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Do Fertilizers and Irrigation Disruption Change Some Physiological Traits of Safflower?

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Abstract
To investigate the effects of nanofertilizers and biofertilizers on the morpho-physiological and biochemical traits of safflower under full irrigation and water deficit stress, this study was carried out as a split-plot experiment based on a Randomized Complete Block Design with three replications at Urmia University in 2015. The main plot was full irrigation (control) and irrigation disruption at heading, flowering, and grain filling stages. Fertilizers, including control (without fertilizer), biofertilizer, water spray, foliar application of nanofertilizers, chemical fertilizers, and combined application of fertilizers, were assigned to the subplot. Plants under full irrigation and combined fertilizers had maximum height and chlorophyll *a*, whereas the lowest ones were obtained in irrigation disruption at the heading stage and control treatments. The maximum oil content (28.41%) was detected in irrigation disruption at the grain filling stage and nanofertilizer treatment, the lowest (21.96%) was obtained at irrigation disruption at the flowering stage and water spray treatment. The highest proline (397.21 μg g^{-1} fresh leaf) was found in irrigation disruption at the grain filling stage and water spray treatment, and the lowest (154.68 μg g^{-1} fresh leaf) was obtained at full irrigation and water spray treatment. Irrigation disruption at the heading stage and control treatments decreased carbohydrate content of fresh leaves by 86.54% compared to full irrigation and the combined fertilizers treatment. Irrigation disruption increases saturated fatty acids (palmitic and stearic acid) and decreases vitamin E and linoleic acid. The combined application of fertilizers significantly increased safflower oil quality. Overall, concerning the obtained highest oil percentage (28.41%), irrigation disruption during grain filling reduced water consumption and application of combined fertilizer via improving oil quality, so it is recommended to farmers.

Keywords Biofertilizer · Chemical fertilizer · Foliar application · Irrigation · Nanofertilizer

Introduction

Safflower (*Carthamus tinctorius* L.) is one of the most important industrial crops in arid and semiarid regions of Iran, where some abiotic stresses such as salinity and drought are prevalent. Safflower is a tap-rooted multipurpose crop that is able to tolerate environmental stresses, so it can be a candidate crop for dryland agro-ecosystems due to its potential for growth under water stress and the economic value in terms of both oil and seed (Yau 2004). One of the important priorities of the agricultural sector in Iran could be enhancing oil yield of safflower because of its lower seed yield as compared to other countries (Haghighati 2010).

Nitrogen is a chemical fertilizer that has an important role in improving the growth and yield of safflower significantly in dry conditions (Kulekci et al. 2009). However, intensive utilization of chemical fertilizers entails several ecological issues and increases the production costs and food insecurity. Integrated nutrient management and irrigation are practically two elements of crop production. The application of biofertilizers is critical in the agricultural sector for sustainability of soil fertility, plant growth and development, and final yield performance (Bhardwaj et al. 2014). Biofertilizers contain living cells or efficient strains of symbiotic and non-symbiotic microorganisms. These beneficial bacterial or fungal inoculants accelerate the uptake of nutrients in the rhizosphere once applied over seed and soil. Various studies have documented that plant growth-promoting rhizobacteria can promote plant growth by various mechanisms such as fixation of atmospheric nitrogen, production of siderophores that chelate metal elements and make them accessible to

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plant roots, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Gusain et al. 2015).

It appears that nanofertilizers have high potential to attain sustainable plant production. Nanofertilizers are a novel agricultural input that can release nutrients into the soil steadily and in a controlled way, thereby evading ecological damages and enhancing the crop yield and profitability (Sekhon 2014). For instance, nanoparticles of titanium dioxide increase chlorophyll synthesis, the activity of Ribulose 1,5-bisphosphate carboxylase enzyme, photosynthesis, plant growth, and health. Moreover, these nanoparticles enhance electron transportation and light energy conversion, prolong the photosynthetic time in the chloroplasts, increase light absorbance, and postpone chloroplast aging (Yang et al. 2006).

In semiarid regions, where water resources are limited, the main challenge for the coming decades would be coping with food needs by less water. The growing interest in increasing water efficiency has resulted in conducting a mild stress irrigation strategy with minimal impact on plant performance (Geerts and Raes 2009). This method that can be applied by farmers is more profitable and will maximize water use efficiency rather than harvest per unit area (Fereres and Soriano 2006).

Fatty acids are generally distributed in plant oils as the primary components (Carvalho et al. 2006). Many studies have shown that polyunsaturated fatty acids (PUFAs), especially linoleic and linolenic acids, play an important role in human health. However, because these fatty acids are not synthesized in the human body, they should be included in the diet to meet the needs of the human body (Yu et al. 2005). Safflower oil is principally composed of palmitic, stearic, oleic and linoleic acids, but the main constituents are oleic and linoleic acids (Coşge et al. 2007). Depending on the genetics of the individual safflower cultivars, different types of oil can be produced which would be high in either linoleic acid or oleic acid or will contain roughly equal quantities of them (Besbes et al. 2005). It has been reported that 60–70% of oil accumulated in safflower seeds is biosynthesized within 22 days after flowering; hence, any nutrition deficiency or stress during this time would have a detrimental impact on the amount and composition of fatty acids (Slack et al. 1985).

The value of safflower cultivars can be improved with the availability of high-quality oils, which depends on the diversity of existed fatty acids in safflower. Standard safflower oil contains fatty acid compounds that have 6–8% palmitic acid, 2–3% stearic acid, 6–20% oleic acid, and 71–75% linoleic acid (Coşge et al. 2007). The Safflower Germplasm Organization has reported substantial variation in the percentage of oleic acid (3.1–90.6%) and linoleic acid (3.9–88.8%) (Knowles 1965; Fernandez-Martinez et al. 1993). Linoleic oil of safflower contains 70–75% polyunsaturated linoleic acid, while oleic oil of safflower contains 75–80% monounsaturated oleic acid, which is identical to olive oil in terms of quality.

Linoleic acid is an essential fatty acid, and its high quantity in safflower oil reduces cholesterol levels in human blood (Herbel et al. 1998). Nevertheless, linoleic acid is easily polymerized after being heated and is not suitable for long frying. It should be noted that safflower oil has high oleic acid levels, so it has high oxidative stability, which makes it resistant to prolonged frying (Fuller et al. 1967). In fact, high unsaturation single bound in oleic acid makes safflower oil apposite to a series of chemical changes to avoid having different properties.

Despite the wealth of information available individually on the foliar application of some nanoparticles and applying biofertilizers on plant growth and development, there is insufficient information about the efficiency of nanofertilizers and biofertilizers in combination with each other and chemical fertilizers under water shortage conditions. Thus, this study was conducted to investigate the effects of nanofertilizers and biofertilizers on the morpho-physiological and biochemical traits of safflower under full irrigation and water deficit stress.

Materials and Methods

The experiment was carried out at the experimental farm of Urmia University, Iran (longitude of 45°41′ E, latitude of 37°32′ N and altitude of 1320 m above the sea level) in 2015. The experiment was laid out as split plot based on a randomized complete block design (RCBD) in three replications. The main plot was assigned to the irrigation regime at four levels including full irrigation, and irrigation disruption at heading, flowering, and grain filling stages. The treatments in the sub-plot were sources of fertilizer comprised of control (without fertilizer), water spray, and foliar application of nanofertilizer, chemical fertilizer, biofertilizer, and combined application of fertilizers. Each plot was 3.5 × 4.5 m and contained seven sowing rows with 50 cm space between rows and 10 cm between plants on the rows. Top 0–60 cm soil samples were randomly collected from the field and analyzed for physicochemical properties (Table 1).

Biofertilizers, i.e., 100 g ha^{-1} Azote Barvar-1, 100 g ha^{-1} Phospho Barvar-2 and 5 kg ha^{-1} Biosulfur with 250 kg mineral sulfur, were applied for 100% biofertilizer treatment. Nanofertilizer, 2 l ha^{-1} NanoChelate Super Micro Plus and 3 kg ha^{-1} Green Micro ($N–P_2O_5–K_2O$, 20–20–20%) were applied for foliar application of chemical fertilizer. For combined fertilizer treatment, one-half was chemical and nanofertilizer together and the other half was biofertilizer. The first irrigation was conducted just after sowing. Hand weeding was carried out 30 and

Table 1 Soil physical and chemical properties of experimental area

Soil texture	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Total N (%)	Organic carbon (%)	EC (dS m^{-1})	pH
Clay loam	11.6	395	0.094	0.94	0.54	7.15

45 days after the sowing date and repeated for 12 weeks every week. Irrigation was conducted every 10 days in the full irrigation treatment. Irrigation disruption treatments in the above-mentioned stages were continued until the appearance of wilt. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation. Foliar treatment was applied at four stages (stem elongation, heading, flowering, and grain filling). For pest control, 2 l of Diazinon in per 1000 l of water was applied at the first heading stage. The central two rows of each plot were harvested at maturity, and different agronomic traits were measured in each plot. Harvested leaves were placed in an ice container, and then, they were transferred to the crop physiology laboratory.

The leaf chlorophyll content was determined by rinsing the leaves in 85% acetone, and the homogenate was centrifuged at 2500 rpm for 10 min. The absorbance of the supernatant was detected at 663 and 645 nm. Using a PD-303 spectrophotometer Arnon formulated Mackinney's work to get chlorophyll concentration shown in Equation (Arnon 1949).

$$C_{\text{chl-}a} = 12.7A_{663} - 2.69A_{645} \quad (1)$$

$$C_{\text{chl-}b} = 22.9A_{645} - 4.68A_{663} \quad (2)$$

Field grown leaves of safflower were sampled; then, free proline was estimated by the following procedure: 200 µl of potassium phosphate extract was mixed with 800 µl of ninhydrin reagent that contained 1% (w/v) ninhydrin in 60% acetic acid solution (Magné and Larher 1992). The mixture was heated at 100 °C for 20 min and then kept cool in ice. One milliliter of toluene was added, and the sample was vigorously shaken for 15 s. The absorbance of the supernatant was, then, read spectrophotometrically at 520 nm. The proline content was expressed in µg g^{-1} FW.

For the measurement of total carbohydrate, fresh leaves were grounded at 4 °C in 0.1 M phosphate buffer (pH 7.5). Then, the homogenate was centrifuged at 12,000 rpm at 4 °C for 15 min. Afterward, the supernatant was utilized to detect carbohydrates. Next, 200 µl of the potassium phosphate extract was mixed with 1 ml of anthrone-sulfuric reagent (0.1% anthrone and 0.1% thiourea in 12.5 N sulfuric acid) and incubated at 100 °C for 10 min. After cooling, the absorbance was read at 625 nm (Yemm and Willis 1954), using glucose as the standard. The results were expressed in milligram glucose equivalents g^{-1} FW.

To determine the oil percentage, the ground seeds were oven-dried at 75 °C for 24 h. Then, an amount of 6 g of each sample was placed in the soxhlet. The samples were washed for 6 h using *N*-hexane, and fats were removed. Then, the samples again were transferred to an oven at 75 °C for 24 h for removing the moisture. After calculating weight loss in the samples, the oil percentage was determined (Horwitz 2000).

The fatty acid composition of the safflower seed oil was determined according to Metcalf et al. (1966) using gas chromatography (Agilent 5973). 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.

Analysis of variance was done by using the general linear model procedure in the statistical analysis system (SAS Institute 2003). Means were separated using the Duncan test at the 95% level of probability.

Results

Analysis of variance showed that the interactive effect of irrigation and different sources of fertilizer was significant on plant height at the 1% probability level (Table 2). The highest plant height (79.5 cm) was observed in full irrigation and the combined fertilizers treatment, whereas the lowest one (62.47 cm) was detected under irrigation disruption at the heading stage and in the control fertilizer treatment (Table 2). The interactive effect of irrigation and different sources of fertilizer was significant on the number of branches per plant at the 1% probability level (Table 2).

Means comparison (Table 2) showed that the highest number of branches per plant (9.53) was found in full irrigation and the combined fertilizers treatment and the lowest number (6.23) was observed in irrigation disruption at the grain filling stage for the control condition (without fertilizer).

The findings revealed that the interaction of irrigation disruption and different sources of fertilizer was significant for stem diameter at the 1% probability level (Table 2). Moreover, means comparison (Table 2) indicated that the highest

Table 2 Mean comparisons and analysis variance of some measured traits of safflower affected by irrigation disruption and fertilizer sources

Irrigation regimes	Fertilizer treatment	Plant height (cm)	Branches number	Stem diameter (cm)	Oil percent (%)	Chlorophyll-a (mg g^{-1} fresh leaf)	Chlorophyll-b (mg g^{-1} fresh leaf)	Carotenoid (mg g^{-1} fresh leaf)	Proline (µg g^{-1} fresh leaf)	Carbohydrate (mg g^{-1} fresh leaf)
FI	Control	64.37l	7.73f–h	0.65h	25.85c–f	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	Water spray	71.57h	7.77f–h	0.71g	25.06d–g	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	FANF	79.50a	7.9e–h	0.86c	28.27ab	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	FACF	76.63c	8.8a–h	0.91b	25.34c–g	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	AB	67.67j	8.1c–h	0.76ef	24.40f–h	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	CANBCF	74.43e	9.53a	0.94a	27.02a–c	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
IDHS	Control	62.47m	6.5j	0.65h	23.77g–j	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	Water spray	64.60l	7.97e–h	0.73fg	24.75eg	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	FANF	73.50f	8d–h	0.82d	25.18c–g	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	FACF	71.43h	7.33g–i	0.78e	23.49g–j	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	AB	62.53m	8.23hi	0.66h	23.84g–i	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	CANBCF	72.77f	8.47c–f	0.83cd	23.66g–j	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
IDFS	Control	69.20i	8.8a–d	0.66h	22.59h–j	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	Water spray	68.83i	9.27ab	0.76ef	21.96j	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	FANF	72.43g	9.37ab	0.86c	26.05c–f	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	FACF	72.17h	8.33c–f	0.86c	26.53b–e	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	AB	69.10i	6.93ij	0.64h	22.49ij	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	CANBCF	73/33f	8.67b–e	0.84cd	24.29f–i	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
IDGFS	Control	66.47k	6.23j	0.73fg	26.80a–d	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	Water spray	69.43i	8.13c–g	0.76ef	25.93c–f	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	FANF	75.23d	8.23c–f	0.84cd	28.41a	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	FACF	77.83b	8.87a–c	0.85cd	24.42f–h	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	AB	64.70l	8.8a–d	0.63h	25.75c–f	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
	CANBCF	72.37g	9.43ab	0.86c	25.92c–f	Figure 1	Figure 1	Figure 1	Figure 2	Figure 2
Replication		ns	ns	ns	ns	ns	ns	ns	ns	ns
Irrigation (I)		**	**	**	**	**	**	**	**	**
Fertilizers (F)		**	**	**	**	**	**	**	**	**
I×F		**	**	**	**	**	**	**	**	**
CV (%)		6.519	5.15	2.38	1.47	5.13	2.54	8.33	1.45	3.95

ns, *, and **: nonsignificant, significant difference in 5% and 1% level, respectively. The same letters in each column show nonsignificant difference at $p \leq 0.01$ by Duncan test

FI full irrigation, *IDHS* irrigation disruption at heading stage, *IDFS* irrigation disruption at flowering stage, *IDGFS* irrigation disruption at grain filling stage, *FANF* foliar application of nanofertilizer, *FACF* foliar application of chemical fertilizer, *AB* application of biofertilizer, *CANBCF* combined application of nanofertilizers, biofertilizers and chemical fertilizers

stem diameter (0.94 cm) was detected in full irrigation and the combined fertilizers treatment and the lowest (0.63 cm) was obtained from irrigation disruption at the grain filling stage in the biofertilizer treatment.

The oil content of seeds (Table 2) in irrigation disruption at the grain filling stage treated with nanofertilizer (28.41%) was significantly higher than that of irrigation disruption at the flowering stage treated with water spray (21.96%). As far as the chlorophyll content of safflower is concerned, significant differences were observed in terms of the interaction of irrigation disruption and different sources of fertilizer on chlorophyll *a* at the 1% probability level (Table 2). In general, full irrigation and combined fertilizers treatment had the highest amounts of chlorophyll *a* (24.07 mg g^{-1} fresh leaf), whereas irrigation disruption at the heading stage in the control fertilizer treatment had the lowest (21.76 mg g^{-1} fresh leaf) (Fig. 1).

ANOVA also showed that irrigation × fertilizer had a significant ($p < 0.01$) effect on chlorophyll *b* (Table 2). The highest chlorophyll *b* (20.51 mg g^{-1} fresh leaf) was observed in irrigation disruption at the grain filling stage as well as the combined fertilizers treatment, whereas the lowest one (13.52 mg g^{-1} fresh leaf) was found at full irrigation and the combined fertilizers treatment (Fig. 1).

Irrigation disruption at the heading stage in control condition was found to have the highest carotenoid concentration so that the carotenoid content was 21.70 mg g^{-1} fresh leaf, while the lowest content (13.46 mg g^{-1} fresh leaf) was found in full irrigation and the combined fertilizers treatment (Fig. 1).

When comparing the combined effects of irrigation disruption and different sources of fertilizer treatment on proline, it was found that there were highly significant differences. It could be seen from the results that the highest proline (397.21 µg g^{-1} fresh leaf) was observed in irrigation disruption at the grain filling stage and water spray treatment, and the lowest (154.68 µg g^{-1} fresh leaf) was at full irrigation and water spray treatment (Fig. 2).

In general, significant differences were observed in total soluble carbohydrates among the interactions of irrigation disruption and different sources of fertilizer treatments. The total soluble carbohydrates content of safflower in full irrigation and the combined fertilizers treatment was the highest (91.69 mg g^{-1} fresh leaf), and the lowest (12.34 mg g^{-1} fresh leaf) was observed at irrigation disruption at the heading stage and in control (Fig. 2).

Analysis showed that the effect of irrigation was significant on oil component at the 1% probability level except oleic acid (Table 3). In addition, the effect of fertilizers on all fatty acids and vitamin E was significant at the 1% probability level (Table 3). The highest palmitic acid (89.05 mg g^{-1}) was observed in irrigation disruption at the flowering stage, whereas the lowest one (70.25 mg g^{-1}) was detected under full irrigation (Table 3).

For stearic acid (40.56 mg g^{-1}), the highest was in irrigation disruption at the heading stage and the lowest (34.56 mg g^{-1}) was detected under full irrigation (Table 3). The maximal oleic acid (110.84 mg g^{-1}), linoleic acid (770.26 mg g^{-1}), vitamin E (0.2747 mg g^{-1}), total unsaturated fatty acids (881.10 mg g^{-1}), and ratio of unsaturated fatty acids to saturated fatty acids (8.47%) were observed in full irrigation, whereas the minimum oleic acid (104.50 mg g^{-1}), vitamin E (0.1994 mg g^{-1}), total unsaturated fatty acids (844.143 mg g^{-1}), and ratio of saturated

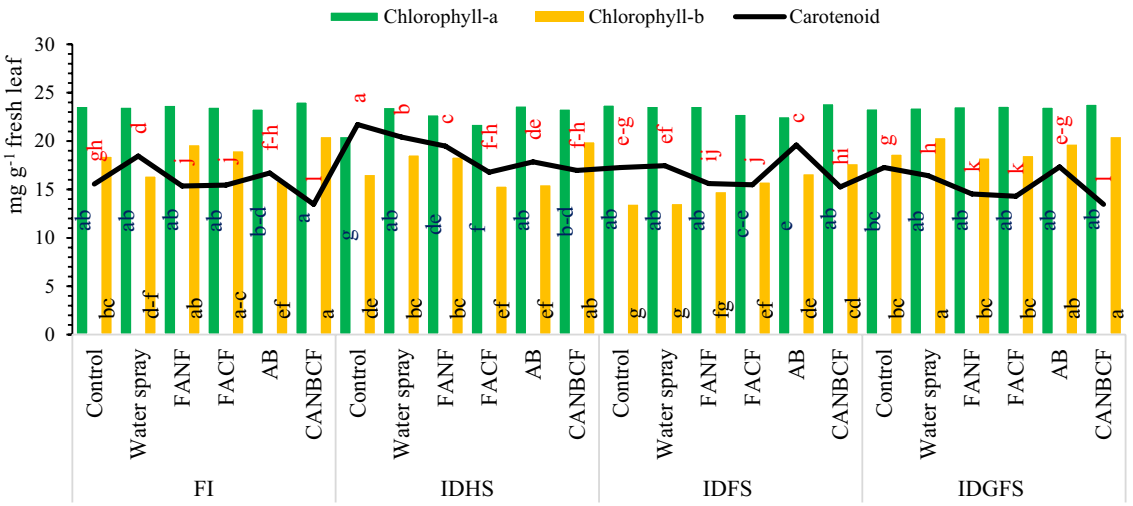

Fig. 1 Means comparison of interaction effects of irrigation disruption and fertilizers sources on chlorophyll *a*, chlorophyll *b* and carotenoid of safflower. *FI* full irrigation, *IDHS* irrigation disruption at heading stage, *IDFS* irrigation disruption at flowering stage, *IDGFS* irrigation disruption at grain filling stage. *FANF* foliar application of nanofertilizer, *FACF* foliar application of chemical fertilizer, *AB* application of biofertilizer, *CANBCF* combined application of nanofertilizers, biofertilizers and chemical fertilizers

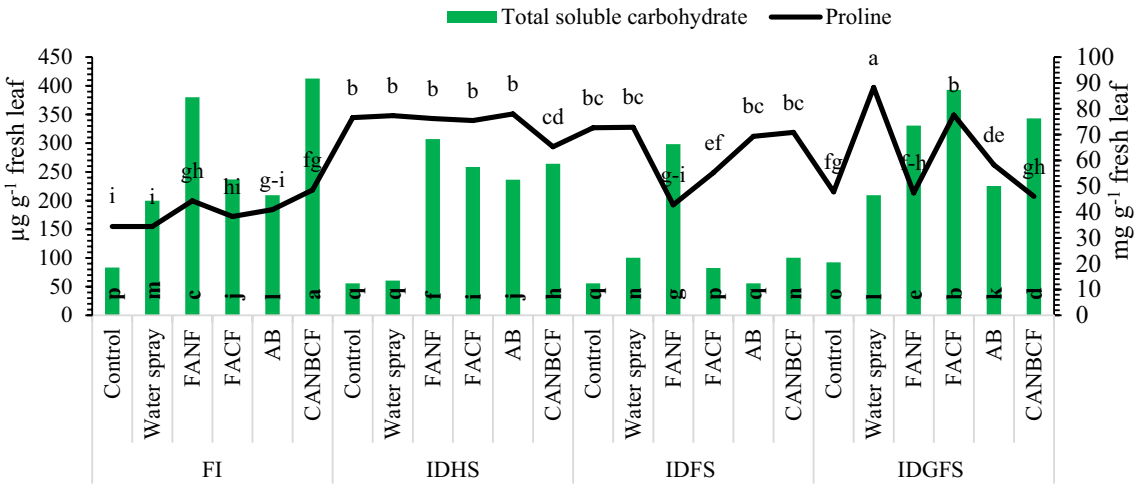

Fig. 2 Mean comparisons of interaction effects of irrigation disruption and fertilizers sources on safflower proline and total soluble carbohydrates. *FI* full irrigation, *IDHS* irrigation disruption at heading stage, *IDFS* irrigation disruption at flowering stage, *IDGFS* irrigation disruption at grain filling stage. *FANF* foliar application of nanofertilizer, *FACF* foliar application of chemical fertilizer, *AB* application of biofertilizer, *CANBCF* combined application of nanofertilizer, biofertilizer and chemical fertilizers

Table 3 Means comparison and analysis variance of fatty acids of safflower seed oils affected by irrigation disruption and fertilizers sources

Irrigation regimes	Palmitic acid (mg g^{-1})	Stearic acid (mg g^{-1})	Oleic acid (mg g^{-1})	Linoleic acid (mg g^{-1})	Vitamin E (mg g^{-1})	Total saturated fatty acids (mg g^{-1}) (S)	Total unsaturated fatty acids (mg g^{-1}) (US)	Ratio of US/S
FI	70.25c	34.56c	110.84a	770.26a	0.2747a	104.80c	881.10a	8.47a
IDHS	75.86b	40.56a	107.99ab	739.25b	0.2002b	116.42b	847.24b	7.30b
IDFS	89.05a	37.19bc	104.50b	739.64b	0.1994b	126.24a	844.143b	6.76c
IDGFS	75.02b	38.41ab	105.52ab	750.29b	0.2150b	113.43b	855.81b	7.65b
Fertilizer treatments								
Control	84.01a	46.03a	95.15d	717.53c	0.1802d	130.03a	812.68c	6.30e
Water spray	79.51ab	42.10b	98.25dc	728.88c	0.1969 cd	121.61b	827.13c	6.85d
FANF	74.30dc	33.93dc	112.06b	772.08a	0.2451b	108.23 cd	884.14a	8.20b
FACF	77.42bc	36.36c	113.76ab	762.34ab	0.2214bc	113.77c	876.11a	7.76bc
AB	77.75bc	36.87c	104.58c	746.29b	0.2069 cd	114.62c	850.87b	7.48c
CANBCF	72.29d	30.81d	119.48a	772.05a	0.2837a	103.10d	891.53a	8.70a
Irrigation	**	**	ns	**	**	**	**	**
Fertilizers	**	**	**	**	**	**	**	**
CV (%)	4.06	6.81	4.05	1.54	10.54	3.60	1.40	3.95

ns, *and**: nonsignificant, significant difference in 5% and 1% level, respectively

The same letters in each column show nonsignificant difference at $p \leq 0.01$ by Duncan test

FI full irrigation, *IDHS* irrigation disruption at heading stage, *IDFS* irrigation disruption at flowering stage, *IDGFS* irrigation disruption at grain filling stage, *FANF* Foliar application of nanofertilizer, *FACF* Foliar application of chemical fertilizer, *AB* application of biofertilizer, *CANBCF* combined application of nanofertilizer, biofertilizer and chemical fertilizers

fatty acids to unsaturated fatty acids (6.76%) were detected under irrigation disruption at the flowering stage. The lowest linoleic acid (739.25 mg g^{-1}) was found under irrigation disruption at the heading stage (Table 3). The highest total saturated fatty acids (126.24 mg g^{-1}) were observed in irrigation disruption at the flowering stage, whereas the lowest (104.80 mg g^{-1}) was under full irrigation (Table 3).

In relation to the fertilizer treatments, the safflower plants in the control condition had the highest palmitic acid (84.01 mg g^{-1}), stearic acid (46.03 mg g^{-1}), and total saturated fatty acids (130.03 mg g^{-1}), whereas the lowest levels (72.29 mg g^{-1}, 30.81 mg g^{-1}, 103.10 mg g^{-1}, respectively) were detected under combined application of nanofertilizer, biofertilizer, and chemical fertilizers, respectively

(Table 3). For linoleic acid, the highest (772.08 mg g^{-1}) was found in foliar application of nanofertilizer and the lowest amount (717.53 mg g^{-1}) was detected under control condition (Table 3). The maximum amount of oleic acid (119.48 mg g^{-1}), total unsaturated fatty acids (891.53 mg g^{-1}), vitamin E (0.2837 mg g^{-1}), and ratio of saturated fatty acids to unsaturated fatty acids (8.70%) were found under combined application of nanofertilizer, biofertilizer, and chemical fertilizers, whereas the lowest ones (95.15 mg g^{-1}, 812.68 mg g^{-1}, 0.1802 mg g^{-1}, and 6.30%, respectively) were in the control condition (Table 3).

Discussion

Our results have shown that some morphological traits (the plant height, the number of branches per plant and stem diameter) of safflower were the highest in full irrigation and combined fertilizer treatments as compared with the other treatments (Table 2). In fact, irrigation disruption at different growth stages of safflower caused reduced morphological traits of safflower (Table 2). Drought stress often reduces the size of the plant (plant height), changes the color of the leaves, leaf surface durability, dry matter production, plant photosynthesis, and the storage of assimilates in the shoot and eventually decreases grain yield (Kumar 2000). This finding confirms the result of Ryan et al. (2012) who suggested that in semiarid regions, especially in areas faced with water shortages, application of just biofertilizers cannot be an eligible nutrient management option. In other words, the use of bacteria causes root development and the better uptake of water and nutrients and is effective on vegetative growth and plant height (Biari et al. 2008). The combined fertilizer indicated 0.31 cm greater stem diameter than biofertilizer, which confirms higher efficiency of combined fertilizer. The superiority of combined chemical and organic fertilizers has been reported too (Bulluck Lii et al. 2002). It seems that the integrated nutrient management strategies involving chemical fertilizers, organic manures, and biofertilizers are the only viable means of bridging the gap between nutrient requirement and supply to improve agricultural production (Zougmore et al. 2014).

The reduction of 22.7% of oil content in terms of irrigation disruption at the flowering stage and application of water spray as compared to irrigation disruption at the grain filling stage with application of nanofertilizer treatment indicated the sensitivity of safflower oil synthesis at the flowering stage (Table 2). The reduction in oil percentage under drought stress may be due to a disorder in the seed metabolism and assimilate transmission to the grain (Bouchereau et al. 1996).

The reaction of photosynthetic pigments was very different in relation to experimental treatments so that the irrigation disruption at the heading and flowering stages caused a decrease in chlorophyll a and b, respectively, under control fertilizer treatment (Fig. 1). In addition, the carotenoid content was the lowest in safflower plants under irrigation disruption at the grain filling stage and combined application of nanofertilizer, biofertilizer and chemical fertilizers (Fig. 1). It can be pointed out that drought stress, due to an increase in free radicals, peroxidation and decomposition of the chlorophyll, caused a reduction in Chl-a contents as compared to their controls (Schutz and Fangmeir 2001). The amount of chlorophyll in living plants is one of the important factors for photosynthesis capacity (Jiang and Huang 2001). There are credible results obtained from several studies for the carotenoid pigmentation response because of irrigation disruption and application of fertilizer. As pointed out by other scholars, plants under the lowest soil moisture level had maximum carotenoid concentration, whereas the highest soil moisture level exhibited the lowest one (Frank and Cogdell 1996). Biofertilizer resulted in increased carotenoid content under drought conditions, indicating that carotenoids have crucial functions in the photosynthesis apparatus and photo-protection. Besides their structural roles, they have antioxidant activity properties through quenching Chl_3 and O_2, restrain lipid peroxidation, and contribute to the maintenance of the membrane (Frank and Cogdell 1996). Zaimenko et al. (2014) stated that utilization of nanoparticles (NPs) in corn and wheat under drought stress improved photosynthetic pigments, accumulation of secondary metabolites and antioxidants activity.

Results showed that at all levels of fertilizer treatments, irrigation disruption increased proline in comparison with full irrigation treatment. In addition, the trend changes in soluble carbohydrates were very different in relation to experimental treatments (Fig. 2). The high quantity of proline in plant cells might be because of higher stimulation of the proline biosynthesis pathway, stabilizing proline enzymes, facilitating cells to maintain their water status, and improving the production of some compounds derived from photosynthesis, which protects plant vital functions against detrimental impacts of drought stress (Schutz and Fangmeir 2001; Zhang et al. 1999). It was demonstrated in other studies that in contrast to soluble carbohydrates, non-reducing and total sugar amounts in shoots of oregano plants were decreased significantly in response to water stress application, and this reduction was most expressed with the increase in the intensity of drought. Concerning total soluble carbohydrates, our results were similar to those of other studies using different plants (Sawhney and Singh 2002; Zhang et al. 2007). The reduction can be assigned to soil water deficit that activates specific chemical stimulus (mostly ABA) through stomata conductance, CO_2 fixation in leaf, electron transport system, and the rate of photosynthesis and ultimately, the quantity of assimilates, thereby

leading to the decline in growth rates. Statistical experiments revealed that biofertilizers significantly enhanced the amount of reducing, non-reducing and total sugars due to boosting carbon fixation and activation of enzymes (Mathur and Vyas 2000; Nelson and Achar 2001).

In comparison with full irrigation, irrigation disruption at the heading and flowering stages increases saturated fatty acids (palmitic and stearic acids), but decreases unsaturated fatty acids (oleic and linoleic acids) and vitamin E in safflower seeds (Table 3). With respect to drought, studies have shown that this stress can cause serious and harmful changes in the content of phospholipid and galactolipid, as well as an increase in neutral lipid content (Navari-Izzo et al. 1989). Water shortages have also been reported to reduce the levels of free sterols (Quartacci et al. 1995). Similar results were observed in canola roots (Svenningson and Liljenberg 1986). Dorenbos and Mullen (1992) found that under drought stress conditions in soybeans seeds, the content of stearic and oleic acid increased; however, linoleic acid was decreased.

Results showed that the combined application of nanofertilizers, biofertilizers, and chemical fertilizers increased oleic and linoleic acid, vitamin E, total unsaturated fatty acids, and the ratio of total unsaturated fatty acids to saturated fatty acids, whereas this treatment reduced palmitic acid, stearic acid, and total saturated fatty acids (Table 3). As stated by Coşge et al. (2007), the chemical composition of safflower oil can be influenced by some factors such as ecology, physiology, genotype, morphology, and fertilization. The quality of safflower oil is attributed to oil constitution including saturated and unsaturated fatty acids. The designation of safflower response to chemical fertilizers and biofertilizers is crucial to enhance safflower yield and economic profitability. Seed inoculation with biofertilizers decreased the amount of saturated fatty acids (palmitic and stearic acids) and boosted the amount of unsaturated fatty acids (linoleic, linolenic, and oleic acids) (Sharifi et al. 2017). Furthermore, Mirzakhani et al. (2009) disclosed that safflower seeds inoculated with *Azotobacter* resulted in yield enhancement. As a biological method for safflower production, grain oil content and yield components of safflower were affected with *Azotobacter chroococcum* and the *Arbuscular mycorrhizal* as well as a desired partial replacement for N and P fertilization (Mirzakhani et al. 2014).

Sharifi et al. (2017) revealed that safflower seeds treated with *P. putida* in optimal N fertilizer had higher plant growth, and oil quantity and quality. Shehata and El-Khawas (2003)'s research on sunflower, Silva et al. (2013)'s study on soybean and Coşge et al. (2007)'s work on safflower indicated similar results for seeds treated with PGPR inoculation. Nitrogen fertilizers, including biofertilizers, improved oil quality by enhancing unsaturated fatty acids and reducing saturated fatty acids. Biofertilizers are microorganisms that are able to alter ineffectual nutritional components to effectual and efficient compositions and this modification is carried out biologically. Manufacture expenditure of biofertilizers is low, and they do not cause soil contamination and environment pollution (Rahimi-Shokooh et al. 2013). Nowadays, engineered nanomaterials have been applied to cultivated plants to raise crop yield and improve crop protection against pathogens (Khot et al. 2012). Hence, nanotechnology is a new and feasible tool that can be used in various ways such as foliar application in the field to increase plant production. Shekhbaglou et al. (2018) reported that the highest rate of oleic acid (20.45%) and linoleic acid (49.47%) in soybean were detected in treatment of 0.75 g l^{-1} of nanoiron oxide that showed significant differences with other treatments and control.

Conclusion

Results of this study demonstrated that the application of nanofertilizers and biofertilizers could increase the production and oil percentage of safflower under irrigation disruption at the grain filling stage. Meanwhile, irrigation disruption during the grain filling stage and the application of combined fertilizers resulted in diminution of water consumption and reduced the impact of drought stress on safflower. Additionally, full irrigation along with combined fertilizer improved plant height and physiochemical traits such as chlorophyll *a, b*, and carbohydrate content of fresh leaves. However, irrigation disruption increased total saturated fatty acids (palmitic and stearic acid), decreased vitamin E, and total unsaturated fatty acids. The results can be explained by the fact that due to the production of oil and importance of safflower as an oilseed crop, applying irrigation disruption at the grain filling stage and nanofertilizer can increase oil yield. It is significant because the proposed method has the potential to increase the product value by reducing water consumption, obtaining appropriate yield and oil per unit area. It is suggested that in terms of food safety, the accumulation of the nano-particles in plant tissue is a vital point. Therefore, in future trials, valuable information can be derived from determining their quantity.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this article.

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