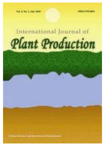

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Effect of irrigation water salinity, manure application and planting method on soil ions variation and ions uptake by saffron (*Crocus sativus* L.)

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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 ions variation in soil and their uptake by saffron. A split-split plot arrangement was conducted in a randomized complete 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 subplot and planting method (basin (P_1) and in-furrow (P_2)) as the sub-subplot with three replications. Results showed that the concentration of sodium (Na^+), calcium (Ca^{2+}), chloride (Cl^-), potassium (K^+), sulphate (SO_4^{2-}) ions in soil was increased significantly with increasing water salinity levels. These variations were in accordance with EC_e variations that were 2.6 times in S_4 compared with S_1 treatment. However, the soil nitrate (NO_3-N) decreased in the highest irrigation salinity level by about 30% compared with the lowest salinity level in two growing seasons. These element concentrations were significantly higher in F_2 treatment in comparison with F_1 due to addition of these ions by higher application rate of cow manure to soil. There was no significant difference between element concentrations in soil for two planting methods. Increasing salinity to the highest level significantly increased the saffron leaf concentration of Na^+, Ca^{2+} and Cl^- by about 4.0, 1.4 and 1.5 times, respectively. Increasing salinity resulted in decrease in K^+, nitrogen (N) and phosphorus (P) concentration in saffron leaf by about 30, 20 and 39% under the highest water salinity level, respectively. The in-furrow planting method significantly led to increase in K^+, N and P concentration in plant by about 10, 3 and 8% in comparison with the basin planting, respectively. Also, higher manure application rate as 60 Mg ha^{-1} significantly increased plant N and P concentration by about 12 and 20% in two growing seasons, respectively.

Keywords: Fertilizer level; Ions concentration; Irrigation water salinity; Planting method; Saffron; Soil salinity.

Introduction

Salinity is one of the major constraints in crop production in arid and semi-arid regions. Salinity stress imposes ionic and osmotic influences in plants (Adolf et al., 2013). The osmotic effects of salinity can be observed immediately after salt application, resulting in inhibited cell expansion and cell division, as well as stomatal closure (Flowers, 2004). Salinity dominated by sodium salts not only reduces calcium availability but decreases calcium transport and mobility to growing organs of the plant, which affects the quality of both vegetative and reproductive growth. Salinity can directly affect nutrients uptake by plant, such as high accumulation of sodium in plant, reducing potassium uptake or high chloride uptake and reducing nitrate uptake. Salinity can also cause a combination of complex interactions that affect plant metabolism, susceptibility to injury or internal nutrient requirement (Grattan and Grieve, 1999). Higher concentration of Na^+ and lower concentration of K^+ in saline conditions resulted in lower K^+/Na^+ ratio in plant tissues. Furthermore, ratio of K^+/Na^+ in plant decreased when electrical conductivity and sodium adsorption ratio (SAR) of soil increased (Porcelli et al., 1995; Ashraf et al., 2012).

Nutrient imbalance may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for that essential element (Grattan and Grieve, 1999). Specific ion toxicities such as high accumulation of Na^+, Cl^- or SO_4^{2-} could decrease the uptake of essential nutrients from soil like P, K^+, N and Ca^{2+} (Zhu, 2002). The appropriate ion ratios could be helpful to systematize the physiological response of a plant in relation to its growth and development (Wang et al., 2002).

Appropriate management of saline water and choice of salt-tolerant genotypes would lessen harmful effects of salts on soil characteristics and crop yield. Proper conditions for plant growth are provided by in-furrow planting method due to higher soil moisture, reduction in evaporation from the soil surface, higher salt leaching and lower salt concentration in root zone (Li et al., 2010; Quanqi et al., 2012; Shabani et al., 2013). Dong et al. (2008) observed that Na^+ accumulation of leaf in in-furrow planting method was lower in comparison with flat planting method for cotton. Shabani et al. (2015) stated that the in-furrow planting method is preferred for rapeseed planting or other sensitive crops in saline water and soil condition due to increase in plant Cl^- and soil Na^+ threshold for yield reduction.

It has been proved that soil amendments such as manure and organic matter could mitigate the impacts of water salinity stress on crops (Mahmoodabadi et al., 2010; Ouni et al., 2014). The humic substances enhance plant growth significantly due to the increasing cell membrane permeability, respiration, photosynthesis, oxygen and P uptake and supplying root cell growth (Gulser et al., 2010; Pizzeghello et al., 2013). Evidences showed that application of enough animal manure, supplied not only nutrient requirements of plant, but also improved soil fertility. This lead to the minimization of the use of chemical fertilizers (organic system) and consistently affected quantity and quality of crop yield such as saffron (Koocheki et al., 2006). The effect of animal manure is attributed to enhancement of the physical criteria of the soil including aeration, water holding capacity, nutrients balance and availability for plant in soil solution and improvement of nutrients exchange in soil (Zebarth et al., 1999).

Saffron (*Crocus sativus* L.) is a strategic export crop and the most expensive spice in the Islamic Republic of Iran. It is produced largely in the Khorasan and Fars Provinces with arid and semi-arid climates, respectively (Abrishami, 1987). Because of saffron low water requirement and high income it should be considered in sustainable agriculture. Effects of planting methods and corm density (Behnia, 2008; Naderi-Darbaghshahi et al., 2009), chemical and organic fertilizers (Arslan et al., 2009; Omidi et al., 2009), corms weight and size (Nassiri-Mahallati et al., 2007) and effects of salinity, irrigation regimes, fertilizer levels and planting methods (Sepaskhah and Yarami, 2009; Yarami and Sepaskhah, 2015a; Yarami and Sepaskhah, 2015b) on the yield and physiological growth of saffron have been investigated. However, investigation of toxic and essential ions uptake by saffron in different conditions is limited. Therefore, the objectives of this study were to study the effects of irrigation water salinity, cow manure levels and different planting methods on ions variation in soil and ions concentration in saffron (*Crocus sativus* L.).

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 Badjgah 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 1. 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 2.

Table 1. Physico-chemical properties of the soil at experimental site.

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

* Silty clay loam.

Table 2. Chemical analysis of the fresh and saline irrigation water used in the experiment (average of two years).

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 randomized complete block design with salinity levels of irrigation water as the main plot, cow manure fertilizer levels as the subplot and planting method as the sup-subplot 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 which were applied at the beginning of each growing seasons. Some chemical properties of the cow manure fertilizer are presented in Table 3. The planting methods were basin (P_1) and in-furrow (P_2) planting.

Table 3. Chemical properties of the cow manure.

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

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 fertilizer 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. In the second growing season half of the initial levels of cow manure fertilizer were added to the plots before the first irrigation.

All plots were irrigated on October 27 at the first and second growing seasons (2011 and 2012) with fresh and saline water, respectively. 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 before the first irrigation was measured by gravimetric method. After the first irrigation a 1.0 m long aluminum access tube was installed at the center of the plots of two replications for measuring the soil water content by neutron scattering method. Saffron flowering started about 10 days after the first irrigation after breaking the soil surface crust. The flowering period lasted for 3-4 weeks. After flower harvest in the first year, second irrigation was applied with different experimental salinity levels.

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 water depth as:

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

Where I is the irrigation water depth (m), Θ_{FCi} and Θ_i is 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.

Measurements and calculations

Soil samples of each 0.30 m increment down to 0.9 m depth were collected in 73, 107, 161 and 193 days after first irrigation (DAFI) in the first growing season (2011-2012) and 54, 93, 158 and 195 DAFI in the second growing season (2012-2013). The samples air dried and passed through 2 mm sieve for chemical analysis including electrical conductivity of soil saturation extract (EC_e) using method described by the U. S. Salinity Laboratory Staff (USDA, 1954), soluble Na^+ and K^+ by flame photometer, soluble Ca^{2+} and Mg^{2+} by EDTA solution titrating, Cl^- by $AgNO_3$

titration and NO_3-N using the method presented by Chapman and Pratt (1961). SO_4^{2-} were measured by titrating the saturation extract against EDTA solution for the samples taken at the end of each growing season. Sodium adsorption ratio (SAR) was then calculated as follows:

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}} \tag{2}$$

Where Na^{+}, Ca^{2+} and Mg^{2+} as meq L^{-1} are the concentrations of sodium, calcium and magnesium, respectively.

Plant samples of saffron (as leaf dry matter, oven dried at 70 °C to a constant weight) were taken in 70, 102, 135, 173 and 209 DAFI in the first growing season and 51, 81, 113, 140, 172 and 210 DAFI in the second growing season. Total nitrogen concentration of saffron leaf were determined by the Kjeldahl method based on the method presented by Chapman and Pratt (1961). The concentrations of K^{+} and Na^{+} in leaf were measured by flame photometer. Also, the concentrations of Ca^{2+}, Cl^{-} and total P were measured by EDTA (Kalra, 1998), silver nitrate titration (Chapman and Pratt, 1961) and Chapman and Pratt (1961) method, respectively.

Relationships between relative saffron yield that reported by Yarami and Sepaskhah (2015a) and mean values of soil Na^{+} and Cl^{-} and plant Na^{+}, Cl^{-} and K^{+} concentrations during two growing seasons determined by linear regression analysis as follows:

$$\frac{Y_a}{Y_m} = 1 - b(IC_{s/p} - IC_{th}) \tag{3}$$

Where Y_a is the actual saffron yield (kg ha^{-1}), Y_m is the maximum saffron yield (kg ha^{-1}), $IC_{s/p}$ is the ion concentration in soil (meq l^{-1}) or plant (%) and IC_{th} and b are the threshold value (meq l^{-1} or %) and saffron yield reduction coefficient (%/meq l^{-1} or %/%) due to increase in soil or plant ion concentration, respectively.

Results and Discussion

Soil saturation extract salinity

Comparison between the results of soil saturation extract salinities and ions for two growing seasons showed that there was a significant effect of year on the measured parameters. Therefore, measured data of two growing seasons were separately evaluated and discussed.

The EC_e averaged in root zone (60 cm soil depth) during two growing seasons are presented in Table 4. EC_e increased significantly with increasing irrigation salinity levels and its value in the highest saline condition was about 2.6 times of that obtained in no saline condition in two years. Salt accumulation was significantly higher in F_2 treatment in comparison with F_1 as about 8% in two years. Also, there was no significant difference between EC_e of P_1 and P_2 treatments in two years.

Table 4. Mean values of soil salinity (EC_e) and ions concentration in different irrigation water salinities and fertilizer levels and planting methods for two growing seasons.

Measured parameter	Year	Salinity levels, dS m^{-1}				Fertilizer levels, Mg ha^{-1}		Planting methods	
		S_1=0.45	S_2=1.0	S_3=2.0	S_4=3.0	F_1=30	F_2=60	P_1: Basin	P_2: In-furrow
EC_e (dS m^{-1})	1^{th}	0.67^{d*}	1.05^{c}	1.44^{b}	1.71^{a}	1.16^{b}	1.27^{a}	1.24^{a}	1.20^{a}
	2^{th}	0.62^{D}	1.00^{C}	1.29^{B}	1.64^{A}	1.10^{B}	1.18^{A}	1.13^{A}	1.16^{A}
Na^+ (meq L^{-1})	1^{th}	3.51^{c}	5.07^{b}	7.63^{a}	9.20^{a}	6.10^{a}	6.60^{a}	6.45^{a}	6.26^{a}
	2^{th}	3.14^{D}	5.15^{C}	6.75^{B}	8.97^{A}	5.61^{B}	6.40^{A}	5.99^{A}	6.01^{A}
Ca^{2+} (meq L^{-1})	1^{th}	5.21^{c}	7.08^{b}	9.41^{a}	10.71^{a}	7.77^{b}	8.44^{a}	8.18^{a}	8.02^{a}
	2^{th}	4.83^{D}	6.85^{C}	8.61^{B}	11.06^{A}	7.58^{B}	8.09^{A}	7.79^{A}	7.89^{A}
Cl^- (meq L^{-1})	1^{th}	5.50^{d}	8.46^{c}	12.55^{b}	15.45^{a}	9.87^{b}	11.11^{a}	10.58^{a}	10.40^{a}
	2^{th}	4.86^{D}	7.44^{C}	11.56^{B}	16.00^{A}	9.59^{B}	10.33^{A}	9.87^{A}	10.06^{A}
K^+ (meq L^{-1})	1^{th}	0.51^{d}	0.79^{c}	1.12^{b}	1.41^{a}	0.89^{b}	1.02^{a}	0.98^{a}	0.94^{a}
	2^{th}	0.43^{D}	0.68^{C}	0.97^{B}	1.24^{A}	0.80^{B}	0.86^{A}	0.82^{A}	0.84^{A}
SAR	1^{th}	1.71^{c}	2.11^{b}	2.78^{a}	3.15^{a}	2.39^{a}	2.48^{a}	2.47^{a}	2.40^{a}
	2^{th}	1.59^{D}	2.23^{C}	2.59^{B}	3.10^{A}	2.25^{B}	2.49^{A}	2.38^{A}	2.37^{A}
SO_4^{-2} (meq L^{-1})	1^{th}	0.97^{d}	1.08^{c}	1.23^{b}	1.39^{a}	1.12^{b}	1.21^{a}	1.20^{a}	1.14^{a}
	2^{th}	1.72^{D}	1.88^{C}	2.05^{B}	2.27^{A}	1.95^{A}	2.01^{A}	1.96^{A}	2.00^{A}
NO_3-N (kg ha^{-1})	1^{th}	106.93^{a}	103.17^{a}	95.99^{a}	78.03^{b}	87.93^{b}	104.12^{a}	96.92^{a}	95.14^{a}
	2^{th}	115.11^{A}	108.92^{B}	98.15^{B}	78.43^{C}	88.23^{B}	112.08^{A}	99.88^{A}	100.43^{A}

* Means followed by the same letters in rows for each factor and each trait are not significantly different at 5% level of probability by t-test.

Seasonal variation of EC_e averaged in root zone under different experimental treatments for the first and second growing seasons are presented in Figures 1a, b, respectively. In the first year, it is shown in Figure 1.a that EC_e at 107 DAFI declined and after that an increasing trend was observed for all treatments. EC_e in F_2 treatments were higher than F_1 treatments. The reason for this finding is probably due to higher application of saline cow manure (Table 3) to the soil. Mohammad et al, 2012 also reported that application of cow manure (20 to 30 Mg ha^{-1}) to the soil increased significantly (P=0.05) the soil EC_e in comparison with control treatment and chemical N-fertilizer application.

Regardless of cow manure levels, P_2 treatments showed lower EC_e compared with P_1, there was an exception in S_4 salinity and F_1 cow manure levels that EC_e in P_2 treatments were higher than P_1. According to Figure 1.b, similar patterns as Figure 1.a was observed in EC_e of experimental treatments for the second year. Because of rainfall during the two growing seasons, there was no salinity build up and due to higher rainfall in the second year its EC_e was significantly lower than the first year.

The EC_e of the all salinity levels were about equal or less than electrical conductivities of irrigation water (EC_{iw}). This is due to the fact that before irrigation, soil was not saline and rainfall in winter leached the salts and decreased the irrigation requirement, therefore soil salinity was not high. Although salt accumulation was significantly higher in F_2 treatment in comparison with F_1 as about 8% in two years, however; higher cow manure application (F_2 level) mitigated the effect of irrigation salinity and caused to higher saffron yield by promoting soil fertility and providing nutrient requirements.

Results showed there was no significant difference between EC_e of basin and in-furrow planting method in two years, while Shabani et al. (2013) reported that salt accumulation was higher in on-ridge planting in comparison with in-furrow planting in rapeseed field. The salts accumulated in the top 30 cm layer of the soil profile (data not shown) in all treatments showed higher EC_e than the bottom layers (60 and 90 cm depths). In fact, with short term application of saline water, the salts had not enough opportunity to be transferred to deep layers and the saline water-soil equilibrium condition is not established. Such distribution patterns were also reported by Amer (2010) and Azizian and Sepaskhah (2014) in soil profile of the maize field and Noshadi et al. (2013) in tomato field.

Monitoring soil salinity during two years indicated that EC_e decreased in winter (about 100 DAFI) because of rainfall occurrences (about 170 and 280 mm rainfall in the first and second years, respectively) and no need for irrigation and after that average of soil salinity of root zone increased under all experimental treatments. In general, salt accumulation at the second year was lower than the first year because of higher rainfall (363 and 445 mm rainfall in the first and second years, respectively) and fewer irrigation events.

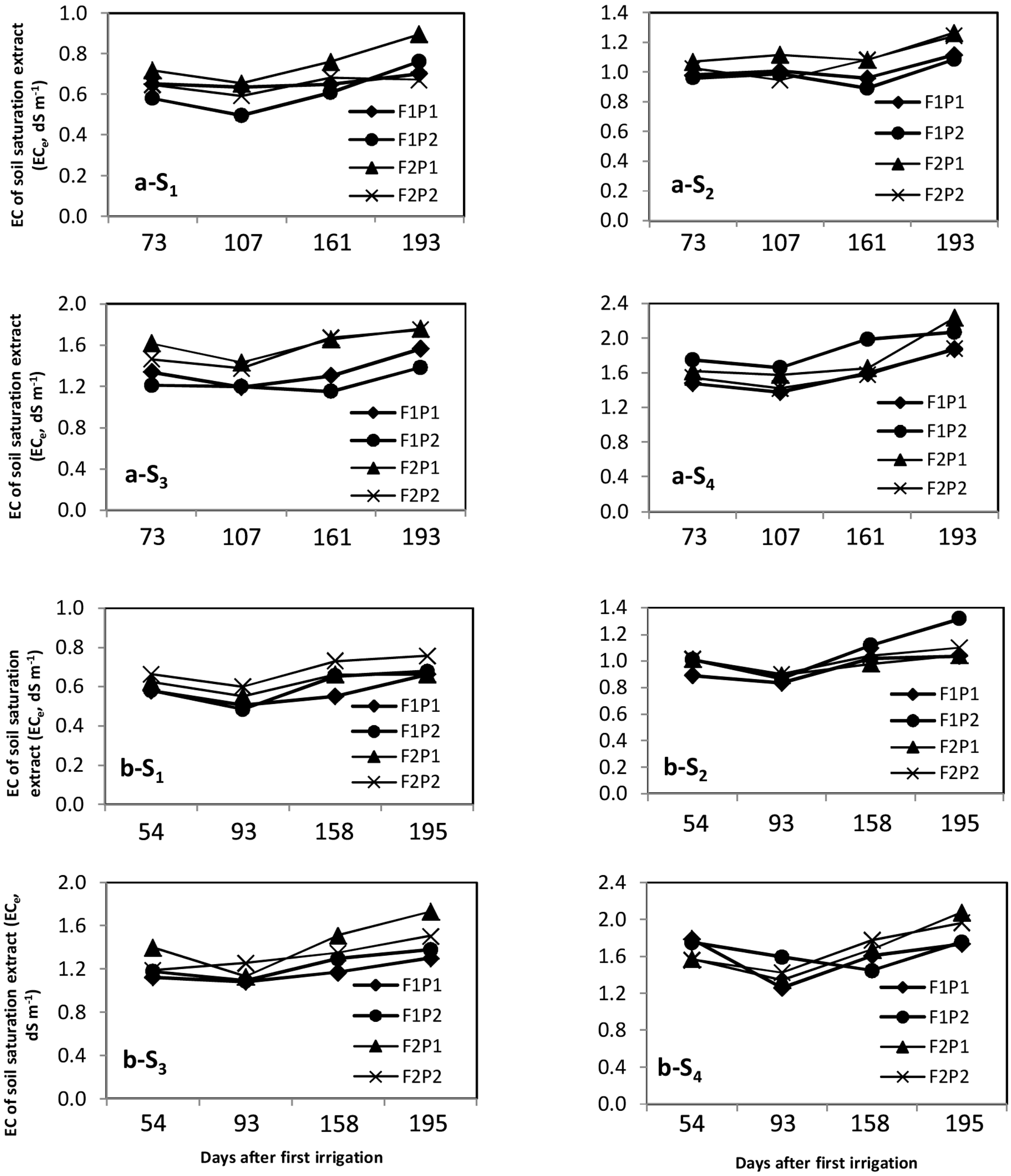

Figure 1. Seasonal variation of soil saturation extract salinity (EC_e, dS m^{-1}) at different irrigation water salinity (S_1=0.45, S_2=1.0, S_3=2.0 and S_4=3.0 dS m^{-1}), cow manure levels (F_1=30, F_2=60 Mg ha^{-1}) and planting methods(P_1: basin and P_2: in-furrow) in the first (a) and the second growing seasons (b).

Soil saturation extract ions

The concentration of Na^+, Ca^{2+}, Cl^-, K^+ and NO_3-N in soil saturation extract averaged in root zone (60 cm soil depth) during two growing seasons for experimental treatments are presented in Tables 4. Also, the concentration of SO_4^{2-} in soil saturation extract averaged in root zone at the end of each growing season is shown in Table 4.

The concentration of measured ions almost increased significantly with increasing salinity levels. The concentration of Na^+ and K^+ were 2.7 and 2.8 times in S_4 compared with S_1 treatment for two growing seasons, respectively. These variations were in accordance with EC_e variations that was 2.6 times in S_4 compared with S_1. The corresponding values for Ca^{2+}, Cl^- and SO_4^{2-} were 2.2, 3.1 and 1.4 times, respectively. Generally, increase in irrigation water salinity increased the soil ions due to increase in accumulation of ions in soil. The soil ions concentration were significantly higher by 8-15% in F_2 treatment in comparison with F_1 in two years. The reason for this finding is probably due to addition of these ions by higher application rate of cow manure to the soil. Furthermore, there was no significant difference between ion concentrations in two planting methods (P_1 and P_2) in two growing seasons.

Relative concentration of Na^+ ion compared to Ca^{2+} and Mg^{2+} is calculated as SAR index and used for evaluating infiltration capacity of soil in companion with EC_e. The values of SAR index increased significantly with increasing irrigation salinity levels by about 1.9 times in S_4 compared with S_1 treatment for two growing seasons (Table 4). SAR value was significantly higher in F_2 compared to F_1 treatment by about 11% in the second growing season. However, different planting methods showed no significant effect on SAR. The values of SAR index in all treatments were below than the critical value (SAR<13 for soil, Soil Science Society of America, 2001). Gibbs (1970) stated there is an interesting relationship between salinity and the ratio of $Na^+/(Na^+ + Ca^{2+})$ in water. According to Table 2, this ratio for all water salinity levels of this study were less than 0.7 that indicate Ca^{2+} is a major contributor to the salinising media. Therefore, because of the high concentration of calcium in the irrigation water, no difficulties are expected with soil structural degradation and infiltration problem in the soil.

Soil NO_3-N content showed an inverse relationship with increase in irrigation water salinity in two growing seasons (Table 4). The soil NO_3-N decreased in the highest irrigation salinity level (S_4) by 27% and 32% compared with the lowest salinity level (S_1) in the first and second growing seasons, respectively. This might be due to the fact that under highest salinity levels, the activity of soil microorganisms for NO_3-N production decreased. Tate (1995) reported that increasing salt concentrations may have a detrimental effect on soil microbial populations as a result of direct toxicity as well as through osmotic stress. Also, experiments have indicated that the presence of soluble salts and the reduction in pH as a result of high levels of ammonia could also suppress the activity of soil microorganisms (Ibekwe et al., 1997). There was no significant differences between the soil NO_3-N content of S_1, S_2 and S_3 salinity levels in the first year and between S_2 and S_3 levels in the second year. This may be due to complex behavior of NO_3-N in salinity condition. Higher application rate of cow manure as 60 Mg ha^{-1} (F_2) increased significantly the soil NO_3-N content by 18 and 27% in the first and second growing seasons in comparison with F_1 treatment, respectively. This result showed that higher application rate of cow manure can provide higher available form of nitrogen for plant. Different planting methods indicated no significant effect on the soil NO_3-N concentration in two growing seasons.

Soil indices based on yield-ion relationship

Relationships between relative saffron yield and mean values of soil Na^+ and Cl^- concentrations during two growing seasons determined by linear regression analysis for different fertilizer levels and planting methods based on two growing seasons data. The results are shown in Figures 2 and 3. The threshold values and also yield reduction coefficients due to increase in soil ions concentration were calculated using these linear models (Table 5). Saffron yield and soil Na^+ and Cl^- concentration were negatively correlated. Hence, it is expected that the response of saffron yield to increasing concentration of these ions showed a declining trend after a threshold value.

Table 5. Threshold values of Na^+ and Cl^- concentration in soil saturation extract and saffron yield reduction coefficients at different fertilizer levels and planting methods.

Soil ion	Fertilizer levels Mg ha^{-1}	Planting methods			
		P_1: Basin		P_2: In-furrow	
		Threshold	Slope	Threshold	Slope
		meq L^{-1}	%/meq L^{-1}	meq L^{-1}	%/meq L^{-1}
Na^+					
	F_1=30	2.70	11.2	3.12	6.5
	F_2=60	3.96	8.2	5.87	6.8
Cl^-					
	F_1=30	4.05	6.2	5.38	3.9
	F_2=60	5.17	4.1	9.40	3.5

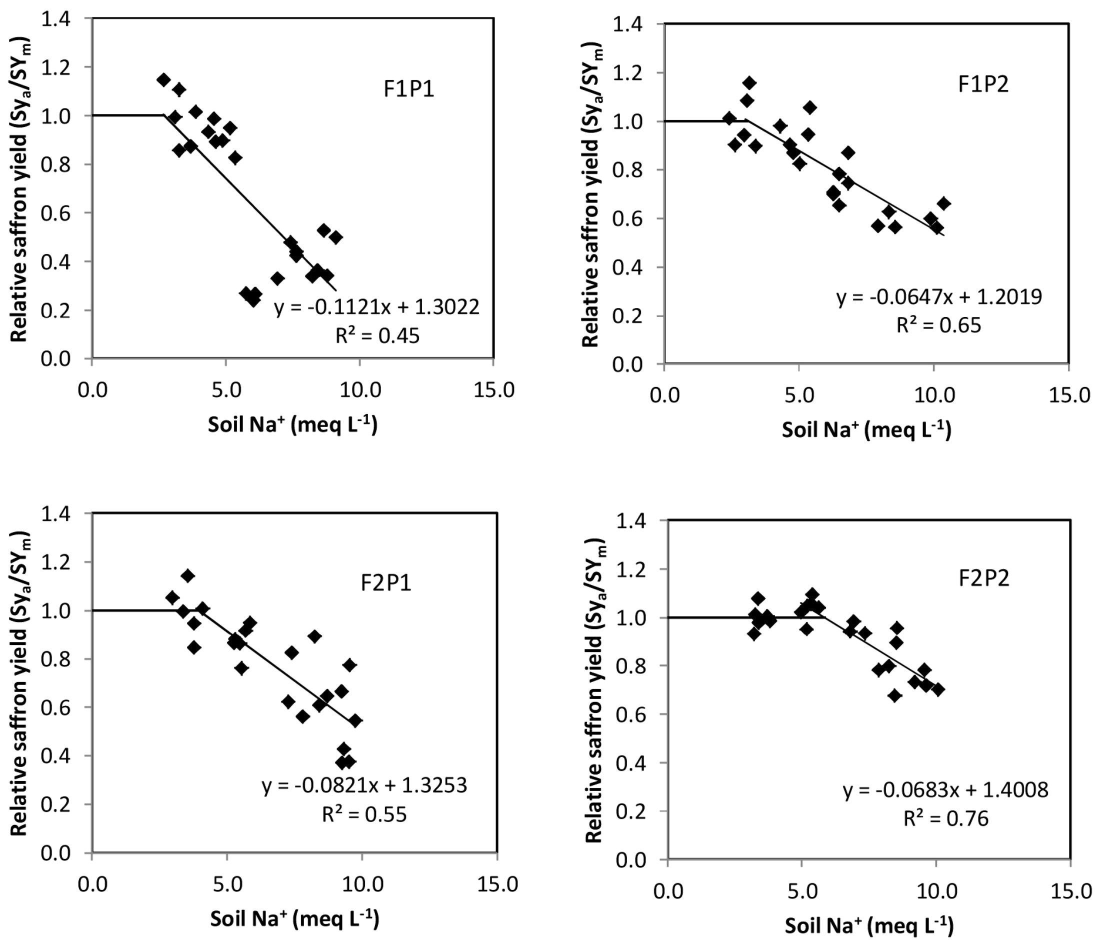

Figure 2. Relationship between relative saffron yield (SY_a/SY_m) and mean values of soil Na^+ concentration under different fertilizer levels and planting methods.

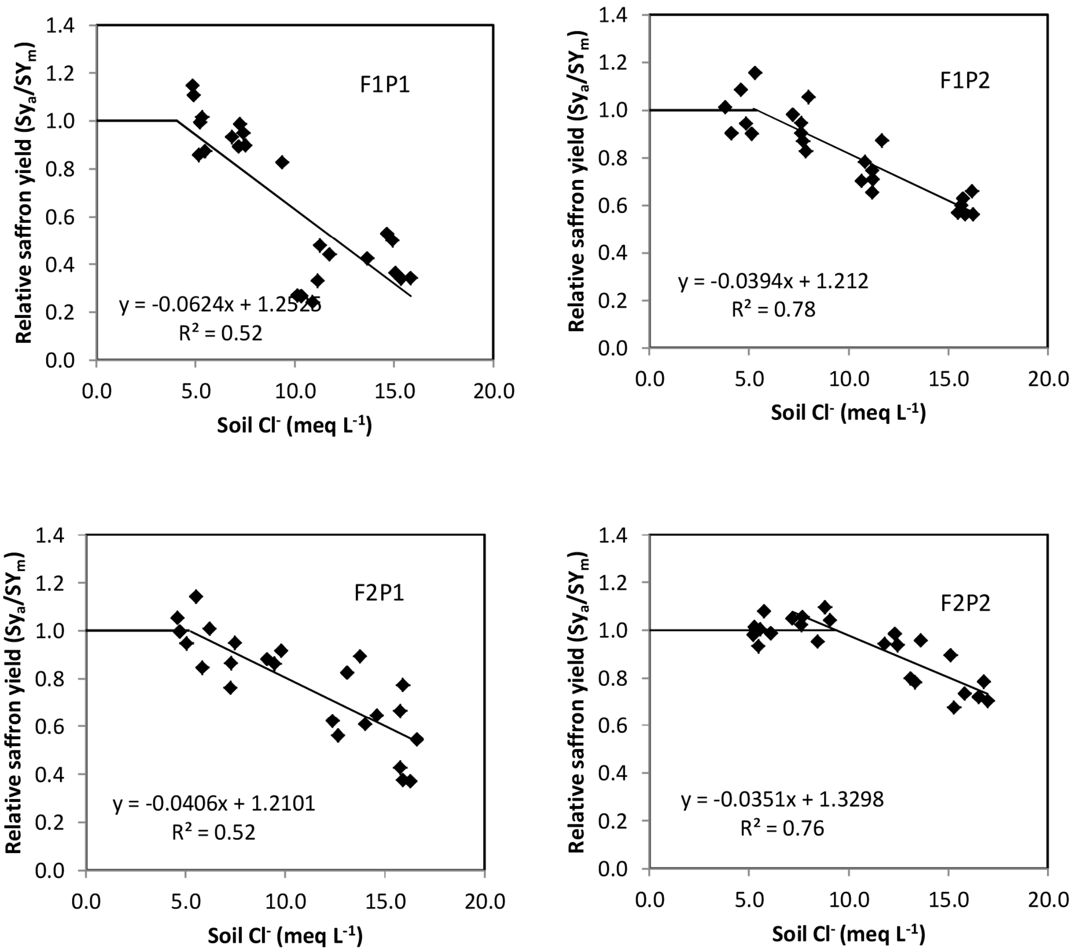

Figure 3. Relationship between relative saffron yield (SY_a/SY_m) and mean values of soil Cl^- concentration under different fertilizer levels and planting methods.

Na^+ and Cl^- threshold for saffron yield (2.70 meq L^{-1} in F_1P_1 to 3.96 meq L^{-1} in F_2P_1 and 3.12 meq L^{-1} in F_1P_2 to 5.87 meq L^{-1} in F_2P_2 for Na^+ threshold and 4.05 meq L^{-1} in F_1P_1 to 5.17 meq L^{-1} in F_2P_1 and 5.38 meq L^{-1} in F_1P_2 to 9.40 meq L^{-1} in F_2P_2 for Cl^- threshold) increased from 28% to 88% with increasing manure application rate under each planting methods. In each fertilizer level, Na^+ and Cl^- threshold in the in-furrow planting method was higher than that in the basin method. The Na^+ thresholds for saffron yield reduction (2.70 meq L^{-1} in F_1P_1 to 3.12 meq L^{-1} in F_1P_2 and 3.96 meq L^{-1} in F_2P_1 to 5.87 meq L^{-1} in F_2P_2) were increased by 16% and 48% for in-furrow planting method in comparison with the basin method under F_1 and F_2 fertilizer levels, respectively. The corresponding increases for Cl^- threshold (4.05 meq L^{-1} in F_1P_1 to 5.38 meq L^{-1} in F_1P_2 and 5.17 meq L^{-1} in F_2P_1 to 9.40 meq L^{-1} in F_2P_2) were 33% and 82% for F_1 and F_2 treatments, respectively. These results indicated that saffron was more sensitive to Na^+ and Cl^- under the basin planting method. Saffron yield reduction coefficient for Na^+ and Cl^- in soil decreased by 27% and 34% by increasing manure application rate under the basin planting method, respectively. However, these reduction coefficients were about the same under the in-furrow planting method (6.5%/meq L^{-1} in F_1P_2 and 6.8%/meq L^{-1} in F_2P_2 for Na^+ and 3.9 %/meq L^{-1} in F_1P_2 and 3.5%/meq L^{-1} in F_2P_2 for Cl^-).

According to Table 5, the threshold and slope values for Cl^- were higher and lower than those values for Na^+, respectively. Therefore, Na^+ accumulation in soil was more detrimental than Cl^- accumulation for saffron irrigated with saline water. Generally, saffron is considered less sensitive to toxic ions in soil such as Na^+ and Cl^- with application of 60 Mg ha^{-1} cow manure under two planting method. Similar results were obtained for the in-furrow planting method compared with the basin planting method, regardless of cow manure levels.

Plant ions concentration

Comparison between the results of saffron leaf ions for two growing seasons showed that there was a significant effect of year on plant ions. Therefore, measured data of two growing seasons were separately analyzed and discussed.

Plant Na^+

Saffron leaf Na^+ concentration under different irrigation water salinities, fertilizer levels and planting methods are presented in Table 6. Leaf Na^+ concentration in S_3 and S_4 treatments were statistically higher than those values obtained in S_1 and S_2 salinity levels in the first growing season. Increasing salinity level from S_2 to S_4 resulted in significant increase in Na^+ concentration of saffron leaf by about 3 times in the second growing season. These results were due to higher addition of Na^+ by saline irrigation water to soil and its higher uptake by plant. Excess Na^+ accumulation in plant might have toxic effect on plant. However, in this study, no foliar injury symptoms were observed on the saffron leaves.

There was no significant difference between the effect of different fertilizer levels and two planting methods on Na^+ concentration in saffron leaf. However, the plant Na^+ concentration was lower in the in-furrow planting method in comparison with the basin planting method. This result was in agreement with the findings of Dong et al. (2008) for cotton and in contrast to those reported by Shabani et al. (2014) that stated the plant Na^+ in the in-furrow planting method was higher than that in the on-ridge planting for rapeseed. Dong (2012) stated that recirculation of Na^+ from shoot to the low-salinity side of root through the phloem is an important mechanism for reducing leaf Na^+ accumulation in leaves by furrow-bed seeding.

Plant Ca^{2+}

Increase in irrigation water salinity resulted in significantly enhancement of Ca^{2+} in saffron leaf due to higher Ca^{2+} concentration in soil and irrigation water (Table 6). Similar results were reported by Francois (1994) and Shabani et al. (2015). Planting method showed no significant effect on the Ca^{2+} in plant during two growing seasons. Shabani et al. (2015) also reported that in-furrow and on-ridge planting methods showed no significant effect on the Ca^{2+} concentration in rapeseed. Higher application rate of cow manure as 60 Mg ha^{-1} increased significantly the saffron leaf Ca^{2+} concentration by 10 and 12% in the first and second growing seasons, respectively. Higher leaf calcium concentration due to higher application rate of cow manure could play a regulatory role in response of saffron to saline environment, whereas, higher saffron yield were reported by Yarami and Sepaskhah (2015a) under saline irrigation water by higher cow manure application rate.

Table 6. Mean values of saffron leaf ions concentration in different irrigation water salinities and fertilizer levels and planting methods for two growing seasons.

Measured parameter	Year	Salinity levels, dS m^{-1}				Fertilizer levels, Mg ha^{-1}		Planting methods	
		S_1=0.45	S_2=1.0	S_3=2.0	S_4=3.0	F_1=30	F_2=60	P_1: Basin	P_2: In-furrow
Na^+ (%)	1^{th}	0.045^{b*}	0.061^b	0.106^a	0.189^a	0.099^a	0.101^a	0.115^a	0.086^a
	2^{th}	0.048^C	0.058^C	0.109^B	0.175^A	0.101^A	0.094^A	0.099^A	0.096^A
Ca^{2+} (%)	1^{th}	1.01^d	1.06^c	1.32^b	1.46^a	1.15^b	1.27^a	1.21^a	1.21^a
	2^{th}	1.19^C	1.20^C	1.54^B	1.67^A	1.32^B	1.48^A	1.40^A	1.40^A
K^+ (%)	1^{th}	1.22^a	1.11^b	0.99^c	0.93^d	1.05^a	1.07^a	1.02^b	1.10^a
	2^{th}	1.39^A	1.20^B	1.02^C	0.89^D	1.12^A	1.14^A	1.06^B	1.19^A
K^+/Na^+	1^{th}	33.22^a	22.32^b	13.90^c	7.34^d	20.30^a	18.09^a	17.01^b	21.38^a
	2^{th}	36.42^A	27.63^B	16.59^C	7.95^D	22.00^A	22.30^A	20.61^A	23.69^A
Cl^- (%)	1^{th}	1.20^c	1.27^c	1.66^b	1.82^a	1.45^a	1.52^a	1.53^a	1.44^a
	2^{th}	1.11^B	1.15^B	1.58^A	1.61^A	1.29^B	1.44^A	1.40^A	1.33^A
N (%)	1^{th}	1.84^a	1.83^a	1.51^b	1.43^c	1.51^b	1.79^a	1.63^b	1.67^a
	2^{th}	2.36^A	2.33^A	2.09^B	1.86^C	2.01^B	2.31^A	2.14^B	2.18^A
P (%)	1^{th}	0.222^a	0.216^a	0.152^b	0.125^c	0.159^b	0.198^a	0.171^b	0.186^a
	2^{th}	0.258^A	0.249^B	0.197^C	0.173^D	0.204^B	0.235^A	0.213^B	0.225^A

* Means followed by the same letters in rows for each factor and each trait are not significantly different at 5% level of probability by t-test.

Plant Cl^-

The main effects of water salinity, fertilizer level and planting method are shown in Table 6 on saffron leaf Cl^- ($P \leq 0.05$) in both years. Chloride concentration in plant was increased with enhancement of irrigation water and soil saturation extract salinity that resulted in higher accumulation of Cl^- in plant. Chloride ion accumulated statistically higher in saffron leaf under S_3 and S_4 treatments compared with S_1 and S_2 salinity levels in two growing seasons. There was no significant difference between Cl^- concentration of plant in different planting methods. Similar results were reported by Shabani et al. (2015) for rapeseed. However, chloride concentration in plant was lower in the in-furrow planting method in comparison with the basin planting method. Higher application rate of cow manure (F_2) increased significantly the plant Cl^- concentration by about 12% compared with F_1 treatment in the second growing season. The reason for this result is probably due to addition of higher Cl^- concentration to soil by higher application rate of cow manure and hence, uptake increasing and accumulation of this ion in saffron leaves.

Plant K^+ and K^+/Na^+ ratio

Saffron leaf K^+ concentration showed significantly inverse relationship with increase in soil and irrigation water salinity in two growing seasons (Table 6). The plant K^+ concentration decreased in the highest irrigation salinity level (S_4) by 24% and 36% compared with the lowest salinity level (S_1) in the first and second growing seasons, respectively. K^+ in plant decreased as a result of increase in Na^+ in soil. Sodium can be substituted for potassium due to similar mechanisms of uptake for both ions (Rameeh et al., 2004). Reduction in K^+ uptake in plants by Na^+ increase is a competitive mechanism and occurs regardless of whether the solution is dominated by Na^+ salts of Cl^- or SO_4^{2-}. However, plant species may differ in response to each of these salinising systems (Grattan and Grieve, 1999). Different fertilizer levels showed no significant effect on plant K^+ concentration in two growing seasons. The in-furrow planting method increased significantly plant K^+ concentration by about 8% and 12% in comparison with the basin planting method in the first and second growing seasons, respectively. However, Shabani et al. (2015) reported that there was no significant difference between the effect of planting method on K^+ concentration of rapeseed.

Whereas, potassium uptake is impaired by higher Na^+, higher K^+ concentrations in plant tissue are required for higher shoot growth. On the other hand, increase in leaf Na^+ concentration may help to maintain plant turgor pressure whereas, Na^+ cannot completely substitute for K^+ that is specifically required for protein synthesis and enzyme activation (Marschner, 1995). Therefore, the K^+/Na^+ ratio should be considered. Plant K^+/Na^+ ratio can be applied as selection criteria for assessing salinity tolerance of different crop species. In this study, with increase of irrigation water salinity, the plant K^+/Na^+ ratio decreased significantly in two growing seasons (Table 6). This reduction between the highest and lowest salinity levels was about

78% for two growing seasons. Rameeh et al. (2004) stated that soil and irrigation water salinity increased Na^+ influx and K^+ efflux. Therefore, higher Na^+ uptake and lower K^+ uptake in high salinity level resulted in dramatically decrease in plant K^+/Na^+ ratio. There was no significant difference between the effect of fertilizer levels on saffron leaf K^+/Na^+ ratio. Plant K^+/Na^+ ratio in the in-furrow planting method was higher than that in the basin planting method due to lower Na^+ concentration and higher K^+ concentration in saffron leaves. These increases were 26% and 15% for the first and second growing seasons, respectively. Comparison between two years indicated that plant K^+/Na^+ ratio in the second year was higher than those in the first year due to higher K^+ concentration in plant in the second year compared with the first year. This finding may be due to higher release of K^+ concentration as an essential nutrient element from cow manure during the second growing season and hence higher K^+ uptake by saffron.

Plant nitrogen

Total nitrogen (N) concentration of saffron leaf under different irrigation water salinities, fertilizer levels and planting methods are shown in Table 6. Plant N concentration decreased significantly by increasing of irrigation water salinity levels and dropped to the least amount in the highest salinity levels which was about 22% less than that obtained in the lowest salinity level in two growing seasons. The reduction in plant N concentration with increased salinity level was accompanied by the soil and plant Cl^- concentration increase according to Tables 4 and 6. Results of other studies indicated that the N uptake or accumulation in the plants shoot may be reduced under saline conditions (Savvas and Lenz, 1996; Grattan and Grieve, 1999; Azizian and Sepaskhah, 2015).

Higher application rate of cow manure (60 Mg ha^{-1}) increased significantly the saffron leaf N concentration by 19 and 15% in the first and second growing seasons, respectively. This result indicated that higher cow manure application (F_2) mitigated the effect of irrigation salinity and resulted in higher plant N concentration by promoting soil fertility and providing essential plant nutrient requirements. This finding is in agreement with the result of Mohammad et al. (2012), who reported that application of 30 Mg ha^{-1} cow manure increased significantly the saffron leaf N concentration by 23% in comparison with the control treatment.

On the other hand, the in-furrow planting method increased significantly plant N concentration by about 3% in comparison with the basin planting method in two growing seasons. However, the effect of higher manure application on N concentration was dramatically higher than that the planting methods. Comparison between the results of two years indicated that plant N concentration in the second year was higher than those in the first year for all experimental treatments. This result revealed that cow manure is a good source of plant nutrients due to providing long-term soil fertility.

Plant phosphorus

Saffron leaf phosphorus (P) concentration showed significantly inverse relationship with increase in soil and irrigation water salinity in two growing seasons (Table 6). The plant P concentration decreased in the highest irrigation salinity level by 44 and 33% compared with no salinity in the first and second growing seasons, respectively. Reduction in plant P concentration by salinity is a result of reduced activity of P in the soil solution due to the high ionic strength of the media and low solubility of Ca-P minerals. In most studies, salinity decreased the concentration of P in plant tissue (Sharpley et al., 1992); however, the results of some studies indicated salinity increased or showed no effect on plant P uptake. Plant growing conditions, plant type and even cultivar play a large role in P accumulation under salinity condition (Grattan and Grieve, 1999).

Higher application rate of cow manure (F_2) increased significantly the plant P concentration by about 25 and 15% compared with F_1 treatment in the first and second growing seasons, respectively. This is due to the nutritional effect of higher cow manure application on saffron. Mohammad et al. (2012) also reported 28% increase in leaf saffron P concentration by application of 30 Mg ha^{-1} cow manure compared with no application of manure. The in-furrow planting method increased significantly plant P concentration by about 9 and 6% in comparison with the basin planting method in the first and second growing seasons, respectively. Also, plant P concentration in the second year was higher than those in the first year in all treatments due to providing long-term soil fertility by manure application.

Plant-ions indices

Relationships between relative saffron yield and mean values of plant Na^+, Cl^- and K^+ concentrations during two growing seasons were determined by linear regression analysis based on data of two growing seasons for different fertilizer levels and planting methods (Figures 4 to 6). The threshold values and also yield reduction coefficients due to increase in these ions concentration in plant were calculated by these models (Table 7). Relative saffron yield and plant Na^+ and Cl^- concentration were negatively correlated because accumulation of these ions in saffron beyond a threshold value resulted in yield reduction. Plant Na^+ threshold for saffron yield increased with increasing manure application rate under the in-furrow planting method (0.024% in F_1P_2 to 0.059% in F_2P_2). The plant Na^+ threshold for saffron yield reduction under F_2 fertilizer level (0.038% in F_2P_1 to 0.059% in F_2P_2) were increased by 55% for in-furrow planting method in comparison with the basin method. Saffron yield reduction coefficient for plant Na^+ concentration was very high because of high sensitivity of saffron to accumulation of sodium especially under the basin planting method (1724.8%/% and 427.9 %/% in F_1 and F_2 fertilizer levels).

Table 7. Threshold values of plant Na^+, Cl^- and K^+ concentration and saffron yield reduction coefficients at different fertilizer levels and planting methods.

Plant ion	Fertilizer levels Mg ha^{-1}	Planting methods			
		P_1: Basin		P_2: In-furrow	
		Threshold	Slope	Threshold	Slope
		%	%/%	%	%/%
Na^+					
	F_1=30	0.054	1724.8	0.024	273.9
	F_2=60	0.038	427.9	0.059	233.2
Cl^-					
	F_1=30	0.91	67.4	0.98	63.1
	F_2=60	0.82	40.8	1.38	55.4
K^+					
	F_1=30	1.21	166.6	1.29	116.5
	F_2=60	1.20	146.4	1.15	128.5

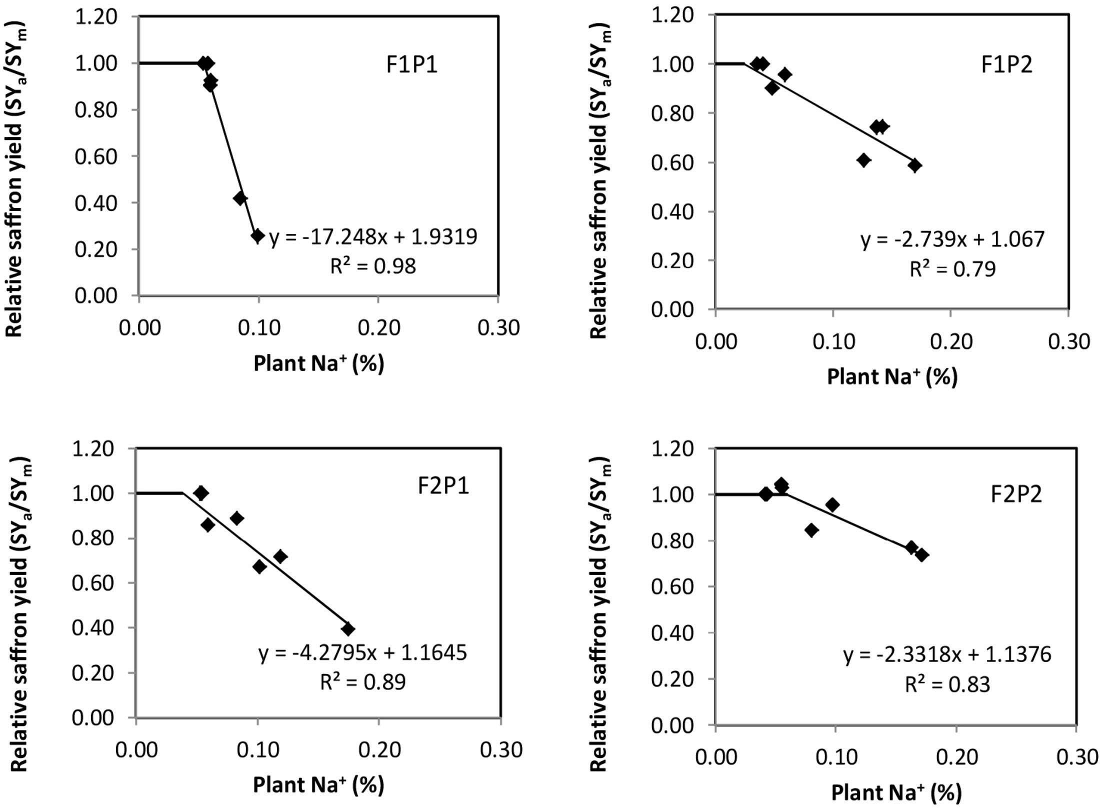

Figure 4. Relationship between relative saffron yield (SY_a/SY_m) and mean values of plant Na^+ concentration under different fertilizer levels and planting methods.

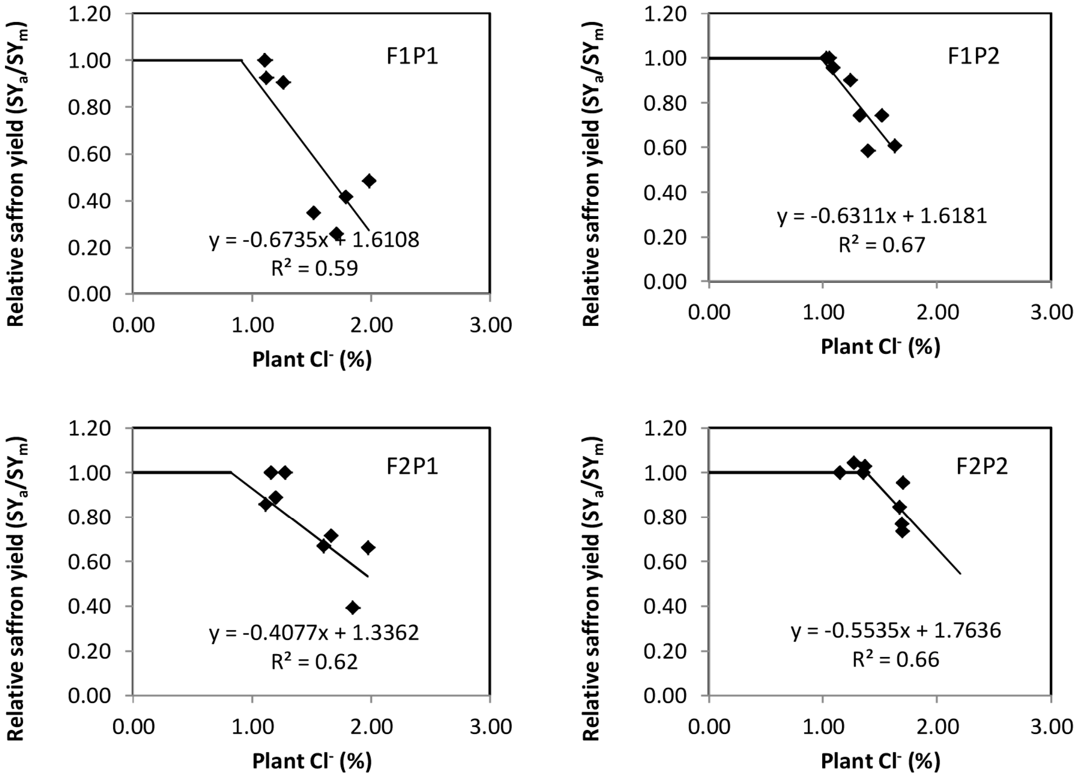

Figure 5. Relationship between relative saffron yield (SY_a/SY_m) and mean values of plant Cl^- concentration under different fertilizer levels and planting methods.

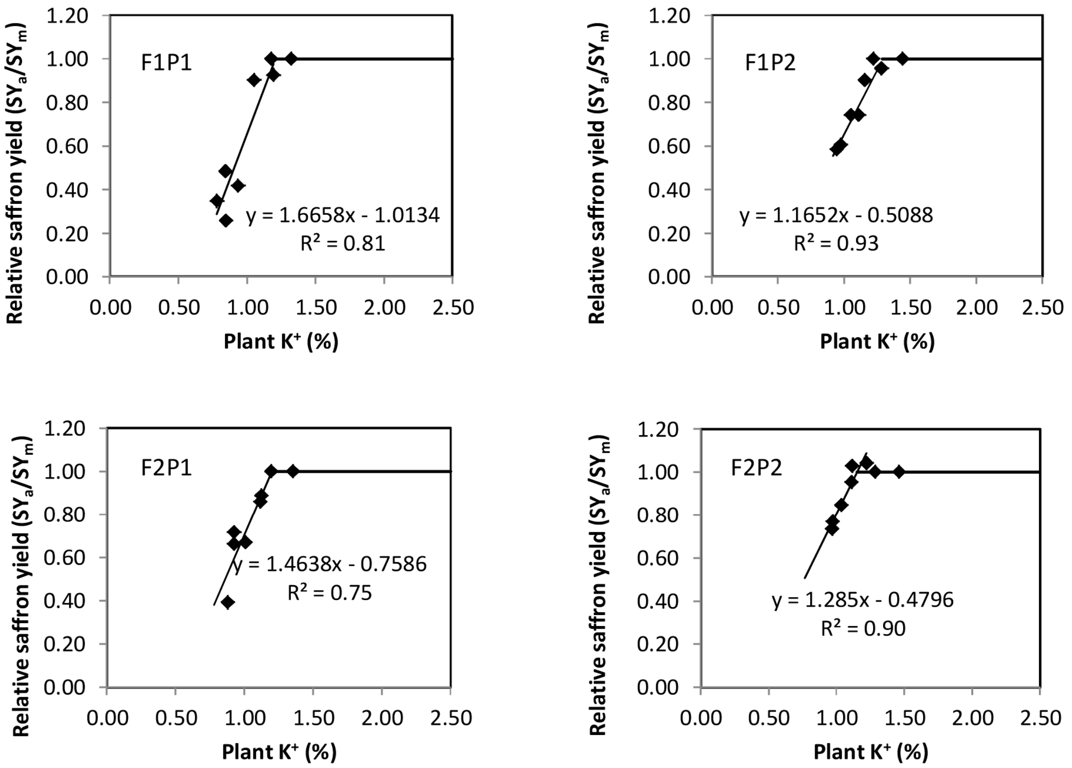

Figure 6. Relationship between relative saffron yield (SY_a/SY_m) and mean values of plant K^+ concentration under different fertilizer levels and planting methods.

In each fertilizer levels, plant Cl^- threshold in the in-furrow planting method is higher than that in the basin method. The plant Cl^- thresholds for saffron yield reduction (0.91 % in F_1P_1 to 0.98 % in F_1P_2 and 0.82 % in F_2P_1 to 1.38 % in F_2P_2) were increased by 8% and 68 % for in-furrow planting method in comparison with the basin method under F_1 and F_2 fertilizer levels, respectively. Hence, saffron was more sensitive to Cl^- accumulation under the basin planting method. Saffron yield reduction coefficient for plant Cl^- concentration (67.4%/% in F_1P_1 to 40.8%/% in F_2P_1 and 63.1%/% in F_1P_2 to 55.4%/% in F_2P_2) decreased by 39% and 12% by increasing manure application rate under the basin and in-furrow planting methods, respectively. According to Table 7, the threshold values for plant Cl^- (0.82%-1.38%) were higher than those values for plant Na^+ (0.024%-0.059%). Therefore, Na^+ accumulation in plant was more detrimental than Cl^- accumulation for saffron irrigated with saline water which is in agreement with the results of Azizian and Sepaskhah (2015) and Isla and Aragüés (2010) for maize as a salinity sensitive crop.

Because of the Na^+ and K^+ discrimination found in most plants grown in saline conditions, relative saffron yields were positively correlated with plant K^+ concentration. Hence, growth is reduced with K^+ concentration lower than the threshold value of plant K^+ (Figure 6). According to Table 7, there was no clear variation occurred in the threshold values for plant K^+ under different fertilizer levels and planting methods. However, saffron was more tolerant to K^+ deficit under higher manure application (F_2) and the in-furrow planting method (P_2). This is due to the fact that the value of K^+ of which saffron yield was reduced, was lower under these treatments.

Conclusions

Results of this study showed that application of saline irrigation water led to salts accumulation (soil EC_e) higher than 1.6 times of that for no saline water. Similar pattern was observed for soil Na^+, Ca^{2+}, K^+, SO_4^{2-} and Cl^- concentrations by application of saline water. Monitoring of soil salinity during two years indicated that EC_e decreased in winter (about 100 days after first irrigation) because of rainfall occurrence and no need for irrigation and after that average of soil salinity of root zone increased under all experimental treatments. In general, salt accumulation at the second year was lower than the first year because of higher rainfall and fewer irrigation events. Soil NO_3-N decreased in the highest irrigation salinity level (S_4) by 27% and 32% compared with the lowest salinity level (S_1) in the first and second growing seasons, respectively. Results showed there was no significant difference between EC_e of basin and in-furrow planting method in two years. Also, there was no significant difference between ions concentration in soil for two planting methods. Furthermore, salt accumulation was significantly higher in F_2 treatment in comparison with F_1 by about 8% in two years; however, higher cow manure application (F_2) mitigated the effect of irrigation water salinity and resulted in higher saffron yield by promoting soil fertility and providing nutrient requirements. Increasing salinity to the highest level significantly increased the saffron leaf concentration of Na^+, Ca^{2+} and Cl^- by about 4.0, 1.4 and 1.5 times, respectively. However, increasing salinity levels resulted in reduction of K^+, N and P concentration in saffron leaf by about 30, 20 and 39% under the highest water salinity level, respectively. Therefore, salinity can directly affect on uptake of major nutrient elements by plant. The in-furrow planting method significantly increased the 2-year mean values of K^+, N and P concentrations by about 10, 3 and 8% in comparison with the basin planting, respectively. Also, higher manure application rate (60 Mg ha^{-1}) significantly increased the 2-year mean of plant N and P concentration by about 12 and 20%, respectively. According to the threshold and yield reduction coefficient values, the detrimental effects of Na^+ accumulation in soil on cells metabolism for saffron is higher than other ions such as Cl^- by application of saline water. Soil Na^+ and Cl^- threshold for saffron yield increased with increasing manure application rate under each planting methods. Therefore, slow release of nutrients from cow manure during growing season and hence low leaching of the nutrients could be important criteria for cow manure application, which improved growth and yield of saffron plant.

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