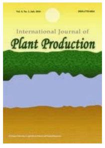

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Effect of irrigation water salinity, manure application and planting method on qualitative compounds of saffron (*Crocus sativus* L.)

N. Yarami, A.R. Sepaskhah*

Irrigation Department, Shiraz University, Shiraz, I.R of Iran.
*Corresponding author. E-mail: sepas@shirazu.ac.ir

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Abstract

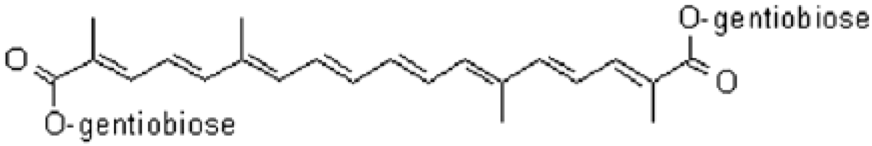
The objective of the present study is to investigate the effects of irrigation water salinity, cow manure levels and different planting methods on saffron quality compounds including crocin (coloring strength), picrocrocin (bitterness) and safranal (aromatic strength). A split-split plot arrangement was conducted in complete randomized block design with irrigation water salinity levels (0.45 (fresh water, S_1), 1.0 (S_2), 2.0 (S_3) and 3.0 (S_4) dS m^{-1}) as the main plot, cow manure levels (30 (F_1) and 60 (F_2) Mg ha^{-1}) as the sub plot and planting method (basin (P_1) and in-furrow (P_2)) as the sub-sub plot in three replications. Results showed that the saffron coloring strength, bitterness and aromatic strength in higher salinity level decreased by 9, 13 and 18% in comparison with the lowest salinity level, respectively. However, saffron (stile/stigmas) yield declined significantly as about 42% by increasing water salinity to highest level. The saffron crocin and picrocrocin concentration for the in-furrow planting method were significantly higher than the basin planting method by about 4 and 8%, respectively. Higher application rate of cow manure (60 Mg ha^{-1}) did not promote the saffron quality compounds. Furthermore, planting methods showed no significant effect on saffron aromatic strength. Correlation analysis indicated that saffron quality compounds showed negative relationship with leaf calcium, sodium and chloride and positive relationship with leaf phosphorus, nitrogen and potassium at 0.01 and 0.05 significant levels, Furthermore, a positive correlation between crocin and picrocrocin and saffron yield components (leaf dry matter, corm, flower and saffron yields) was observed; however, there was no significant correlation between safranal and yield components. Generally, the saffron quality compounds variations were concurrent with the saffron quantity variations.

Keywords: Crocin; In-furrow planting; Picrocrocin; Saffron; Safranal; Saline water.

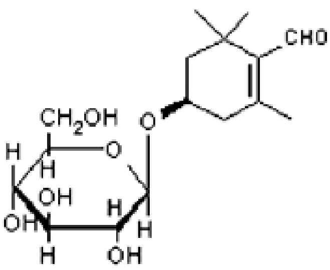
Introduction

Saffron (*Crocus sativus* L.) is native in the Mediterranean condition and is adapted to cool winters and warm dry summers. It is an excellent crop for arid and semi-arid regions with limited water resources due to the fact that the growing period is mostly occurred during the winter and spring. Saffron as a strategic export crop and most expensive spice in the Islamic Republic of Iran, is mostly produced in the Khorasan and Fars Provinces with arid and semi-arid climates, respectively (Abrishami, 1987). The three-branch style of saffron flower is the most important economic part of the plant. Saffron is used as spice, natural food color and showed several important properties in traditional medication. Further, its leaves are used as animal feed.

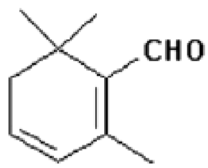
The main compounds of dried red stigmas of saffron including crocin, picrocrocin and safranal (Figure 1) are responsible for its coloring strength (color), bitterness (taste) and aromatic strength (odor), respectively (Basker, 1999). The amounts of these compounds are used to express the quality of saffron. The higher amounts of these compounds in saffron, means higher quality of saffron (Tarvand, 2005). Many methods for determining saffron quality compounds have been described (Tarantilis et al., 1995). The chemical composition of saffron samples from many countries indicated that the reported values are strongly dependent on the methods employed for drying, extraction and analysis (Kanakis et al., 2004; Zareena et al., 2001).



Crocin



Picrocrocin



Safranal

Figure 1. Chemical structures of crocin, picrocrocin and safranal.

The international organization for standardization (ISO) has set a classification for saffron quality based on minimum requirements of each quality compounds (ISO/TS 3632). According to this classification, they established four categories (Table 1); whereas, saffron with the best quality belongs to category I (Tarvand, 2005). The mentioned compounds of saffron quality are determined by specifications described within the ISO/TS-3632 standard that established the spectrophotometric quantification of crocin, picrocrocin and safranal in 1% aqueous solution of dried saffron by direct reading of absorbance at 440, 257 and 330 nm wavelengths, respectively (Cagliani et al., 2014). Akbarian et al. (2013) reported that the highest values of crocin (40.8%), picrocrocin (68.9%) and safranal (82.8%) for saffron cultivated in Bam region of Iran were obtained from a treatment by application of 10 Mg ha^{-1} cow manure, 100 kg ha^{-1} urea fertilizer and 100 kg ha^{-1} triple superphosphate fertilizer.

Table 1. ISO classification for saffron quality (Tarvand, 2005).

Characteristic	Saffron in filaments	Saffron in Powder form	Test method
Bitterness, expressed as direct reading of the absorbance of picrocrocin at about 257 nm, minimum value requirement			ISO 3632-2, clause 13
Category I	70	70	
Category II	55	55	
Category III	40	40	
Category IV	30	30	
Safranal, expressed as direct reading of the absorbance at about 330 nm			ISO 3632-2, clause 13
Minimum	20	20	
Maximum	50	50	
Coloring strength, expressed as direct reading of the absorbance of crocin at about 440 nm, minimum value requirement			ISO 3632-2, clause 13
Category I	190	190	
Category II	150	150	
Category III	110	110	
Category IV	80	80	

Lage and Cantrell (2009) studied the variation of the main saffron compounds (crocin, picrocrocin and safranal) in some Moroccan agricultural areas with low and erratic rainfalls during three years. Their results showed that crocin was stable under each specific environment tested ($P>5\%$) for three years of study. Meanwhile, there was a large variability in safranal concentration for the same period ($P<0.05$). Furthermore, their analysis of environmental impact on saffron quality showed that the altitude affected crocin. Caballero-Ortega et al. (2004) compared the chemical compositions of saffron extracts from four countries (Azerbaijan, Iran, Spain and India) by using HPLC method. They found that the total concentration of carotenoids in Azerbaijanian and Iranian saffron samples were higher in comparison with other samples.

Akbarian et al. (2012) studied the effect of foliar application of potassium (K), zinc (Zn) and iron (Fe) on saffron quality and quantity characteristics. They found that the amounts of crocin and safranal were reduced by increasing the application rate of elements. However, use of nutrients especially Fe showed positive effects on saffron quantity characteristics. Maghsoodi et al. (2012) evaluated four different dehydration methods including Iranian traditional method (room temperature drying), dehydration with electrical oven at different temperatures and dehydration in microwave oven at different powers on saffron quality. Their results showed that the highest coloring strength (crocin) was obtained when saffron treated in higher temperatures and lower durations. Furthermore, the higher amount of safranal and crocin was obtained at high temperature. There was no significant difference between the amounts of picrocrocin in different temperatures in all drying methods.

Abdullaev et al. (2007) reported that the values of saffron quality compounds were related to different environmental conditions and cultivation practices. Gresta et al. (2008) showed that an earlier planting time (end of July) resulted in higher quality of saffron stigmas compared with later planting (end of August). Zarinkamar et al. (2011)

stated that saffron samples from Ghaen, Iran (region with altitude of 1400 m and lower average air temperature) compared with samples from Tabas, Iran (region with altitude of 700 m and higher average air temperature) contained higher concentration of crocin, picrocrocin and safranal. However, investigations on saffron quality compounds under saline conditions and different planting methods are limited. Therefore, the objectives of this study were to study the effects of irrigation water salinity, cow manure levels and different planting methods on saffron (*Crocus sativus* L.) quality compounds including crocin, picrocrocin and safranal.

Materials and Methods

Site description

This research was conducted in 2011-2012 and 2012-2013 at Experimental Station of Agricultural College, Shiraz University located in Bajgah region at 29° 43’ N, 52° 35’ E and 1810 m above the mean sea level, in southwest of Iran with a semi-arid climate. Long-term average air temperature, relative humidity and precipitation of the region are 13.4 °C, 52.2% and 387 mm, respectively. Some physico-chemical properties of soil in the experimental site are presented in Table 2. The soil was classified as silty clay loam down to 0.9 m depth. Chemical analysis of the fresh and saline irrigation water is also shown in Table 3.

Table 2. Physico-chemical properties of the soil at experimental site.

Characteristic	Soil depth, cm			Characteristic	Soil depth, cm		
	0-30	30-60	60-90		0-30	30-60	60-90
Field capacity (%)	32	33	35	Texture	SCL*	SCL	SCL
Permanent wilting point (%)	17	19	19	EC (dS m^{-1})	0.74	0.51	0.49
Bulk density (g cm^{-3})	1.40	1.47	1.51	Cl^- (meq l^{-1})	5.31	3.05	2.90
%Sand	11	10	16	Na^+ (meq l^{-1})	3.29	1.97	1.91
%Silt	56	51	50	Ca^{2+} (meq l^{-1})	5.43	4.16	4.07
%Clay	33	39	34	Mg^{2+} (meq l^{-1})	3.50	2.88	2.84

* Silty clay loam.

Table 3. Chemical analysis of the fresh and saline irrigation water used in the experiment.

Characteristic	Fresh water	Saline water		
EC (dS m^{-1})	0.45	1.0	2.0	3.0
pH	7.31	7.24	7.12	7.00
Cl^- (meq l^{-1})	3.75	15.00	24.25	38.25
Na^+ (meq l^{-1})	0.57	5.67	11.60	18.17
Ca^{2+} (meq l^{-1})	3.00	5.40	11.80	18.20
Mg^{2+} (meq l^{-1})	2.80	2.60	3.40	3.70
HCO_3^- (meq l^{-1})	6.2	2.20	1.60	1.40
SO_4^{2-} (meq l^{-1})	0.45	0.65	0.85	1.45

Experimental design and treatments

Experimental design was a split-split plot arrangement in complete randomized block design with salinity levels of irrigation water as the main plot, cow manure levels as the subplot and planting method as the sup-sub plot in three replications. The salinity treatments of irrigation water consisted of 0.45 (fresh water, S_1), 1.0 (S_2), 2.0 (S_3) and 3.0 (S_4) dS m^{-1}. The fertilizer levels were 30 (F_1) and 60 (F_2) Mg ha^{-1} of cow manure for first growing season and 15 and 30 Mg ha^{-1} for the second growing season that were applied at the beginning of each growing seasons. According to the local farmers practice, the manure application rate in the second year is half of that in the first year. Some chemical properties of the cow manure are presented in Table 4. The planting methods were basin (P_1) and in-furrow (P_2) planting.

Table 4. Chemical properties of the cow manure.

Characteristic	value	Characteristic	value
EC (dS m^{-1}) in 1:5 solution	10.63	Mg^{2+} (meq l^{-1}) in 1:5 solution	17.50
pH in 1:5 solution	8.50	K^{+}(meq l^{-1}) in 1:5 solution	79.17
Cl^{-} (meq l^{-1}) in 1:5 solution	72.50	Total phosphorous (%)	0.80
Na^{+} (meq l^{-1}) in 1:5 solution	20.73	Total nitrogen (%)	2.10
Ca^{2+} (meq l^{-1}) in 1:5 solution	21.50		

First irrigation of the first growing season was applied with fresh water for plants establishment and after that, saline water treatments were applied. Saline water was obtained by addition of NaCl and $CaCl_2$ to the fresh water, in equal equivalent proportion.

In the first growing season, after deep plowing and field leveling in early September 2011, plots were constructed manually with dimension of 1.5×2 m and 1.0 m distance between two adjacent plots. The cow manure levels and 100 kg ha^{-1} triple superphosphate as chemical fertilizer were added to the soil at plot construction time. Saffron corms were planted with 15 Mg ha^{-1} density on September 9 in five rows with 30 cm spacing in 15-20 cm soil depth in each plot. This rate was used to obtain a good stand in the first year of planting.

All plots were irrigated on October 27 at the first growing season (2011) with fresh water. The amount of first irrigation water was determined based on increasing soil water content to the field capacity for 40 cm soil depth. Soil water content at 0.3, 0.6 and 0.75 m depths was measured with neutron scattering method before each irrigation event. During periods with no sufficient rain, irrigation water was applied at 24 days interval that is the best interval for saffron irrigation in the study area (Azizi-Zohan et al., 2006). Soil water content in the root zone before irrigation (Θ_i) was used to determine the irrigation depth as:

$$I = \sum_{i=1}^{n} (\theta_{FCi} - \theta_i) \times \Delta z_i \quad (1)$$

Where I is the irrigation water depth (m), Θ_{FCi} and Θ_i are the volumetric soil water content in layer i at field capacity (m^3 m^{-3}), Δz_i is the thickness of each soil layer (m) and n is the number of soil layers and before irrigation, respectively.

Leaching fraction used for each irrigation was 15% to prevent salt accumulation in the root zone. The weeding was performed manually during the growing seasons as needed. Total amount of irrigation water applied was 207 and 263 mm for the first and second growing seasons, respectively. Total rainfall was also 363 and 445 mm during the growing periods in 2011-2012 and 2012-2013, respectively. The first irrigation was applied on October 27 at both growing seasons.

Measurements and calculations

During the flowering periods of two growing seasons, flowers were harvested every morning from the entire plot and weighted immediately. Then, the style and stigmas were separated from flowers and air dried in room shadow environment. At the end of flowering stage, total weights of style and stigmas (as saffron yield) were determined. Saffron samples of the second growing season were powdered and passed through a 0.5 mm mesh sieve. The main quality compounds of saffron were determined according to ISO 3632 trade standard (ISO/TS 3632-2, 2003) method in terms of picrocrocin, safranal and crocin concentration using a UV/VIS spectrophotometer.

The direct reading of absorbance were obtained according to ISO/TS 3632-2 at 440, 330 and 257 nanometers (nm) wavelengths corresponding to the maximum absorbance of the coloring strength (crocin), the aromatic strength (safranal) and the bitterness (picrocrocin), respectively. Each value of these compounds (the coloring strength, aromatic strength and bitterness) as $A_{1cm}^{1\%}(\lambda_{\max})$ was calculated as follows:

$$A_{1cm}^{1\%}(\lambda_{\max}) = \frac{D \times 10000}{m \times (100 - W_{MV})} \tag{2}$$

Where D is the specific absorbance, m defined as the mass of saffron sample in gram and W_{MV} is the moisture and volatile content of the sample, expressed as a mass fraction. A 1.0 cm quartz cell was used as the sampling unit in the spectrophotometer.

Several saffron samples were placed in an oven (at 103±2 °C) and kept for 16 h. Then, the samples were cooled down in desiccator. After reaching room temperature, the samples were precisely weighted. The moisture and volatile matter content, *WMV*, was calculated as percentage of the initial sample weight as follows:

$$W_{MV} = \frac{(m_0 - m_1) \times 100}{m_0} \tag{3}$$

Where m_0 is the mass of the initial sample (g) and m_1 is the mass of dry sample (g). The mean value of the moisture and volatile matter content of saffron samples was about 7%.

Data analysis and statistics

Measured data were analyzed by MSTATC software. Duncan´s method was used to find out the differences among means with probability level of 5% (P≤0.05). Pearson's correlation coefficients were calculated by using SPSS software and graphs were performed by Excel software.

Results and Discussion

Coloring strength (crocin)

The main effect of irrigation water salinities and planting methods were statistically significant on crocin concentration at P≤0.05 (Table 5). Value of saffron coloring strength was statistically the same in S_3 and S_4 treatments; however, it significantly decreased in S_2 (1.0 dS m^{-1}) and dropped to the least amount at S_4 treatments by 9%. It may be due to reducing enzymatic activities of saffron that are led to lower quality under saline conditions.

Table 5. Mean values of saffron quality compounds including crocin, picrocrocin and safranal in different irrigation water salinities, fertilizer levels and planting methods.

Measured parameter	Crocin, $A_{1cm}^{1\%}(\lambda_{440})$	Picrocrocin, $A_{1cm}^{1\%}(\lambda_{257})$	Safranal, $A_{1cm}^{1\%}(\lambda_{330})$
	Salinity levels, dS m^{-1}		
S_1=0.45	197.3[a*]	76.3[a]	32.6[a]
S_2=1.0	192.7[b]	75.2[a]	31.3[b]
S_3=2.0	181.2[c]	71.1[b]	28.4[c]
S_4=3.0	179.1[c]	69.4[b]	26.7[d]
	Fertilizer levels, Mg ha^{-1}		
F_1=30	188.4[a]	73.1[a]	30.0[a]
F_2=60	186.8[a]	72.9[a]	29.5[a]
	Planting methods		
P_1: Basin	183.8[b]	70.2[b]	29.5[a]
P_2: In-furrow	191.4[a]	75.8[a]	30.0[a]

* Means followed by the same letters in columns for each factor and each trait are not significantly different at 5% level of probability, using Duncan's multiple range test.

Fertilizer levels showed no significant effect on crocin concentration; however, higher application rate of cow manure as 60 Mg ha^{-1} decreased this compound in comparison with application of 30 Mg ha^{-1}. Comparison of results showed that planting method had significant effect on crocin concentration. This quality compound for the in-furrow planting method was significantly higher than the basin planting method by about 4% that is similar to the saffron yield (Yarami and Sepaskhah, 2015a). This result indicated that the in-furrow planting method is efficient method for promoting the coloring strength of saffron.

Triple interaction effect of experimental factors on crocin is presented in Table 6. Results showed that the coloring strengths under all salinity levels in the in-furrow planting were significantly higher compared with the basin planting method. At each salinity level for the basin planting method, higher application rate of cow manure (60 Mg ha^{-1}) almost resulted in a higher coloring strength. However, higher application rate of cow manure decreased this quality compound in the in-furrow planting method in all salinity levels. It may be concluded that higher application rate of cow manure was more efficient in improving of coloring strength in the basin planting method compared with that in in-furrow planting method.

Table 6. Interaction effect of salinity, cow manure levels and planting methods on saffron quality factors including crocin, picrocrocin and safranal.

Salinity levels, dS m^{-1}	Planting methods							
	P_1: Basin				P_2: In-furrow			
	S_1=0.45	S_2=1.0	S_3=2.0	S_4=3.0	S_1=0.45	S_2=1.0	S_3=2.0	S_4=3.0
Fertilizer levels, Mg ha^{-1}	Crocin, $A_{1cm}^{1\%}(\lambda_{440})$							
F_1=30	192.9^{abcd*}	183.7^{def}	174.9^{f}	179.7^{ef}	202.9^{a}	200.2^{a}	188.6^{bcde}	184.3^{def}
F_2=60	195.8^{abc}	187.8^{cde}	178.5^{ef}	177.4^{ef}	197.7^{abc}	199.2^{ab}	182.9^{def}	175.0^{f}
	Picrocrocin, $A_{1cm}^{1\%}(\lambda_{257})$							
F_1=30	74.1^{abcde}	73.2^{bcde}	68.8^{def}	65.7^{f}	78.9^{ab}	77.7^{abc}	75.0^{abcd}	71.6^{cdef}
F_2=60	72.4^{cde}	70.6^{def}	68.5^{ef}	68.5^{def}	79.8^{a}	79.2^{ab}	72.3^{cde}	71.9^{cdef}
	Safranal, $A_{1cm}^{1\%}(\lambda_{330})$							
F_1=30	31.4^{abcd}	30.8^{abcde}	28.3^{defg}	26.3^{fg}	33.1^{ab}	32.2^{ab}	30.3^{bcde}	27.8^{efg}
F_2=60	32.0^{abc}	30.9^{abcde}	28.6^{cdef}	27.8^{efg}	34.0^{a}	31.3^{abcd}	26.5^{fg}	25.0^{g}

* Means followed by the same letters in rows for each factor and each trait are not significantly different at 5% level of probability, using Duncan's multiple range test.

There was significant interaction effect between fertilizer level and planting method (F×P) on saffron coloring strength; whereas, there was no significant interaction effect between irrigation water salinity (S), fertilizer levels (F) and planting methods (P), (S×F×P) on crocin concentration.

Variation of saffron coloring strength versus irrigation water salinity at different fertilizer levels and planting methods are shown in Figure 2a,b. Maximum crocin concentration was occurred in S_1F_1 treatment in the in-furrow planting method (Table 6). Coloring strength was higher at fresh water treatment (S_1, 0.45 dS m^{-1}) and it decreased by increasing water salinity levels. Regardless of irrigation water salinity levels, crocin concentration was higher in the in-furrow planting method (P_2) in comparison with the basin planting method (P_1). Nevertheless, there was no clear and significant difference between two fertilizer levels. It could be concluded that salinity stress and planting method showed higher effect on saffron coloring strength in comparison with the fertilizer levels.

According to ISO criteria for saffron quality (Table 1), the coloring strength values of S_1 level in the basin planting method and S_1 and S_2 levels in the in-furrow planting method belonged to category I (higher than 190) that is the best quality. Other salinity levels of two planting methods classified as category II (between 150-190). In spite of saline water application, saffron coloring strength showed low reduction in high salinity levels (S_3 and S_4); whereas, all values of this quality compound lied between the ISO standard range. Nevertheless, the in-furrow planting method has almost compensated the coloring strength reduction under saline conditions. Yadollahi et al. (2007) reported that the coloring strength values of saffron for India, Kashmir (115.8) and Iran, Birjand area (96.9) lied between the standard range for coloring strength values (80-190); whereas the UK, England-East Midlands value (65.0) was lower than the minimum standard value. These differences could be explained by different crop production methods and ecophysiological climatic conditions (e.g. the number of sunny days in a year) that is higher in Iran and Kashmir.

Bitterness (picrocrocin)

The main effect of irrigation water salinity on picrocrocin concentration was statistically significant ($P \leq 0.05$) between S_1-S_2 and S_3-S_4 levels (Table 5). The saffron bitterness decreased in higher salinity level by 13% in comparison with the lowest salinity level. The differences between S_1 and S_2 treatments and S_3 and S_4 levels were not significant. Fertilizer levels showed no significant effect on the saffron bitterness. The saffron picrocrocin concentration significantly increased in the in-furrow planting method by about 8% compared with that in the basin planting method. Therefore, by using the in-furrow planting method saffron with higher bitterness quality was produced.

Triple interaction effect of experimental factors on picrocrocin is presented in Table 6. Results showed that there was no significant interaction effect between experimental treatments on picrocrocin concentration. Regardless of irrigation water salinities and fertilizer levels, the bitterness values of saffron were higher in the in-furrow planting method compared with the basin planting method (Table 6).

Variation of saffron bitterness versus irrigation water salinity at different fertilizer levels and planting methods (Figure 2c,d) showed that bitterness was higher in the lowest irrigation salinity level (0.45 dS m^{-1}) and by increasing water salinity levels, bitterness decreased significantly. In all irrigation water salinity levels, picrocrocin compound of saffron was higher in the in-furrow planting method (P_2) in comparison with the basin planting method (P_1). Nevertheless, there was no clear difference between two fertilizer levels (F_1 and F_2) in different salinity levels.

Regardless of fertilizer levels, the bitterness values of S_1 and S_2 levels in the basin planting method and all irrigation water salinities in the in-furrow planting method belonged to category I (higher than 70) that is the best quality. The bitterness compound of S_3 and S_4 salinity levels in basin planting method lied in category II (between 55-70) according to ISO classification for saffron quality (Table 1). Despite of saline water application, saffron bitterness showed low reduction in high salinity levels (S_3 and S_4); whereas, all values of this quality compound lied between the ISO standard range. On the other hand, the in-furrow planting method increased the saffron bitterness under saline conditions. Generally, based on ISO criteria, standard values of saffron bitterness varied between 30 to 70. The results of Yadollahi et al. (2007) indicated that the bitterness values for Indian (75.6) and Iranian (76.2) saffron were slightly higher than that of the maximum value in the bitterness standard range (category I). Otherwise, the bitterness value for UK saffron (50.0) lied between the standard range of category III. This might be due to higher number of sunny days in Iran and Kashmir.

Aromatic strength (safranal)

The main effect of salinity and fertilizer levels and also planting methods on aromatic strength (safranal) of saffron is shown in Table 5, the main effect of salinity levels on safranal was significant ($P \leq 0.05$). Safranal concentration statistically decreased with increasing salinity levels and dropped to the least amount in S_4 level by 18% in comparison with S_1 level. Cow manure levels and planting methods showed no significant effect on saffron aromatic strength.

Triple interaction effect of experimental treatments on safranal is presented in Table 6. There was no significant effect of triple interaction of salinity, fertilizer levels and planting methods on safranal. Maximum safranal concentration was occurred in S_1F_1 and S_1F_2 treatment in the in-furrow planting method. Results showed that there was a significant interaction effect between fertilizer level and planting method (F×P) on saffron aromatic strength; whereas, there was no significant interaction effect between irrigation water salinity (S), fertilizer levels (F) and planting methods (P), (S×F×P) on safranal concentration.

Figure 2e,f showed the variation of saffron aromatic strength versus irrigation water salinity at different fertilizer levels and planting methods. Aromatic strength of saffron was higher in the least salinity level (S_1, 0.45 dS m^{-1}) and decreased by increasing water salinity levels. There was no significant difference between safranal concentration in two fertilizer levels and planting methods. According to Figure 2e,f, it is concluded that salinity stress showed higher effect on saffron aromatic strength in comparison with the fertilizer levels and planting methods.

The values of safranal concentration varied between minimum and maximum (20-50) values of ISO classification. In spite of saline water application, saffron aromatic strength showed low reduction in high salinity levels (S_3 and S_4); whereas, all values of this quality compound lied between the standard range of ISO classification. Yadollahi et al. (2007) stated that the aromatic strength values of saffron from India (44.0), Iran (40.7) and UK (33.3) lied between the range of the ISO standard values; however, its value was higher in Iran and Kashmir due to higher number of sunny days.

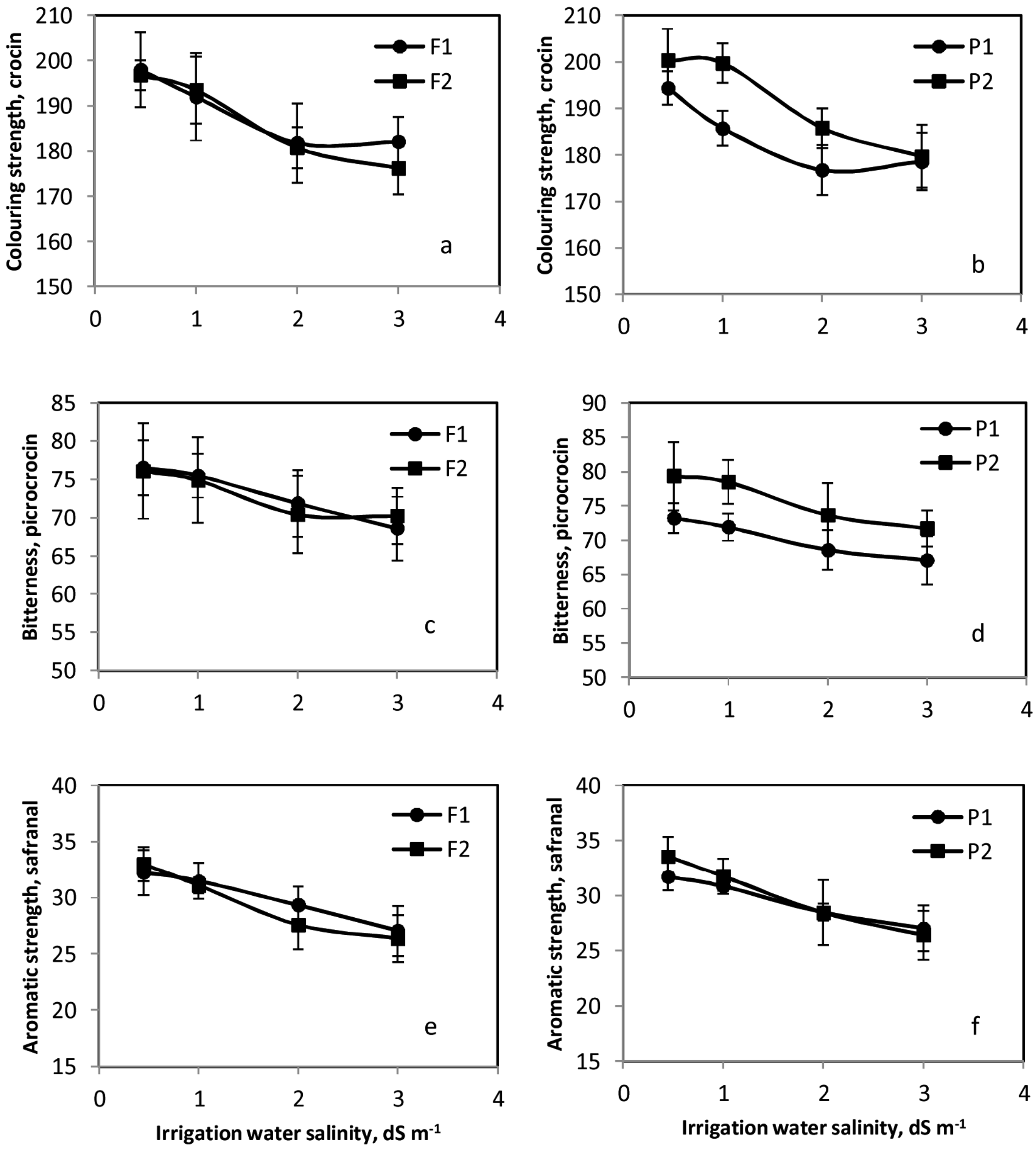


Figure 2. Saffron quality compounds variation in different irrigation water salinities, different fertilizer levels (F_1=30 Mg ha^{-1} and F_2=60 Mg ha^{-1} cow manure) and planting methods (P_1: basin and P_2: in-furrow).

Correlation analysis

The correlation coefficient is one of the most often used statistical tools for analyzing the associations among traits. Therefore, in order to assess the association or coherence between saffron quality compounds and yield quantity, Pearson's correlation coefficient was calculated by using SPSS software. The Pearson's correlation coefficient matrix of all the measured variables (including yield components (leaf dry matter, corm, flower and saffron yields, Yarami and Sepaskhah, 2015a and b) and saffron leaf ions concentration (Yarami and Sepaskhah, 2016) and quality compounds (crocin, picrocrocin and safranal) is reported in Table 7. Results showed that only in very few cases significant correlations were not found; however, in other cases there were positive or negative significant correlation at 0.01 (P_{value}<0.01) or 0.05 (P_{value}<0.05) level. It was observed negative correlation between saffron quality compounds and plant calcium, sodium and chloride concentration. It is concluded that excess accumulation of these ions in plant under saline conditions might have toxic effect on plant and lead to decrease in saffron quality compounds. On the other hand, there was significant positive correlation between saffron quality compounds and plant nutrient elements (phosphorous, nitrogen and potassium).

Table 7. Correlation matrix of Pearson's coefficients between saffron quality traits and its yield components and leaf ions.

Traits	Crocin, $A_{1cm}^{1\%}(\lambda_{440})$	Picrocrocin, $A_{1cm}^{1\%}(\lambda_{257})$	Safranal, $A_{1cm}^{1\%}(\lambda_{330})$
Saffron leaf phosphorus (%)	0.75**	0.71**	0.78**
Saffron leaf nitrogen (%)	0.60*	0.58*	0.65**
Saffron leaf calcium (%)	-0.79**	-0.63**	-0.88**
Saffron leaf sodium (%)	-0.73**	-0.66**	-0.85**
Saffron leaf potassium (%)	0.88**	0.84**	0.87**
Saffron leaf K/Na ratio	0.86**	0.76**	0.91**
Saffron leaf chloride (%)	-0.84**	-0.66**	-0.86**
Leaf dry matter (kg ha^{-1})	0.70**	0.90**	0.49^{ns}
Corm yield (Mg ha^{-1})	0.71**	0.90**	0.52*
Flower yield (kg ha^{-1})	0.67**	0.86**	0.40^{ns}
Saffron yield (kg ha^{-1})	0.65**	0.85**	0.37^{ns}
Crocin, $A_{1cm}^{1\%}(\lambda_{440})$	1.00	0.85**	0.87**
Picrocrocin, $A_{1cm}^{1\%}(\lambda_{257})$		1.00	0.74**
Safranal, $A_{1cm}^{1\%}(\lambda_{330})$			1.00

** Correlation is significant at 0.01 level.
* Correlation is significant at 0.05 level.
^{ns} Correlation is not significant.

There was no significant correlation between safranal and yield components (leaf dry matter, flower and saffron yields). On the other hand, according to the correlation analysis among all pairs of variables, there was appropriate significant correlation between quality compounds (crocin and picrocrocin (r=0.85, P<0.01), crocin and safranal (r=0.87, P<0.01) and picrocrocin and safranal (r=0.74, P<0.01)). Lage and Cantrell (2009) evaluated the correlation coefficients between saffron quality compounds and environmental parameters in Morocco conditions. They found significant positive correlation between altitude and crocin; soil texture (clay) and safranal (P<0.05). They reported higher crocin concentrations (above 250) for regions with altitude over 1000 m. Our experimental station was located in region with 1810 m above the mean sea level. Lower crocin concentrations in our site (between 170-205) may be due to differences between other environmental parameters compared with Morocco conditions. Furthermore, they observed higher safranal concentrations (between 40-50) in soils with clay content higher than about 20%. Our soil contained about 35% clay; however, we observed lower safranal concentrations (between 25-35) because of different environmental conditions, soil physico-chemical properties and saffron drying conditions. Safipouriyan et al. (2011) studied the effects of the time of corms lifting (harvesting) and foliar nutrition on some morphological and chemical yields of the saffron. They observed the most crocin and picrocrocin concentration in stigmas by using 7% of foliar nutrition (percentage of nutritional elements not mentioned). Furthermore, the most concentration of safranal and maximum stigma height were shown by using 5% foliar nutrition level. They reported linear correlation between the saffron quality compounds (crocin, picrocrocin, safranal) and stigmas height and corm dry weight.

The relationships between saffron quality compounds (crocin, picrocrocin and safranal) and yield components (leaf dry matter, corm, flower and saffron yields) were determined (Table 8). Based on these relationships, it is possible to predict saffron quality compounds by determining the yield components. Data analysis indicated that these relationships were significant at $P<0.05$ level and their slopes were positive; therefore, saffron quality compounds increased by increasing saffron yield components. According to Table 8, crocin and picrocrocin showed significant linear relationships with all yield components; however, safranal showed only significant relationship with corm yield. There were stronger relationships between picrocrocin concentration and yield components regarding to R^2 coefficients and P_{values} in comparison with crocin and safranal.

Table 8. Relationships between saffron quality compounds (crocin, picrocrocin and safranal) and saffron yield ($Y_{saffron}$), flower yield (Y_{flower}), corm yield (Y_{corm}) and leaf dry matter (leaf DM).

Quality compounds	Equation	R^2	SE	P_{value}
	Quality compounds vs. saffron yield (kg ha^{-1})			
Crocin	Crocin=1.09×$Y_{saffron}$+177.36	0.43	7.41	6.00E-03
Picrocrocin	Picrocrocin=0.63×$Y_{saffron}$+67.07	0.72	2.33	3.56E-05
Safranal	-	-	-	ns
	Quality compounds vs. flower yield (Mg ha^{-1})			
Crocin	Crocin=10.05×Y_{flower}+177.56	0.45	7.27	5.00E-03
Picrocrocin	Picrocrocin=5.77×Y_{flower}+67.24	0.74	2.24	2.04E-05
Safranal	-	-	-	ns
	Quality compounds vs. corm yield (Mg ha^{-1})			
Crocin	Crocin=0.83×Y_{corm}+168.80	0.51	6.87	2.00E-03
Picrocrocin	Picrocrocin=0.47×Y_{corm}+62.41	0.80	1.94	2.49E-06
Safranal	Safranal=0.17×Y_{corm}+25.90	0.28	2.32	3.70E-02
	Quality compounds vs. leaf dry matter (Mg ha^{-1})			
Crocin	Crocin=11.25×Leaf DM+169.69	0.49	6.98	2.00E-03
Picrocrocin	Picrocrocin=6.49×Leaf DM+62.67	0.82	1.87	1.50E-06
Safranal	-	-	-	ns

[ns] Regression is not significant.

Conclusions

Results showed that the saffron coloring strength, bitterness and aromatic strength in higher salinity level significantly decreased by 9, 13 and 18% in comparison with the lowest salinity level, respectively. These results may be due to reducing enzymatic activities of saffron under saline conditions that are led to lower qualitative compounds of saffron. However, saffron (stile/stigmas) yield declined significantly by about 42% by increasing water salinity to highest level. Therefore, the saffron yield quantity was more affected by increasing irrigation water salinity compared with the saffron quality.

The saffron crocin and picrocrocin concentrations for the in-furrow planting method were significantly higher by about 4 and 8% than the basin planting method, respectively. However, planting methods showed no significant effect on saffron aromatic strength. On the other hand, higher application rate of cow manure (60 Mg ha^{-1}) could not promote any of the saffron quality compounds.

According to ISO criteria for saffron quality, the coloring strength values of S_1 level in the basin planting method and S_1 and S_2 levels in the in-furrow planting method classified as the best quality category (with values higher than 190). In terms of bitterness, its values in S_1 and S_2 levels in the basin planting method and all irrigation water salinities in the in-furrow planting method belonged to category I (higher than 70) that is the best quality. Furthermore, all values of safranal concentration varied between minimum and maximum (20-50) values of ISO classification. By saline water application, saffron quality compounds showed low reductions in high salinity levels (S_3 and S_4); whereas, all values of the quality compounds lied between the standard range of ISO classification. On the other hand, the in-furrow planting method compensated the quality compounds reductions under saline conditions.

Correlation analysis indicated that saffron quality compounds showed negative correlation with saffron leaf calcium, sodium and chloride and positive correlation with phosphorus, nitrogen and potassium. It is concluded that excess accumulation of toxic ions in plant in saline conditions might have negative effect on plant and lead to decrease saffron quality compounds; however, increasing of nutrition elements concentration in plant can improve the quality compounds because of increasing enzymatic activities. Furthermore, it was observed positive correlation between crocin and picrocrocin with saffron yield components (including leaf dry matter, corm, flower and saffron yields); however, there was no significant correlation between safranal and yield components (including leaf dry matter, flower and saffron yields).

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