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Response of safflower Seed quality characteristics to different soil fertility systems and irrigation disruption

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Abstract

To study the quality of safflower seed in response to plant nutrition under water deficit stress condition, an experiment was carried out at the Research Farm of Faculty Agriculture of Urmia University, Urmia-Iran, during 2010. The experimental design was split-plot, laid out in Randomized Complete Block with three replicates. The main plots were well-irrigation (I_1), irrigation disruption at vegetative growth stage (I_2), and irrigation disruption at reproductive growth stage (I_3). The subplot included seven levels of soil fertilization: Control (C), Urea (U), Humix as organic fertilizer (O), Biofertilizers (Nitroxin (N), Biosoulphour (B)), integrated fertilize treatments: (Urea + Humix + Nitroxin) (T_1), and (Urea + Humix + Biosoulphour) (T_2). Results showed that the highest and lowest seed yield was obtained from the I_1 and I_2 irrigation regimes, respectively. Whereas T_2 and control fertilization treatments produced the maximum and minimum seed yield, respectively. Oil and protein percentages were significantly influenced by the "irrigation regimes × fertilization" interaction. So, the highest (28.41 %) and lowest (21.01 %) protein percent was obtained from the I_3B and I_1O treatment, respectively. I_1B and I_3C fertilization treatments had the highest (26.92 %) and the lowest (20.68 %) oil percentage. The highest (75.20 %) linoleic and oleic acid (13.95 %) were obtained in plants under well irrigation regimes. But the lowest of them were observed in plants under I_2 and I_3 irrigation regimes treatments, respectively. The existence of a water deficit strikingly decreased the oil percentage, oleic acid and linoleic acid concentrations of the seeds. However, it increased the protein percentage, palmitic acid, and stearic acid in safflower seeds.

Keywords: Biofertilizer, Fatty acid, Oil, Organic fertilizer, Protein.

Introduction

In recent years, there has been an increasing demand for agricultural products with specific qualities (jalilian et al., 2012). The demand for vegetable oils for food purposes has entailed a considerable expansion of oilseed crops all over the world (Corleto et al., 1997). Vegetable oil is one of the fundamental components in foods and has important functions regarding human health and its nutritional physiology (Necdet camas et al., 2007). Particularly, consumers have demanded healthier oils, naturally low in saturated fat such as olive, safflower, canola and sunflower oils. The quality of oils is associated with their fatty acids (FA) composition, particularly with respect to the percentages of oleic (omega-9), linoleic (omega-6) and linolenic (omega-3) acids (jalilian et al., 2012). Safflower oil contains the saturated fatty acids palmitic (C16:0) and stearic (C18:0) and the unsaturated fatty acids oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) (Camas and Esendal, 2006). Oleic acid has desirable frying characteristics of stability and a bland flavor (Yeilaghi et al., 2012), while linoleic acid reduces the cholesterol level in the blood (Wilson et al., 2006).

Standard safflower oil contains about 6–8% palmitic acid, 2–3% stearic acid, 16–20% oleic acid, and 71–75% linoleic acid (Velasco and Fernandez-Martinez, 2001). Consumption of oils with a high concentration of unsaturated FA has been found to have a positive effect on human health (Hu et al., 2001).The seeds of safflower contain 35-50% oil, 15-20% protein and 35-45% hull fraction (Rahamatalla et al., 2001).Oil quality and yield are both dependent upon the genotype of a plant and its interaction with the environment (jalilian et al., 2012). Among the factors responsible for increasing crop yield and quality, irrigation (Blum, 1997) and fertilizer (Reddy et al., 2003) are the most important. The growth, development, and spatial distribution of plants are severely restricted by various environmental stresses. Among the different stresses that plants encounter, water deficit stress is one of the most serious conditions that affect crop productivity (Boyer, 1982). Water deficit disturbs the balance of the nutritional characteristics of plants. In the context of plant nutrition, nitrogen deficiency represents the most important limit for safflower production, which is also true for most other crops (Dordas and Sioulas, 2008).Despite of the important role of N in the productivity of crops, the use of N fertilizer is associated with economic and environmental risks, especially when poor N fertilizer management is employed (Sylvester-Bradley, 1993).

Beneficial microbes are living cells of different types of microorganisms that stimulate plant growth (Vessey, 2003). Positive interactions between free living, nitrogen-fixing rhizosphere bacteria belonging to the genera *Azotobacter* and *Azospirillum* and various field-grown crops have been recorded in a number of studies (Pandey and Kumar, 1989; Dobereiner and Pedrosa, 1987; De Freitas, 2000). Several studies have shown that beneficial microbes, such as *Azotobacter* and *Azospirillum,* are not only effective in terms of nitrogen fixation but also exhibit other favorable properties, including production of growth hormones (Remus et al., 2000), fungicidal substances (Lakshminarayana, 1993), and siderophores (Shah et al., 1992), and the ability to solubilize phosphate (Kumar and Narula, 1999). The beneficial effects of *Azospirillum* on plants are enhanced when it is coinoculated with other microorganisms. Apparently, coinoculation allows plants to obtain a better balance nutrition. Furthermore, the absorption of nitrogen, phosphorous and other mineral nutrients is improved (Rokhzadi and Toashih, 2011).

Plant growth can be increased by dual inoculation with *Azospirillum* and phosphate-solubilizing bacteria (Belimov et al., 1995). Wu et al. (2005) reported that the application of biofertilizer containing beneficial microbes (*Azotobacter chroococcum*, *Bacillus megaterium*, and *Bacillus mucilaginosus*) growth-promoting on maize, and improve the soil properties in a greenhouse study. The effects of beneficial microbes on several crops have been described previously (Zahir et al., 1998; Hatch et al., 2007). However, little work has been done to elucidate the effects of BM on safflowers, especially under water shortage conditions. The objectives of this study were to evaluate the effects of different soil fertility systems, as alone and combined application form, on safflower seed quality and quantity characteristics under water shortage conditions.

Materials and Methods

Field experiment was conducted at the Research Farm of Faculty Agriculture of Urmia University, (37° 32´ N and 45° 41´ E) with an altitude of 1320 m, West Azarbaijan province, Iran, during 2010 growing seasons (May to September) table 1 showed the some environmental condition during growing season. One week before sowing, soil samples were collected to determine soil characteristics.

The soil had a clay loam texture. The soil characteristics are presented in Table 2 for soil conditions at the start of growing seasons.

The experimental design was a randomized complete block design with a split-plot arrangement of treatments in three replicates. The treatments were three levels of irrigation regime levels [well-irrigation (I_1),irrigation disruption at vegetative growth stage (I_2) and irrigation disruption at reproductive growth stage (I_3)in the main plot] and seven types of fertilization [control (C), Urea (U), Humix as organic fertilizer (O), Biofertilizers (Nitroxin (N), Biosoulphour (B)), integrated fertilize treatments: (Urea + Humix + Nitroxin) (T_1), and (Urea + Humix + Biosoulphour) (T_2).in subplots].

Nitrogen was applied as urea. Half the dose of recommended N (100 kg ha^{-1}) was applied at sowing time, and the remaining N was topdressed at the 5-6 leaf stage.

Tow strains of bacteria, including *Azospirillum lipoferum, Azotobacter chroccoccum*, were used in this study as Nitroxin biofertilizer for seed inoculation of safflowers (2 liters ha^{-1}). Also biofertilizer of Biosoulphour containing sulfur oxidizing microorganisms (strains of *Thibacillus*) and the rate of (5 kg ha^{-1}) was used in this study in soil. Mehr Asia Biotechnology Co. (MABCO), Iran, supplied biofetilizers. Seed inoculation involved placement of the seeds in bacterial suspensions at 10^9 CFU ml^{-1} for 30 min before planting (Jalilian et al., 2012).

Humix as organic fertilizer (containing 12% folic acid, 68% humic acid and 15-13% potassium) were used in this study for seed inoculation of safflowers (2 kg for 1 ton of seed).

Inoculated seeds of safflower (*Carthamus tinctorius* L., cv. Goldasht) were hand sown at a 3 cm depth in the middle of rows on 31 May 2010. Individual subplots were 2 m long and consisted of 8 rows 0.55 m apart.

Safflower was over-planted and thinned to the recommended plant density (11.8 plants m^2) when most plants had 3–4 leaves. Weed controlled manually during the growing season.

All plants were irrigated uniformly with locally recommended (once irrigation per 8 days) (I_1), irrigation disruption at vegetative growth stage (V_{15}), until reducing soil moisture to 70% of field capacity (I_2) and irrigation disruption at reproductive growth stage ($R_{3.2}$) until reducing soil moisture to 70% of field capacity (I_3).Growth stage were determined according to tanaka et al (1997). The amount of water in each irrigation regimes (that measured by flumes type III (Washington State College)) was as follow:
[(I_1: 7599 m^3 ha^{-1}), (I_2: 6479 m^3 ha^{-1}) and (I_3: 5322 m^3 ha^{-1})].

After the safflower hybrids reached physiological maturity, seed yield was determined by harvesting of one m^2 the four central rows in the 23 September 2010. Plants were hand harvested from 4 m of row (one meter lengths of four rows) in each plot after leaving 0.5 m on each end of the row as borders. Subsamples of air dried seeds were left in bags at −10 °C until the oil and FA compositions were determined. A sample comprised of 50 g of clean seeds from each plot was isolated to measure the oil and protein concentrations. Soxhlet extraction was employed to determine the total oil concentration of the safflower seed. In the Soxhlet extraction procedure, 10 g of the milled seeds (20 mashes) was packed in a paper extraction thimble and the oils were extracted using 300ml of petroleum benzene (bp 40–60 ◦C, obtained from Merck Chemical Co., Germany) in a Soxhlet extractor for 4 h and the solvent was then evaporated. Oils were filtered and dehydrated by Whatmanno. 2 filter paper, anhydrous sodium sulfate, Buchner funnel, suction flask and vacuum pump. Based on whole seed, the oil concentration was expressed as mg g−1 (Movahhedy- Dehnavy et al., 2009).

The Seed protein content was calculated by multiplying total nitrogen content with factor 6.25. Total nitrogen content was determined by the micro-Kjeldahl method (Jackson et al., 1973). The oil and protein concentrations were reported as percent of the seed weight standardized to 8.5% moisture. The fatty acids composition of the safflower seed oils was determined according to Metcalf et al. (1966) using an Agilent gas chromatograph. A capillary column (BPX 70, 50 m by 0.25 mm) was used in a gas chromatograph equipped with an FID detector. The carrier gas was nitrogen and hydrogen.
The levels of palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids were determined using a computing integrator. The effects of the independent variables on oil concentration and the palmitic, stearic, oleic, and linoleic acid concentrations in the oil were analyzed on a g 100 g^{-1} total FA basis.

The data were analyzed by analysis of variance using the general linear model procedure in The Statistical Analysis System (SAS Institute, 2003). The UNIVARIATE procedure within SAS was used to examine the residuals for normality and to check for outliers in the data. Means were separated using Fisher's protected least significance difference (LSD) test at the 95% level of probability. The relative contributions of main and interaction effects of each treatment to total non-error variance were considered to simplify the reporting of the results. For determined main and interaction effects of treatments on FA composition descriptive analysis were used.
Descriptive statistics of mean fatty acid composition (mixture of 3 replications) including mean, standard deviation (SD), and range, are shown in Table 8.

Results and Discussion

Seed yield

Analysis of variance showed that there was a significant effect of the irrigation and fertilization treatments on seed yield (Table 3). The highest (2.952 t ha^{-1}) and lowest (2.04 t ha^{-1}) safflower seed yields were obtained from I_1 and I_2, respectively (Table 4). Also Plants that were treated with T_2 and control fertilization treatments had the highest (2830 kg ha^{-1}) and the lowest (1845.4 kg ha^{-1}) seed yield, respectively (table 5). Clavel et al (2005) reported that productivity decreased under water deficit conditions. Also it was found that a high seed yield was obtained when the sufficient irrigation water was applied during heading and flowering periods. In general, oil, protein and seed yield were increased by fertilization application compared with the control treatment (Table 5), the highest safflower productivity obtained from the integrated fertilize treatment.

Increased grain yield and improved growth due to integrated fertilize treatment and plant inoculation with *Azotobacter* and *Azospirillum* have been reported by (Shyalaja and Swarajyalakshmi, 2004; Fulchieri and Frioni, 1994; Jalilian et al., 2012). The stimulating effects of these microorganisms are attributed to their efficiency in supplying growing plants with dissolved immobilized nutrients and producing phytohormones, which could stimulate nutrient and water absorption as well as photosynthesis, leading to increased plant growth and yield (Remus et al., 2000; Kumar and Narula, 1999). Increasing the water deficit level strongly decreased the performance of the beneficial microbes (BM) alone treatment, and the advantage of the using anintegrated nutrition forms increased with increasing water stress level compared with the application of BM or N fertilizer alone. It found that low soil moisture adversely affected the survival and the metabolic functioning of microorganisms (Maot et al., 2002).

Oil concentration and yield

The oil concentration was affected by the interaction of irrigation and fertilization (Table 3). The highest (26.92%) and lowest (20.68%) oil concentrations were obtained in the I_1B and I_3Ctreatments, respectively (Fig. 1). Compared to the control treatment under I_3 condition, the application of Biosoulphour under well irrigation condition (I_1) increased the oil concentration approximately 6.24% (Fig. 1).

Oil yield was significantly affected by fertilization treatment (Table 3). Integrated fertilize treatments (Urea + Humix + Biosoulphour) (T_2) and control fertilization treatments produced the maximum (657.2 kg ha^{-1}) and minimum (455.3 kg ha^{-1}) oil yield (Table 5). Compared to the control treatment, the application of T_2 integrated fertilize treatment, increased the oil yield approximately 201.9 kg ha^{-1} (Table 5). The oil yield was increased approximately 30.72% in the T_2 integrated fertilize treatment compared with the control. Xia et al. (1990) and Babhulkar et al. (2000) found that the oil concentration of rapeseed and safflower, respectively increased in response to BM inoculation.

Protein concentration and yield

The interaction of irrigation and fertilization on protein concentration were significant (Table 3). So, the highest (28.41 %) and lowest (21.01 %) protein percent was obtained from the I_3B and I_1O treatment, respectively (Fig. 1). There was an increase in protein percentage approximately 7.4% with Biosoulphour application under irrigation disruption at reproductive growth stage (I_3) compared to the application of organic fertilizer (O) under well-irrigation condition (I_1) (Fig. 1). The results showed that there was a significant effect of the irrigation and fertilization treatments on protein yield (Table 3). The highest (706.2kg ha^{-1}) and lowest (503.1 kg ha^{-1}) protein yields were obtained from I_1 and I_2, respectively (Table 4). Protein yield decreased approximately 28.75 and 6.11% in irrigation disruption at vegetative growth stage (I_2) in comparison with the well-irrigation (I_1) and irrigation disruption at reproductive growth stage (I_3) regimes, respectively (Table 4). The maximum (674.7 kg ha^{-1}) and minimum (449.6 kg ha^{-1}) amount of protein yield was seen in the Nitroxin and Control fertilizer treatment, respectively (Table 5). The amount of protein yield per hectare (calculated from seed yields and protein concentration) in the Nitroxin treatment compared with the control was increased by 33.36% (Table 5).

Water deficit conditions increase protein and decrease oil concentrations presumably due to reduce the length of the growing season (Henry and MacDonald, 1978). Jalilian et al. (2012) showed that inoculation of sunflower with *Azotobacter chroccoccum* and *Azospirillum lipoferum* produced a higher grain protein concentration compared to a non-inoculated control.

Fatty acid composition

The results showed that the palmitic acid, stearic acid, oleic acid and linoleic acid concentrations were influenced by the irrigation regimes and fertilization treatments (Tables 6 and 7). The highest concentrations of linoleic acid (75.20%) and oleic acid (13.95%) were seen in plants under well irrigation regimes (Table 6). Furthermore the highest and lowest levels of palmitic acid (6.34%) and linoleic acid (74.48%) were obtained from I_2 irrigation regimes, respectively. But the plants that were under I_3 condition yielded the highest (2.44%) and lowest (13.37%) levels of stearic acid and oleic acid respectively (Table 6).

The highest levels of oleic acid (14.36%) and linoleic acid (75.40%) were obtained from control and biosoulphoure treatments, respectively. Whereas, the lowest levels of oleic acid (13.33%) and linoleic (74.15%) were observed in biosoulphoure and control treatments, respectively (Table 7). control plants and Plants receiving T_1 fertilization treatments produced the highest levels of palmitic acid (6.38%) and stearic acid (2.47%), respectively (Table 7).

Jalilian et al. (2012) showed that water deficit increased levels of saturated FA and decrease levels of unsaturated FA in sunflower (*Helianthus annuus* L.). Also reported that water deficit stress increased the proportions of saturated fatty acids in caraway (*Carum carvi* L.) (Laribi et al., 2009). Flagella et al. (2002) found that irrigation resulted in an increase in linoleic acid concentration, a concurrent decrease in oleic acid level, a slight increase in palmetic acid and a slight decrease in stearic acid. Finally, Salera and Baldini (1998) found no effect of water management on the oleic acid concentration both in standard and high oleic genotypes. These diverse results are likely due to the different environmental conditions in which sunflowers were grown (Fernández-Moya et al., 2002; Izquierdo et al., 2009).

The Plants receiving biosoulphoure produced the highest level of L and lowest level of O (Table 7). Gao et al. (2010) reported that nutrient application decreased the levels of oleic acid and increased the levels of linoleic acid in canola seeds. Zheljazkov et al. (2008) found that application of nitrogen fertilizer at a higher rate significantly increased oleic acid level at one location.

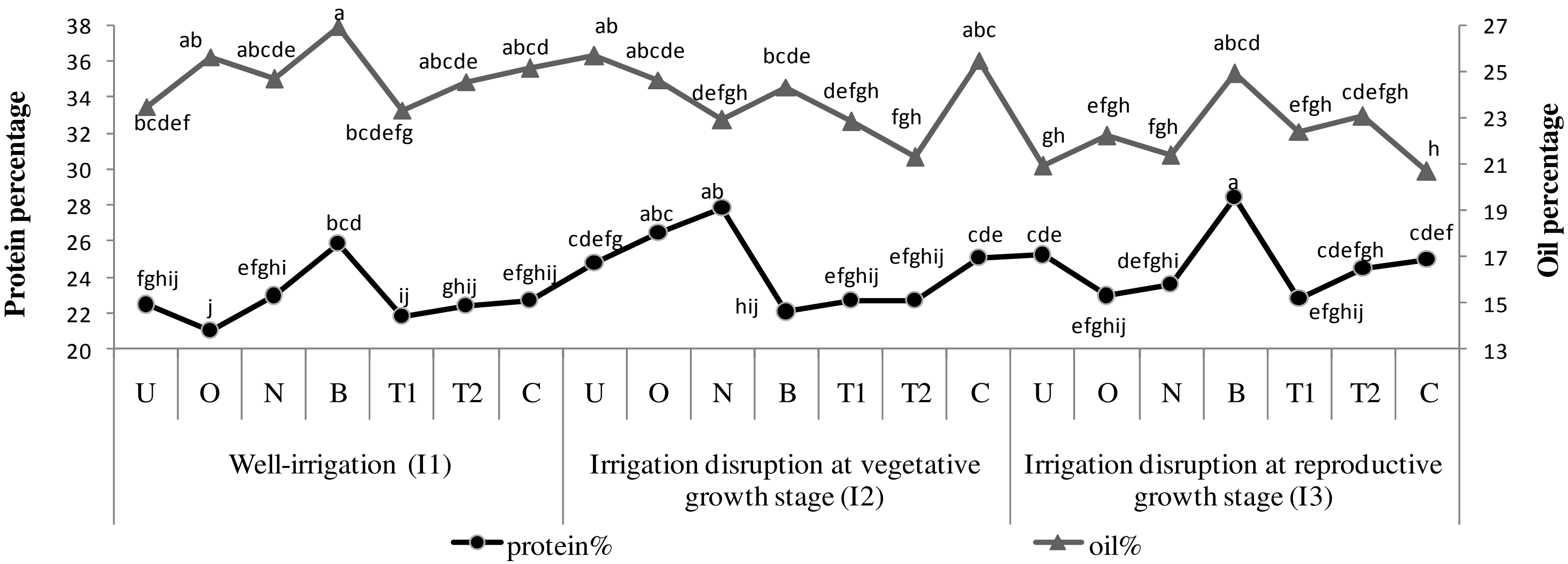

Figure1. Mean comparisons for interaction between Irrigation regimes and fertilization treatments on oil and protein content of safflower seeds. The means followed by same letter are not significant at p= 0.05 (LSD Test).

100 kg.ha^{-1} Urea (U), Humix as organic fertilizer (O), Biofertilizers (Nitroxin (N), Biosoulphour (B)), integrated fertilize treatments: (Urea + Humix + Nitroxin) (T_1), (Urea + Humix + Biosoulphour) (T_2), and Control (C).

Table 1. Monthly temperature and precipitation during the growing season in 2010–2011

Months	May	Jun	Jul	Aug	Sep
M.A.Max.T.(^{0}C)	28.87	32.61	32.51	29.58	25.33
M.A.Min.T. (^{0}C)	12.80	15.67	15.06	15.16	8.53
Rainfall (mm)	7.82	0	0	27.2	3.62

M.A.Max.T. = mean air maximum temperature; M.A.Min.T. = mean air minimum temperature
Safflower planting and harvesting dates were on 31 May and 23 September 2010, respectively.

Table 2. Physico-chemical properties of the soil

Soil depth (cm)	pH	E.C (ds m^{-1})	Soil texture	Organic C. (%)	N (%)	P (ppm)	K (ppm)	F.C* (% vol)	P.W.P* (% vol)
0-30	7.15	0.54	Clay loam	0.94	0.094	11.6	395	27.99	14.5

*F.C (field capacity) and P.W.P (permanent wilting point) are water content at 10 and 1500 kPa matric potential.

Table 3. Analysis of variance of yield, oil percent, protein percent, oil and protein yield of safflower (*Carthamus tinctorius* L.) affected by irrigation disruption under different soil fertility.

Source of variation	df	Mean square				
		Seed yield (kg ha^{-1})	Oil concentration (%)	Protein concentration (%)	oil yield (kg ha^{-1})	protein yield (kg ha^{-1})
Block (B)	2	31926.19	1.94	3.84	0.036	7557.28
Irrigation (I)	2	4605921.34 **	11.02 $^{n.s}$	6.77 $^{n.s}$	181177.9 $^{n.s}$	249739.28 **
B × I	4	214573.64	9.84	3.29	30056.60	11078.23
Fertilization (F)	6	1291913.30 **	12.04 **	9.21 **	54457.21 **	65112.32 **
I × F	12	96456.46 $^{n.s}$	7.44 **	14.02 **	8854.79 $^{n.s}$	9810.58 $^{n.s}$
Error	36	94373.31	2.20	2.36	7861.94	6318.59
CV (%)		12.61	6.27	6.41	15.5	13.66

ns, * and ** are non , Significant at p≤0.05 and p≤0.01, respectively

Table 4. Mean comparisons of seed and protein yield of safflower affected by irrigation disruption.

Irrigation regimes	Traits	
	Seed yield(kg ha^{-1})	Protein yield(kg ha^{-1})
I_1	2952.5^a	706.26^a
I_2	2040^b	503.14^b
I_3	2311.5^b	535.91^b

Within columns, means followed by same letter are not significantly at p= 0.05 (LSD Test).
Well-irrigation (I_1), irrigation disruption at vegetative growth stage (I_2), and irrigation disruption at reproductive growth stage (I_3).

Table 5. Mean comparisons of seed, oil and protein yield of safflower affected by different soil fertility.

Fertilization treatments	Traits		
	oil yield(kg ha^{-1})	protein yield(kg ha^{-1})	seed yield(kg ha^{-1})
U	606.01^a	636.8^a	2630.5^{ab}
O	631.93^a	608.77^a	2635.5^{ab}
N	630.12^a	674.73^a	2743.2^a
B	510.87^b	515.73^b	2015.5^c
T_1	512.31^b	526.6^b	2344.1^b
T_2	657.24^a	660.13^a	2830^a
C	455.33^b	449.62^b	1845.4^c

Within columns, means followed by same letter are not significantly at p= 0.05 (LSD Test).
100 kg.ha^{-1} Urea (U), Humix as organic fertilizer (O), Biofertilizers (Nitroxin (N), Biosoulphour (B)), integrated fertilize treatments: (Urea + Humix + Nitroxin) (T_1), (Urea + Humix + Biosoulphour) (T_2), and Control (C).

Table 6. Mean comparisons of descriptive analysis, for fatty acids composition of safflower seeds affected by irrigation disruption.

Irrigation regimes	Traits			
	Palmitic acid (percent in total fatty acid)	Stearic acid (percent in total fatty acid)	Oleic acid (percent in total fatty acid)	linoleic acid (percent in total fatty acid)
I_1	6.15	2.37	13.95	75.20
I_2	6.34	2.440	13.82	74.48
I_3	6.04	2.444	13.37	74.62

Well-irrigation (I_1), irrigation disruption at vegetative growth stage (I_2), and irrigation disruption at reproductive growth stage (I_3).

Table 7. Mean comparisons of descriptive analysis, for fatty acids composition of safflower seeds affected by different soil fertility.

Fertilization treatments	Traits			
	Palmitic acid (percent in total fatty acid)	Stearic acid (percent in total fatty acid)	Oleic acid (percent in total fatty acid)	linoleic acid (percent in total fatty acid)
U	6.18	2.45	13.50	75.10
O	6.183	2.31	13.68	74.69
N	6.083	2.41	13.58	75.17
B	6.100	2.46	13.33	75.40
T_1	6.237	2.47	13.82	74.44
T_2	6.120	2.39	13.80	74.42
C	6.380	2.41	14.31	74.15

100 kg.ha^{-1} Urea (U), Humix as organic fertilizer (O), Biofertilizers (Nitroxin (N), Biosoulphour (B)), integrated fertilize treatments: (Urea + Humix + Nitroxin) (T_1), (Urea + Humix + Biosoulphour) (T_2), and Control (C).

Table 8. Descriptive statistics analysis of mean fatty acid composition (mixture of 3 replications) affected by irrigation disruption under different soil fertility.

Fatty acids	Minimum	Maximum	Mean	Std. Deviation
Palmitic (C16:0)	5.85	6.84	6.18	0.207
Stearic (C18:0)	2.23	2.57	2.41	0.853
Oleic (C18:1)	12.87	14.90	13.72	0.551
Linoleic (C18:2)	72.57	76.19	74.77	0.909

Conclusion

The results of this study showed the Water deficit conditions increase protein and decrease oil concentrations. The lowest level of unsaturated fatty acids (palmetic and stearic acid) and highest level of saturated fatty acids (oleic and lenoleic acid) were observed in the water deficit conditions. Combined application of organic fertilizer and biofertilizers (nitroxin and biosoulphoure) with N fertilizer improved safflower productivity compared to their individual application. The highest protein and oil yields were obtained from T_2 and nitroxin fertilization treatments, respectively. In general, the application of biofertilizers increased the levels of unsaturated fatty acid (linoleic acid) and decrease saturated fatty acid (palmetic acid).

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