizer could partially replace P fertilizers without yield reduction. Moreover, Shabani et al. (2015) applied a combination of P-solubilizing bacteria and showed a synergistic impact on the plant's P concentrations. Their observation was explained by the positive contribution of plant growth-promoting rhizobacteria (PGPR) to nutrient plant uptake, improving the intake of mineral elements, including P. Also, Zarabi et al. (2011) identified a positive influence of micro-organisms capable of P solubilization on elevated growth and P uptake in maize.

To better understand the effects of bio-fertilizers on sunflower, we carried out a study to investigate the impact of bio-fertilizer compounds. We investigated the quantitative and qualitative yields of sunflower by the employment of bio-, chemical, and no-fertilizers. The primary objectives of this research were to determine an optimal application of N and P chemical fertilizers as well as N-fixing and P-solubilizing bio-fertilizers at rates used in this experiment, and to compare the effect of chemical and bio-fertilizers on the quantitative and qualitative yield of sunflower.

MATERIALS AND METHODS

Field Crop Specifications

The field research was carried out in the Ekbatan Research Station at the Agriculture and Natural Resources Research Center of Hamedan in the 2017–2018 crop year. The laboratory analysis was performed in the Central Laboratory of the College of Aburaihan at the University of Tehran, Iran. The studied region is semi-arid based on De Martonne's climatic classification. Soil sampling was taken from 0 to 30 cm depths and was composited for analysis. The soil texture was determined as loam consisting of clay (21.8%), silt (29.2%), and sand (49%) with a high pH of 8. Total N was 0.08%, available P and K were 8.2 and 39.4 ppm respectively; and organic carbon was 0.81%. Means of temperature and precipitation during the growing season at the experimental site were 24 ^{o}C and 302 mm, respectively.

Experimental Design

The research was conducted as a combination of two factorial experiments the application of (a) N and P chemical fertilizers (as a 3×3 design with nine treatments) and (b) the application of N-fixing (BN) and P-solubilizing (BP) bio-fertilizers (as a 3×3 design with nine treatments). Each experiment was arranged in a randomized complete block design with three replications using 1900 m^2 land. The chemical factorial treatments included three rates of chemical N fertilizers (0, 45, and 90 kg N ha^{-1}) from a urea source and three rates of chemical P fertilizers (0, 40, and 80 kg P ha^{-1}) from a Triple Superphosphate source. The bio-fertilizer factorial treatments included three rates of bio-fertilizers with N-fixing bacteria (0, 0.5, and 1 L ha^{-1}) from a Nitroxin source and three rates of bio-fertilizers containing P-solubilizing bacteria (0, 0.5, and 1 L ha^{-1}) from a Biophosphorus source. Each experimental plot comprised four ridges with 60-cm spacing and one cultivated row on each ridge. Each plot was 6 m long, with 120 plants per plot. We spaced plots 120 cm from each other, and the distance between the blocks was 2 m.

Land Preparation

The land preparation, including surface tillage, disking, and levelling was performed 5 days before cultivation. A longitudinal furrow was created beside the ridges for the placement of chemical fertilizers. According to the experimental treatments, P fertilizer was added to specified plots at the time of cultivation. Nitrogen fertilizer was applied in two parts, one at the planting time and the other at the 6–8 leaf stage.

Sunflower Seed Cultivation

The sunflower seeds used in this research study were of the *Eroflor Hybrid* type. Seed planting was performed manually on the rows at a 2–3 cm depth and 20 cm spacing via the ridge and furrow method (plough with an iron plow twice and then form ridges and furrows, using three ridges). Three to four seeds were planted in each hole. Irrigation (pH of irrigation water was 6.8) was carried out immediately after cultivation to ensure the even germination of seeds.

Bio-Fertilizer Treatments

The Nitroxin bio-fertilizer, as the inoculant (0, 0.5, and 1 L ha^{-1}), is a combination of N-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 (0, 0.5, and 1 L ha^{-1}), is a combination of P-solubilizing bacteria. It comprises of *Pseudomonas* and *Bacillus* genera, with 10^7 live and active bacteria per milligram of inoculant for each of the bacteria genera. The proper amounts of bio-fertilizers for each of the treatments were calculated and added to the seeds. Inoculation was carried out by dipping the sunflower seeds in the cell suspension for 4 hours at 28 °C before sowing according to the manufacturer's recommendation. Seed inoculation was carried out an hour before cultivation and drying in the shade.

Chemical Treatments

We performed pre-cultivation fertilization of 87 and 174 kg ha^{-1} to provide the respective 40 kg and 80 kg ha^{-1} from the triple superphosphate with a purity degree of 46% (46% P_2O_5). Also, we added the urea with a purity degree of 46% (46% N) to the soil in two times, once in pre-cultivation and again in the 6–8 leaf stage at 98 and 196 kg urea ha^{-1}, for the target treatments as a strip next to the rows.

Maintenance

We carried out the crop maintenance comprising of irrigation, weeding, and thinning (at the 3–4 leaf stage) upon the field requirements.

Harvesting

The harvesting process was carried out from the two middle rows of each plot in a 2 m length after eliminating the marginal effects from both sides of the cultivation lines. The final harvesting was carried out with the appearance of signs of complete maturity in sunflower (browning of the undersides of the receptacle and the involucral bracts) and the physiological maturity of seeds on September 30, 2017.

Laboratory Measurements

We cut the plants above the ground to determine their dry weight. Samples were oven-dried at 70°C for 72 hours to estimate yield and yield components. Then the dried weight was measured by using a calibrated digital scale. We used three random samples to determine the dry weight of the plant, thousand-grain weight, and grain yield for each replicate plot. The thousand-grain weight was counted with a laboratory seed counter. After yield determination, the seeds were analyzed for their physical characteristics (kernel percentage and hull-kernel ratio) and chemical characteristics (oil and protein percentages). To calculate the hull-kernel ratio and kernel percentage, seed samples of each treatment were weighed and peeled manually, then the seed kernel and hull were weighed and the percentage was calculated. The oil percentage was determined via a Soxhlet device (Büchi Universal Extraction System B-811, Germany) and an ether solvent according to the AOCS method (AOCS 1993). We calculated the oil yield by multiplying the oil percentage by the grain yield in each treatment. The grain protein percentage was measured via the Bradford protein assay and using a SPECT device. The harvest index (HI) was calculated by dividing grain yield (Y) by total biomass (Yt) as HI = Y/Yt.

Statistical Analysis

We analyzed data with the analysis of variance (ANOVA) procedure of the statistical analysis system, SAS (Ver 8.2) and MSTATC. The significance of differences among main treatment effects and their interaction were determined by Duncan's multiple range test (DMRT) with a significance testing level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Analysis of Variance of the Measured Characteristics

Table 1 presents the variance analysis of the measured parameters with different fertilizers. Evaluating the interaction effect of chemical fertilizers versus bio-fertilizers revealed that only the protein characteristic was significant at a 1% probability.

Thousand-Grain Weight

The variance analysis for chemical N (0–90 kg ha^{-1}) and P (0–80 kg ha^{-1}) rates showed an increase in the thousand-grain weight (Table 2). Also, the change of the chemical N and P on the thousand-grain weight was significant at a 1% probability. Gharali and Asl (2018) investigated the effects of nitrogen on sunflower. They showed there is a direct relationship between nitrogen and the hundred-grain weight, which is consistent with our current study.

The comparison of Nitroxin and Biophosphorus rates showed that the increase of bio-fertilizers rates from 0 to 1 L ha^{-1} increased the thousand-grain weight. Effects of N and P bio-fertilizers on the thousand-grain weight were significant at a 1% and 5% probability, respectively (Table 1). The application of bio-fertilizers considerably increased the thousand-grain weight of sunflower (Gharali and Asl 2018). The group comparison in Table 1 also showed that there is no difference between the chemical and bio-fertilizer treatments in terms of thousand-grain weight. The maximum thousand-grain weight was obtained for N_2P_2 (65.33 g) of the chemical treatments and BN_2BP_2 (65 g) of the bio-fertilizer treatments.

Hull-Kernel Ratio

The variance analysis in Table 1 showed that the effect of chemical N and Nitroxin on the hull-kernel ratio was significant at a 1% probability. The variance analysis from the group comparison of the chemical and bio-fertilizer treatments did not show a significant difference

Table 1. Variance analysis of measured parameters of the yield of sunflower for the chemical and bio-fertilizer treatments.

Source of variance	d.f	Means of square								
		1000-grain weight	Hull-kernel ratio	Plant dry weight	Kernel percentage	Protein percentage	Oil percentage	Oil yield	Harvest index	Grain yield
Rep		7.447	0.003	1.993	9.805	0.453	1.8002	0.004	6.843*	0.128
Chemical fertilizer										
N	2	36.614**	0.0077**	4.63**	26.418**	9.291*	11.928*	0.044	6.536*	0.752**
P	2	56.569**	0.0023	2.46**	3.195	8.269*	1.766	0.039	6.57*	0.36*
N × P	4	4.906	0.0002	0.21	0.414	0.81	0.159	0.002	4.104*	0.013
Bio-fertilizer										
BN	2	47.925**	0.007**	3.191*	31.775**	8.594*	3.748	0.072	15.761**	0.412*
BP	2	16.119*	0.0009	0.147	6.657	2.112	8.683	0.019	4.606*	0.653**
BN × BP	4	11.932	0.0006	0.639	3.072	0.193	0.645	0.02	0.416	0.137
Chemical vs bio-fertilizers	1	0.012	0.0022	0.043	15.388	16.555**	0.546	0.029	1.513	0.135
Error	34	6.936	0.0009	0.692	5.069	2.181	4.134	0.036	1.356	0.113
CV (%)		4.33	10.714	6.233	2.889	8.62	4.55	9.26	3.22	6.14

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

Table 2. The average measured characteristics of yield of sunflower for the chemical and bio-fertilizer treatments.

Treatments	1000-grain weight (g)	Hull- kernel ratio	Plant dry weight (t ha^{-1})	Kernel (%)	Protein (%)	Oil (%)	Oil yield (t ha^{-1})	Harvest index (%)	Grain yield (t ha^{-1})
Chemical fertilizer									
N_0	58.47b	0.297a	12.905a	77.11a	16.54b	45.76a	2.34	35.28b	5.12b
N_1	61.29ab	0.291a	13.02a	78.15a	17.94ab	45.04a	2.42	36.93a	5.39ab
N_2	62.38a	0.243b	14.20b	80.46b	18.52a	42.84b	2.48	36.46ab	5.8a
P_0	58.03b	0.295a	12.97a	77.93a	16.66b	45.25a	2.35	35.12ab	5.21b
P_1	61.12ab	0.272a	13.18a	78.68a	17.76ab	44.44a	2.41	36.43b	5.39ab
P_2	63a	0.264a	13.96b	79.11a	18.57a	43.94a	2.48	37.12a	5.61a
Bio-fertilizer									
BN_0	58.63a	0.32a	12.67a	75.30a	15.56b	45a	2.36	34.6a	5.19b
BN_1	60.25b	0.288b	13.43ab	77.47b	16.61ab	44.87a	2.48	35.82b	5.63ab
BN_2	63.18b	0.262b	13.84b	79.05b	17.51a	43.83a	2.54	37.24c	5.88a
BP_0	59.51a	0.298a	13.244a	76.68a	16.10a	45.65a	2.41	35.22b	5.26b
BP_1	60.40a	0.293a	13.245a	76.88a	16.51a	44.31a	2.49	35.79ab	5.54a
BP_2	62.14a	0.278a	13.46a	78.26a	17.07a	43.74a	2.48	36.65a	5.8 a

Means in each column, followed by similar letter are not significantly different at the 5% level of probability (by Duncan's test)
N_0, N_1 and N_2 = 0, 45 and 90 kg ha^{-1}, respectively BP_0, BP_1 and BP_2 = 0, 0.5, 1 L ha^{-1}, respectively
P_0, P_1 and P_2 = 0, 40, 80 kg ha^{-1}, respectively BN_0, BN_1 and BN_2 = 0, 0.5, 1 L ha^{-1}, respectively

(Table 1). The maximum hull-kernel ratio was observed in the N_0P_0 (0.306) and BN_0BP_0 (0.32) in chemical and bio-fertilizer treatments, respectively (Table 3). Since the main purpose of sunflower cultivation is oil extraction, the use of fertilizers can reduce the ratio of the hull-kernel in this plant and increase oil yield (Akbari et al. 2009). The highest hull-kernel ratio is related to the control treatment and other treatments followed a downward trend (Javanmard and Shekari 2016), which is consistent with the results of the present study.

Plant Dry Weight

According to Table 1, the effect of applying chemical N and P rates on the dry weight showed a significant difference at a 1% probability, and a significant difference at a 5% probability was also observed for bio-fertilizer containing N-fixing bacteria. The maximum dry weight yield was that of the N_2P_2 treatment at 14.52 ton ha^{-1} (Table 3). The comparison of means showed that an increase in Nitroxin and Biophosphorus from 0 to 1 L ha^{-1} increased the dry weight (Table 2). The maximum dry weight of 14.21 t ha^{-1} was obtained for the bio-fertilizer treatments of BN_2BP_0 combination (Table 3). Kandil et al. (2004) reported that the use of biological fertilizers in sugar beet significantly increased plant dry weight. Zarei et al. (2012) stated that bio-fertilizers had a positive impact on plant growth and increased plant dry weight.

Kernel Percentage

The effect of various chemical N and Nitroxin rates significantly increased kernel percentage (Table 1). However, the effect of various rates of chemical P and Biophosphorus was not significant. The comparison of means for the chemical and bio-fertilizers revealed that by increasing their rates of application, the kernel percentage showed an increasing trend (Table 2). The maximum kernel percentage, obtained for the chemical and biological treatments, was that of the N_2P_2 and BN_2BP_2 combination with the average values of 81.12 and 81.16, respectively, and the minimum number of kernel percentage was that of the N_0P_0 and BN_0BP_0 control treatments with the average values of 76.6 and 75.68, respectively (Table 3). The group comparisons between the chemical and bio-fertilizer treatments didn't show a significant difference (Table 1). Roshdi Mohsen et al. (2019) reported that the use of N fertilizers increased the percentage of grain kernels in sunflower cultivars due to the availability of nutrients. Abbasdokht et al. (2017) found that increasing the level of N fertilizer from 60 to 120 kg ha^{-1} increased the kernel percentage.

Harvest Index

The variance analysis in Table 1 showed that the effect of chemical N and P on the harvest index was significant.

Table 3. The mean comparison of yield characteristics of sunflower for the chemical and bio-fertilizer treatments.

Treatments	1000-grain weight (g)	Hull-kernel ratio	Plant dry weight (t ha^{-1})	Kernel (%)	Oil (%)	Oil yield (t ha^{-1})	Harvest index (%)	Grain yield (t ha^{-1})	Protein (%)
Chemical fertilizer									
N_0P_0	56.66	0.306	12.54	76.60	46.3	2.24	33.88	4.84	15.65de
N_0P_1	59.55	0.293	12.48	77.31	45.54	2.34	35.67	5.15	16.33abcde
N_0P_2	59.22	0.292	12.68	77.43	45.44	2.44	36.28	5.38	17.6abcde
N_1P_0	58.66	0.32	12.66	77.78	45.43	2.39	37.61	5.26	16.37abcde
N_1P_1	60.78	0.284	12.70	77.88	45.24	2.42	35.91	5.36	18.5abc
N_1P_2	64.44	0.269	13.69	78.78	44.46	2.46	37.28	5.55	18.87ab
N_2P_0	58.78	0.26	14.34	79.41	44.03	2.43	36.86	5.53	17.9abcde
N_2P_1	63.05	0.238	13.73	80.84	42.56	2.46	34.7	5.68	18.39abcd
N_2P_2	65.33	0.232	14.52	81.12	41.93	2.55	37.82	5.9	19.2a
Bio-fertilizer									
$BN_0 BP_0$	55.66	0.32	12.40	75.68	46.21	2.22	34.25	4.81	15.33e
$BN_0 BP_1$	58.33	0.317	13.29	74.63	44.83	2.48	34.15	5.53	15.52de
$BN_0 BP_2$	61.89	0.31	12.32	75.6	43.97	2.39	35.4	5.44	15.82cde
BN_1BP_0	60	0.294	13.11	76.79	46	2.41	34.75	5.25	16.14cbde
$BN_1 BP_1$	61.21	0.288	13.54	77.07	44.95	2.55	36.11	5.68	16.63abcde
$BN_1 BP_2$	59.55	0.281	13.64	78.01	43.66	2.5	36.62	5.7	17.07abcde
$BN_2 BP_0$	62.89	0.278	14.21	77.57	44.83	2.59	36.68	5.81	16.85abcde
$BN_2 BP_1$	61.66	0.275	13.56	78.41	43.16	2.46	37.12	5.7	17.36abcde
$BN_2 BP_2$	65	0.23	13.76	81.16	43.58	2.57	37.92	5.9	18.32abcd

Different letters indicate significant differences at the 5% level.
N_0, N_1 and N_2 = 0, 45 and 90 kg ha^{-1} N, respectively BP_0, BP_1 and BP_2 = 0, 0.5, 1 L ha^{-1}, respectively
P_0, P_1 and P_2 = 0, 40, 80 kg ha^{-1} P, respectively BN_0, BN_1 and BN_2 = 0, 0.5, 1 L ha^{-1}, respectively

Table 2 shows that the minimum and maximum harvest indices were obtained for the 0 and 45 kg ha^{-1} for chemical N at 35.28% and 36.93%, respectively. The effect of various N and P bio-fertilizers on harvest index was significant at a 1% and 5% probability, respectively (Table 1). The comparison of the average harvest index also showed that this index increased by the increase of the Nitroxin and Biophosphorus from 0 to 1 L ha^{-1} (Table 2). A study by Raissi et al. (2012), showed that the use of P-solubilizing bacteria increased the harvest index of maize by increasing grain yield, as compared to biological yield. Therefore, it can be stated that growth-promoting bacteria increase the harvest index by influencing the apportionment of shrub dry weight and allocating more dry weight to the grain, which is consistent with the results of the present study. Phosphate solubilizing bacteria increase plant growth through the production of plant hormones such as indole acetic acid, in this way, they affect the early stages of plant growth and the roots occupy a larger volume of soil, then root surface increases for nutrients uptake (Mehnaz and Lazarovits 2006). The chemical fertilizer treatments compared with the bio-fertilizers treatments did not show a significant difference at a 5% probability (Table 1). The maximum harvest index belonged to the N_2P_2 (37.82%) among the chemical treatments and the BN_2BP_2 (37.92%) among the bio-fertilizer treatments (Table 3).

Grain Protein

The variance analysis showed that the effect of chemical N and P on the protein percentage was significant, whereas their interaction effects were not significant. The results showed that the increase of chemical N and P from 0 to 90 kg ha^{-1} and 0 to 80 kg ha^{-1} respectively, increased the grains' protein percentage (Table 2). Different studies (Li et al. 2017; Mojaddam 2017) reported similar results showing N fertilizers significantly affect the protein content and increase its value (Li et al. 2017), also increasing grain yield and protein percentage (Mojaddam 2017). Adding chemical P increased sunflower protein content which could be due to improved N uptake by the crop because of P's role in root development. Sharma (2002) reported that one of the advantages of feeding the plants with P is to create deeper and more abundant roots. Phosphorus causes early ripening in plants, decreasing grain moisture, and improving crop quality Malakooti (2000).

The variance analysis showed that various Nitroxin significantly increased protein content. Although the increase of Biophosphorus improved the protein content, the results were not statistically significant. The results showed that the increase in Nitroxin and Biophosphorus from 0 to 1 L ha^{-1} leads to an increase in grain protein content (Table 2). In general, it can be concluded that the synergistic effect of bio-fertilizers may have resulted from their ability to increase the availability of N and P in soil, which improves seed quality (Saeidi et al. 2018).

The variance analysis from the group comparison of chemical treatments showed a significant difference compared with the bio-fertilizer treatments. It indicates that the bio-fertilizer treatments (16.56%) increase the protein content at a lower rate compared with the chemical treatments (17.67%). The maximum protein content obtained was for N_2P_2 (19.2%) between the chemical treatments (Table 3) and the BN_2BP_2 (18.32 %) for the bio-fertilizer (Table 3).

Grain Oil

Table 1 shows that the chemical N significantly affected grain oil content. The increase of N and P from chemical and biological sources decreased the grain oil content (Table 2). Investigating the effect of fertilizer treatments and the qualitative characteristics of sunflower, Khodaei-Joghan et al. (2018) reported a negative, significant correlation between the protein percentage and the grain oil percentage. The physiological reason for a negative correlation between oil and protein synthesis is the competition for carbon during the metabolism of carbohydrates because the amount of carbohydrates required for protein synthesis is lower than that of oil synthesis. Therefore, the employment of N fertilizer increases the synthesis of proteins compared to the synthesis of fatty acids (Mohammadi et al. 2013).

The result showed that the increase of the Nitroxin and Biophosphorus from 0 to 1 L ha^{-1} resulted in reduced grain oil percentage (Table 2). The oil reduction from the abundant application of chemical N fertilizers has been reported in other studies. For example, Munir et al. (2007) found that the minimum oil percentage exists in an integrated treatment and the maximum oil percentage is in the control treatment. The variance analysis from the group comparison of the chemical treatments with the bio-fertilizer treatments did not show a significant difference (Table 1). The maximum grain oil amount was in the N_0P_0 (46.3%) between the chemical treatments and the BN_0BP_0 (46.21%) between the bio-fertilizer treatments (Table 3). Comparing the bio-fertilizer and chemical treatments did not show a significant difference (at a 5% probability) (Table 1). Yousefpoor and Youdi (2014) reported that the increase of chemical fertilizers initially increases the oil content to a certain extent but eventually decreases.

Grain Oil Yield

The result showed that the increase of the chemical N and P did not significantly change grain oil yield, though oil yield showed an increasing trend. The group comparison of chemical and bio-fertilizer treatments did not show a statistically significant difference (Table 1). More details have been provided in Zamanian and Yazdandoost (2021). When N increased, grain protein content and oil yield increased; however, grain oil percentage decreased (Ali and Ullah 2012; Heidari and Bagheri 2016). Rastgo et al. (2014) reported that the application of N fertilizers reduces the oil percentage and increases the oil yield and grain protein percentage in safflower, which is consistent with the results of the present study.

Grain Yield

The effect of various amounts of bio-fertilizer on grain yield was significant for the Nitroxin and Biophosphorus (Table 1) and the use of N and P from chemical and biological sources increased grain yield (Table 2). More details have been provided in Zamanian and Yazdandoost (2021). Fazeli Kakhki et al. (2014) reported that the use of P-solubilizing bacteria in sesame plants significantly increased seed yield and yield components compared to the control treatment. Hashemi and Mojaddam (2015) showed that both triple superphosphate fertilizer and Barvar2 bio-fertilizer significantly increased the grain yield. Investigations declared that the results of the present study are consistent with those of previous studies in the related fields.

Simple Correlation Analysis of Characteristics

Evaluating the correlation between the measured characteristics comprising grain yield, oil yield, oil percentage, protein percentage, thousand-grain weight, hull-kernel ratio, kernel percentage, plant dry weight, and the grain yield showed a positive correlation between harvest index and all assessed characteristics, except for hull-kernel ratio and oil percentage. As evident in Table 4, the oil yield characteristic (0.95) had the highest rate

Table 4. Simple correlation analysis of yield characteristics of sunflower for the chemical and bio-fertilizer treatments.

Treatments		1	2	3	4	5	6	7	8	9
1000-grain weight	1	1								
Hull-kernel ratio	2	-0.74**	1							
Plant dry weight	3	0.60**	-0.78**	1						
Kernel percentage	4	0.68**	-0.93**	0.66**	1					
Protein percentage	5	0.73**	-0.85**	0.61**	0.86**	1				
Oil percentage	6	-0.70**	0.69**	-0.67**	-0.65**	-0.60**	1			
Oil yield	7	0.75**	-0.60**	0.75**	0.48*	0.54*	-0.64**	1		
Harvest index	8	0.61**	-0.52*	0.53*	0.64**	0.65**	-0.53*	0.61**	1	
Grain yield	9	0.81**	-0.70**	0.80**	0.60**	0.62**	-0.84**	0.95**	0.64**	1

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

of positive and significant correlation with grain yield. The results showed a significant positive correlation between oil yield and the thousand-grain weight, kernel percentage, plant dry weight, protein percentage, grain yield, and harvest index characteristics. The highest negative correlation was found between the hull-kernel ratio and kernel percentage (-0.93). The harvest index also showed a significant negative correlation with the oil percentage and hull-kernel ratio.

CONCLUSION

The protein content of sunflower was most promising for 80 kg ha^{-1} of P and 90 kg ha^{-1} of N, compared with all other rates. Rates of 90 kg ha^{-1} N was also the best for the plant dry weight and kernel content. Furthermore, the 1 L ha^{-1} of Nitroxin bio-fertilizer exhibited the most suitable for the thousand-grain weight and harvest index. The highest grain yield was obtained for N_2P_2 and BN_2BP_2 treatments. The effect of fertilizer treatments showed a significant negative correlation between the protein percentage and the grain oil percentage. The application of *Azotobacter* and *Azospirillum* with and without *Pseudomonas* and *Bacillus* increased the thousand-grain weight, plant dry weight, kernel percentage, protein percentage, oil yield, grain yield, and harvest index. Among treatments, the interactions between chemical fertilizer and bio-fertilizer were significant only for grain protein content. This study strongly supports the positive impact of N-fixing and P-solubilizing bio-fertilizers on grain yield, harvest index, and thousand-grain weight. To better identify the efficiency of fertilizers in different conditions for future research, we suggest a change in the fertilizer amount and more diverse sources of chemical and bio-fertilizers.

CONFLICT OF INTEREST

We have not received funds for this article.

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