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Assessment of changes in secondary metabolites and growth of saffron under organic fertilizers and drought

Roohollah Saeidi Aboueshaghi[a], Heshmat Omidi[a], and Amir Bostani[b]

[a]Department of Agronomy, Faculty of Agriculture Science, Shahed University, Tehran, Iran; [b]Deparment of Soil Sciences, Faculty of Agriculture Science, Shahed University, Tehran, Iran

ABSTRACT

Saffron (*Crocus sativus* L.) as the most expensive spice, needs low water for growth. However, its yield reduces under drought and nutrient stresses. A two-year field experiment was aimed to evaluate the improvement of saffron growth and quality by nutrition supply on drought conditions in 2018 and 2019. Irrigation regimes were included control (field capacity) and drought (66% depletion of soil water). Fertilizer treatments were included control (without fertilizing), chicken manure, chemical fertilizer, 25% chicken manure + 75% chemical fertilizer ($Ch_{25}M_{75}$), 50% chicken manure + 50% chemical fertilizer ($Ch_{50}M_{50}$), and 75% chicken manure + 25% chemical fertilizer ($Ch_{75}M_{25}$). Drought reduced the saffron growth characteristics, chlorophyll content, relative water content (RWC), and yield. On the contrary, the amount of proline and soluble carbohydrates (SC) increased. However, the content of crocin, picrocrocin, and safranal increased by drought. Mineral nutrition deficit reduced the saffron growth characteristics, yield, and secondary metabolites content under both irrigation regimes. The combined use of chemical fertilizers and chicken manure was more effective in improving the growth and biochemical traits of saffron in both irrigation regimes than using them alone. The greatest saffron growth was acquired by replacing 75% of chemical fertilizer with chicken manure. However, the highest quality of saffron was obtained when the share of chemical fertilizer was higher than chicken manure (3:1). Therefore, reducing chemical fertilizer and using chicken manure instead is recommended to improve the yield and quality of saffron in drought conditions.

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chicken manure; crocin; drought; fertilizer; proline; saffron

Introduction

Drought is the most momentous restricting agent in crop production worldwide. Drought is estimated to reduce crop yields by 55–70% by the late 21st century (Chai et al. 2016). Meanwhile, global food demand is forecasted to increase by at least 70% by 2050. Hence, access to food security would be the main challenge over the world (Keating et al. 2014). Drought also challenges the production of medicinal plants and herbs. Although drought often leads to the accumulation of secondary metabolites, it may reduce their dry matter. Therefore, the value of medicinal plants decreases due to the reduction in secondary metabolites yield. Hence, many agronomic practices such as irrigation management, soil nutrient management, and the development of drought-resistant species need to be improved to overcome this problem (FAO 2017).

Saffron (*Crocus sativus* L.) as the most expensive spice is a perennial plant that belongs to the Iridaceae family. Saffron has low water requirements and high economic capacity. Hence, it is

CONTACT Heshmat Omidi heshmatomidi@yahoo.com; omidi@shahed.ac.ir Department of Agronomy, Faculty of Agriculture Science, Shahed University, Tehran, Iran

widely cultivated in the arid and semi-arid regions of Iran such as the Khorasan and Fars provinces (Oftadeh et al. 2017). The saffron quality grade is determined based on the rate of yellow color, bitter taste, and aroma in the stigma. They are respectively dependent on the amount of Crocin, Picrocrocin, and Safranal, which are affected by genetic factors, fertilizer type, environmental stress, and drying procedure (Ahmad et al. 2011; Jami et al. 2020). The saffron water requirement is lower than other common crops during the first growing season (about 3600 m^3 ha^{-h}) (Fallahi and Mahmoodi 2018). However, adequate irrigation has an impressive effect on saffron yield increment (Fallahi et al. 2016). Aghhavani Shajari et al. (2020) reported that irrigation management is an important factor in obtaining a higher stigma yield of saffron.

Providing mineral nutrition is another important factor to determine saffron yield (Amiri 2008). Some studies have reported that there is a positive correlation between soil organic matter and saffron yield. This is probably due to the release of nutrients, particularly phosphorus and nitrogen, and the improvement of soil physical properties (Negbi 1999). Leaf nitrogen amount has a positive correlation with saffron yield, although safranal and crocin contents have a high correlation with the leaf phosphorus content (Naghdi Badi et al. 2011; Parsa et al. 2018). Therefore, a sufficient nutrition supply can play an effective role in improving saffron yield and quality. In agriculture, soil nutrient deficiencies are compensated by the use of chemical, organic, and biological fertilizers (Selim 2020). In recent years, chemical fertilizer consumption has exponentially risen around the world. This is responsible for a 50% increment in agricultural production over the 20th century (Aziz et al. 2015). On the other, the excessive use of synthetic fertilizers causes serious problems, e.g., deterioration of soil physio-chemical properties, soil nutrient deficiency, soil erosion, eutrophication, and food contamination (Wu and Ma 2015; Selim 2020). The consciousness of organic fertilizer ability has lately increased to replace a part of chemical fertilizers. Because Integrated Nutrition Management (INM) benefits are more known for improving plant growth and agroecosystem sustainability (Suge, Omoniyi, and Omani 2011; Selim 2020). Organic manure use along with chemical fertilizers improves the physical, chemical, biological, and hydrological properties of the soil. Its use reduces drought detrimental effects and helps to access a more volume of water and nutrients by spreading the roots in the soil (Fallahi and Mahmoodi 2018; Selim 2020). Saffron yield has been reported that extremely affected by organic and chemical fertilizers (Jahan and Jahani 2007; Koocheki and Seyyedi 2015). However, chemical fertilizers especially nitrogen and phosphate have a significant impact on increasing the saffron stigma yield (Behzad, Razavi, and Mahajeri 1992). Koocheki and Seyyedi (2015) reported that the application of organic (cattle manure composts 25 Mg ha^{-}) and chemical fertilizers (N 300 kg ha^{-} + P 100 kg ha^{-+}) increased the flower numbers (FNs) and stigma yield.

Drought is decreased uptake, redistribution, and transport of mineral nutrition in plants. Besides, diffusion and mass flow of mineral nutrition in the soil were reduced by a water deficiency (Gessler, Schaub, and McDowell 2017). A meta-analysis study revealed that drought reduced nitrogen and phosphorus contents in the plant tissue (He and Dijkstra 2014). Moreover, some studies have reported that drought can reduce nutrient absorption from the soil (Sardans and Peñuelas 2012; Bista et al. 2018). Despite some studies performed to evaluate saffron response to irrigation or fertilizer management, limited studies have been evaluated fertilizer management's effect on improving the saffron yield and quality in drought conditions. Thus, this study presumed that the utilization of appropriate chemical and organic fertilizers can alleviate the drought's detrimental impact on saffron growth and secondary metabolites content.

Materials and methods

Farm location and treatments

A two-year field experiment was conducted in Shahrekord, Iran (50° 39′ N and 52° 24′ E, 2300 m elevation) during 2018 and 2019. Experimental treatments included irrigation regimes as the

Table 1. Chemical properties of used chicken manure.

OM (%)	EC (ds m^{-1})	pH	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
65.88	5.87	7.4	2.11	1.65	1.82	6.1	0.92	0.36	714.2	296.4	68.4	411

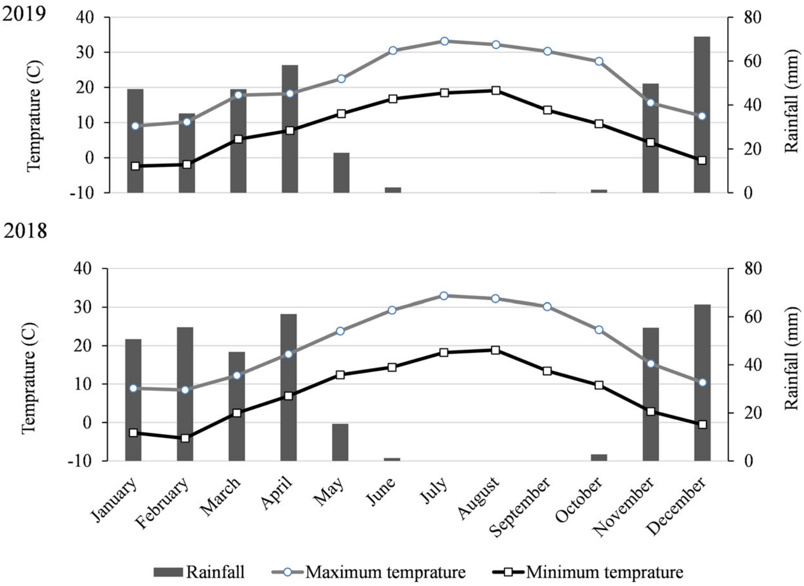

Figure 1. Change of minimum and maximum temperature and rainfall in different months between 2018 and 2019.

main plot and six fertilizer combinations as the subplot. They were arranged as split-plot based on a randomized complete block design with three replicates. Irrigation regimes included field capacity as control (soil water potential at −0.5 atm) and 66% soil water depletion from the field capacity as drought (soil water potential at −6.5 atm). The drought was applied after the end of flowering (beginning on November 10 and ending on May 10). Fertilizer combinations included control (without fertilizing), chicken manure (3 Mg ha^{-}), chemical fertilizer (100 kg $ha^{-,}$ urea + 150 kg $ha^{-ur}K_2SO_4$ + 45 kg ha^{-+} $FeSO_4$ + 15 kg ha^{-+} $ZnSO_4$ + 20 kg ha^{-+} $MnSO^4$), 25% chicken manure + 75% chemical fertilizer, 50% chicken manure + 50% chemical fertilizer, and 75% chicken manure + 25% chemical fertilizer. Some chemical properties of the chicken manure are shown in Table 1. The fertilizer treatments were applied after preparing the experimental plots. The soil water content was determined by the Equitensiometer equipment. This apparatus was placed in the adjacent saffron corms before the irrigation was implemented. Then, the soil water potential was calculated daily from the recorded data. Therefore, irrigation intervals were determined for each irrigation regime. Biennial meteorological data including monthly minimum and maximum temperatures changes and precipitation amount are presented in Figure 1.

Farm operation and sampling

The operations for preparation experimental farms included deep plowing, crushing great lumps, and smoothing the surface. The experimental plots were created with 4×2 m dimensions in September 2017. Each plot was contained 20 cultivating rows with a 20 cm distance from each other. Saffron corms were cultivated on the row with a 10 cm interval on 7 October 2017. The first irrigation was done for all plots on October 18. The sample of flowers was taken up randomly from each plot on 1 November 2018 and 2019. The leaf sampling was done before irrigation between 10 and 20

Table 2. Physical and chemical properties of experimental farm soil.

Texture	OC (%)	EC (ds m^{-1})	pH	N (%)	P (ppm)	K (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	B (ppm)	Mn (ppm)
Sandy clay	1.6	0.56	7.6	0.13	27.6	164	2.15	0.93	0.66	2.27	8.31

March 2018 and 2019. A part of the samples was immediately frozen in liquid nitrogen and was held for the biochemical analysis at −80 °C. Other samples were transferred to the laboratory and were dried in the shade after measuring fresh weight (FW). The physical and chemical properties of soil were investigated by sampling before field preparation (Table 2).

Flower traits

FN was measured in a square meter of each plot. Stigma and style after measuring flower fresh weight (FFW) separated from the flower and was measured their length. The flowers and stigmas were dried in shadow conditions at ambient temperature for 7 d.

Chlorophyll and carotenoid measurement

The chlorophyll and carotenoid amounts were measured by Arnon (1949) method. An appropriate amount of frozen leaf (1 g) was homogenized with 10 mL acetone 80% by a mortar and pestle. The suspension solution was filtered and then the residual was washed to remove its color. Then, the absorbance of the extract was measured at 663, 645, 510, and 480 nm, respectively. The pigments content was calculated by the following equations and was expressed as mg g^{-a} FW:

$$\text{Chlorophyll a}(\text{Ch}_\text{a}) = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000W} \quad (1)$$

$$\text{Chlorophyll b}(\text{Ch}_\text{b}) = 22.9\ (A_{645}) - 2.69\ (A_{663}) \times \frac{V}{1000W} \quad (2)$$

$$\text{Chlorophyll Total}(\text{Ch}_\text{T}) = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times \frac{V}{1000W} \quad (3)$$

$$\text{Carotenoid}(\text{Car}) = 7.6\ (A_{480}) - 14.9\ (A_{510}) \times \frac{V}{1000W} \quad (4)$$

RWC

RWC was determined by the leaf immersion method presented by Kwasniewski et al. (2016). Fresh leaves were cut into 3 cm sections and were immediately weighted as FW. The leaf pieces were immersed in a petri dish containing deionized water (15 mL) and were maintained in a dark place for 24 h. Then, the swollen leaf pieces take up from the water and were weighted as turgid weight (TW). The leaf pieces were dried at 70 °C for 24 h until was obtained a constant weight and was recorded as dry weight (DW). The RWC was measured as the following equation:

$$\text{RWC}(\%) = \left[\frac{(TW - FW)}{(TW - DW)}\right] \times 100 \quad (5)$$

Proline content measurement

The proline content was measured *via* frozen leaf crushing in sulfosalicylic acid by a mortar and pestle. The homogenate solution was centrifuged at 13,000 *g* for 12 min. The extract (2 mL) was combined with an equal volume of Ninhydrin reagent and glacial acetic acid in a test tube. The reaction was completed by heating the test tube in a boiling water bath for 1 hr. Then, toluene was added to the reaction solution after cooling. The absorbance of the upper layer was read at 520 nm. The proline content was computed by proline standard curves and reported as µM g^{-} FW (Bates, Waldren, and Teare 1973).

Sugar content measurement

The sugar content was assayed according to DuBois et al. (1956) method. The appropriate amount of frozen leaf was extracted with distilled water at 4 °C. The extract was filtered and reached 10 mm volume. Then, 1 mL of extract was reacted by aqueous phenol (5%) and sulfuric acid in a test tube, respectively. The reaction solution absorbance was read at 480 nm. Sugar amount was calculated by the glucose standard curve and was expressed as mg g^{-} FW.

Crocin, picrocrocin, and safranal assay

Crocin, picrocrocin, and safranal were measured by ISO 3632-2 method (ISO 3632–2 Technical Specification 2014). Briefly, dried stigma (50 mg) was extracted by distilled water (90 mL) in a shaker for 1 hr. The extract was then reached 100 mL volume by distilled water and was incubated at ambient temperature in darkness for 24 hr. Finally, the extract was diluted with distilled water (1:20) and filtered quickly to obtain a clear solution. The absorbance of picrocrocin, safranal, and crocin was recorded at 257, 330, and 440 nm, and their content was measured as the following equation:

$$E\ 1\% = \frac{(D \times 10000)}{(m(100 - h))} \tag{6}$$

where D is the absorbance rate at 257, 330, and 440 nm; m is sample weight (g); h is moisture content of the sample.

Statistical analysis

Data analysis was performed by SAS version 9.4 software (SAS Institute, Cary, NC, USA). The Bartlett test was applied to evaluate the variance homogeneity. Duncan's multiple range test was used for main factors comparison at a 5% confidence interval. The least-square means procedure was used for the comparison of the interaction of the main factors.

Results

Saffron flower characteristics

Drought and different fertilizers applications had a significant effect ($p \leq 0.01$) on saffron flower characteristics including stigma length (SL), FN, FFW, stigma fresh weight (SFW), flower dry weight (FDW), and yield. The flower traits and saffron yield in 2019 were higher than in 2018. FNs per square meter and SL were reduced to 12.3% and 7.1%, respectively, by decreasing soil water potential to −6.5 atm. Moreover, flower fresh and DWs were decreased to 11.7% and 13.7% by drought compared with the control. Meanwhile, the saffron yield was reduced to 13.2% under drought conditions (Table 3).

The saffron yield or stigma DW had a high positive correlation with SL ($r = 0.91$, $p \leq 0.01$), FN ($r = 0.99$, $p \leq 0.01$), FFW ($r = 0.99$, $p \leq 0.01$), SFW ($r = 0.98$, $p \leq 0.01$), and FDW ($r = 0.99$, $p \leq 0.01$). However, the stepwise regression showed that 99% changes in saffron yield were justified by flower DW in both irrigation regimes (Figure 2). Therefore, it can be concluded that the stigma DW was dependent directly on the flower DW and indirectly on the SL, FN, and FFW.

Table 3. Mean comparison of some saffron characteristics at different years and irrigation regimes.

	SL (mm)	FN	FFW (g m^{-2})	SFW (g m^{-2})	FDW (g m^{-2})	Yield (g m^{-2})	Ch_a (mg g^{-1} FW)	Ch_b (mg g^{-1} FW)
2018	69.8^b	82.8^b	33.67^b	3.65^b	8.9^b	0.6^b	3.37	3.5
2019	70.7^a	97.2^a	38.75^a	4.24^a	10.05^a	0.67^a	3.61	3.75
S.E.M.	3.62	–	–	–	–	–	–	–
Control	72.9^a	95.6^a	38.47^a	4.21^a	10.16^a	0.68^a	4.44^a	4.29^a
Drought	67.7^b	83.8^b	33.95^b	3.69^b	8.77^b	0.59^b	2.535^b	2.97^b

Means in each column with the same letters have no significant difference at $p \leq 0.05$.

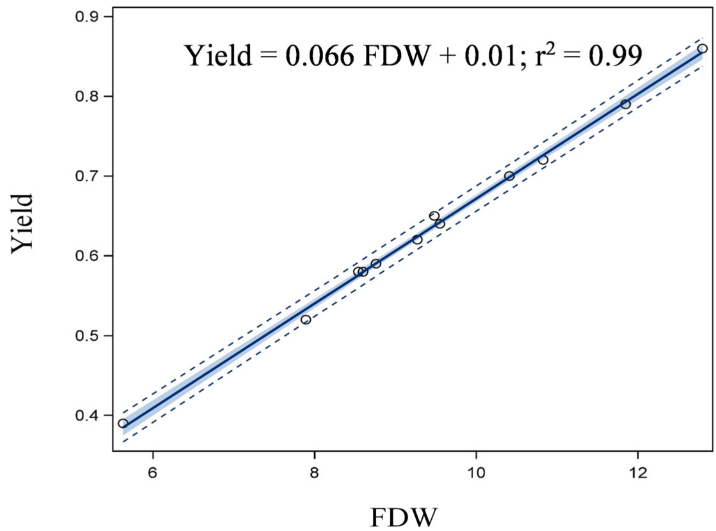

Figure 2. Saffron yield changes associated with the change of flower dry weight.

The flower traits include SL, FN, SWF, FFW, and FDW reduced without fertilizer application in both irrigation regimes. Although, this reduction was further in drought conditions and was 15%, 21.7%, 14.9%, 15.9%, and 28.6% for those traits, respectively. The saffron yield was also reduced down to 25% by water plus nutrition deficiency. The chicken manure and chemical fertilizer was the same influence on saffron SL. However, other flower traits and yields in saffron fertilized by chicken manure were higher than in saffron fertilized by chemical fertilizers. The combined fertilizer use including organic and chemical fertilizers significantly increased the flower traits and yield in both irrigation regimes than using them alone. Albeit, the efficiency of combined fertilizer is reduced by decreasing the chicken manure share. Thus, the greatest flower traits and saffron yield were observed in $Ch_{25}M_{75}$ under both irrigation regimes. This treatment was more efficient in drought stress than in optimal irrigation. Therefore, $Ch_{25}M_{75}$ respectively increased the SL, FN, FFW, SFW, FDW, and yield in deficit irrigation by 63.6%, 78.9%, 79.7%, 78.9%, 110.3%, and 103.1% compared with the control. However, these traits respectively increased by $Ch_{25}M_{75}$ to 45.6%, 61%, 61.3%, 63.1%, 63.3%, and 64% in the optimal irrigation. Reducing the share of chicken manure had more deleterious impacts in drought conditions than in good irrigation regimes. So, the flower traits and saffron yield more reduced by decrement chicken fertilizer share from 75% to 50% in drought stress (on average 50%) (Table 4 and Figure 3).

Photosynthetic pigments content

The amount of leaf photosynthetic pigments was identical in 2018 and 2019 (Table 3). Drought and different fertilizers applications had a significant effect ($p \leq 0.01$) on the amount of

Table 4. Mean comparison of saffron yield and flower characteristics at different fertilizers and irrigation regimes.

		SL (mm)		FN		SFW (g m^{-2})		FDW (g m^{-2})		Yield (g m^{-2})	
Drought	Fertilizer	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control (−0.5 atm)	Control	59.25^{i}	62^{h}	67.5^{i}	82.5^{f}	2.95^{j}	3.54^{g}	7.32^{h}	8.46^{g}	0.48^{i}	0.56^{g}
	Manure	66.75^{g}	68.5^{f}	85.5^{d}	109^{c}	3.78^{d}	4.75^{c}	9.52^{d}	11.31^{c}	0.65^{c}	0.75^{c}
	Chemical	67^{g}	68.75^{f}	80^{f}	101^{d}	3.53^{f}	4.39^{d}	8.63^{f}	10.48^{d}	0.59^{f}	0.7^{d}
	$Ch_{25}M_{75}$	88^{a}	88.5^{a}	111.5^{a}	130^{a}	4.92^{a}	5.67^{a}	12.11^{a}	13.49^{a}	0.82^{a}	0.9^{a}
	$Ch_{50}M_{50}$	77.25^{c}	82^{c}	87.5^{c}	115.5^{b}	3.97^{c}	5.07^{b}	9.7^{c}	11.97^{b}	0.63^{d}	0.8^{b}
	$Ch_{75}M_{25}$	72.25^{e}	74^{e}	76^{g}	101.5^{d}	3.46^{h}	4.45^{d}	8.45^{g}	10.51^{d}	0.59^{fg}	0.71^{d}
Drought (−6.5 atm)	Control	52.25^{j}	50.75^{i}	58.5^{j}	65^{g}	2.69^{k}	2.83^{h}	4.61^{i}	6.66^{h}	0.33^{j}	0.45^{h}
	Manure	63.75^{h}	62^{h}	83^{e}	90.5^{e}	3.29^{i}	3.96^{e}	9.2^{e}	9.35^{e}	0.62^{e}	0.63^{e}
	Chemical	63.25^{h}	63.25^{g}	77^{g}	84^{f}	3.52^{fg}	3.65^{f}	8.55^{fg}	8.65^{fg}	0.58^{gh}	0.59^{fg}
	$Ch_{25}M_{75}$	84.25^{b}	84.25^{b}	104.5^{b}	116.5^{b}	4.76^{b}	5.12^{b}	11.55^{b}	12.15^{b}	0.77^{b}	0.82^{b}
	$Ch_{50}M_{50}$	74.75^{d}	76^{d}	82.5^{e}	86.5^{ef}	3.6^{e}	3.76^{ef}	8.57^{fg}	8.96^{ef}	0.58^{gh}	0.6^{ef}
	$Ch_{75}M_{25}$	69^{f}	69.25^{f}	73^{h}	84^{f}	3.47^{gh}	3.66^{fg}	8.44^{g}	8.63^{fg}	0.57^{h}	0.58^{fg}

Means in each column with the same letters have no significant difference at $p \leq 0.05$.

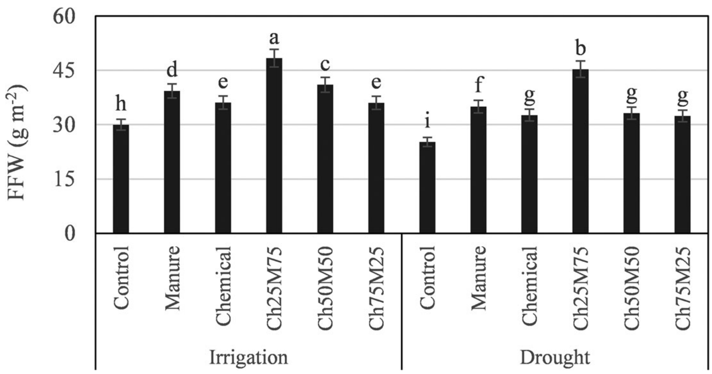

Figure 3. The effect of chemical and organic fertilizers on flower fresh weight under different irrigation regimes.

photosynthetic pigment. Drought reduced the amount of Ch_a, Ch_b, and Ch_T by about 44% to 47% in both years; however, the Car amount had no significant change. The content of total chlorophyll and Ch_a improved by using the chemical fertilizer or chicken manure in both irrigation regimes and two years. However, they had no significant effect on the Ch_b content compared with the control. The chemical fertilizers were more efficient than the chicken manure to increase the average content of Ch_a in a good irrigation regime. However, they were no significant differences in drought conditions. The combined use of chemical fertilizer and chicken manure further increased the content of Ch_T, Ch_a, and Ch_b in both irrigation regimes. However, the highest amount of chlorophyll was obtained by using various proportions of chemical fertilizer and chicken manure in each irrigation regime. So that, a higher amount of chicken manure was required in drought stress. The greatest Ch_T amount was obtained in optimal irrigation conditions (10.16 mg g^{-1} FW) by replacing the used chemical fertilizer 25% with chicken manure. Besides, the greatest Ch_T content obtained in drought conditions (5.96 mg g^{-1} FW) has required to replace the used chemical fertilizer 50% with chicken manure. The highest amount of Ch_a in drought conditions was observed in $Ch_{25}M_{75}$ (on average, 2.95 mg g^{-1} FW). In drought conditions, all combinations of chemical fertilizer and chicken manure significantly increased the Ch_b rate in both years (Table 5). Fertilizing operations were reduced carotenoid content in drought conditions. However, only $Ch_{25}M_{75}$ (1.72 mg g^{-1} FW) and $Ch_{50}M_{50}$ (1.75 mg g^{-1} FW) significantly reduced carotenoid content in a good irrigation regime compared with the control (1.93 mg g^{-1} FW) (Figure 4).

Table 5. Mean comparison of some saffron characteristics at different fertilizers and irrigation regimes.

		Ch_a (mg g^{-1} FW)		Ch_b (mg g^{-1} FW)		Crocin (%)		Picrocrocin (%)		Safranal (%)	
Drought	Fertilizer	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control (−0.5 atm)	Control	3.42^{d}	3.66^{d}	4.00^{bc}	4.28^{bc}	23.28^{h}	22.86^{h}	63.18^{k}	62.65^{g}	35.56^{g}	32.97^{g}
	Manure	4.12^{c}	4.41^{c}	3.89^{cd}	4.16^{cd}	23.96^{g}	24.11^{g}	67.49^{i}	67.07^{e}	36.38^{f}	35.5^{f}
	Chemical	4.48^{ab}	4.79^{ab}	3.61^{cde}	3.86^{cde}	25.99^{d}	26.46^{e}	70.78^{f}	70.4^{c}	38.27^{d}	38.78^{c}
	$Ch_{25}M_{75}$	4.40^{bc}	4.71^{bc}	3.93^{bcd}	4.20^{bcd}	25.27^{f}	25.93^{f}	69.8^{h}	69.69^{d}	37.57^{e}	37.6^{d}
	$Ch_{50}M_{50}$	4.55^{ab}	4.87^{ab}	4.39^{b}	4.69^{b}	25.59^{ef}	26.6^{e}	70.72^{f}	70.46^{c}	37.51^{e}	36.59^{e}
	$Ch_{75}M_{25}$	4.80^{a}	5.13^{a}	5.02^{a}	5.38^{a}	26.08^{d}	27.22^{d}	71.25^{e}	72.51^{b}	39.95^{c}	40.38^{b}
Drought (−6.5 atm)	Control	1.83^{g}	1.96^{g}	2.32^{h}	2.48^{h}	23.76^{g}	24.15^{g}	64.77^{j}	65.35^{f}	35.61^{g}	35.69^{f}
	Manure	2.41^{f}	2.57^{f}	2.62^{gh}	2.80^{gh}	25.75^{de}	26^{f}	70.08^{g}	70.15^{cd}	38.42^{d}	38.48^{c}
	Chemical	2.37^{f}	2.54^{f}	2.56^{gh}	2.74^{gh}	27.08^{c}	27.29^{cd}	73.81^{c}	73.05^{b}	41.48^{a}	41.44^{a}
	$Ch_{25}M_{75}$	2.85^{e}	3.05^{e}	2.97^{fg}	3.17^{fg}	27.31^{c}	27.53^{bc}	72.54^{d}	72.64^{b}	40.55^{b}	40.59^{b}
	$Ch_{50}M_{50}$	2.70^{ef}	2.89^{ef}	3.53^{de}	3.78^{de}	27.77^{b}	27.69^{b}	74.4^{b}	74.52^{a}	40.58^{b}	40.59^{b}
	$Ch_{75}M_{25}$	2.54^{ef}	2.71^{ef}	3.23^{ef}	3.45^{ef}	29.05^{a}	28.99^{a}	74.75^{a}	74.81^{a}	41.73^{a}	41.8^{a}

Means in each column with the same letters have no significant difference at $p \leq 0.05$.

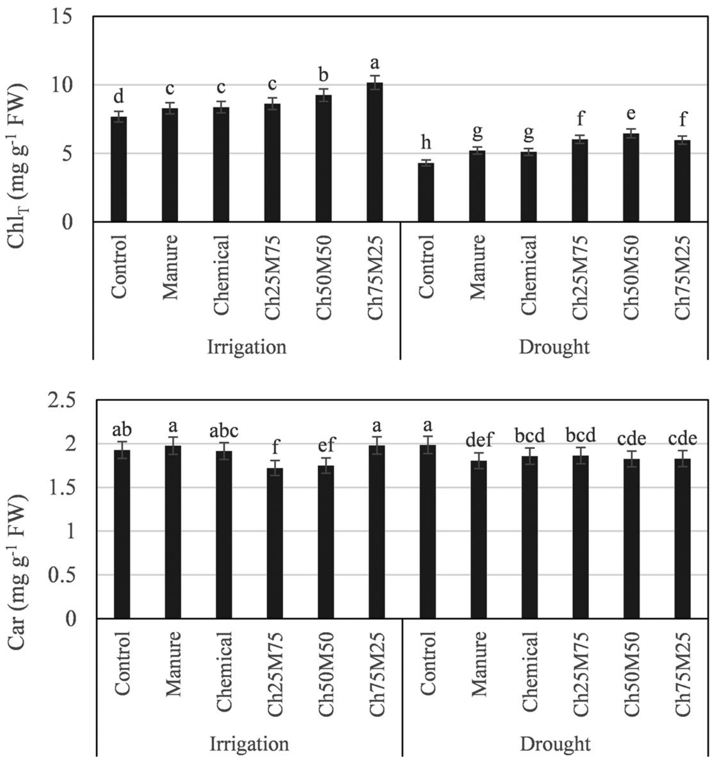

Figure 4. The effect of chemical and organic fertilizers on photosynthetic pigments content under different irrigation regimes.

RWC, proline, and soluble carbohydrates (SC)

The amount of RWC, proline, and soluble carbohydrates (SC) was similar in 2018 and 2019. Drought and different fertilizers applications had a significant impact ($p \leq 0.01$) on RWC, proline, and SC rates. Drought significantly increased proline content (2.3 times) and SC amount (63%) compared with good irrigation regimes. In contrast, RWC reduced to 24.2% under drought conditions. The lowest content of proline and SC and the highest RWC value were observed in $Ch_{25}M_{75}$ in the drought regime. So, in drought conditions, the average amount of proline and SC in $Ch_{25}M_{75}$ was, respectively, 21.8% and 32.7% less than the control, on the contrary, RWC was 15.9% more than the control (Figure 5).

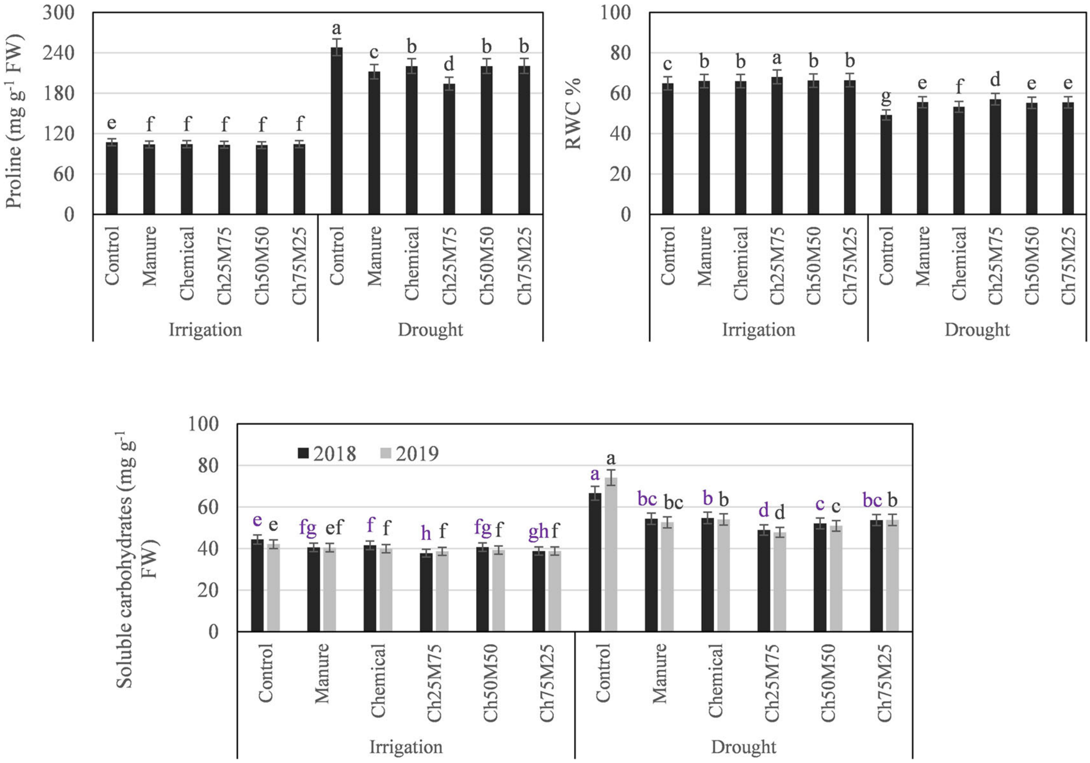

Figure 5. The effect of chemical and organic fertilizers on RWC, proline, and soluble carbohydrates rates under different irrigation regimes.

Crocin, picrocrocin, and safranal amounts

Crocin amount was higher in 2018 (26.2%) than in 2019 (25.9%). However, the picrocrocin and safranal rates were identical in 2018 and 2019. Drought and different fertilizers utilization had a significant effect ($p \leq 0.01$) on the content of crocin, picrocrocin, and safranal. Drought slightly improved the content of crocin (3.8%), picrocrocin (3.4%), and safranal (4.04%). Besides, the results showed that the highest amount of crocin, picrocrocin, and safranal was observed in $Ch_{75}M_{25}$ in drought (on average, 29.02%, 74.8%, and 41.8%, respectively) and a good irrigation regime. However, reducing the share of chemical fertilizers in the combined fertilizer reduced the amount of crocin, picrocrocin, and safranal compared to the use of chemical fertilizers alone (Table 5).

Discussion

Saffron growth was more in 2019 than in 2018, while the amount of photosynthesis pigments, RWC, proline, and SC were similar in both years. The rainfall amount in February 2019 was lower than in 2018. In contrast, the temperature average in March 2019 was higher than in 2018. Since the soil moisture was adjusted in this study, the two-year difference in rainfall will not be the main factor in the saffron growth. Besides, RWC amount and osmolytes accumulation in two years did not have significant differences which indicated water accessibility for treatments was similar in two years. Therefore, higher temperatures in 2019 caused an increase in the growth of saffron.

The features of saffron flower have a slight decrease by drought stress which indicates that saffron is a resistant plant to drought. The results were confirmed by other studies (Koocheki et al. 2015; Dastranj and Sepaskhah 2019). Koocheki, Seyyedi, and Eyni (2014) reported that the number of saffron flowers, fresh DW, and stigma DW was significantly reduced by supplying 50% of saffron water requirement. Decreased access to water causes reduced cell turgor pressure, growth, and division (Kim et al. 2018). Therefore, drought can impose an adverse impact on saffron SL by decreasing cellular elongation and division. In contrast, water availability promotes more development of primordia in the corm, thus the appearance of leaves and flowers per stem and the area unit was increased.

Fertilizer application improved the growth and yield of saffron in both irrigation regimes. However, the combined fertilizers were more effective than using them alone. Organic fertilizer share determined the efficiency of fertilizer management because the saffron yield is reduced by decreasing the chicken manure in both irrigation regimes. However, this schedule was more efficient in drought stress. Saffron plant needs mineral elements for optimal growth, which must be provided from chemical, organic, and biological sources. Jahan and Jahani (2007) reported that saffron yield augmented with increasing manure consumption. However, the favorable manure impact elevated in the following years. Yarami and Sepaskhah (2015) showed that cow manure use increased the saffron yield and harvest index. Therefore, the improvement of flower traits and saffron yield may be due to meeting the saffron nutritional needs through chemical or chicken fertilizers. Chicken manure used in this study had lower amounts of nitrogen, potassium, iron, zinc, and manganese than chemical fertilizers but had more phosphorus content. Therefore, it can be concluded that phosphorus can be a rate-limiting agent for saffron growth. Besides, chicken manure may have been effective in improving the morphological traits and saffron yield due to having a higher amount of organic matter, water retention ability, and cation exchange capacity. In contrast, Rezvani-Moghaddam et al. (2007) reported that chemical and cow manure had a greater impact on the saffron flower and stigma yield than chicken manure. While Ghanbari et al. (2019) reported that the use of compost had a greater effect on improving FN and flower DW than chemical fertilizers.

Drought reduced photosynthesis pigment amount in saffron leaves, however, the content of Ch_b less decreased than the rate of Ch_a. Other studies have also reported that drought stress reduced chlorophyll content in various plant species (Chen et al. 2015; Abd Elbar, Farag, and Shehata 2019; Afsahi et al. 2020). Abd Elbar, Farag, and Shehata (2019) reported that water deficit in *Thymus vulgaris* L. reduced Ch_a and Ch_b to 25.5% and 19.5%, respectively. They expressed that drought stress reduced the chlorophyll content by preventing chlorophyll biosynthesis or chloroplast degradation. However, the latter has reported the result of increment reactive oxygen species (ROS) and lipid peroxidation. Moreover, they explained that less reduction of Ch_b content is related to its protective role for the photosynthetic apparatus under drought regime. This may be the reason for the lower percentage reduction of Ch_b and carotenoids in saffron leaves compared to Ch_a under drought stress.

Fertilizer using improved the content of total chlorophyll and Ch_a, however, had no significant effect on the Ch_b content. Reducing chemical fertilizer share in the combined fertilizer further increased the content of Ch_T, Ch_a, and Ch_b in both irrigation regimes. However, a higher amount of chicken manure was required in drought stress. So that, replacing 25% of the used chemical fertilizer with chicken manure in the good irrigation regimes and replacing 50% of it with chicken manure in drought conditions resulted in a higher number of photosynthetic pigments. These findings were confirmed by other studies (Jami et al. 2020; Rahimi et al. 2019; Afsahi et al. 2020). It is reported that vermicompost application as 24 tons ha^{-1} increased the total chlorophyll content in saffron (Jami et al. 2020). Heydari, Besharati, and Maleki-Farahani (2014) confirmed that chlorophyll content in saffron leaves had no significant reduction by replacing half amount of chemical fertilizer with biological fertilizer. Nasarudin et al. (2018) reported that plants fed by integrated fertilizing had more N uptake and chlorophyll synthesis. Hence, light absorption capacity, photoassimilate synthesis, growth, and yield are increased. Moreover, adding livestock manure to the soil increases the water maintenance ability, thus the duration of the plant exposure to drought is reduced. Besides, drought stress reduced nutrient availability *via* reducing root spread, soil minerals mass flow, soil microbial activity, etc. (Selim 2020). Fertilizing regardless of the chemical or organic source facilitates the access of mineral elements and increases plant growth. However, an appropriate proportion of chemical and organic fertilizers can reduce abiotic stress negative effects by providing adequate nutrients in plant time required and more water retention. Fertilizing operations were reduced Car content in saffron leaves in drought conditions. The carotenoid content increased in drought stress to reduce photo-oxidative stress. However, carotenoid biosynthesis was decreased by plant fertilization and alleviating the drought stress negative effects. In contrast, chlorophyll synthesis increased, which could be due to reduced free radical production and chlorophyllase enzyme (Rahimi et al. 2019).

This study showed that proline and SC content accumulated in the leaf of saffron exposure to drought. Accumulation of proline and soluble sugars in saffron was associated with RWC increment. Besides, a significant negative correlation was found between RWC with proline ($r = -0.98$, $p \leq 0.01$) and soluble sugars ($r = -0.95$, $p \leq 0.01$). Some authors reported that proline accumulation is a general indicator for leaf water loss and is related to drought susceptibility (Dien, Mochizuki, and Yamakawa 2019). Xu et al. (2015) revealed that soluble sugar content significantly increased in roots and leaves of rice varieties exposure to drought, however, the RWC is reduced less. It is demonstrated that increasing the content of SC and proline have strongly been associated with osmotic adjustment in plants. This can help to leaf water potential declining and protection of macromolecules and membrane integrity under drought stress (Arteaga et al. 2020). Due to the sharp increase in proline content than SC, it is assumed that proline is a key osmolyte in saffron response to drought. SC (such as glucose, fructose, etc.) are used as an accessible source of energy, a precursor to the synthesis of other compounds, etc. Hence, their low accumulation may be due to greater catabolism (Dien, Mochizuki, and Yamakawa 2019; Arteaga et al. 2020).

Generally, the rates of proline and SC were reduced by the fertilizer application in both irrigation regimes, and on the contrary, the RWC increased. Chicken manure reduced the amount of proline more than chemical fertilizer and thus increased the amount of RWC further. Similar results are obtained by other authors (Heidari, Mousavinik, and Golpayegani 2011; Mondal, Datta, and Mondal 2017). Salehi, Tasdighi, and Gholamhoseini (2016) reported that RWC enhancement in vermicompost-treated chamomile plants might be due to soluble sugar concentration increment in the leaves. Mohammadi et al. (2019) reported that bio-fertilizer application more reduced proline content compared to chemical fertilizers utilization under drought conditions. Reduction in nutrient availability is an impact of soil water shortage on plants. Hence, plants will face nutrient deficiency when a long period is exposed to drought (Bista et al. 2018). Therefore, meeting plant nutrition requirements by using appropriate amounts of fertilizer can improve plant resistance to drought conditions. On the other hand, livestock manure can mitigate the negative effects of drought due to more water retention. Besides, Potassium ion plays a vital role in regulating plant water relations. Chicken manure used in this study was 62.2% more potassium than chemical fertilizer used.

Water shortage slightly increased the content of crocin, picrocrocin, and safranal in the stigma of saffron. Studies on other plants have shown that drought increases secondary metabolites (Caser et al. 2017; Mandoulakani, Eyvazpour, and Ghadimzadeh 2017). Abd Elbar, Farag, and Shehata (2019) reported that the total phenolic content of *Thymus vulgaris* L. increased under drought stress. Selmar and Kleinwachter (2013) explained that the stomata are closed in drought conditions and prevent CO_2 from entering into the leaves. Then, a fewer amount of CO_2 has been fixed in the Calvin cycle and less NADPH is oxidized and re-reduced. This is responsible for the oxidation and reduction imbalance in the plant tissues toward a greater reduced status. Hence, the plant employs all routes to NADPH consumption such as secondary metabolites biosynthesis. Crocin, picrocrocin, and safranal in saffron are derived from lycopene, which itself is synthesized from geranyl-geranyl diphosphate. Therefore, more pyruvate may be shifted to the Mevalonic acid and MEP pathways in saffron faced by drought stress. Besides, the genes involved in the carotenoid biosynthesis pathway may be overexpressed. Radwan, Kleinwächter, and Selmar (2017) reported similar results in *Salvia officinalis*. They revealed that over-reduction status, plant growth regulators biosynthesis, biochemical pathways shift, and main genes up-expression involved in terpenoid synthesis are responsible for increasing monoterpene biosynthesis in drought conditions.

Every fertilizer utilization increased the amount of crocin, picrocrocin, and safranal in both irrigation regimes in 2018 and 2019. Rezaie, Feizi, and Moradi (2019) oppositely reported that the use of fertilizer reduced the content of saffron secondary metabolites. Soil nutrients have an important effect on the content of plants' secondary metabolites. However, other factors, such as soil microbial community, soil water content, environmental stresses, etc., affect the amount of them (Jami et al. 2020). There are no mineral nutrients in the chemical structure of crocin, picrocrocin, and safranal. However, the use of fertilizer may be increased their amount due to improving photosynthesis conditions and increasing carbon fixation, glucose production, protein synthesis, etc. The impact of chemical and organic fertilizers has not been well studied on the saffron secondary metabolites except in a few studies (Ghanbari et al. 2019). This study showed that the use of chemical fertilizer was more effective than chicken manure in incrementing the amount of crocin, picrocrocin, and safranal in each irrigation regime. Feli, Maleki Farahan, and Besharat (2018) similarly reported that chemical fertilizer (100 kg ha^{-1} urea) more improved safranal content than vermicompost and biofertilizer. However, there was no significant impact on crocin and picrocrocin amounts. In addition, a study showed that the amount of crocin and picrocrocin improved by utilization of 25 Mg ha^{-1} cow manure (Rezaian and Paseban 2006). It has been expressed that biofertilizers have a positive impression on saffron quality. Because they improve nutrients supplying, hormones and vitamins biosynthesis, soil microorganisms synergistic, secondary metabolites biosynthesis (Heydari, Besharati, and Maleki-Farahani 2014). Thus, it can be concluded that supplying saffron-required nutrients increases plant growth and plays a main role in improving its quality.

The combined fertilizer was more effective to improve saffron quality than using chemical fertilizer or chicken manure alone. So that, replacing 25% of chemical fertilizer with chicken manure was the best option for improving saffron quality. Similar results were obtained by other authors. Rasooli, Maleki-Farahani, and Besharati (2013) explained that the simultaneous use of biofertilizers and chemical fertilizers has the most effect on the number of saffron apo-carotenoids. Gholizadeh, Aminifard, and Sayari (2016) revealed that the utilization of compost, biofertilizers, nitrogen fertilizer, and the combination of the fertilizers had a significant effect on the content of saffron picrocrocin. The reason can be related to ameliorating soil physical and biological properties, more water retention, development of cation exchange capacity, carbon to nitrogen ratio improvement, etc., by chicken manure, and rapid supply of saffron-required elements by chemical fertilizers.

Conclusions

Drought stress reduced the growth and chlorophyll content of saffron. However, the amount of proline and SC was increased and the RWC was decreased. Supplying the saffron-required minerals increased growth, chlorophyll content, and yield under drought or good irrigation regimes. Combined fertilizers involving different proportions of chicken manure and chemical fertilizers were more effective to improve saffron growth and biochemical traits than using them alone. Thus, the highest amount of these traits was acquired when 75% of chemical fertilizer was replaced with chicken manure. Besides, drought increased the content of crocin, picrocrocin, and safranal, on the contrary, mineral nutrition deficit reduced their content. So that, supplying the saffron-required nutrients improved the secondary metabolites content. Furthermore, the use of combined fertilizer has increased the secondary metabolites in stigma. However, the use of higher proportions of chemical fertilizers in the combined fertilizer had a greater effect to increase secondary metabolites rates. Unlike the growth traits, the highest quality of saffron was obtained when the share of chemical fertilizer was higher than chicken manure (3:1). Thus, it can be concluded that under drought stress, the use of combined fertilizer further increased saffron growth, yield, and quality.

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Disclosure statement

The authors declare that they have no conflict of interest.

ORCID

Heshmat Omidi http://orcid.org/0000-0002-2728-1469

Author contributions

Roohollah Saeidi Aboueshaghi Performed lab experiments, wrote the article; Heshmat Omidi: Designed experiments, ecotypes collector, edited article; Amir Bostani: Performed farm experiment, analyzed data, co-wrote the article.

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