

Evaluation of changes in fatty acid profile, grain, and oil yield of *Carthamus tinctorius* L. in response to foliar application of polyamine compounds under deficit irrigation conditions

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ABSTRACT

Safflower is an oilseed plant rich source of omega 3 and 6 whose fatty acids composition may be affected by water deficit stress. Thus, field experiments were conducted in Tehran, Iran during 2017 and 2018 growing seasons to investigate the influence of foliar application polyamines on fatty acid compositions and agronomic traits under deficit irrigation stress as a split plot with four replications. The studied treatments included irrigation at two levels of normal irrigation (I1, 50 %) and deficit irrigation at flowering stage (I2, 75 %) (irrigation after depletion of 50 % and 75 % moisture of field capacity, respectively) as the main plot and foliar application treatments at 10 levels included (water foliar application or control, foliar application putrescine, spermidine and spermine each in concentrations (50, 100, 200 $\mu\text{mol L}^{-1}$)) as the subplot. Foliar application of putrescine under I2 enhanced grain and oil yield by 721.7 and 120.7 kg ha^{-1} , respectively, on average in the two years of experiment compared with the control treatment. The total polyunsaturated and unsaturated fatty acids (ΣPUFA and ΣUFA), UFA (omega 3 and 6), ratio of ΣPUFA to saturated fatty acids (ΣSFA), harvest index and oil content rose under I2 conditions in both years of the experiment with spermine foliar application. Foliar application of putrescine also increased omega 6, ΣPUFA and ΣUFA under I1 conditions in both years. The results of this experiment indicate that foliar application of polyamines can improve grain and oil yield and quality of fatty acids of safflower under irrigation regimes.

1. Introduction

The content and composition of fatty acids have been considered as important determinants of the nutritional value of some oils (Karami et al., 2018). Safflower (*Carthamus tinctorius* L.) is one of the most important oilseed crops not only because of its high quality oil (Kim et al., 2016) but also its clinical application to reduce the level of blood cholesterol, osteoporosis and rheumatism (Lee et al., 2002). Safflower oil contains two unsaturated fatty acids of oleic acid (Cis, Omega-9) (C18: 1) and linoleic acid (Cis, Omega-6) (C18: 2), which accounts for about 90 % of the total fatty acids, with the remaining 10 % palmitic acid (C16: 0) and stearic acid (C18: 0). Safflower standard oil contains 6–8 % palmitic acid, 2–3 % stearic acid, 16–20 % oleic acid and 71–75 % linoleic acid (Velasco and Fernández-Martínez, 2001). Thus, safflower

oil can be eaten as a good fat effective on lowering cholesterol level (Karami et al., 2018). Generally, drought stress adversely affecting yield and quality of many crops including oilseeds will affect plants through changes in plant growth and physiology and metabolic activities (Alqudah et al., 2011). It's illustrated that irrigation regime, planting time, growth temperatures and abiotic stresses may also change the oil content and composition of plant oil (Bagheri et al., 2012). Generally, the reproductive stage is more sensitive to drought than the vegetative stage, and drought at the reproductive stage reduces the number of flowers and pods in the plants, which leads to a reduction in the number of seeds (Pushpavalli et al., 2015). Also, it will affect gametogenesis, fertilization, embryogenesis, limiting seed growth and thereby reducing crop yield (Farooq et al., 2014, 2009).

Other researchers have been stated that seed and oil yield were

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affected by drought stress more than other traits except oil content in different safflower genotypes (Nazari et al., 2017). The results of an experiment by Akbari et al. (2020) on safflower also showed that with increasing water shortage stress (irrigation after the depletion of 85 % field capacity moisture) at 50 % flowering stage, the oil content and palmitoleic acid reduced. One method to minimize drought stress-induced yield reduction has been foliar application with polyamine compounds (Baloglu et al., 2012). In recent years, in order to increase plant resistance to stress, various methods are used such as the exogenous use of growth regulators. There are also different reports of polyamines (PAs) and environmental stresses (Bouchereau et al., 1996).

Polyamines (PAs) are low molecular weight aliphatic amines that have two or more amino groups and high biological activity (Vuosku et al., 2018) and widely found in eukaryotic and prokaryotic cells (Liu et al., 2018; Mustafavi et al., 2018).

It's demonstrated that in high-chain fatty acids' oil plants species, polyamines can affect the activity of enzymes involved in lipid metabolism (Tomosugi et al., 2006). Other researchers have also stated that polyamines affect fixed constituents of seed oil through activating the biosynthesis of some enzymes related to fatty acid metabolism (Talaat and El-Din, 2005). For example, Ullah et al. (2012) reported that foliar application of salicylic acid (SA) and putrescine (PUT) growth regulators during flowering stage under drought stress had a positive effect on the growth and quality of rapeseed (*Brassica napus* L.) oil and prevented oleic acid reduction in rapeseed under stress. The effect of exogenous polyamines on plant physiological processes, growth traits and yield has also been well documented (Farooq et al., 2009). Furthermore, Gupta and Gupta (2011) reported that different concentrations of PUT enhanced grain yield under both non-stress and stress conditions in wheat (*Triticum aestivum* L.). Also Ahmed et al. (2015) studied the effect of foliar application of PUT and SPD polyamines at different concentrations on yield components and grain yield of maize (*Zea mays* L.) under stress conditions during the two years of experiment reporting that foliar application of PUT and SPD at a concentration of 100 mg L⁻¹ improved the grain yield and HI in maize, respectively.

Based on earlier studies, there have been few studies on the effect of polyamines on fatty acids changes in oilseeds, but no information has been found on the effect of irrigation regime (normal and deficit irrigation) and the use of polyamines (PUT, SPD and SPM) as exogenous use on fatty acids composition, \sum UFA ratio \sum SFA in safflower seed oil. Accordingly, the present study aimed to evaluate the different compounds of polyamines by irrigation regime on grain and oil yield and fatty acid composition in safflower seed oil. We hypothesized that using different compounds of polyamines improved oil quality, and safflower grain and oil yield under deficit irrigation conditions.

2. Materials and methods

2.1. Experimental design

In order to study the effect of irrigation regime and foliar application of polyamine compounds and their interactions on fatty acid profile and grain and oil yield of safflower, a field experiment was performed at the research farm of the Faculty of Agriculture, Shahed University, located at Tehran (35° 33' N and 51° 20' E) during two crop years (2016–2017 and 2017–2018) (hereinafter referred to as 2017 and 2018) with an elevation of 1190.8 m above the sea level in Iran.

The meteorological data for both years are shown in Fig. 1. This area with a 30-year average rainfall of 238.9 mm and a temperature of 17.7 °C is classified as a dry climate with relatively hot and dry summers according to Koppen climate classification (Akhavan et al., 2018). In 2017, in addition to higher rainfall during the growing season, compared with 2018, there was 44 percent more rainfall in March and May (38.8 and 33.2 mm, respectively). On average, the highest average temperature difference in 2017 and 2018 was 5.9 °C and 2.5 °C, respectively, in March and July. The average temperature in 2017 was

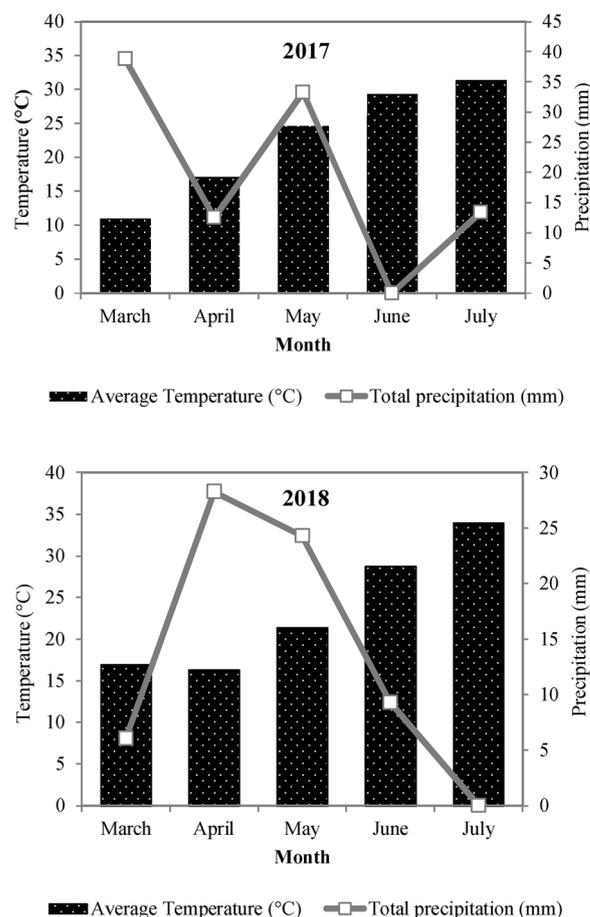


Fig. 1. Variation of temperature and rainfall in Shahed University, Tehran meteorology station during 2017 and 2018 growing seasons.

about 1 °C cooler than in 2018, but in June 2018, which coincided with the flowering and pollination stages of safflower, the temperature was 0.6 °C lower in 2017.

The chemical and physical properties of the soil and water of the experiment site are given in Table 1. Two weeks before planting, soil samples were prepared from 0–45 cm. In order to measure saturation percentage, organic carbon, electrical conductivity (EC) and pH, the samples were first air dried and grinded and then measurements were performed using sulfuric acid using the method of Walkley and Black (1934).

Total nitrogen (N) in soil was measured at three general stages of digestion, distillation and titration using Kjeldahl method (Bremner, 1960). Moreover, available phosphorus (P) was measured by Olsen

Table 1

Physico-chemical properties of the soil and characteristics of water used for irrigation.

Soil properties	Values	Water characteristics	Values
EC (dS m ⁻¹)	2.19	EC (μS cm ⁻¹)	2100
pH	7.79	pH	7.44
Organic carbon (%)	0.83	Ca ²⁺ (mequiv. l ⁻¹)	1.5
Total N (%)	0.08	Mg ²⁺ (mequiv. l ⁻¹)	0.6
Available P (ppm)	25.6	Na ⁺ (mequiv. l ⁻¹)	18.2
Available K (ppm)	326.6	Cl ⁻ (mequiv. l ⁻¹)	15.5
Fe (ppm)	6.01	SO ₄ (mequiv. l ⁻¹)	1.9
Zn (ppm)	1.91	SAR	17.76
Mn (ppm)	15.9	HCO ₃ (mequiv. l ⁻¹)	2.9
Cu (ppm)	1.52	TDS (ppm)	1060
B (ppm)	1.06	SSP (%)	89.65

The soil and water characteristics were determined according to Tandon (1995).

method (Olsen et al., 1954), potassium available (K) by film meter (Mehlich, 1953), boron (B) by azomethine H colorimetric method (Parker and Gardner, 1981) and Fe, Zn, Mn and Cu were analyzed by atomic absorption spectrometry (Tandon, 2005). In addition, water parameters such as pH, EC, Na, Fe, Cu, Mg, Cl, Ca, and sodium absorption ratio (SAR) were measured using the method of (Tandon, 2005).

The experiment was performed as a split plot in a randomized complete block design with four replications. The main factors included irrigation at the two levels of normal irrigation (I1) and deficit irrigation at flowering and pollination stage (I2) (irrigation after 50 % and 75 % depletion of field capacity moisture content, respectively) and sub-factor at 10 levels including water foliar application (control), foliar application of PUT1 (50 $\mu\text{mol L}^{-1}$ Putrescine), PUT2 (100 $\mu\text{mol L}^{-1}$ Putrescine), PUT3 (200 $\mu\text{mol L}^{-1}$ Putrescine), SPD1 (50 $\mu\text{mol L}^{-1}$ Spermidin), SPD2 (100 $\mu\text{mol L}^{-1}$ Spermidin), SPD3 (200 $\mu\text{mol L}^{-1}$ Spermidin), SPM1 (50 $\mu\text{mol L}^{-1}$ Spermin), SPM2 (100 $\mu\text{mol L}^{-1}$ Spermin), and SPM3 (200 $\mu\text{mol L}^{-1}$ Spermin).

Polyamines were prepared from Sigma-Aldrich Chemical Company. With foliar application of polyamine compounds, Tween-20 (5% V / V) was used as a surfactant (to increase contact surface area and leaf absorption). We employed the foliar application by a 12-liter back-up sprayer with a flood nozzle (for a more uniform spraying and no wind blowing). Before the foliar application, the sprayer was first calibrated to reach 1,000 L per hectare. Safflower cultivar used in this experiment was Goldasht and was prepared from Karaj Seed and Breeding Institute in Iran. This is an early-spring cultivar and cold tolerant with 25–30 % oil content, which is suitable for cultivation in warm and temperate climates. Before planting and based on soil test results, 350 kg ha⁻¹ of nitrogen (from urea source) and 100 kg ha⁻¹ of potassium sulfate were added to the soil. Seeding was performed manually in both years on March 14. The area of each plot was 10 m² (5 × 2 m) with 4 planting rows 50 cm apart. The length of each row was 5 m and for each plot we considered a row of planting. The plant spacing was 50 cm in rows and the final density was 400,000 plants ha⁻¹. The seeds were sterilized with fungicide before planting and weeds and insects were effectively controlled.

2.2. Determination of soil moisture content and the volume of irrigation

Biologische Bundesantalt, Bundessortenamt and Chemische Industrie (BBCH) (safflower phenological stages) was consulted to determine the timing of irrigation regimes according to Flemmer et al. (2015) method. BBCH is an accurate and simple method for identifying the stages of plant phenology development using a code to identify different stages of plant species growth (Lancashire et al., 1991). Consequently, at 50 % flowering stage according to BBCH code (65), all the plots were irrigated simultaneously; also, after the depletion of 50 % field capacity moisture content (50 % FC), there was a light simultaneous irrigation of plots after the depletion of 75 % field capacity moisture content (75 % FC). Accordingly, Putrescine [NH₂ (CH₂)₄NH₂], Spermidin [NH₂ (CH₂)₃NH (CH₂)₃NH₂] and Spermine [NH₂ (CH₂)₃NH (CH₂)₃NH (CH₂)₃NH₂] were applied at the flowering stage and in deficit irrigation treatment. Soil weight, moisture content in potential points of FC, permanent wilting (PW), after FC 50 % and FC 75 % moisture content depletion were determined using (Saxton and Rawls, 2006) method by soil moisture curve (Fig. 2). The soil moisture content at FC points (23.3 % volumetric moisture %V), after FC 50 % and FC 75 % moisture content depletion was 17.5 % and 14.5 %, respectively with wilting point (WP) measured at 11.6 %. Soil water potential was also calculated for FC, after FC 50 % and FC 75 % moisture content depletion irrigation and WP (-0.034, -0.15, -0.38 and -1.19 MPa) (Table 2). Notably, the soil of the experiment site had a loamy texture with 50 % sand, 16 % clay and 34 % silt and a bulk density of 1.45 g cm⁻³. Gravimetric weight method was used to accurately determine irrigation time for each treatment by Souza et al. (2000). For this purpose, 48 h after irrigation, the field soil

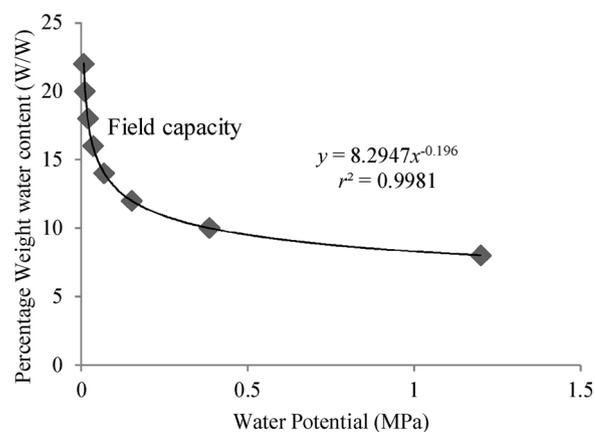


Fig. 2. The soil moisture curve of farm. Field capacity, 50 and 75 % soil moisture depletion of field capacity of the soil was around respectively, 23.3 %, 17.5 % and 14.5 % humidity. Wilting point was around 11.6 % humidity in the soil.

Table 2

Field capacity (FC), 50 and 75 % soil moisture depletion of field capacity, respectively, Wilting point (WP) values for the studied soil for the safflower.

Point	Percentage Weight water content (W/W)	Percentage volumetric water content	Water potential (MPa)
Estimated FC	16	23.3	-0.034
Estimated depletion FC 50 %	12	17.5	-0.15
Estimated depletion FC 75 %	10	14.5	-0.38
Estimated WP	8	11.6	-1.19

was sampled from 45 cm depth of root development. The samples were immediately weighed and dried to determine soil moisture content in the oven at 100 °C. Accordingly, irrigation time for normal (I1) and deficit irrigation (I2) treatments when the soil moisture at 45 cm depth was 12 and 10 %, respectively (Fig. 2). The amount of water given to the soil at each irrigation round (d) (cm) depends on the pre-irrigation moisture (Θ_2) (in terms of weight moisture content) and the depth of root development (d) (cm), which that was calculated according to the following equation.

$$d = (\Theta_{FC} - \Theta_i) \times pb \times D / 100$$

In this equation, Θ_{FC} is weight soil moisture content (field capacity), pb stands for soil bulk density in g cm⁻³ (measured for soil at the experiment site (1.45 g cm⁻³)), and D signifies root development depth (cm) (measured for safflower (45 cm)). Then the irrigation volume using the following equation $d/100 \times 10,000 \text{ m}^2$ was calculated. In this way, the required volume of water at each irrigation stage was calculated in each experiment and uniformly distributed based on 90 % water distribution efficiency using pump and volume meter. The respective amounts of 1458.8 and 1312.9 m³ ha⁻¹ as an average for the two-year I1 and I2 treatments (irrigation after 50 and 75 % of field capacity moisture content depletion, respectively) at safflower flowering stage were used. Thus, irrigation volume of deficit irrigation treatment (I1) was 10 % lower than the normal irrigation (I1).

2.3. Determination of oil concentration and fatty acid profile

After harvesting, oil extraction and percentage of fatty acids were measured. Soxhlet method and petroleum gasoline as a solvent were employed for safflower seed oil extraction for 4.5 h

(Movahhedy-Dehnavy et al., 2009). The following formula was used to calculate oil content.

Oil percentage (%) = weight of extracted oil / weight of seed sample \times 100

To degrade fatty acids, initially isolated and methylated fatty acids in the structure of triacylglycerols (TAGs) molecules and other molecules. To do so, the method of (Metcalf et al., 1966) was used, and applied 2% (Me-NaOH), 20 % (BF₃), sodium chloride (NaCl), and sodium sulfate (Na₂SO₄) for the analysis of seed fatty acids in the oil samples. In this experiment, UNICAM 4600 (Cambridge, England) with a BPX70 capillary column 30 mm long and 0.25 mm in diameter was utilized for the isolation of fatty acids. The column temperature was fixed at 180 °C, injection site temperature was set at 240 °C, and the flame ionization detector (FID) temperature was 200 °C. Helium carrier gas pressure was set at 18 PSI and air pressure was 25 PSI. The injection volume of ester sample measured 0.1 μ L. The fatty acid standard sample was injected into the device and the content of each fatty acid was calculated as reported by (Movahhedy-Dehnavy et al., 2009).

2.4. Productivity (Grain yield and harvest index)

BBC code 99 was adopted to determine the best harvesting time of safflower. In order to determine grain yield, from each plot, we considered an area of 4 m² by removing marginal effects. After crushing and separating the seeds by sieve, the grain yield was measured. Oil yield was also calculated by multiplying the grain yield by oil content. The harvest index (HI) was also calculated by the following formula.

Harvest index (HI %) = (seed economic yield) \times (biological yield⁻¹) \times 100

2.5. Calculation statistical methods

For the calculations and running the analysis of variance (ANOVA), we used software SAS Ver. 9.1 (SAS Institute Inc, 2004) and GLM method. Analysis of variance was run after performing homogeneity analysis of variance of two years for each trait (Bartlett test). According to the results of Bartlett test, the measured traits in this experiment were identified as non-homogeneous, so all non-homogenous traits are presented in a simple (separate) analysis. LSMEANS or PDIF method for the mean comparison was used. PROC CORR to calculate the correlation coefficients in SAS and Excel to plot the graphs were employed. The orthogonal comparisons were performed using SAS, and the relationships between traits and polyamines were also obtained from response curves.

3. Results and discussion

The irrigation regime \times foliar application polyamine interaction effect was significant for all measured traits except stearic acid (C18: 0) and total saturated fatty acids (Σ SFA).

3.1. Changes in (Grain Yield, Harvest Index, and Oil Yield) under irrigation regime and polyamines

The average grain yield (on average between irrigation levels) in 2017 and 2018 was 2854 and 2275 kg ha⁻¹, respectively with the difference of 25.4 % (Table 3). The lower grain yield in 2018 could be due to adverse weather conditions. Rainfall during March, April and May in 2017 was about 44.3 % higher than that in 2018 (Fig. 1). Therefore, the rainfall during safflower vegetative growth period resulted in higher plant dry matter production and consequently higher grain yield in 2017 than in 2018. These results are consistent with the study results of Movahhedy-Dehnavy et al. (2009) and Koutroubas et al. (2009). They found that higher yield resulted from higher rainfall. Singh et al. (2016)

also stated that higher rainfall at vegetative growth stage due to higher biomass production would increase safflower grain yield. The study results also showed that in spite of low rainfall and temperature during June 2018 at safflower reproductive stage (Fig. 1), it seems that higher rainfall and lower temperature had a negative impact on grain yield during the reproductive stage. In both years, grain yield changes in each irrigation regime were similar in response to polyamine foliar application treatments (Table 3). In both years (on average between foliar application levels) in deficit irrigation treatment (I2), putrescine (PUT) (2939.3 kg ha⁻¹) compared with spermin (SPM) (2716.6 kg ha⁻¹), water foliar application (2526.7 kg ha⁻¹) and spermidin (SPD) (2493.6 kg ha⁻¹) enhanced the grain yield by 8.2, 16.3 and 17.8 %, respectively. Under normal irrigation conditions (I1), the highest grain yield (on average between foliar application levels) was obtained from PUT (2772.5 kg ha⁻¹), which increased by 5.7, 20.4 and 28.4 %, respectively compared with SPM (2622.3 kg ha⁻¹), water foliar application (2302.6 kg ha⁻¹) and SPD (2159 kg ha⁻¹) (Table 3). Given that grain yield was mainly affected by grain yield components, therefore the three yield components in safflower i.e., 1000 grain weight, number of capitula per plant and number of seeds per capitula on they are effective components in regulating grain yield. In this experiment as well, the grain yield had a positive correlation (data not shown) with 1000 grain weight ($r = 0.60$ **, $P < 0.01$), number of capitula per plant ($r = 0.42$ **, $P < 0.01$) and number of seeds per capitula ($r = 0.40$ **, $P < 0.01$) (data not shown). Johnson et al. (2012) also reported that higher yield of safflower was associated with high amount of capitula and seeds per plant. Also, during two years of experiment in their study on maize Ahmed et al. (2015) reported that increasing the concentration of PUT and SPD polyamines to 100 mg L⁻¹ significantly elevated the maize grain yield.

Also, the enhancement of the yield using polyamines may be due to several reasons. For example, studies have shown that polyamines (PUT, SPD, and SPM) can regulate potassium channel size and pore size in the plasma membrane of the guard cells and thereby tightly regulate the opening and closing of the pores of the stomata, thus allowing polyamines to control the loss of water in plants (Liu et al., 2000). It seems that polyamine compounds may affect stomatal conductance and photosynthesis, especially under water shortage conditions, and finally increase the yield and its components in safflower on the other hand, polyamines are involved in a wide range of developmental processes such as cell division, embryogenesis, flowering, fruit ripening and response to environmental stresses (Öztürk and Demir, 2003). Sood and Nagar (2003) reported that peroxidase and cellulose activities are delayed by polyamines, and their biosynthetic inhibitors are accelerated by polyamines, which elevated the yield and yield components. Many observations have also shown that polyamines in the plant also prevent ethylene synthesis through inhibiting ACC synthase, and converting 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene, delaying the aging (Li et al., 2004). Studies have revealed that polyamines improve cellular processes as opposed to ethylene through the growth and the delay in aging (Bitrián et al., 2012; Torrigiani et al., 2012). Growth regulators are effective in enhancing crop yields by improving source and Sink relationships and mobility of photosynthesis to seeds (Solaimalai et al., 2001). Therefore, it seems that exogenous application of polyamines may increase seed filling time through improving the processes involved in growth and regulation of stomata as well as delaying aging and increasing leaf area durability, which have increased the yield and its components.

The results of regression analysis of the two years of experiment showed that safflower grain yield was significantly correlated with the interaction between irrigation and foliar application (Table 6). Under deficit irrigation conditions (I2), the relationship between safflower grain yield and PUT foliar application level was cubic, so that the maximum grain yield obtained from this experiment was 142.8 μ mol L⁻¹ per PUT. Therefore, it can be concluded that to achieve the maximum grain yield of safflower under deficit irrigation conditions (I2) in this experiment, increasing PUT foliar concentration above 142.8 μ mol L⁻¹

Table 3

Mean comparison of the effect of interaction irrigation regime (IR) and Foliar application Polyamine (FAP) on grain yield (kg ha⁻¹), oil yield (kg ha⁻¹), C16:0 (Palmitic acid), C18:0 (Stearic acid), and C18:1 (oleic acid) of safflower in 2017 and 2018 growing seasons.

IR	FAP	Grain yield (kg ha ⁻¹)		Oil yield (kg ha ⁻¹)		Palmitic acid (C16:0) (%)		Stearic acid (C18:0) (%)		Oleic acid (C18:1, omega- 9) (%)	
		2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
I1	Control	2816.6 ± 149.1 b-d	1788.6 ± 35.1 e	645.67 ± 30.01 a-e	420.11 ± 5.3 cd	7.00 ± 0.05 a-c	6.87 ± 0.1 bc	2.46 ± 0.1 a	2.31 ± 0.1 b	14.42 ± 0.1 a-c	15.30 ± 0.1 d
	PUT1	2675.4 ± 62.06 b-d	2721.3 ± 130.08 a	604.44 ± 12.5 b-e	511.52 ± 24.02 b	7.12 ± 0.07 ab	6.74 ± 0.1 c	2.58 ± 0.2 a	2.39 ± 0.1 ab	14.90 ± 0.1 a	15.28 ± 0.1 d
	PUT2	3180.8 ± 333.4 ab	2146.0 ± 51.2 cd	659.62 ± 67.1 a-d	459.93 ± 10.2 c	6.85 ± 0.1 c	7.43 ± 0.2 a	2.56 ± 0.1 a	2.60 ± 0.1 ab	14.18 ± 0.1 bc	16.51 ± 0.1 c
	PUT3	3499.6 ± 447.9 a	2412.4 ± 65.5 b	739.07 ± 98.4 a	515.57 ± 12.7 b	6.90 ± 0.08 bc	7.10 ± 0.1 a-c	2.36 ± 0.1 a	2.38 ± 0.1 ab	14.42 ± 0.1 a-c	15.53 ± 0.1 d
	SPD1	2280.3 ± 169.3 d	1196.0 ± 31.6 f	529.18 ± 39.8 e	257.55 ± 6.1 e	6.90 ± 0.1 bc	7.47 ± 0.1 a	2.52 ± 0.1 a	2.46 ± 0.1 ab	14.65 ± 0.1 ab	17.51 ± 0.1 b
	SPD2	2552.8 ± 221.04 cd	2086.2 ± 30.5 d	553.82 ± 51.1 c-e	445.91 ± 8.1 c	6.91 ± 0.1 bc	7.25 ± 0.1 ab	2.46 ± 0.2 a	2.50 ± 0.1 ab	14.37 ± 0.1 bc	14.71 ± 0.1 e
	SPD3	2520.7 ± 119.6 cd	2318.5 ± 44.4 bc	548.61 ± 22.5 de	529.41 ± 9.7 b	6.96 ± 0.09 a-c	6.85 ± 0.1 bc	2.55 ± 0.1 a	2.41 ± 0.1 ab	14.39 ± 0.2 bc	15.51 ± 0.1 d
	SPM1	3063.4 ± 278.8 a-c	2220.5 ± 85.2 b-d	678.52 ± 62.2 a-c	520.96 ± 21.1 b	6.85 ± 0.1 c	7.07 ± 0.1 a-c	2.41 ± 0.1 a	2.57 ± 0.1 ab	14.14 ± 0.1 c	15.25 ± 0.1 d
	SPM2	2897.9 ± 184.5 bc	2712.0 ± 129.9 a	710.75 ± 50.03 ab	633.66 ± 26.4 a	6.92 ± 0.1 bc	7.23 ± 0.2 a-c	2.58 ± 0.1 a	2.68 ± 0.1 a	13.93 ± 0.1 c	16.57 ± 0.1 c
	SPM3	3128.5 ± 195.2 ab	1711.5 ± 55.1 e	707.36 ± 45.7 ab	377.49 ± 12.3 d	7.19 ± 0.1 a	7.13 ± 0.1 a-c	2.92 ± 0.3 a	2.50 ± 0.1 ab	14.67 ± 0.1 ab	18.09 ± 0.07 a
I2	Control	2781.7 ± 29.8 b	2271.7 ± 45.7 cd	633.66 ± 4.6 ab	502.39 ± 9.2 c	7.21 ± 0.1 a	6.79 ± 0.1 a-c	2.47 ± 0.1 a	2.52 ± 0.00 a	14.68 ± 0.1 a-c	14.81 ± 0.1 a
	PUT1	2884.8 ± 64.2 ab	2375.6 ± 150.4 c	614.21 ± 8.6 a-c	523.01 ± 33.5 c	7.09 ± 0.09 a-c	6.76 ± 0.1 a-c	2.50 ± 0.1 a	2.48 ± 0.1 a	14.08 ± 0.1 d	13.69 ± 0.1 c
	PUT2	3430.0 ± 250.3 a	3066.9 ± 222.3 a	708.39 ± 51.6 a	669.13 ± 52.1 a	6.95 ± 0.07 b-d	6.93 ± 0.1 a-c	2.46 ± 0.1 a	2.61 ± 0.1 a	14.32 ± 0.2 b-d	14.29 ± 0.1 b
	PUT3	2602.1 ± 114.07 b	1990.8 ± 58.09 de	515.05 ± 21.3 c	425.61 ± 13.9 de	7.16 ± 0.09 ab	6.74 ± 0.1 a-c	2.56 ± 0.2 a	2.54 ± 0.1 a	15.06 ± 0.1 a	14.40 ± 0.1 b
	SPD1	2967.3 ± 42.08 ab	2285.9 ± 247.6 cd	570.37 ± 10.1 bc	491.41 ± 54.06 cd	6.87 ± 0.08 cd	7.05 ± 0.07 a	2.48 ± 0.2 a	2.85 ± 0.5 a	14.48 ± 0.1 b-d	14.48 ± 0.1 ab
	SPD2	3004.4 ± 144.9 ab	2275.1 ± 107.5 cd	579.55 ± 28.08 bc	492.08 ± 24.6 cd	7.00 ± 0.04 a-d	6.89 ± 0.1 a-c	2.47 ± 0.1 a	2.44 ± 0.1 a	14.14 ± 0.1 d	14.52 ± 0.1 ab
	SPD3	2576.4 ± 137.3 b	1852.8 ± 91.9 e	542.13 ± 29.4 bc	380.44 ± 20.3 e	6.97 ± 0.1 a-d	6.97 ± 0.08 ab	2.46 ± 0.1 a	2.47 ± 0.1 a	14.45 ± 0.1 b-d	14.41 ± 0.1 ab
	SPM1	2908.0 ± 473.2 ab	2553.8 ± 260.6 bc	535.63 ± 82.1 bc	558.46 ± 56.7 bc	7.05 ± 0.1 abc	6.82 ± 0.1 a-c	2.61 ± 0.1 a	2.63 ± 0.1 a	14.75 ± 0.1 ab	13.78 ± 0.1 c
	SPM2	2542.1 ± 173.4 b	2799.2 ± 237.1 ab	558.45 ± 41.9 bc	636.33 ± 52.8 a	6.90 ± 0.1 cd	6.59 ± 0.1 bc	2.57 ± 0.1 a	2.52 ± 0.1 a	14.38 ± 0.2 b-d	13.65 ± 0.1 c
	SPM3	2770.4 ± 121.4 b	2726.1 ± 43.9 b	537.41 ± 26.9 bc	602.87 ± 8.8 ab	6.80 ± 0.06 d	6.56 ± 0.1 c	2.48 ± 0.1 a	2.58 ± 0.1 a	14.27 ± 0.1 cd	13.69 ± 0.1 c
P-Value		*	**	**	**	*				**	**

I1 and I2 = 50 and 75 % soil moisture depletion of field capacity, respectively.

Control = water foliar application, PUT1, PUT2 and PUT3 = 50, 100 and 200 µmol L⁻¹ concentration Putrescine. SPD1, SPD2 and SPD3 = 50, 100 and 200 µmol L⁻¹ concentration Spermidin. SPM1, SPM2 and SPM3 = 50, 100 and 200 µmol L⁻¹ concentration Spermin.

For a given year LSMEANS (along with ± standard error) within each column of each section followed by the same letter are not significantly different ($P < 0.05$). * Significance at P level of 0.05, ** significance at P level of 0.01.

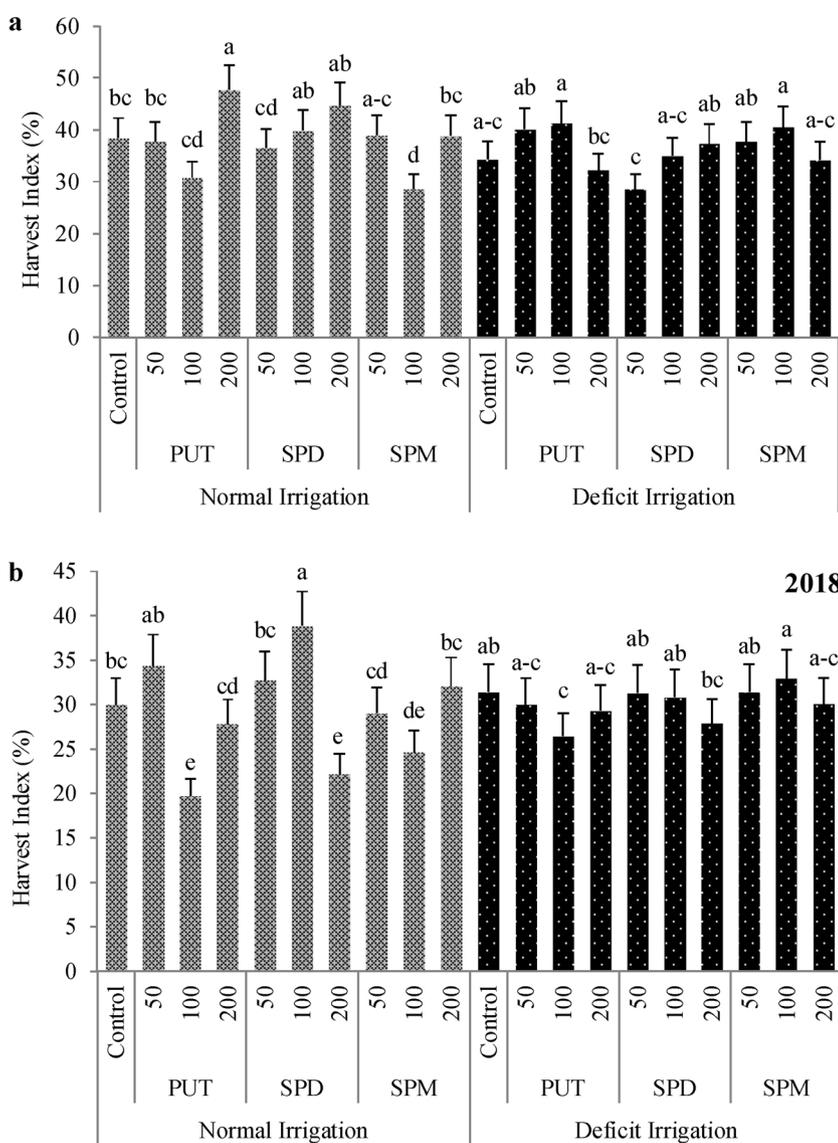
did not affect the grain yield. Evaluation of simple linear regression equation both under normal irrigation conditions (I1) illustrated that each 1 $\mu\text{mol L}^{-1}$ increase in PUT concentration elevated the grain yield by 2.8 kg ha^{-1} (Table 6).

The mean comparison showed that PUT foliar application had a significant effect on oil yield in both years under deficit irrigation (I2) conditions (Table 3). Foliar application of 100 $\mu\text{mol L}^{-1}$ of PUT increased oil yield by 21.2 %. In general, in both years, SPD foliar application at each irrigation level showed low oil yield. However, under normal irrigation conditions (I1) SPM2 (100 $\mu\text{mol L}^{-1}$ with a mean of 672.2 kg ha^{-1}) and PUT3 (200 $\mu\text{mol L}^{-1}$ with a mean of 627.3 kg ha^{-1}) treatments enhanced the oil yield by 26.1 and 17.7 %, respectively (Table 3).

The results of regression analysis in the two years of experiment showed that oil yield was significantly correlated with irrigation interaction and foliar application treatments (Fig. 5). At each irrigation level, the change in safflower oil yield was quadratic. The results of this experiment showed that under deficit irrigation conditions (I2), PUT, 87.4 $\mu\text{mol L}^{-1}$ should be used for foliar application to achieve the

maximum oil yield. During normal irrigation (I1), the highest oil yield was obtained when SPM foliar application concentration was 104.4 $\mu\text{mol L}^{-1}$ (Fig. 5). According to Hussain et al. (2018), in most cases, the reduction in oil yield is lower than the reduction in grain yield, which indicates richer oil content. However, severe drought at flowering and germination stages reduces the oil yield more than the grain yield, probably caused by a reduction in seed oil content. They also reported that low soil moisture (water deficit stress) associated with high temperatures during flowering to seed filling stages significantly lowered grain yield and quality of Sunflower (*Helianthus annuus L.*) oil. According to the study results, it seems that polyamine compounds enhanced the oil yield by improving relationships between source and reservoir and increasing leaf area durability and seed filling time.

In this study, a correlation was found between oil yield with grain yield ($r = 0.93^{**}$, $P < 0.01$), oil content ($r = 0.16$ ns), number of seeds per capitula ($r = 0.37^*$, $P < 0.05$), number of capitula per plant ($r = 0.35^*$, $P < 0.05$) and 1000 grain weight ($r = 0.51^{**}$, $P < 0.01$) (data not shown). This is consistent with the study results of (El-Lattief, 2012) on the correlation between the above traits.



2017 Fig. 3. Mean comparison of the effect interaction of irrigation regime and foliar application Polyamine on Harvest Index (HI) in 2017 (a) and 2018 (b) growing seasons. normal irrigation and deficit irrigation = 50 and 75 % soil moisture depletion of field capacity, respectively. Control = water foliar application, PUT1, PUT2 and PUT3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration putrescine. SPD1, SPD2 and SPD3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration spermidin. SPM1, SPM2 and SPM3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration spermin. LSMEANS within each column of each section followed by the same letter are not significantly different ($p \leq 0.05$).

Fig. 3.

HI was reduced by 20.2 % in 2018 compared with that in 2017. HI is an important physiological index which indicates the percentage of photosynthetic material transferred from vegetative organs to seeds. For evaluating the interaction between irrigation regime and foliar application, it was found that polyamine foliar application at each irrigation level caused a change in HI. On average, in the two years in normal irrigation (I1) treatment, SPD2 treatment ($100 \mu\text{mol L}^{-1}$) with a mean of 39.3 % had the highest harvest index compared with the control (water foliar application), SPM2 and PUT2 ($100 \mu\text{mol L}^{-1}$) by 15, 48 and 55.7 %, respectively. However, when applying deficit irrigation (I2) treatment at flowering stage, SPM2 treatment ($100 \mu\text{mol L}^{-1}$) had a greater effect on HI than other foliar application treatments and increased 11.6, 11.3 and 2% HI compared with the control, SPD2 ($100 \mu\text{mol L}^{-1}$) and PUT2 ($100 \mu\text{mol L}^{-1}$) treatments (Fig. 3). The photosynthetic substances in the plant were reduced for various reasons under water stress condition, so the share of each seeds in these nutrients is reduced, which eventually leads to a decrease in HI. Thus, the increase in HI indicates a higher percentage of the produced photosynthetic material has been

transferred to the seeds. Foliar application of polyamines, especially in drought stress conditions, seems to have increased the allocation of photosynthetic materials to the seeds by delaying aging and continuity of leaf area and increasing the length of grain filling period, thus increasing HI. An increase in harvest index in wheat with PUT foliar application has also been reported (Karimi, 2016).

3.2. Changes in oil content under irrigation regime and polyamines

The mean oil content in both years of the experiment was 21.6 %. The change range of safflower oil content in spring cultivation varied from 21.2–25.8% as reported by (Knowles and Ashri, 1995). The lowest and highest oil content belonged to I2 × SPM1 (18.4 %) and I1 × SPM2 (24.5 %) treatments, respectively. The trend of oil content changes at each irrigation levels in both years of the experiment was similar to the amount and type of foliar application of polyamines. On average, the highest oil content in both years was obtained from I1 × SPM2 (23.9 %) and I2 × SPM2 (22.2 %) treatments, which increased by 3.1 and 6.7 %,

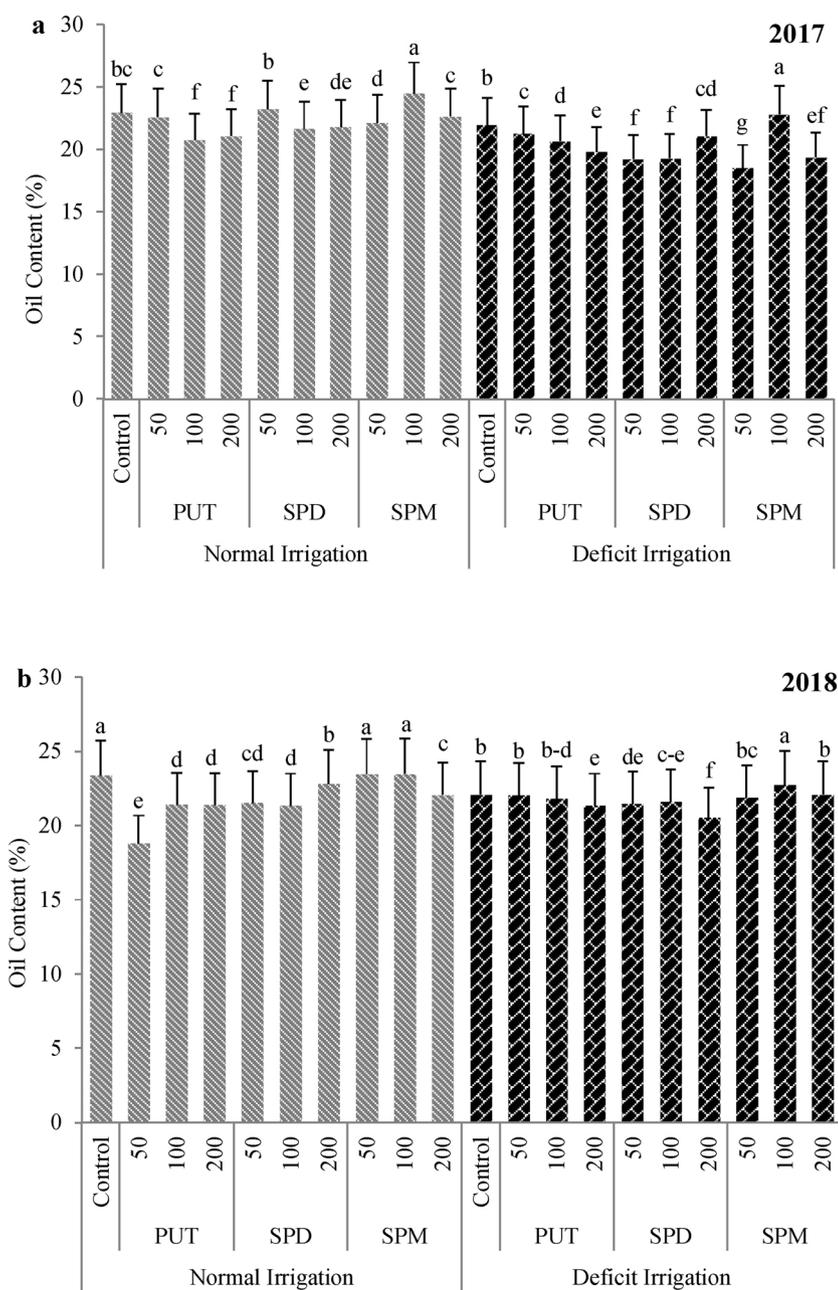


Fig. 4. Mean comparison of the effect interaction of irrigation regime and foliar application Polyamine on oil content in 2017 (a) and 2018 (b) growing seasons. normal irrigation and deficit irrigation = 50 and 75 % soil moisture depletion of field capacity, respectively. Control = water foliar application, PUT1, PUT2 and PUT3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration putrescine. SPD1, SPD2 and SPD3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration spermidin. SPM1, SPM2 and SPM3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration spermin. LSMEANS within each column of each section followed by the same letter are not significantly different ($p \leq 0.05$).

respectively, compared with water foliar application (control) treatment (Fig. 4). The results of the experiment by Deotale et al. (2016) also showed that 100 ppm PUT foliar application significantly increased soybean [*Glycine max* (L.) Merr.] oil content before flowering stage and 10 days after flowering stage. Ashrafi and Razmjoo (2010) found that safflower oil content was strongly affected by different levels of irrigation. They also reported that the oil content of safflower cultivars was significantly reduced under deficit irrigation. Given that the oil content in oilseed plants plays an important role in the human diet, its stability under deficit irrigation conditions is critical. Therefore it seems that richer oil content under both normal and deficit irrigation conditions with foliar application of polyamines is due to improved photosynthetic capacity and increased mobility of stored nutrients to developing reservoirs.

The results of regression analysis in the two years of experiment showed that safflower oil content was significantly correlated with irrigation interaction and foliar application of polyamines (Table 7). The trend of oil content changes was similar in different SPM foliar application treatments at both irrigation levels. The cubic regression relationship between seed the oil content and SPM levels indicated that an increase in SPM foliar application concentration in each irrigation regime (I1 and I2) in the range of 10–150 $\mu\text{mol L}^{-1}$ enhanced the oil content linearly. Moreover, it results in a less rich oil content. This indicates that increasing SPM foliar application levels from 150 to 200 $\mu\text{mol L}^{-1}$ at both irrigation levels did not further increase the oil content in this experiment (Table 7).

3.3. Changes in the level of saturated fatty acids (SFA) under irrigation regime and polyamines

The mean palmitic acid (C16: 0) in both years was 6.9 %, which is consistent with the value reported by Sabzalian et al. (2008). In this experiment, in both years was the lowest and highest levels of palmitic acid (C16: 0) (on average between foliar application levels) under deficit irrigation conditions (I2) belonged to SPM and water foliar application treatments, respectively. However, under normal irrigation conditions (I1), water foliar application (control) had a greater effect on reducing the level of palmitic acid (C16: 0) (Table 3).

The mean stearic acid (C18: 0) in both years of the experiment was 2.5 %. Guan et al. (2008) also reported stearic acid content in safflower between 1.5 and 2.7 %.

In both years of the experiment, the level of stearic acid (C18: 0) was not altered by the interaction between irrigation regime and polyamine (Table 3). However, in each irrigation regime (on average between foliar application levels), water foliar application (control) could more

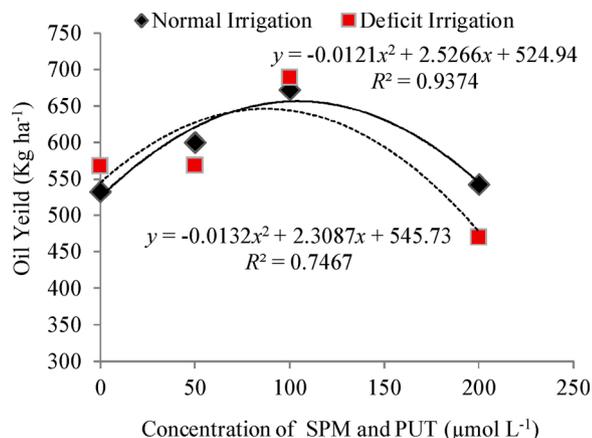


Fig. 5. Regression between application concentrations of spermine (SPM) and putrescine (PUT) in normal irrigation and deficit irrigation = 50 and 75 % soil moisture depletion of field capacity, respectively with oil yield.

markedly reduce the level of stearic acid (C18: 0), i.e., the level and type of foliar application of polyamines had no impact on it (Table 3). Arslan and Küçük (2005) also stated that stearic and palmitic acid were less affected by stress in safflower.

In 2017, $\sum\text{SFA}$ was not affected by the interaction between irrigation and polyamine (Table 5). The mean $\sum\text{SFA}$ value in both years was 9.2, which is consistent with the study results of (Akbari et al., 2020). The study results demonstrated that in both years of the experiment (on average between foliar application levels) under normal irrigation conditions (I2) water foliar application had a stronger effect on $\sum\text{SFA}$ reduction. However, the results showed that increasing SPM foliar application levels in both years resulted in a reduction in $\sum\text{SFA}$ under deficit irrigation conditions. In both years (on average between foliar application levels) SPM was able to reduce $\sum\text{SFA}$ by 1.2, 1.4 and 1.5 % compared with PUT, SPD and control treatments, respectively (Table 5). It's indicated that water deficit stress caused an increase in total saturated fatty acids in evening primrose (*Oenothera biennis* L.) (Mohammadi et al., 2018). Research has also revealed that polyamines affect the compounds of seed oils by activating the synthesis of some fatty acid metabolism enzymes. Also Talaat and El-Din (2005) stated that the activity of some fatty acid synthesizing enzymes in rapeseed fatty acids depends on polyamines. Given that polyamines are involved in many metabolic processes of plant and seed development, it seems that foliar application of polyamines may possibly through the activity of some enzymes involved in the synthesis of fatty acids have changed the seed quality in terms of oil content and composition of fatty acid.

3.4. Changes in the amount of monounsaturated fatty acids (MUFA) under irrigation regime and polyamines

Palmitoleic content (C16: 1, Omega-7) in the 2018 experiment was 19.1 % higher than that in 2017. On average, in both years deficit irrigation (I2) reduced palmitoleic (C16: 1, Omega-7) by 41.5 % (data not shown). Our results are consistent with the study results of Akbari et al. (2020) on the effect of drought stress on safflower flowering stage on palmitoleic reduction (C16: 1, Omega-7). The mean difference of this trait at irrigation levels was high among foliar application treatments, with the mean palmitoleic content (C16: 1, Omega-7) ranging from 0 to 0.16 %. The effect of SPM foliar application on palmitoleic content (C16: 1, Omega-7) was significant under normal irrigation conditions (I1). The results of mean comparison showed that (on average between foliar application levels) in the two years SPM (with a mean of 0.09 %) as compared with SPD (with a mean of 0.06 %), PUT (with a mean of 0.052 %) and water foliar application (with a mean of 0.03 %) treatments increased palmitoleic acid content by 55.7, 82.6 % and 196.8 %, respectively (C16: 1, Omega-7). The study results showed that in both years (on average between foliar application levels) water foliar application had a significant effect on elevating palmitoleic content (C16: 1, Omega-7) under deficit irrigation conditions (I2) and all foliar application treatments of polyamines reduced (41.6–213%) palmitoleic acid under these conditions (Table 4).

In 2018, the mean oleic acid content (C18: 1, Omega-9) was 4.5 % higher than the amount in 2017. Notably, the mean oleic acid content (C18: 1, Omega-9) in this study was 14.7 %. Guan et al. (2008) also reported a mean oleic acid of between 7.9 and 32.9 %. In both years (on average between foliar application levels), water foliar application (control) reduced oleic acid (C18: 1, Omega-9) when reducing soil moisture depletion to 50 % FC (I1). Nevertheless, SPM foliar application elevated the level of oleic acid (C18: 1, Omega-9) compared with water, PUT and SPD foliar application treatments by 3.9, 2 and 1.6 %, respectively. Most changes in oleic acid content (C18: 1, Omega-9) were caused by water foliar application when increasing soil moisture depletion to 75 % FC (I2) (Table 3). Temperature has a great effect on fatty acid composition of safflower oil (Samanci and Ozkaynak, 2003) during the growing season. Studies have shown that oleic acid rises with higher temperature during seed filling, while linoleic acid and linolenic

Table 4

Mean comparison of the effect of interaction irrigation regime (IR) and Foliar application Polyamine (FAP) on C18:2 (linoleic acid), C18:3 (α -linolenic acid), C16:1 (palmitoleic acid), C20:1 (eicosenoic acid), and C20:3 (eicosatrienoic acid) of safflower in 2017 and 2018 growing seasons.

IR	FAP	Linoleic acid (C18:2, omega-6) (%)		α -Linolenic acid (C18:3, omega-3) (%)		Palmitoleic acid (C16:1, Omega-7) (%)		Eicosenoic acid (C20:1, Omega-9) (%)		Eicosatrienoic acid (C20:3, Omega-3) (%)		
		2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	
I1	Control	75.58 ± 0.1 bc	74.21 ± 0.5 b	0.306 ± 0.00 ab	0.312 ± 0.01 c-e	0.000 ± 0.00 g	0.063 ± 0.00 de	0.000 ± 0.00 g	0.119 ± 0.00 d	0.174 ± 0.00 c	0.242 ± 0.00 b	
	PUT1	78.66 ± 0.1 a	74.86 ± 0.1 ab	0.278 ± 0.01 a-c	0.312 ± 0.01 c-e	0.057 ± 0.00 c	0.031 ± 0.00 h	0.137 ± 0.00 b	0.116 ± 0.00 d	0.220 ± 0.00 b	0.235 ± 0.00 bc	
	PUT2	75.56 ± 0.1 bc	72.68 ± 0.09 c	0.292 ± 0.00 a-c	0.355 ± 0.01 ab	0.057 ± 0.00 c	0.059 ± 0.00 ef	0.123 ± 0.00 c	0.067 ± 0.00 ef	0.248 ± 0.00 a	0.171 ± 0.00 e	
	PUT3	75.73 ± 0.1 bc	74.44 ± 0.1 ab	0.292 ± 0.01 a-c	0.309 ± 0.01 c-e	0.058 ± 0.00 c	0.050 ± 0.00 g	0.092 ± 0.00 e	0.053 ± 0.00 f	0.214 ± 0.00 b	0.202 ± 0.00 d	
	SPD1	75.30 ± 0.2 c	71.97 ± 0.1 de	0.263 ± 0.02 c	0.295 ± 0.01 d-f	0.047 ± 0.00 ef	0.071 ± 0.00 cd	0.091 ± 0.00 e	0.078 ± 0.00 e	0.215 ± 0.00 b	0.186 ± 0.00 de	
	SPD2	75.53 ± 0.1 bc	74.91 ± 0.08 a	0.292 ± 0.01 a-c	0.284 ± 0.01 ef	0.051 ± 0.00 de	0.065 ± 0.00 de	0.079 ± 0.00 f	0.085 ± 0.00 e	0.218 ± 0.00 b	0.223 ± 0.00 c	
	SPD3	75.45 ± 0.2 bc	74.57 ± 0.1 ab	0.313 ± 0.01 a	0.259 ± 0.01 f	0.077 ± 0.00 b	0.054 ± 0.00 fg	0.081 ± 0.00 f	0.084 ± 0.00 e	0.206 ± 0.00 b	0.228 ± 0.00 bc	
	SPM1	75.91 ± 0.1 b	74.35 ± 0.1 ab	0.266 ± 0.01 bc	0.333 ± 0.02 b-d	0.054 ± 0.00 cd	0.115 ± 0.00 b	0.101 ± 0.00 d	0.153 ± 0.00 c	0.246 ± 0.00 a	0.230 ± 0.01 bc	
	SPM2	75.74 ± 0.1 bc	72.37 ± 0.1 cd	0.284 ± 0.01 a-c	0.394 ± 0.01 a	0.042 ± 0.00 f	0.169 ± 0.00 a	0.091 ± 0.00 e	0.350 ± 0.01 a	0.205 ± 0.00 b	0.280 ± 0.00 a	
	SPM3	74.70 ± 0.1 d	71.33 ± 0.2 e	0.290 ± 0.01 a-c	0.337 ± 0.02 bc	0.112 ± 0.00 a	0.077 ± 0.00 c	0.165 ± 0.00 a	0.226 ± 0.01 b	0.213 ± 0.00 b	0.284 ± 0.00 a	
	I2	Control	75.08 ± 0.08 bc	75.20 ± 0.1 d	0.263 ± 0.02 cd	0.286 ± 0.01 bc	0.065 ± 0.00 b	0.078 ± 0.00 b	0.067 ± 0.00 i	0.128 ± 0.00 a	0.175 ± 0.00 f	0.237 ± 0.01 ab
		PUT1	75.69 ± 0.1 a	76.28 ± 0.1 a	0.288 ± 0.01 b-d	0.342 ± 0.01 a	0.033 ± 0.00 d	0.058 ± 0.00 c	0.111 ± 0.00 b	0.094 ± 0.00 c	0.216 ± 0.00 c	0.232 ± 0.00 bc
PUT2		75.61 ± 0.1 a	75.49 ± 0.1 cd	0.248 ± 0.01 d	0.318 ± 0.01 ab	0.000 ± 0.00 e	0.101 ± 0.00 a	0.188 ± 0.00 a	0.079 ± 0.00 f	0.154 ± 0.00 g	0.194 ± 0.00 e	
PUT3		74.62 ± 0.1 d	75.68 ± 0.1 bc	0.280 ± 0.01 b-d	0.239 ± 0.01 c	0.061 ± 0.00 b	0.000 ± 0.00 g	0.104 ± 0.00 d	0.070 ± 0.00 g	0.241 ± 0.00 b	0.202 ± 0.00 de	
SPD1		75.65 ± 0.1 a	75.55 ± 0.1 cd	0.249 ± 0.01 d	0.277 ± 0.01 bc	0.000 ± 0.00 e	0.042 ± 0.00 d	0.086 ± 0.00 f	0.108 ± 0.00 b	0.186 ± 0.00 ef	0.219 ± 0.01 b-d	
SPD2		75.77 ± 0.1 a	75.62 ± 0.1 bcd	0.263 ± 0.01 cd	0.294 ± 0.01 ab	0.049 ± 0.00 c	0.000 ± 0.00 g	0.076 ± 0.00 h	0.094 ± 0.00 c	0.195 ± 0.00 de	0.229 ± 0.01 bc	
SPD3		75.43 ± 0.1 ab	75.57 ± 0.1 cd	0.351 ± 0.01 a	0.340 ± 0.02 a	0.049 ± 0.00 c	0.000 ± 0.00 g	0.080 ± 0.00 g	0.080 ± 0.00 ef	0.206 ± 0.00 cd	0.227 ± 0.01 bc	
SPM1		74.89 ± 0.1 cd	76.07 ± 0.1 ab	0.312 ± 0.01 ab	0.316 ± 0.01 ab	0.000 ± 0.00 e	0.033 ± 0.00 ef	0.090 ± 0.00 1e	0.086 ± 0.00 de	0.215 ± 0.00 c	0.210 ± 0.00 c-e	
SPM2		74.62 ± 0.1 d	76.46 ± 0.1 a	0.323 ± 0.01 ab	0.322 ± 0.01 ab	0.066 ± 0.00 b	0.037 ± 0.00 de	0.110 ± 0.00 b	0.128 ± 0.00 a	0.207 ± 0.00 cd	0.259 ± 0.00 a	
SPM3		75.66 ± 0.1 a	76.47 ± 0.1 a	0.311 ± 0.01 a-c	0.324 ± 0.01 ab	0.072 ± 0.00 a	0.030 ± 0.00 f	0.108 ± 0.00 c	0.091 ± 0.00 cd	0.264 ± 0.00 a	0.224 ± 0.00 b-d	
P-Value		**	**	*	**	**	**	**	**	**	**	**

I1 and I2 = 50 and 75 % soil moisture depletion of field capacity, respectively.

Control = water foliar application, PUT1, PUT2 and PUT3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration Putrescine. SPD1, SPD2 and SPD3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration Spermidin. SPM1, SPM2 and SPM3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration Spermin.

For a given year LSMEANS (along with \pm standard error) within each column of each section followed by the same letter are not significantly different ($P < 0.05$). * Significance at P level of 0.05, ** significance at P level of 0.01.

Table 5
Mean comparison of the effect of interaction irrigation regime (IR) and Foliar application Polyamine (FAP) on total saturated fatty acids (\sum SFA), total mono unsaturated fatty acids (\sum MUFA), total unsaturated fatty acids (\sum UFA), total poly unsaturated fatty acids (\sum PUFA), and ratio of \sum PUFA to \sum SFA (P/S) of safflower in 2017 and 2018 growing seasons.

IR	FAP	\sum SFA (%)		\sum MUFA (%)		\sum UFA (%)		\sum PUFA (%)		Ratio of \sum PUFA to \sum SFA (P/S)	
		2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
I1	Control	9.46 ± 0.2 b	9.18 ± 0.2 bc	14.42 ± 0.1 b-d	15.42 ± 0.1 d	90.49 ± 0.01 b	90.26 ± 0.5 ab	76.06 ± 0.1 bc	74.77 ± 0.5 a	8.05 ± 0.2 a	8.16 ± 0.2 ab
	PUT1	9.71 ± 0.1 ab	9.14 ± 0.3 c	15.04 ± 0.1 a	15.39 ± 0.1 d	94.26 ± 0.3 a	90.83 ± 0.09 a	79.16 ± 0.1 a	75.40 ± 0.09 a	8.15 ± 0.1 a	8.27 ± 0.2 a
	PUT2	9.41 ± 0.3 b	10.03 ± 0.07 a	14.30 ± 0.1 cd	16.58 ± 0.2 c	90.46 ± 0.00 b	89.85 ± 0.08 b	76.10 ± 0.1 bc	73.21 ± 0.1 b	8.10 ± 0.2 a	7.29 ± 0.06 d
	PUT3	9.26 ± 0.06 b	9.48 ± 0.02 a-c	14.51 ± 0.1 b-d	15.58 ± 0.1 d	90.81 ± 0.3 b	90.58 ± 0.3 ab	76.24 ± 0.1 bc	74.95 ± 0.1 a	8.22 ± 0.08 a	7.90 ± 0.03 abc
	SPD1	9.43 ± 0.2 b	9.94 ± 0.03 a	14.74 ± 0.1 a-c	17.59 ± 0.1 b	90.57 ± 0.09 b	90.12 ± 0.3 ab	75.78 ± 0.2 c	72.45 ± 0.1 cd	8.05 ± 0.2 a	7.28 ± 0.01 d
	SPD2	9.38 ± 0.08 b	9.76 ± 0.03 ab	14.45 ± 0.1 b-d	14.80 ± 0.1 e	90.55 ± 0.3 b	90.29 ± 0.2 ab	76.04 ± 0.1 bc	75.42 ± 0.09 a	8.10 ± 0.05 a	7.72 ± 0.03 b-d
	SPD3	9.51 ± 0.2 ab	9.26 ± 0.3 bc	14.47 ± 0.2 b-d	15.59 ± 0.1 d	90.53 ± 0.4 b	90.71 ± 0.3 a	75.97 ± 0.2 bc	75.06 ± 0.1 a	8.00 ± 0.2 ab	8.12 ± .2 ab
	SPM1	9.26 ± 0.07b	9.65 ± 0.2 a-c	14.24 ± 0.1 cd	15.40 ± 0.1 d	90.73 ± 0.04 b	90.44 ± 0.04 ab	76.43 ± 0.1 b	74.92 ± 0.1 a	8.25 ± 0.08 a	7.77 ± 0.2 a-d
	SPM2	9.51 ± 0.2 ab	9.91 ± 0.08 a	14.03 ± 0.1 d	16.92 ± 0.1 c	90.31 ± 0.2 b	90.13 ± 0.02 ab	76.23 ± 0.1 bc	73.04 ± 0.1 bc	8.03 ± 0.2 a	7.36 ± 0.07 d
	SPM3	10.11 ± 0.2 a	9.64 ± 0.3 a-c	14.83 ± 0.1 ab	18.31 ± 0.08 a	90.16 ± 0.2 b	90.35 ± 0.3 ab	75.21 ± 0.1 d	71.95 ± 0.2 d	7.45 ± 0.1 b	7.49 ± 0.2 cd
I2	Control	9.69 ± 0.03 a	9.32 ± 0.00 ab	14.74 ± 0.1 a-c	14.94 ± 0.1 a	90.33 ± 0.2 a	90.75 ± 0.03 a	75.52 ± 0.06 b	75.72 ± 0.1 c	7.79 ± 0.02 a	8.12 ± 0.00 ab
	PUT1	9.59 ± 0.2 a	9.25 ± 0.3 ab	14.20 ± 0.1 c	13.79 ± 0.1 c	90.43 ± 0.1 a	90.70 ± 0.06 a	76.20 ± 0.08 a	76.85 ± 0.1 a	7.96 ± 0.2 a	8.33 ± 0.2 a
	PUT2	9.41 ± 0.1 a	9.54 ± 0.2 ab	14.51 ± 0.2 bc	14.37 ± 0.2 b	90.53 ± 0.3 a	90.48 ± 0.04 a	76.01 ± 0.1 a	76.00 ± 0.1 c	8.07 ± 0.08 a	7.98 ± 0.2 ab
	PUT3	9.73 ± 0.3 a	9.29 ± 0.06 ab	15.16 ± 0.1 a	14.47 ± 0.1 b	90.50 ± 0.1 a	90.60 ± 0.01 a	75.14 ± 0.1 b	76.12 ± 0.1 bc	7.74 ± 0.2 a	8.19 ± 0.03 ab
	SPD1	9.36 ± 0.2 a	9.90 ± 0.5 a	14.57 ± 0.1 a-c	14.59 ± 0.1 ab	90.66 ± 0.3 a	90.69 ± 0.05 a	76.09 ± 0.1 a	76.05 ± 0.2 c	8.14 ± 0.2 a	7.72 ± 0.3 b
	SPD2	9.47 ± 0.2 a	9.33 ± 0.00 ab	14.40 ± 0.2 c	14.61 ± 0.1 ab	90.69 ± 0.1 a	90.76 ± 0.3 a	76.23 ± 0.1 a	76.14 ± 0.2 bc	8.05 ± 0.1 a	8.15 ± 0.02 ab
	SPD3	9.43 ± 0.03 a	9.45 ± 0.2 ab	14.53 ± 0.1 bc	14.49 ± 0.1 b	90.57 ± 0.01 a	90.63 ± 0.3 a	75.99 ± 0.1 a	76.13 ± 0.1 bc	8.05 ± 0.01 a	8.06 ± 0.1 ab
	SPM1	9.67 ± 0.2 a	9.45 ± 0.2 ab	15.03 ± 0.3 ab	13.87 ± 0.1 c	90.45 ± 0.4 a	90.50 ± 0.2 a	75.42 ± 0.1 0b	76.60 ± 0.1 ab	7.81 ± 0.2 a	8.11 ± 0.2 ab
	SPM2	9.47 ± 0.2 a	9.11 ± 0.02 b	14.49 ± 0.2 bc	13.78 ± 0.1 c	90.66 ± 0.07 a	90.86 ± 0.3 a	76.09 ± 0.1 a	77.04 ± 0.1 a	8.04 ± 0.2 a	8.45 ± 0.01 a
	SPM3	9.28 ± 0.1 a	9.14 ± 0.02 b	14.38 ± 0.1 c	13.78 ± 0.1 c	90.69 ± 0.04 a	90.83 ± 0.3 a	76.24 ± 0.1 a	77.02 ± 0.1 a	8.21 ± 0.1 a	8.41 ± 0.03 a
P-Value				**	**	**		**	**		*

I1 and I2 = 50 and 75 % soil moisture depletion of field capacity, respectively.

Control = water foliar application, PUT1, PUT2 and PUT3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration Putrescine. SPD1, SPD2 and SPD3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration Spermidin. SPM1, SPM2 and SPM3 = 50, 100 and 200 $\mu\text{mol L}^{-1}$ concentration Spermin.

For a given year LSMEANS (along with \pm standard error) within each column of each section followed by the same letter are not significantly different ($P < 0.05$). * Significance at P level of 0.05, ** significance at P level of 0.01.

Table 6

Regression between application concentrations of putrescine (PUT) and grain yield in irrigation regime.

Variable	Normal irrigation (I1)	Deficit irrigation (I2)
Grain yield (kg ha ⁻¹)	$y = 2.8881x + 2402.4^{**}$ $r^2 = 0.8419$	$y = -0.0012x^3 + 0.2896x^2 - 9.2987x + 2526.7^{**}$ $R^2 = 1$

** Significance at *P* level of 0.01.

Table 7

Regression between application concentrations of spermine (SPM) and oil content in irrigation regime.

Variable	Normal irrigation (I1)	Deficit irrigation (I2)
Oil content (%)	$y = -3E-06x^3 + 0.0007x^2 - 0.0383x + 23.22^{**}$ $R^2 = 1$	$y = -6E-06x^3 + 0.0017x^2 - 0.104x + 21.95^{**}$ $R^2 = 1$

** Significance at *P* level of 0.01.

acid concentrations are reduced. In our study, the higher mean oleic acid in 2018 than 2017 could also be due to the high temperature during seed filling period. In addition, Kane et al. (1997) found a negative relationship between rainfall during seed filling and oleic acid content. In this study, rainfall during seed filling period in 2017 may have reduced oleic acid compared with 2018. The reduction in cellular water can also change permeability of the cell membrane and fluidity (Marrink et al., 1996). A reduction in unsaturated fatty acids means increased membrane layer strength (De Paula et al., 1990). The ability to regulate membrane fluidity by changing the levels of unsaturated fatty acids is a characteristic of stress-tolerant plants that is mainly mediated by activities regulated by fatty acid desaturases. The change in membrane fluidity leads to a suitable environment for the function of essential proteins, such as the mechanism of photosynthetic system during stress (Blée, 2002). The negative impact of drought stress on oleic acid content in rapeseed (Aslam et al., 2009) and safflower (Ashrafi and Razmjoo, 2010) has also been reported. In a study on the effect of drought stress at flowering stage with foliar application of salicylic acid (SA) and putrescine (PUT) growth regulators at a concentration of 10^{-5} mol L⁻¹ on two rapeseed cultivars for 10 days, both growth regulators significantly improved oleic acid content (C18: 1); however, the application of growth regulators had no effect on linoleic acid content (C18: 2) in rapeseed cultivars under stress conditions (Ullah et al., 2012). They also reported that PUT improved the ratio of oleic acid to linoleic acid under drought stress due to the effect of growth regulators on the enzyme involved in PUFA (Ullah et al., 2012). According to the results, it seems that polyamine compounds (PUT, SPD and SPM) influence oil content and fatty acid composition. This may be due to heightened enzymatic activity and metabolite transfer to safflower seeds.

Eicosenoic acid (C20: 1, Omega-9) content in the 2018 experiment was significantly higher than that in 2019. The content of eicosenoic acid (C20: 1, Omega-9) in the experiment varied from 0 to 0.3 % which is consistent with the study results of Akbari et al. (2020). The mean eicosenoic acid (C20: 1, Omega-9) was 0.1 % during the two years of experiment. In both years (on average between foliar application levels), eicosenoic acid (C20: 1, Omega-9) rose significantly with SPM treatment (Table 4). The increase in eicosenoic acid (C20: 1, Omega-9) in SPM (0.09 %), SPD (0.08 %) and water foliar application (0.06 %) treatments were 84.6 %, 118 % and 201.6 %, respectively. When soil moisture reduced by 75 % FC (I2) at flowering stage, PUT foliar application treatment had a greater effect on eicosenoic acid content (C20: 1, Omega-9). In both years (on average between foliar application levels), PUT treatment increased eicosenoic acid (C20: 1, Omega-9) by 5.8 %, 10.2 % and 24.1 % in comparison to SPM, SPD and control treatments, respectively (Table 4).

The results of regression analysis in the two years of experiment showed that the content of eicosenoic acid (C20: 1, Omega-9) was significant due to the interaction between irrigation and foliar application

treatments (Fig. 6). The increasing trend of the content of eicosenoic acid (C20: 1, Omega-9) was almost similar in irrigation treatments. Higher SPM and PUT foliar application concentrations in irrigation treatments (I1 and I2) significantly enriched the content of eicosenoic fatty acid (C20: 1, Omega-9) linearly with a gentle gradient (Fig. 6).

The mean \sum MUFA during the two years of testing was 14.8 %. The results of the interactions between polyamine irrigation regime (Table 5) demonstrated that in both years, on average the lowest and highest \sum MUFA under normal irrigation conditions (I1) belonged to water (control) and SPM foliar application treatments, and increasing the concentration of SPM from 50 to 200 μ mol L⁻¹ led to an enhancement of 11 % \sum MUFA but by elevating soil moisture depletion to FC 75 % (I2) the effect of water foliar application appeared more on \sum MUFA (Table 5).

3.5. Changes in polyunsaturated fatty acids (PUFA) levels under irrigation regime and polyamines

The level of linoleic acid (C18: 2, Omega-6) in 2017 was significantly higher than that in 2018. The level of linoleic acid (C18: 2, Omega-6) during the two years of experiment varied from 71.3–78.6% in irrigation and polyamine treatments, and the mean in two years was 75.1 %. The range of changes between 62.7 and 83.7 % has also been reported (Guan et al., 2008; Velasco et al., 2001). In both years (on average between foliar application levels) increasing soil moisture depletion to FC 75 % (I2) SPM and water foliar application treatments had the highest and lowest effect on linoleic acid (C18: 2, Omega-6) on the other hand, PUT < SPD < SPM treatments increased linoleic acid (C18: 2, Omega-6) by 0.7, 0.6 and 0.5 %, compared with water foliar application treatment respectively (Table 4). The highest level of linoleic acid (C18: 2, Omega-6) (75.3 %) in both years (on average between foliar application levels) was obtained from normal irrigation conditions with PUT foliar application, which compared with the control, SPD and SPM treatments was 0.5, 0.9 and 1.7 % higher, respectively (Table 4). As mentioned earlier, temperature during the growing season has a great effect on the composition of safflower oil fatty acids (Samanci and Ozkaynak, 2003). Studies have shown that the concentration of linoleic acid fell with increasing the temperature during seed filling while oleic acid concentration rose (Oliva et al., 2006). The lower linoleic acid concentration in 2018 could be due to high temperatures during seed filling stage. High temperature may have negative impacts on seed filling stage and reduced linolenic acid by reducing the transfer of assimilates to seeds.

The results of the present study illustrated that the highest significant positive correlation was found between linoleic acid (C18: 2, Omega-6)

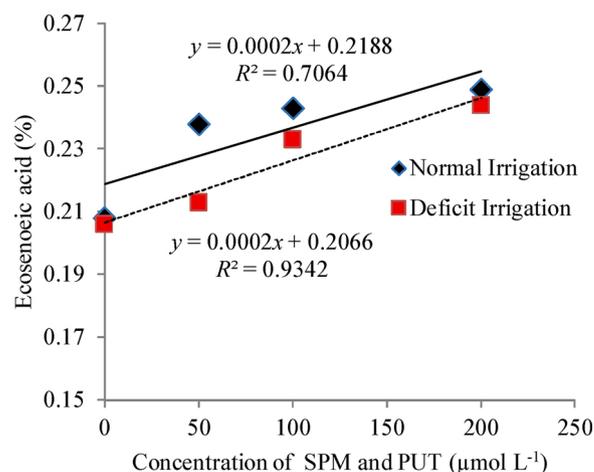


Fig. 6. Regression between application concentrations of spermine (SPM) and putrescine (PUT) in normal irrigation and deficit irrigation = 50 and 75 % soil moisture depletion of field capacity, respectively with eicosenoic acid.

and \sum UFA ($r = 0.631^{**}$, $P < 0.001$) and \sum PUFA ($r = 0.99^{**}$, $P < 0.001$). Also, the highest significant negative correlation was observed between linoleic acid and oleic acid ($r = -0.86^{**}$, $P < 0.001$) and MUFA ($r = -0.87^{**}$, $P < 0.001$). Linoleic acid was correlated with palmitic acid ($r = -0.57^{**}$, $P < 0.001$), palmitoleic ($r = -0.41^{**}$, $P < 0.001$), α -linolenic acid ($r = -0.36^{*}$, $P < 0.05$), eicosenoic acid ($r = -0.36^{*}$, $P < 0.05$), and eicosatrienoic acid ($r = -0.86^{*}$, $P < 0.05$) (data not shown).

In this study, the trend of α -linolenic acid (C18: 3, Omega-3) changes at each irrigation levels was similar to that of polyamine foliar application (on average between foliar application levels) in both years of the experiment (Table 4). When increasing soil moisture depletion from 50 to FC 75 % (I2) by SPM foliar application (0.3 %) compared with SPD (0.2 %), PUT (0.2 %) and water foliar application treatments (0.2 %) α -linolenic acid (C18: 3, Omega-3) increased by 15.6 %, 11.1 % and 7.4 %, respectively, and had the least effect on increasing the level of α -linolenic acid (C18: 3, Omega-3) in water foliar application. When reducing soil moisture depletion to FC 50 % (I1), SPM foliar application also had the greatest effect on increasing α -linolenic acid (C18: 3, Omega-3) and SPM treatment increased the amount of α -linolenic acid (C18: 3, omega-3) by 2.9, 7.4 and 9.6 % compared with water, PUT and SPD treatments, respectively (Table 4). There have been numerous reports of increased linolenic acid in safflower (Akbari et al., 2020) and its reduction in rapeseed (Moghadam et al., 2011) under drought stress. In general, the composition of fatty acids (FAs) is determined by the activity of the enzymes involved in their biosynthesis (Wallis and Browse, 2002). Numerous reports show that environmental stress (cold, heat, dry and salinity) lead to changes in fatty acid composition mainly in linolenic acid content (C18: 3) (Graham and Patterson, 1982).

Studies have shown that linoleic acid biosynthesis (C18: 3) is caused by sequential desaturases of (C18: 1) and (C18: 2) in the leaves, roots, and seeds of many high plants (Cherif et al., 1975). Therefore, the reduction in α -linolenic acid in deficit irrigation treatment may be due to reduced desaturase enzymes activities and assimilates limited to be seeds. Linolenic acid is critical for maintaining the membrane integrity and function of integrated membrane proteins, such as proteins that enhance the mechanism of photosynthesis system. As a result, a reduction in the concentration has a major effect on photosynthesis (Upchurch, 2008). Therefore, it seems that exogenous application of polyamines, especially in deficit irrigation conditions, has a positive effect on the quality of grain fatty acids by increasing the transport and assimilation to the grains.

The mean eicosatrienoic acid (C20: 3, Omega-3) was 0.2 % during the two years of experiment. The study results showed that increasing the concentration of SPM from 50 to 200 $\mu\text{mol L}^{-1}$ at each irrigation level significantly increased the level of eicosatrienoic acid (C20: 3, Omega-3) (Table 4). Additionally, the interaction revealed that (on average between foliar application levels) SPM in I1 treatment of eicosatrienoic acid (C20: 3, Omega-3) compared with PUT, SPD and water foliar application treatments rose by 11.5, 14 and 16.8 %, respectively. While increasing soil moisture depletion up to FC 75 % (I2), the highest eicosatrienoic acid (C20: 3, Omega-3) was also observed in I2 \times SPM (0.230 %) > I2 \times SPD (0.210 %) > I2 \times PUT (0.207 %) < water content (control) (0.206 %); SPM increased eicosatrienoic acid (C20: 3, Omega-3) by 9.5, 11.1 and 11.6 % in comparison with SPD, PUT and control treatments (Table 4).

Unsaturated fatty acids (\sum UFA) changed slightly in the two years. The range of \sum UFA changes was from 89.8–94.2%. The study results are consistent with the study results of Akbari et al. (2020). On average, in both years PUT1 (50 $\mu\text{mol L}^{-1}$) increased \sum UFA by 2.4 % under normal irrigation (I1) conditions. However, under deficit irrigation (I2) conditions at flowering stage, increasing SPM foliar application concentration increased \sum UFA, although no significant difference was observed between SPM3 (200 $\mu\text{mol L}^{-1}$) and SPM2 (100 $\mu\text{mol L}^{-1}$) treatments (Table 5). The reduction in unsaturated levels due to water shortage can be associated with the inhibition of unsaturated fatty acid biosynthesis and desaturase activities (Anh et al., 1985). In general, a reduction in

unsaturated levels leads to a reduction in membrane fluidity. Boucher-eau et al. (1996) showed that water stress at flowering stage affected oil concentration and fatty acid composition of rapeseed seed. Also Aslam et al. (2009) stated that drought stress in rapeseed elevated palmitic acid and reduced UFA.

Regression analysis in the two years of experiment showed a significant interaction between irrigation and foliar application treatments on \sum UFA (Table 8). During normal irrigation (I1), the relationship between \sum UFA and PUT foliar application was cubic. The results of this experiment showed that the highest level of \sum UFA was obtained from normal irrigation (I1) when PUT concentration was about 39 $\mu\text{mol L}^{-1}$ and increasing PUT concentration reduced it. During deficit irrigation (I2), the relationship between \sum UFA and SPM foliar application was linear (Table 8).

The level of \sum PUFA in 2018 was lower than that in 2017. During the two years of the experiment, the level of \sum PUFA ranged from 71.9–79.1%. In both years of the experiment, the lowest level of \sum PUFA was obtained when the soil moisture content was reduced to FC 75 % (I2) with water foliar application (control). Nevertheless, increasing SPM concentration from 50 to 200 $\mu\text{mol L}^{-1}$ (I2 \times SPM3) caused a 1.3 % increase in \sum PUFA. When the soil moisture content was reduced to FC 50 % (I1), the lowest PUT concentration (I1 \times PUT1) caused a 2.4 % increase in \sum PUFA, though (Table 5).

Unsaturated lipids contribute to the stability of the cell membrane and the optimal activity of membrane-bound proteins (Barkan et al., 2006; Vijayan et al., 2002). Thus, an increase in the level of \sum PUFA can help maintain the fluidity and permeability of membrane lipids under stress conditions (Torres-Franklin et al., 2009). The ability to regulate membrane lipid fluidity by changing the levels of unsaturated fatty acids is a consistent and deleterious characteristic of the plant that often enhances membrane fluidity (Upchurch, 2008). Many reports have shown that plants improve environmental stress tolerance by developing unsaturated fatty acid components, especially under heat, cold and drought stresses (Liu et al., 2008; Sui et al., 2007). Therefore, it seems that the exogenous application of polyamine compounds, especially under deficit irrigation conditions, delayed the aging process of plants and boosted the rate of assimilation and transfer to the seed, thus positively affecting the quality of seed fatty acids.

The results of regression analysis in the two years of experiment also showed that a significant relationship was found between irrigation and foliar application treatments in terms of \sum PUFA (Table 8). Increasing the concentration of PUT foliar application significantly increased the level of \sum PUFA during normal irrigation (I1) as cubic. The results of this experiment showed that increasing PUT concentration to 38.5 $\mu\text{mol L}^{-1}$ enhanced the level of \sum PUFA and reduced the level of \sum PUFA thereafter. Based on linear regression equation between \sum PUFA and SPM foliar application concentration during deficit irrigation (I2), it was found that \sum PUFA increased with higher SPM foliar application concentration and per 1 $\mu\text{mol L}^{-1}$ SPM concentration, \sum PUFA increases by 0.005 units (Table 8).

Ratio of total poly unsaturated fatty acids to saturated fatty acids (P/

Table 8

Regression between application concentrations of putrescine (PUT) and spermine (SPM) in normal irrigation and deficit irrigation respectively with total unsaturated fatty acids (\sum UFA), total poly unsaturated fatty acids (\sum PUFA), and ratio of total poly unsaturated fatty acids (\sum PUFA) to total saturated fatty acids (\sum SFA) (P/S).

Variable	Normal irrigation (I1)	Deficit irrigation (I2)
\sum UFA	$y = 6E-06x^3 - 0.0019x^2 + 0.121x + 90.377^{**}$ $R^2 = 1$	$y = 0.0014x + 90.521^{**}$ $r^2 = 0.6272$
\sum PUFA	$y = 7E-06x^3 - 0.0019x^2 + 0.1153x + 75.418^{**}$ $R^2 = 1$	$y = 0.005x + 75.77^{**}$ $r^2 = 0.8089$
\sum PUFA / \sum SFA (P/S)	$y = 1E-06x^3 - 0.0003x^2 + 0.0138x + 8.107^{**}$ $R^2 = 1$	$y = 0.002x + 7.9468^{**}$ $r^2 = 0.8272$

** Significance at P level of 0.01.

S) in 2017 was higher than that in 2014. P/S ratio ranged from 7.2–8.4, which is consistent with the study results of Akbari et al. (2020). Under deficit irrigation conditions (I2), enhancing SPM foliar application concentration had a significant effect on P/S ratio, and SPM3 (200 $\mu\text{mol L}^{-1}$) foliar application with a mean of 9.8 % increased P/S ratio by 3.4 % compared with the control with a mean of 9.5 %. Under normal irrigation (I1) conditions, PUT1 (50 $\mu\text{mol.L}^{-1}$) boosted P/S ratio by 1.3, 2.5 and 7.1 %, respectively, compared with SPM1 (50 $\mu\text{mol.L}^{-1}$), SPD1 (50 $\mu\text{mol.L}^{-1}$) and control treatments (Table 5). P/S index is an important parameter for determining the nutritional value of some oils. Several studies have found that higher values of P/S index reduced fat deposition in the body (Kostik et al., 2013). In plants it is also specified that unsaturated lipids in addition to cell membrane stability also contribute to the optimal activity of membrane-bound proteins, an increase in ΣPUFA can help maintain proper fluidity and permeability of membrane lipids under stress conditions (Torres-Franklin et al., 2009). In contrast, Hamrouni et al. (2001) reported that the major changes in safflower leaves lipids caused by water shortage stress were due to the reduction in PUFAs in favor of SFAs. The results of the present study illustrated that the exogenous application of polyamine compounds can be effective on elevating P/S index.

Based on the results of regression analysis of the two years of experiment, P/S ratio showed a significant relationship between the interaction and foliar application treatments (Table 8). The cubic regression relationship between P/S ratio and PUT concentration during normal irrigation (I1) demonstrated that the highest P/S ratio was obtained from PUT foliar application at 26.5 $\mu\text{mol.L}^{-1}$ concentration under these conditions and the concentration increased more than that value reduces P/S ratio. However, during deficit irrigation (I2) the relationship between P/S ratio and SPM concentration was linear. Accordingly, increasing every 1 $\mu\text{mol L}^{-1}$ SPM in the experiment increased P/S ratio by 0.002 units (Table 8).

Many oilseed plants accumulate Triacylglycerols (TAGs) in their seeds, consisting primarily of 5 major fatty acids, saturated fatty acids (palmitate and stearate), and unsaturated fatty acids (oleate, linolate, and α -linolenate). In species of oilseed plants that contain unique fatty acids such as epoxy, hydroxy and long chain fatty acids, polyamines can affect the activity of enzymes involved in lipid metabolism and can also regulate the synthesis of Triacylglycerols (TAGs) (Tomosugi et al., 2006). For example, polyamines regulate the activity of the enzymes Glycerol 3-phosphate (G3P) and Lysophosphatidic acid (LPA) acyltransferase (Ichihara, 1984; Ichihara et al., 1987) of safflower seed. It was found that polyamines are also essential for seed development (Urano et al., 2005). It was revealed in the studies conducted on safflower seed that G3P and LPA acyltransferase activities are enhanced by polyamines (these enzymes are involved in lipid synthesis from G3P to TAG) (Ichihara, 1984; Ichihara et al., 1987). LPA acyltransferase has a precise and selective property for unsaturated fatty acids found in many oilseed plants (Griffiths et al., 1985; Ichihara et al., 1987). It has also been found that during the ripening process of safflower seed, seed TAG content increased and the content of phosphatidic acid (PA), phosphatidylcholine (PC), diacylglycerol (DAG) reduced. The results of the experiment by Tomosugi et al. (2006) on castor bean (*Ricinus communis* L.) also showed that polyamines are essential for the synthesis of PA from LPA and the activity of LPA-acyltransferase enzyme was affected more by PUT < SPD < SPM. Several mechanisms have also been reported on the stimulating effect of polyamines on LPA-acyltransferase (Tomosugi et al., 2006). Therefore, it seems that polyamines play a positive role in safflower fatty acid quality and oil content in this study under both normal and deficit irrigation conditions.

4. Conclusion

We found that foliar application of spermine and putrescine had a great effect on increasing agronomic traits and improving unsaturated fatty acids at each irrigation level in both years of the experiment. Foliar

application of putrescine had a significant effect on safflower growth (number of seeds per capitula, and grain and oil yield) under deficit irrigation conditions in both years. Under deficit irrigation conditions, foliar application of spermine increased the number of capitula per plant, oil percentage and harvest index in both crop years. When increasing soil moisture (normal irrigation), spermine foliar application increased oil yield and content. The combination of unsaturated and saturated fatty acids was also affected by the foliar application of polyamines under deficit irrigation conditions to FC 75 % so that in every two years spermine foliar application increased Omega-3, 6, Σ PUFA, Σ UFA and Σ PUFA to Σ SFA ratio and reduced SFA (palmitic acid). Also, PUT foliar application can be used to improve the quality of unsaturated fatty acids Omega-6, Σ PUFA and Σ UFA under normal irrigation conditions. As a conclusion, it can be stated that spermine and putrescine foliar application at flowering stage and in areas with similar climatic conditions can be performed to improve seed and oil yield and enhance the quality of fatty acids of safflower.

CRedit authorship contribution statement

Kayvan Fathi Amirkhiz: Formal analysis, Investigation, Resources, Software, Writing - original draft, Visualization. **Majid Amini Dehaghi:** Validation, Resources, Supervision, Funding acquisition, Project administration, Writing - review & editing. **Seyed Ali Mohammad Modares Sanavy:** Conceptualization, Resources, Formal analysis, Visualization. **Alireza Rezazadeh:** Methodology, Resources, Software.

Declaration of Competing Interest

The authors report no declarations of interest.

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