International Research Journal of Agricultural Science and Soil Science (ISSN: 2251-0044) Vol. 1(10) pp. 421-427 December 2011
Available online http://www.interesjournals.org/IRJAS

Full Length Research Paper

The effect of salt stress on flower yield and growth parameters of saffron (*Crocus sativus* L.) in greenhouse condition

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Accepted 28 November, 2011

***Crocus sativus* L. has been cultivated since ancient times as the source of saffron, a substance renowned for its medicinal and flavoring properties. Iran nearly produces 80% of world's annual saffron production. On the other hand Iran has many arid and semi arid regions with low to moderate salinity. So it's important to investigate how saffron response to salinity. To study the effect of salinity stress on flowering stage of saffron (*Crocus sativus* L.), an experiment was performed in the greenhouse of NKANRRC (Northern Khorasan Agricultural and Natural Resource Research Center) in 2010. The experimental treatments included: salinity at six levels S_0=0, S_1=20, S_2=50, S_3=70, S_4=100 and S_5=120 mM sodium chloride. The results of first year showed that number of days to emergence was min in 50 mM NaCL. Increasing salinity levels decreased plant height, No. of days to flower. Fresh weight of flower increased with increasing salinity levels to 100 mM and the threshold salinity level for stigma fresh weight was 50mM. Results also showed that salinity imposed a significant effect on different growth parameters of saffron at high levels characteristics, but, it had a nutritional effect at 50 mM of NaCl. The best growth parameter and stigma yield was found with 50 mM of NaCl.**

Keywords: Saffron, salinity stress, flower yield and growth parameters.

INTRODUCTION

Water scarcity and soil salinity are two important factors limitations for agricultural production in arid and semi-arid regions like Iran. Salinity and water stress reduce root water uptake. In irrigated soils particularly in arid and semi-arid regions, plants are subjected to both salinity and water stress in different intensities. Renewable, high quality water resources in these areas are limited. The main water source for saffron irrigation is groundwater which suffers increasing salinity and, because of limited water resources, more effective use is emphasized. Therefore, saline water may be used for saffron production (Sepaskhah and Yarami, 2010). The effects of salinity and water stress on saffron yield have been reported by Sepaskhah and Yarami (2009) and Sepaskhah and Kamgar-Haghighi (2009). In these conditions, during an irrigation interval, evapotranspiration depletes the soil water content and consequently the matric potential and osmotic head of the soil solution are reduced and these factors reduce root water uptake. *Crocus sativus* L. corms were grown in perlite and watered by half-strength modified Hoagland nutrient solution containing 0, 50, 100, 150, 200 mM NaCl (Rajaei et al., 2009). Growth parameters and contents of proteins, proline, polyphenols, minerals and saccharides were studied in fibrous roots, contractile roots, corms and leaves. All plants remained alive and did not display any sign of foliar damage even at 200 mM NaCl. However, the salinity decreased growth, relative water content and increased contents of proline and Na^+ in all organs (Rajaei et al., 2009).

Another study was done to determine the interaction

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Table 1. Agricultural soil characteristics after improvement

parameter	Texture	Sand	Silt	Clay	pH	EC	SP	Lime	Organic matter	N	P	K
Unit	-		(%)		-	dS/m			(%)		ppm	ppm
amount	Sandy loam	56	32	12	7.55	5.22	36.06	36.25	3.63	0.186	110.30	1320

effects of salinity with 0.5, 1.7, 2.9, and 4.0 dS m^{-1} electrical conductivity and irrigation regime (2, 4, 6, and 8 day) on flower yield and growth of saffron in a pot experiment under a transparent shelter (Sepaskhah and Yarami, 2009). The results indicated that saffron flower and corm yields were the most and the least sensitive to soil water depletion, respectively. Furthermore, they concluded that corm and root growth were inhibited at field-capacity soil water content and that deficit irrigation was necessary for optimum growth. Dry weight reductions per unit increase in the salinity of the drainage water were 14.2, 10.8, 9.1, and 2.9% for saffron flowers, corms, roots, and leaves, respectively. Threshold values for the EC of the drainage water ranged between 0.08 and 0.67 dS m^{-1} for flowers and roots, respectively. Also, dry weight reductions per unit increase in salinity of the irrigation water for saffron flowers, corms, roots, and leaves were 28.3, 25.0, 21.1, and 7.0%, respectively. The threshold EC values of the irrigation water ranged between 0.13 and 0.48 dS m^{-1} for flowers and roots, respectively. When saline water was used to irrigate saffron, the irrigation interval needed to be more frequent (2-d intervals) to avoid severe water stress (Sepaskhah and Yarami, 2009).

In order to investigate the effect of salinity stress (0, 30, 60 and 90 mM NaCl) and amount of potassium (50, 100 and 150 % of Hoagland solution base) on root and shoot biomass accumulation of saffron, a greenhouse study was carried out in 2008-2009 (Sabet Teimouri et al., 2010). Results indicated that salinity imposed a significant effect on different growth characteristics; leaf dry weight, number of leaf, root dry weight, root volume, root density and physiological characteristics of saffron and potassium significantly controlled the negative effects of NaCl on length and number of roots as well as fresh weight and number of leaves per plant. Potassium and NaCl interactions were also significant. At different levels of NaCl adding K to the root medium caused an increasing in root numbers per plant. But this trend was observed up to 60 mM of NaCl and at 90 mM NaCl, potassium showed no modifying effects. Relative water content and electrolyte leakage were drastically decreased by increasing NaCl salinity. There was a reduction at 90 mM compared to the control respectively. The best growth parameter and root to shoot ratio was found with 30 mM of NaCl (Sabet Teimouri et al., 2010).

This study was conducted to evaluate flower yield and some growth parameters of saffron (*Crocus sativus* L.) in response to salt stress and we determined the effects of salinity stress on saffron tolerance threshold.

Also, there is little publicly available information about the trend of salinity tolerance threshold and flower yield of saffron with time. So, we intend to find it.

MATERIALS AND METHODS

In order to study the effect of salinity stress on saffron (*Crocus sativus* L.), an experiment as RCBD (Randomize Complete Block Design) in three replications was performed in the greenhouse at Northern Khorasan Agricultural and Natural Resource Research Center in 2010. The experimental treatments concluded: salinity at six levels S_0=0, S_1=20, S_2=50, S_3=70, S_4=100 and S_5=120 mM sodium chloride. The 18 pots, with 25 and 20 cm diameter and height respectively were prepared for cultivation of saffron corms. For filling pots of soil with the desired soil, sampling operation was carried out and then they were analyzed in soil and water laboratory of NKANRRC. To improve the desired soil texture, the ratio of 1:1:2 of soil, animal manure (cow) and sand were mixed (Amended soil characteristics for cultivation has been expressed in Table 1). The base of each pot was covered with wire netting for better drainage. All of the pots were filled with the amendment soil at the same amount (about 3 kg). Then all pots were irrigated. The soil pots were reached to the field capacity limit 48 hours later; two young saffron corms were planted in each pot. Finally, each pot was uniformly irrigated with water treatments (0, 20, 50, 70, 100 and 120 mM sodium chloride). Saline solution (Treatments) was made by laboratory NaCl. Pots were irrigated with net water requirement of saffron (Saffron maximum water requirement is in March and April of about 15 to 20 litters per m^2 per irrigation period) (Saffron Manual for Afghanistan, 2007). All agricultural operations such as weeds removal, irrigation with salt water, were done until the end of growth period (about 3 months). Date to germinate and seedling emergence in each pot was recorded. Pots were kept in controlled greenhouse conditions (maximum and minimum average greenhouse temperatures were 41.9 and 10.7 ° C and average relative humidity about 60%) until the end of growth period.

Some parameters of saffron were measured and recorded during experiment time. At the end of physio-

Table 2. Effect of different salinity levels on yield and some growth parameters of saffron

Treat.	No. of days to Germinate	Plant Height (cm)	The No. of Plants per Pot	No. of Tiller	Dry Weight of Flower (mg)	Dry Weight of Stigma (mg)
S_0	12.33 c	5.967 b	2.000 a	6.667 a	32.67 b	4.333 b
S_1	15.67 a	8.600 a	1.667 a	3.333 c	59.33 a	6.667 a
S_2	8.667 d	5.600 b	2.000 a	4.000 bc	60.00 a	7.667 a
S_3	13.67 b	5.867 b	2.000 a	4.667 b	31.00 b	3.333 b
S_4	16.33 a	5.833 b	2.000 a	3.333 c	60.33 a	7.667 a
S_5	16.33 a	6.067 b	1.667 a	3.000 c	25.67 c	4.333 b
P_{value}	0.0000	0.0000	-	0.0006	0.0000	0.0000

S_0=0, S_1=20, S_2=50, S_3=70, S_4=100 and S_5=120 mM sodium chloride
*Different letters in each column is indicated significant difference at 5% level.

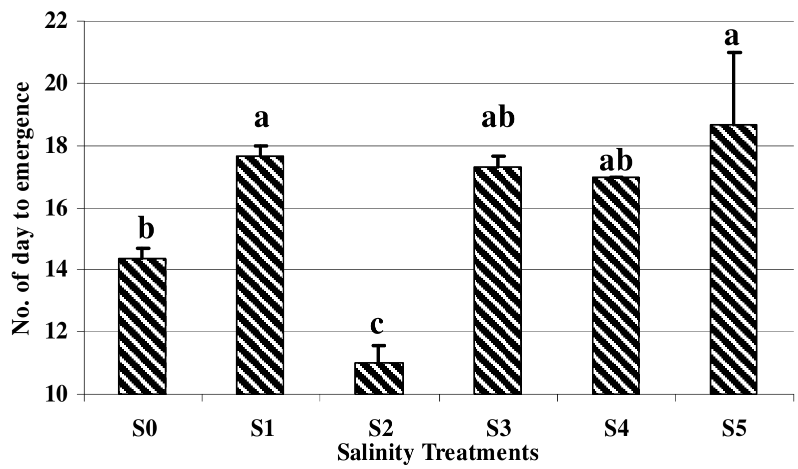

Figure 1. The Effect of different salinity levels on No. of days to emergence

logical growth stage (flowering stage) in the first year of the experiment, plants were harvested from the soil surface in each pot and fresh weight of flower and stigma were separately measured. Each of them was separately dried for 48 hours in the oven at 72°C and then their weight was recorded. The data were analyzed with statistical software (MSTAT-C) and mean data were compared by Duncan Multiple Test Range in 0.05 confidence level.

RESULTS

The results of experiment showed that maximum No. of days to germinate was observed with no significant difference in S_4, S_5 and S_1 with 32.4,32.4 and 27 percent with each other but significant increase compared to the control (Table 2). The two remained treatments (S_3 and S_2) had significant difference with each other and control. Compared of them with control (S_0) showed 10.9% increase and 29.7% decrease respectively (Table 2).

Minimum No. of days to emergence was showed in S_2 with 30.3% reduction compared to control (Figure 1). S_5 and S_1 had maximum No. of days to emergence with no significant difference with each other (30.3% and 23.3% increase) (Figure 1). Other treatments did not show any significant difference with each other and control (Fig. 1).

There was no significant difference between treatments or salinity levels in plant height parameter except of S_1 level. This treatment showed 44.1% increase compare to control (Table 2).

No. of days to flower decreased with increasing salinity levels. No. of days to flower was maximum in S_0 (control) and the other treatments had significant difference with it (Figure 2). Maximum reduction was observed in S_5 and S_4 with 48.9% and 47.6 % decrease respectively and no significant difference with each other (Figure 2). S_3, S_1 and S_2 levels showed 39.4%, 23.8% and 22.4% decreases compare to control respectively.

The No. of plants per pot at the end of growth time did not show any significant difference in all salinity levels (Table 2).

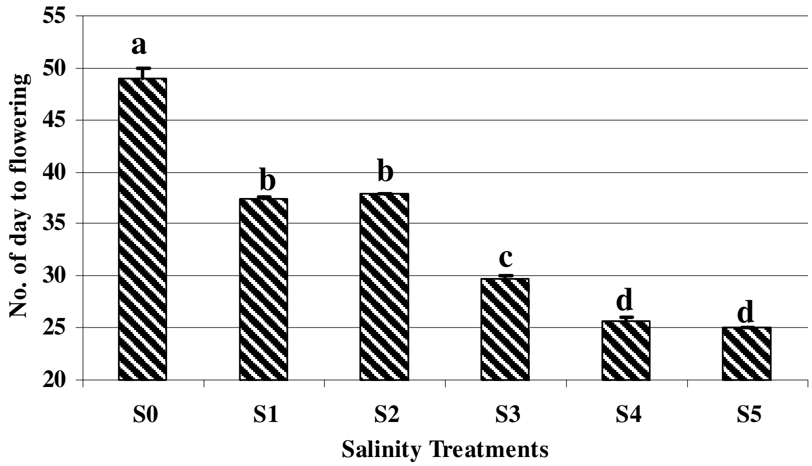

Figure 2. Effect of different salinity levels on No. of days to flower

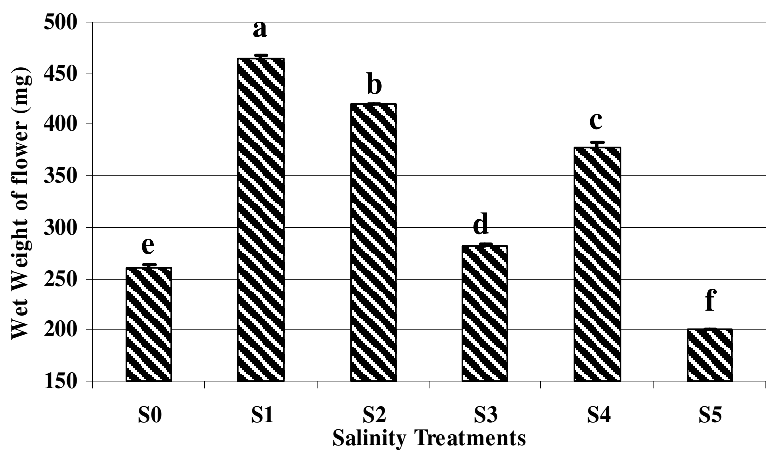

Figure 3. Effect of different salinity levels on fresh weight of flower

Maximum No. of tiller was observed in control (S_0) and the other treatments had significant reduction compare to it. Minimum decrease was observed in S_3 and S_2 with 29.9% and 40% respectively compared to control (Table 2). The other treatments did not show any significant difference with each other.

Fresh weight of flower (stigma +stamens +corolla) in all treatments had significant difference with each other and belonged to separate statistical groups (Figure 3). Maximum of it was observed in S_1, S_2, S_4 and S_3 with 78.6%, 61.2%, 45.4% and 8.6% increases compare to S_0 respectively (Figure 3). Only S_5 treatment had significant decrease (23.2 %) compared to control.

Maximum fresh weight of stigma (Figure 4) was observed in S_2, S_1 and S_4 with 127.4%, 109.6% and 76.7% significant increases than to control respectively (Figure 4). Two remained treatments (S_3 and S_5) had not any significant difference with each other and control (Figure 4).

Maximum dry weight of flower was observed in S_4, S_2 and S_1 with 84.6%, 83.6% and 81.6% increase compare to control respectively (Table 2). Maximum significant reduction was observed in S_5 (21.4 % decrease).

Maximum dry weight of stigma was also observed in S_2, S_4 and S_1 with 76.9%, 76.9% and 53.8% increase compare to control respectively and no significant difference. Two treatments (S_5 and S_3) did not show any significant difference with each other and control.

Correlation between salinity levels and fresh weight of flower (Figure 5) and fresh weight of stigma (Figure 6) showed that there was negative correlation. So, with rising salinity levels, fresh weight of flower and stigma decreased. Correlation between salinity levels with fresh weight of flower was lower than the other correlation and

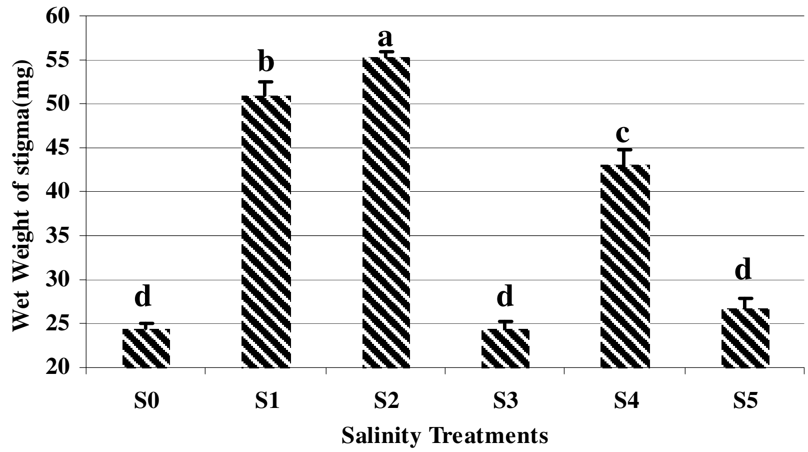

Figure 4. Effect of different salinity levels on fresh weight of stigma

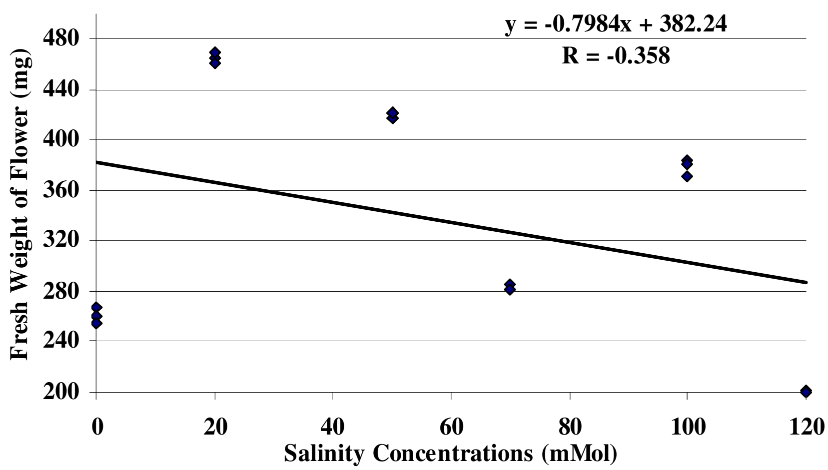

Figure 5. Correlation between salinity levels and fresh weight of flower (mg)

regression coefficient was about 0.358. Regression coefficient for fresh weight of stigma was about 0.514.

DISCUSSION

Saffron is a triploid plant, thus its attractive purple-violet flowers cannot produce any seed. The plant propagation is possible only via corms. Any improvement in crops quality and quantity must emerge from the corm quality. Even though resistance to certain undesirable environmental stress can be achieved by gene transfer, understanding the role of nutritional factors as well as studying their effect on the metabolic and enzymatic properties of the corm might lead to desired flowering quality as well as quantity in this post-genomic era (Keyhani et al., 2004).

High salinity levels (70, 100 and 120 mM) and the lowest salinity level (20 mM) caused a delay in corms germination. The 50 mM salinity level decreased the No. of days to germinate. Indeed, 50 mM salinity level had a nutritional effect on saffron germination. Chloride (Cl^-) is the one of essential nutrients for plant and its lack strongly prevented longitudinal growth of root (Khold Barin and Eslam Zade, 2005). Chloride is also responsible for the emergence of oxygen in the photosystem II (Salardini and Mojtahedi, 1988). Comparison between the need of chloride for growth and the amount of it can be provided by various resources,

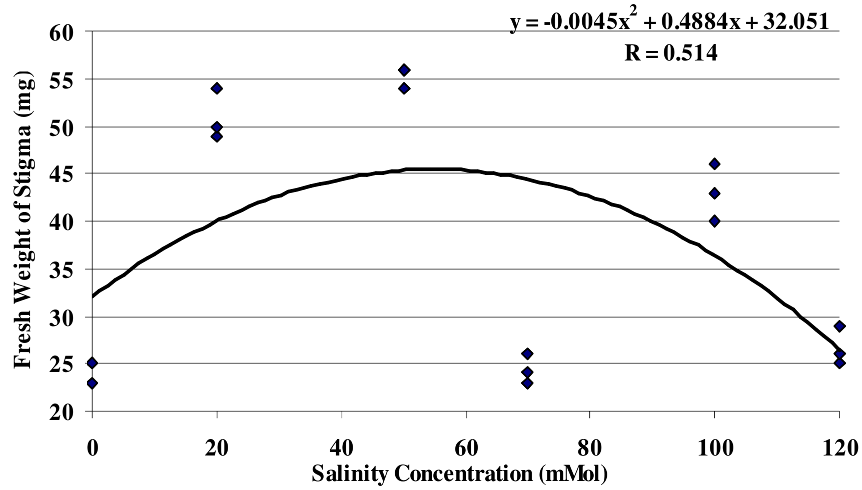

Figure 6. Correlation between salinity levels and fresh weight of stigma (mg)

revealing that under field condition, chloride deficiency rarely occurs. When high amount of chloride exist in root zone, it has non-specific effects on metabolism such as competition with nitrate in plant uptake, balance of cations – anions regulation and metabolisms of organic acids (Khold Barin and Eslam Zade, 2005). Chloride is the one of the active osmotic materials in cell vacuoles and when external osmotic pressure solutions increases than osmotic pressure of plant cells, a disorder osmotic regulation by plant cells accrues and high levels of sodium and chloride have a direct toxic effects on membrane and enzymatic systems (Kafi and Mahdavi Damghani, 2000).

The trend for No. of days to emergence was similar to date to germinate. So, the average salinity level (such as 50 mM) decreased No. of days to emergence. Salt exists in water as ions. Up to a certain amount of the ions can be helpful to plants. During absorption and transpiration of water, plants obtain some of the ions they need to survive and grow. Chloride helps plants to metabolize and sodium is involved in the regeneration of phospho enol pyruvate in CAM and C_4 plants such as saffron. It can also substitute for potassium in some circumstances (Norman and Hopkins, 2009).

With salinity increase plant height decreased except of 20 mM` salinity level. In this treatment plant height was significantly higher than the other treatments and control. Soil salinity reduced plant height and stem diameter of safflower but it accelerated flowering and maturity (Zeinali, 1999). Our results also showed that with increasing salinity the No. of days to flower decreased (Fig. 2). Accelerate flowering was a mechanism of escape from stress condition or adaptation to salinity stress in the most plant such as saffron. Osmotic problem in plants also occurs under drought stress conditions and salinity stress is called a type of physiological drought since 100 years ago (Kafi and Mahdavi Damghani, 2000). The genetically, drought resistance mechanisms can be divided into three categories, which include drought escape, drought avoidance and drought tolerance. However, crops are used usually more than one mechanism for drought resistance. Drought avoidance is the ability of a plant to complete its life cycle before the water deficit in soil and plants. This mechanism involves the fast phenological development (early flowering and earliness), growth flexibility (variation depending on the severity of water deficit during the growing season) and transition pre-flowering photosynthesis to grain products (Iran Nejad and Shahbaziyan, 2005).Maybe, drought avoidance was a mechanism that saffron escaped from salt stress condition in this study. Because, with increasing salinity levels, No of days to flower or time to flowering decreased.

No. of tiller was decreased with increasing salinity levels (Table 2). This result was similar to some researches such as Sabet Teimouri et al. (2010) and Rajaei et al. (2009).

Fresh weight of flower (stigma +stamens +corolla) increased with increasing soil salinity up to certain amount (100 mM) compare to control (Figure 3). In this study, the most important parameter or stigma yield also was increased with increasing in soil salinity up to 50 mM salt concentration than to control (Figure 4). The best growth parameter or stigma yield was found with 50 mM of NaCl. Stigma yield was more sensitive to salinity stress than flower yield. Saffron is very resistant to salinity. Researchers found that bulb production decreased with increasing salinity. When electrical conductivity increased from 1200 to 7000 µs/cm, bulb production reduced from 100 to 35 percent (Regional Saffron Cultivation and Harvesting Techniques in Spain, Greece and Italy, 2011).

Effects of different salinity levels (0, 50, 100, 150 and 200 mM (NaCl)) on some physiological and biochemical parameters in different saffron organs (fibrous root, contractile root, corm and leaf) have been studied (Rajaei et al., 2008). Growth, fresh and dry mass in all organs decrease under salinity and contractile root was the most affected organ. Relative water content decreased significantly when salinity increased more than 100 mM. Flowering and size of flowers severely decreased as salinity increased more than 100 mM (Rajaei et al., 2008).Contractile root and root were more sensitive and corm and leaf were more tolerant organ to salinity.

Our results showed a relatively weak correlation between salinity, flower and stigma saffron yield about 35 and 50 percent respectively and their equation followed from liner and second degree equation respectively (Figure 5 and 6). This was probably due to data were less and the experiment was done in greenhouse and pot condition. Results of other researchers indicated that salinity imposed a significant effect on different growth characteristics, leaf dry weight, number of leaf, root dry weight, root volume, root density and physiological characteristics of saffron (Sabet Teimouri et al., 2010). Mosaferi (2001) indicated that there was a good correlation between irrigation intervals or drought stress and yield of saffron in his research. By reducing irrigation interval, yield was increased for different five age groups farms (1, 2, 3, 4, and 5 years old) and also for the average of all age groups. Higher yield obtained with lower irrigation interval has also been with confirmed elsewhere (Mosaferi, 2001).

There was a good correlation between irrigation interval and yield of saffron in the evaluation of irrigation management of saffron at agro ecosystem scale in dry region of Iran (Behdani et al., 2008). Higher yield obtained with lower irrigation interval. Irrigation with 12 days, interval is the most frequent in Torbat-haydarieh and this could be reason for higher yield in this county, while in Gonabad a 12 day irrigation interval is not practices and in the other counties, the irrigation interval are more less the same.

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