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ORIGINAL ARTICLE

Olive Fruit Quality in Response to Interaction Effects of Irrigation Regimes and Saline Water Levels in Semi-Arid Regions

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ABSTRACT
Olive is considered as a moderately salt-tolerant plant and is cultivated widely in Iran. However, olive trees are new to the pattern of horticulture on fields that are irrigated with saline water. The objective of this study was to investigate the influence of interaction of saline water, SW (S_1, S_2, and S_3) and irrigation regimes (I_1, I_2, I_3, I_4 and I_5) on olive fruit quality parameters in 2013 and 2014. The experiment was considered as randomized complete blocks design with three replications. The highest and the lowest total phenolic content, TPC were observed in I_1S_3 and I_1S_2 respectively in 2013 and in I_5S_1 and I_4S_3 in 2014. Results revealed that similar trend as TPC were found for TFC in I_4S_1 and I_2S_1 in 2013 and in I_4S_1 and I_2S_3 in 2014. The highest (8.73 %) and the lowest (4.79 %) values of RRSA were obtained in I_5S_3 and I_3S_2 in 2013 and in I_3S_1 and I_3S_2 respectively in 2014. It is concluded that the salinity stress increased the olive quality parameters, while water stress decreased phenol and flavonoid content but boosted the antioxidant properties.
Keywords: *Antioxidant, Flavonoid, Olives, Phenol, Salinity stress*

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INTRODUCTION

Olive (*Olea europaea* L.) is an evergreen tree and is one of the oldest plants in the Mediterranean region and in the Middle East. Olive is considered as a moderately salt-tolerant plant [1, 2], albeit with some differences between cultivars [3], and is cultivated widely in Iran. However, olive trees are new to the pattern of horticulture on fields that are irrigated with saline water. So far, there have been no experiments on the evapotranspiration and water requirements of the olive under such conditions, regardless of the geographical location anywhere in the world. Through the past decades, the issue of drought has been the biggest problem in arid and semi-arid areas [4]. The Iranian midlands and also the south of Iran are classified as arid areas. The drought has reached the level of a moderate to severe crisis, especially in the Fars province where this study was conducted in Marv-Dasht city.

Several approaches have been suggested to alleviate the drought. The modification of crop patterns and the breeding of drought-resistant varieties of crops and trees are just two measures that need to be further taken against drought [5] and deficit irrigation [6, 7, 8, 9, 10, 11]. Deficit irrigation reduces olive product yield such as seed, seed oil [9, 8], seed protein content, oil yield [11], leaf osmotic adjustment [12, 13] and leaf-fruit water potential [13]. Modified patterns of cultivating different crops have been a focus in the Fars province of Iran. For example, planting olives and pistachios instead of wheat and alfalfa is deemed a wise policy. More than hundreds of thousands of hectares are cultivated by the olive with 35 different cultivars and the *Olea europaea* is the most common species. For the past three years, the groundwater quality has declined in the present study region and the water quantity has decreased likewise. Therefore, olive trees that are irrigated with saline water will encounter root-zone salinity [14] which can affect the quantity and quality of fruits. Phenols, flavonoids and fatty acids (saturated and unsaturated) are important parameters of quality in olive fruits that change as the salinity and drought stress change with the time.

Several methods have been suggested to reduce the effect of water salinity [15, 4] by causing the salts to leach from the soil during the growing season [16] or cultivating saline-resistant varieties of the plant [17, 18]. Generally, fruit trees are susceptible to salinity; therefore, wide ranges of research results have been published on trees response to saline stress [19, 14, 20]. High salinity did not affect oil content but increased the moisture content and decreased weight of olive fruits [15]. Salinity reduces the viability of pollen, and lowers the mean number of flowers and consequently the fruit set [21] but does not affect flower size and fruit dropping [22]. The threshold of saline toxicity for the olive is reported to be 1.8 dS m^{-1} which is enough to have adverse effects on olive health and yield. Salinity levels beyond 5.6 dS m^{-1} can reduce olive growth and yield by 50 percent [23]. Other reports on irrigation and water quality guidelines for olives indicate that the threshold of saline toxicity is 3-5 dS m^{-1} and that salinity levels above 5.5 dS m^{-1} cause severe problems in growth and yield [4]. Salt tolerance in olive cultivars depend on the effective mechanism of Na^+ and Cl^- uptake by the root [24] and also on the exchange of K-Na at the plasma lemma level [25]. The uptake of salt is regulated by specific proteins crossing the plasma membrane. Cation change is responsible for Na^+ uptake [26] and anion changes are responsible for Cl^- uptake into root cells [27].

Olive growth is limited by moderate to high concentrations of salts [28]. Generally, the total dry weight is less susceptible to salt stress than is the leaf area. In fact, the earliest response to salt stress is the reduction in leaf growth [4]. In spite of this, if olives are irrigated by moderately saline water (electrical conductivity of 4.2 dS m^{-1}) in the long-term, the vegetative growth would still not be affected significantly, in comparison to the control with electrical conductivity of 1.2 dS m^{-1} [29]. High salinity conditions cause increases in the osmotic potential, which lead to reductions in the uptake of water by the olive root. The leaf water potential and relative water content (RWC) also decrease as a result of high salinity. Low RWC can be a sign of high salt concentration in the irrigation water. This can cause osmotic stress and dehydration at the cellular level. Leaf dehydration can explain the drop in leaf water potential when high concentrations of salts prevail, and yet the leaf will have the ability to recover upon relief of stress [4].

Numerous studies have evaluated the interaction between salinity and water stress and their combined effect on crop production [30, 31, 32, 33, 34] and on olive yield [35]. The co-occurrence of saline water and high evaporation in arid areas cause reductions in soil quality [19]. Deficit irrigation by saline water causes reductions in the matric and osmotic potential of water in the soil. These factors reduce the uptake of water by the root [36]. The water content of the soil and there upon the osmotic and matric potentials decrease as a result of evapotranspiration during the intervals between the two consecutive irrigation.

Knowledge is scanty with regard to the interaction between salinity and water stress on the olive and their combined effect on the yield and oil quality of the fruit. Even in the few studies that have been conducted time to time, reports are confined to discussing reductions in the olive fruit yield as a result of high salinity levels. Phenolic compounds exist in almost all higher plants. Depending on the specific environmental condition, for example salinity [37], plants exhibit certain interactions and show increases or decreases in production [14]. Nonetheless, many studies in the past have dealt with the occurrence of phenols and antioxidants in the plant at times of various stresses such as low temperatures, UV irradiation, high light intensity and saline conditions [14]. Accordingly, relevant studies have also been conducted on the olive tree. Olives irrigated with saline water (7.5 dS m^{-1}) have exhibited reductions in the oil (74 to 89 percent) and fresh-fruit yield (68 to 83 percent) in comparison to the control [38]. Water stress [39, 40] and the occurrence of high salinity levels in the irrigation water can increase the total phenol concentration [14] and the total content of saturated fatty acids. However, water stress is known to decrease the unsaturated/saturated fatty acids ratio and the oleic / linoleic acid ratio ([15, 29] regarding olive oil production. Water stress can increase antioxidant activity in olive fruits [40] and in the late vegetative stage of *Origanum majorana* L. [41].

However, the current situations in Iran and in similar arid countries have placed the vegetation in confrontations with water stress and saline water stress. Population growth and distribution, inefficient agriculture management, and mismanagement and thirst for development are three major causes the looking water crisis in the region under study and farmers have no other option than to use well water (WW) to irrigate their crops. Most of wells water are brackish or salty in the study area. Deficit irrigation (lower crop evapotranspiration, ETC) and use of saline water in agriculture is appropriate alternative for increasing water use efficiency. Therefore, the aim of this research was to investigate the phenol, flavonoid and antioxidant profiles of the "Roghani" olive cultivar under the shortage of water supplies and also in the presence of well saline water and well fresh water. These two are examined concurrently under natural conditions where the water is withdrawn from both saline and fresh water wells. The experimental site was located in central part of Marv-Dasht city and was performed on olive seven year's orchard trees.

MATERIALS AND METHODS

Field experiment design and treatments

The experiment was performed from January to October 2013 and 2014 in an olive orchard of the "Roghani" cultivar. The experimental site was located in central part of Marv-Dasht city approximately 15-Km southeast of the ancient Persepolis stronghold. The trees were seven years old from the time of grafting. The spacing between the trees was 5.5 × 5.5 m, adding up to 330 trees ha^{-1}. The soil texture was sandy (Table 1).

Table 1. Soil physical properties (%) in the experiment site

Depth (cm)	Texture	ρ_b ($g.cm^{-3}$)	Clay	Silt	Sand	FC	PWP
0-30	sand	1.42	4.28	10.00	85.72	18.00	8.50
30-70	sand	1.47	5.28	8.00	86.72	16.50	7.00
70-140	sand	1.53	7.28	6.00	86.72	14.00	5.50

The modified method of FAO-Penman-Monteith [42] was used to calculate the reference crop evapotranspiration (ET_o) in $mm.day^{-1}$ by using data obtained from a Persepolis weather station. The evapotranspiration of olive (ET_c) in $mm.day^{-1}$ was estimated by Equation 1.

$$ET_c = (1000(S_r \times S_p) \times P_s) ET_o \times K_c \times P_w \quad \text{Eq. (1)}$$

Where, S_r is row space and S_p is plant space (m), P_s is the percentage of shading level, P_w is the percent wetted area and K_c is modified crop coefficient of the olive [43]. The experimental design was factorial and trees were irrigated in a complete randomized blocks by combination with three replicates per treatments. The experiment was carried out according to a randomized design of three saline irrigation water consisted of S_1 (the saline well water; WW), S_2 (combination of half saline well water; WW and half low saline well (fresh) water; FW) and S_3 (low saline well (fresh) water; FW) were utilized as saline water treatments. Irrigation level treatments consisted of five irrigation levels: I_1 (0.25 ET_c), I_2 (0.5 ET_c), I_3 (0.75 ET_c), I_4 (1.0 ET_c) and I_5 (1.25 ET_c). The Experiment area was 1500 m^2, divided into three blocks selected among the olive trees in the garden. Each block consisted of fifteen trees, (5.5 × 5.5 m). Both of blocks had been separated with a three meter border. Each block (17 × 40 m) consisted of three rows and consisting of all the combinations of irrigation and salinity levels treatments (Figure 1). Before the experiment began, all of the trees were irrigated with the mild saline well water (electrical conductivity (EC) about 2-2.5 dS m^{-1}) by a micro irrigation system with an inlet pressure of one atmosphere. The irrigation was scheduled for every day and the ET_c was set to fulfill the plant's water requirement. Irrigation water was applied to each tree by a lateral loop pattern arrangement of 8 emitters, each with a rate of 4 liters per hour at a distance of 0.8 meter from the tree trunk.

The amount of the irrigation water determined by FAO-Penman-Monteith was controlled by considering the number of emitters, the duration of irrigation, and amount of leaching requirement (LR). Also, three electro pumps and three timers were installed to control the operating pressure of drippers and monitor the irrigation duration. The irrigation water was also measured by a volumetric measuring device. According to the water requirement and based on the I_4 (1.0 ET_c) irrigation level, the trees were irrigated every day and usually at night. The I_4 irrigation level was considered as the control.

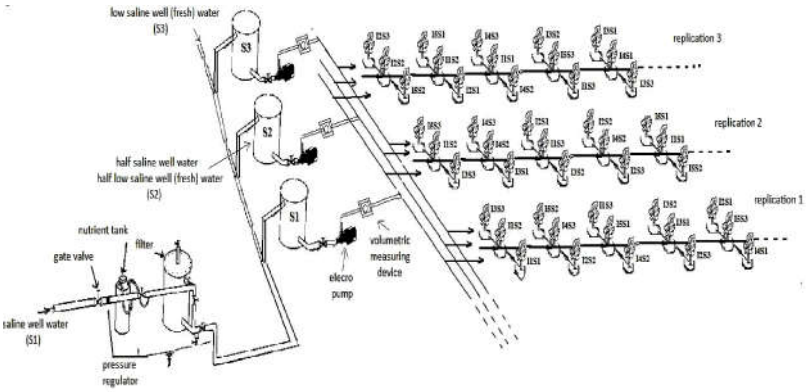

Figure 1. Experimental field layout

Irrigation water quality

Olives' response to salinity depends on the soil salinity from previous years (Hoffman et al., 1990). Drastic weather changes, winter rains and large changes in evaporation can also be determinant. Here we use water from well (S_1 with EC = 2.2-7.7 dS m^{-1} changes with time), low saline well (fresh) water (S_3 with EC = 0.4-0.85 dS m^{-1}) and a combination of the S_1 and S_3 (half-half). All three levels of saline water were applied under natural conditions. The high air temperature of summer causes the water consumption to increase during January to September in 2013 and 2014. The incessant exploitation of water from wells (where wells are the only source of water) the quality of water tends to decrease (EC of irrigation water increases). In other words, the electrical conductivity (EC) of the groundwater changes, proportional to the amount of withdrawal. But the EC variation ranges of S_3 was low (0.4-0.85 dS m^{-1}). The ECs of S_1, S_2 and S_3 were checked on a monthly basis during the experiment, so as to monitor the performance from January to October of 2013 and 2014 (Figure 2).

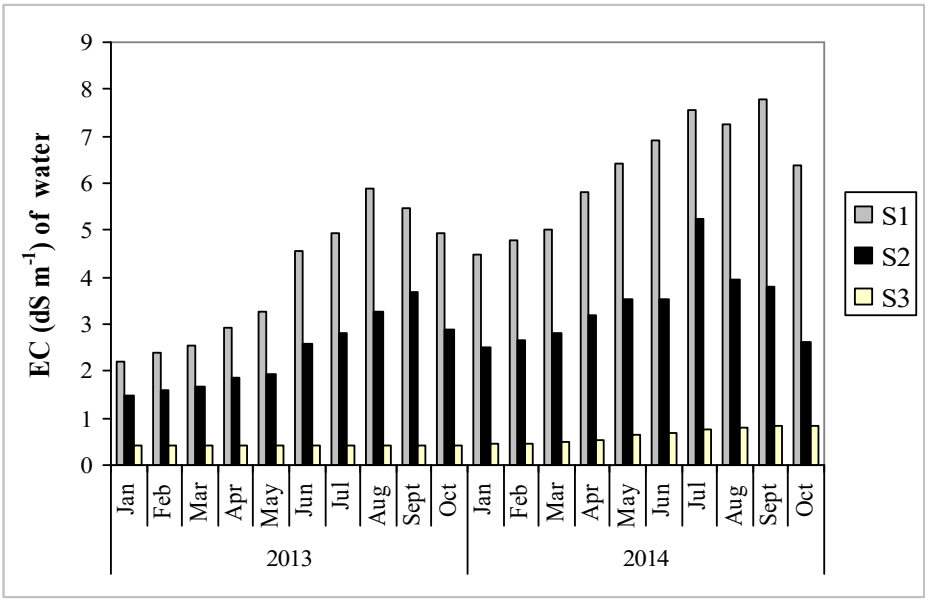

Figure 2. Changes inEC (dS m^{-1}) of three salinity levels of irrigation water (S_1, S_2 and S_3)during two growing periods 2013 - 2014.

Soil salinity was also monitored during the growing season in different depths of the root zone. Soil salinity intensifies over time since saline water adds to the soil's salt concentration. Nevertheless, a mean value of salinity is defined for the soil in the growing season [44]. However, the response of perennial and evergreen trees to salinity over time is very complex and growth and yield can be affected by soil salinity from previous years. Fortunately, before 2013, the EC of S_1 (saline well water) in the studied region had been favourably low (between 2-3 dS m^{-1}) which had not been no limit on olive growth and yield [14].

Olive fruits sample

Olive fruits were harvested at mid-November in 2013 and 2014, when70 percent of the olive fruits had acquired dark purplish colors. The stone in the fruits was separated from the flesh which was later lyophilized, and was stored at low temperatures until chemical extractions were due.

Chemical materials

"2,2-diphenyl-1-picrylhydrazyl" (DPPH) and Ciocalteu-Folin reagent were prepared from Sigma-Aldrich Company (St. Louis, MO, USA). All other chemicals were provided from Merck Company (Darmstadt, Germany).

Phenolic compounds extraction

The extraction of phenolic compounds (Phenolic extraction) was accomplished according to Klen & Vodopivec [45] method with some modifications. The lyophilized olive flesh was grinded in an electric grinder, resulting in olive powder, and 5 gr of each homogenized powder was extracted three times with 50 ml methanol (purity 99.5). In each step, the prepared sample was shaken for 15 min and was then filtered through the filter paper (Watman No. 1). The sample volume was 50 ml with a concentration of 100 mg ml^{-1}. The supernatant was poured into dark containers in a refrigerator (4 °C) until further analysis.

Total phenolic content

Total phenolic content (TPC) was determined by Ciocalteu-Folin reagent. The values are not absolute measurements of the amounts of phenolic compounds but are based on their chemical reducing capacity,

corresponding to an equivalent reducing capacity of Gallic acid [46]. TPC was determined using the method of Lu et al. (2011) [47]. In brief, Ciocalteu-Folin reagent was diluted 10-fold with deionized water and then 0.1 ml of the prepared methanolic extract (100 mg ml^{-1}) was mixed with 0.75 ml of the diluted Ciocalteu-Folin reagent. The solution was stored in darkness after being kept at laboratory temperature (20 ºC) for 10 min. Then, 0.75 ml of 2 percent sodium carbonate (w/v) solution was added. The mixture remained in darkness at 20 ºC for an hour; its absorbance was measured at 765 nm via a UV–Visible spectrophotometer (Unico, China). TPC values were derived from a calibration curve which was prepared by a set of Gallic acid standards (10, 50, 100, 250 and 500 µg ml^{-1}).

Total flavonoid content

Total flavonoid content (TFC) was determined by a method described by Chen, Chung, Chiang, and Lin (2011) [48]. Therefore, 0.5 ml of the methanolic extract (100 mg ml^{-1}) was mixed with 0.1 ml of $AlCl_3$ (10 percent), 0.1 ml of potassium acetate (1 mol) (CH_3CO_2K), and 2.8 ml distilled water. After being kept in darkness for 80 min at laboratory temperature, the absorbance was measured at 415 nm. Quercetin (10, 50, 100, 250 and 500 µg ml^{-1}) dissolved in methanol was employed to establish a calibration curve.

Relative radical scavenging activity

To measure the relative radical scavenging activity (RRSA) of olive fruit, the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPHº) was used. The method of RRSA measuring was described by Sousa, Ferreira, Barros, Bento, and Pereira [49] and is applied here with some modifications (used Ascorbic Acid instead of TBHQ). Antioxidant molecules quench DPPHº and turn into a colorless/bleached DPPH. Exactly 0.1 ml of methanolic extract, having three different concentrations (10, 1 and 0.1 mg ml^{-1}) depending on RRSA intensity was mixed with 1.9 ml methanolic DPPHº solution (0.25 mmol) in triplicates [50]. After an hour of remaining in darkness, the absorbance of methanolic solution was recorded at 517 nm. DPPHº scavenging activity is shown by the IC_{50} (mg ml^{-1}) value, defined as the concentration of the antioxidant required for the 50 percent loss in the DPPHº activity. Equation 2 [50] calculated the percentage of inhibition of DPPHº by the methanolic olive extract.

$$Inhibition(\%) = \frac{AC - AS}{AC} \times 100 \quad \text{Eq.} \quad (2)$$

Where AC is the absorbance of the control sample and AS is the absorbance of the methanolic extract after one hour. To calculate IC_{50} (methanolic extract concentration providing 50 percent inhibition), a graph was illustrated to plot the percentage of the remaining DPPHº against the methanolic olive extract. The Ascorbic acid solution (2000, 1000, 500, 100, 50 and 10 µg ml^{-1}) was used as standard and the IC_{50} of this solution was measured. Furthermore, analyses were directed at calculating the relative radical antioxidant activity (RRAA) of the Ascorbic acid solution, corresponding to the methanolic extracts. The percentage of RRAA (IC_{50} of Ascorbic acid / IC_{50} of sample) was also calculated.

Statistical analysis

Two ways ANOVA was applied to distinguish between the interaction effects of the five irrigation levels and the three salinity levels, with respect to the measured values. Duncan's multiple range tests and a comparison between the mean values were employed to determine significant differences, considering a significant level of 5 percent ($P \leq 0.05$). All statistical analyses were performed by SAS (Version 9.3 for Windows).

RESULTS AND DISCUSSION

(Table 2) shows the combined analysis results to investigate the significant effect between years (2013 and 2014), salinity and deficit irrigation treatments on TPC, TFC and IC_{50}. There was an intense significant difference of the effect of salinity and deficit irrigation on TPC and IC_{50} in 2013 compare to 2014. Therefore, the analysis of data on TPC and IC_{50} were done separately for 2013 and 2014. As well as, there was not significantly difference between the effect of salinity and deficit irrigation on TFC in two years. The variations of TPC, TFC, IC_{50} and RRSA in olive fruits are depicted as they vary due to the interactions between irrigation water levels and salinity levels. Results are shown in (Table 3) for 2 consecutive years.

Table 2. Combined analysis between years (2013-2014), salinity and deficit irrigation treatments on TPC, TFC and IC_{50}

	TPC	TFC	IC_{50}
Yr*	0.0017	0.4323	0.0163
Rep.**(Yr)	<0.0001	0.7432	0.4198
Salt	<0.0001	<0.0001	0.5464
Def. Irrig.***.	<0.0001	1	0.6462
Salt × Def.Irri	<0.0001	0.5521	0.7311
Yr × Salt	<0.0001	0.7093	0.7247
Yr × Def. Irri	<0.0001	0.933	0.8528
Yr × Salt × Def. Irri	<0.0001	0.9277	0.9392

Note: *Year, **Replication, ***Deficit Irrigation

Table 3. Two-way ANOVA analysis of mean values for TPC, TFC, IC_{50} and RRSA of olive samples in two consecutive years 2013 and 2014

Phenolic Compounds	Year	Irrigation Levels	Salinity levels			
			S_1	S_2	S_3	Means
TPC*	2013	I_1	$153.50^{j} \pm 3.57$	$129.60^{k} \pm 3.09$	$473.61^{a} \pm 9.97$	252.26^{d}
		I_2	$153.60^{j} \pm 3.57$	$185.60^{h} \pm 4.21$	$375.60^{c} \pm 8.01$	238.28^{e}
		I_3	$275.60^{e} \pm 6.01$	$319.60^{d} \pm 6.89$	$187.60^{h} \pm 4.25$	260.94^{c}
		I_4	$433.60^{b} \pm 9.17$	$237.60^{f} \pm 5.25$	$171.60^{i} \pm 4.25$	280.94^{b}
		I_5	$465.61^{a} \pm 9.8$	$187.60^{h} \pm 4.25$	$217.60^{g} \pm 4.85$	290.28^{a}
		Means	142.99^{a}	99.76^{b}	64.02^{c}	
	2014	I_1	$97.95^{f} \pm 0.99$	$85.83^{h} \pm 0.86$	$94.92^{g} \pm 0.96$	92.90^{d}
		I_2	$97.95^{f} \pm 0.99$	$111.80^{e} \pm 1.12$	$60.59^{l} \pm 0.61$	89.87^{e}
		I_3	$121.18^{d} \pm 1.22$	$156.52^{c} \pm 1.58$	$48.47^{m} \pm 0.49$	108.72^{b}
		I_4	$171.67^{b} \pm 1.73$	$80.78^{j} \pm 0.8$	$33.32^{n} \pm 0.33$	95.26^{c}
		I_5	$226.20^{a} \pm 2.28$	$64.63^{k} \pm 0.65$	$82.80^{i} \pm 0.83$	124.54^{a}
		Means	296.4^{a}	212.01^{c}	285.21^{b}	
TFC**	2013	I_1	$14.77^{ef} \pm 1.82$	$19.22^{bcd} \pm 2.36$	$18.67^{bcde} \pm 2.28$	17.55^{b}
		I_2	$10.19^{g} \pm 1.27$	$18.24^{bcde} \pm 2.23$	$16.17^{def} \pm 1.98$	14.87^{e}
		I_3	$17.45^{cde} \pm 2.14$	$16.29^{cdef} \pm 2.00$	$13.36^{gf} \pm 1.65$	15.7^{d}
		I_4	$23.49^{a} \pm 2.87$	$20.30^{abc} \pm 2.48$	$21.66^{ab} \pm 2.64$	21.82^{a}
		I_5	$13.12^{gf} \pm 1.62$	$19.59^{bcd} \pm 2.39$	$16.11^{def} \pm 1.98$	16.27^{c}
		Means	15.80^{c}	18.73^{a}	17.195^{b}	
	2014	I_1	$57.38^{ab} \pm 6.32$	$52.26^{b} \pm 5.76$	$32.60^{e} \pm 3.59$	47.41^{b}
		I_2	$51.35^{bc} \pm 5.66$	$49.63^{bcd} \pm 5.47$	$31.68^{e} \pm 3.49$	44.22^{d}
		I_3	$63.22^{a} \pm 6.97$	$37.20^{e} \pm 4.10$	$32.28^{e} \pm 3.56$	44.23^{d}
		I_4	$65.07^{a} \pm 7.17$	$41.54^{cde} \pm 4.58$	$50.47^{bcd} \pm 5.56$	52.36^{a}
		I_5	$48.62^{bcd} \pm 5.36$	$40.65^{de} \pm 4.48$	$47.50^{bcd} \pm 5.23$	45.60^{c}
		Means	57.12^{a}	44.26^{a}	38.91^{b}	
IC_{50} (μg ml^{-1})	2013	I_1	$1.02^{c} \pm 0.01$	$1.05^{b} \pm 0.01$	$0.75^{h} \pm 0.01$	0.94^{a}
		I_2	$1.02^{c} \pm 0.01$	$0.69^{i} \pm 0.01$	$0.75^{h} \pm 0.01$	0.81^{d}
		I_3	$0.66^{j} \pm 0.01$	$1.16^{a} \pm 0.01$	$0.85^{f} \pm 0.01$	0.89^{b}
		I_4	$0.89^{e} \pm 0.01$	$0.95^{d} \pm 0.01$	$0.74^{h} \pm 0.01$	0.85^{c}
		I_5	$0.83^{g} \pm 0.01$	$0.83^{g} \pm 0.01$	$0.64^{k} \pm 0.01$	0.76^{e}
		Means	0.88^{b}	0.93^{a}	0.74^{c}	
	2014	I_1	$0.73^{b} \pm 0.01$	$0.69^{d} \pm 0.01$	$0.44^{k} \pm 0.01$	0.62^{a}
		I_2	$0.71^{c} \pm 0.01$	$0.33^{m} \pm 0.01$	$0.32^{m} \pm 0.00$	0.45^{d}
		I_3	$0.30^{n} \pm 0.00$	$0.82^{a} \pm 0.01$	$0.56^{g} \pm 0.01$	0.56^{c}
		I_4	$0.66^{e} \pm 0.01$	$0.62^{f} \pm 0.00$	$0.54^{h} \pm 0.01$	0.61^{b}
		I_5	$0.46^{j} \pm 0.01$	$0.50^{i} \pm 0.01$	$0.38^{l} \pm 0.01$	0.45^{e}
		Means	0.57^{b}	0.59^{a}	0.45^{c}	
RRSA (%)	2013	I_1	$5.47^{j} \pm 0.05$	$5.30^{k} \pm 0.05$	$7.39^{e} \pm 0.07$	6.05^{d}
		I_2	$5.47^{j} \pm 0.05$	$8.07^{c} \pm 0.08$	$7.39^{e} \pm 0.07$	6.98^{b}
		I_3	$8.35^{b} \pm 0.08$	$4.79^{l} \pm 0.04$	$6.45^{g} \pm 0.06$	5.64^{c}
		I_4	$6.26^{h} \pm 0.06$	$5.85^{i} \pm 0.05$	$7.56^{d} \pm 0.08$	6.55^{c}
		I_5	$6.69^{f} \pm 0.07$	$6.69^{f} \pm 0.07$	$8.73^{a} \pm 0.08$	7.37^{a}
		Means	6.45^{b}	6.14^{c}	7.52^{a}	
	2014	I_1	$7.84^{n} \pm 0.08$	$8.37^{l} \pm 0.08$	$13.04^{e} \pm 0.13$	9.75^{d}
		I_2	$8.13^{m} \pm 0.08$	$17.57^{c} \pm 0.17$	$17.94^{b} \pm 0.18$	14.49^{a}
		I_3	$19.10^{a} \pm 0.19$	$7.02^{o} \pm 0.07$	$10.26^{i} \pm 0.10$	12.12^{c}
		I_4	$8.67^{k} \pm 0.09$	$9.30^{j} \pm 0.09$	$10.64^{h} \pm 0.11$	9.54^{e}
		I_5	$12.60^{f} \pm 0.13$	$11.49^{g} \pm 0.11$	$15.11^{d} \pm 0.15$	13.07^{b}
		Means	11.27^{b}	10.75^{c}	13.40^{a}	

Note: *μg of gallic acid equivalents g^{-1} of sample; **μg of quercetin equivalents g^{-1} of sample

Mean values ± SD (n = 3)which do not have letters in common are significantly different at$P \leq 0.05$ by Duncan's multiple range tests

Phenol changes in olive fruits

The effects of interactions between water and salinity stress on total phenol concentration (TPC; μg ml^{-1}) of olive fruits ("Roghani" cultivar) are shown to be significantly different ($P \leq 0.05$) in (Table 3) ($R^2 = 0.99$) in two consecutive years. In 2013, the highest and the lowest mean values of TPC were observed in the S_1 and the S_3 salinity levels respectively with significant differences across all irrigation levels ($R^2 = 0.99$). Regardless across all salinity levels, the highest TPC was in I_5 ($R^2 = 0.99$) but decreased by reducing the irrigation level. In 2014, the highest TPC were observed in the S_1 salinity level, while the lowest TPC was seen in the S_2 salinity level ($R^2 = 0.99$). Regardless across all salinity levels the variation of TPC values between irrigation levels were similar to sinusoidal pattern. The highest TPC was observed at I_1 in combination with the S_3 in 2013, but were at I_5 in combination with the S_1 in both sequential years 2013 and 2014. The lowest TPC was observed in the I_1 in combination with the S_2 salinity level and also in the I_1 and I_2 irrigation levels in combination with the S_1 salinity level in 2013, although they were at I_4, I_3 and I_2 in combination with the S_3 salinity level in 2014. Data analysis indicated that each irrigation level resulted in a significantly different outcome. There were no significant differences between the I_1 and I_2 irrigation levels when combined with the S_1 salinity level in two successive years. Under low salinity level (fresh water; S_3) condition, the highest TPC decreased significantly as the irrigation level increased up to reach I_4 but then the TPC managed to recover in I_5 for two consecutive years. The TPC increased significantly at S_1 salinity level as irrigation levels increased in 2013 and 2014. The TPC significantly decreased before and after of I_3 irrigation level applied at S_2 salinity level in 2013 and 2014.

Flavonoid changes in olive fruits

Significant differences ($P \leq 0.05$, $R^2 = 0.99$) in total flavonoid concentrations (TFC, μg ml^{-1}) of olive fruits ("Roghani" cultivar) are shown in (Table 3) in two successive years 2013 and 2014. Across all irrigation levels, the lowest and the highest mean values of TFC were in S_1 and S_2 salinity levels respectively in 2013, with significant differences ($R^2 = 0.99$) in contrast, the highest TFC were occurred in S_1 salinity level and the lowest was in S_3 salinity levels in 2014. Across all salinity levels, the highest TFC was observed in the I_4 irrigation level in 2013 and 2014. Diverging from the I_4 level caused the TFC to decrease significantly, but differences was not significant in I_3 and I_2 in 2014, although the I_2 caused the lowest mean TFC. Generally, in 2013, the highest and the lowest TFC were observed in the I_4 and I_2 irrigation levels respectively combined with the S_1 salinity level. In 2014, the highest TFC were observed in the I_4 combined with the S_1 salinity level and the lowest TFC were occurred in the I_2 combined with S_3 salinity level, although there was not seen significant differences of TFC in the I_1, I_2 and I_3 combined with S_3 salinity level. Results of all irrigation levels at each salinity level treatments depicted a sinusoidal pattern in two consecutive years 2013 and 2014. The I_4 irrigation level caused the highest TFC through all three levels of salinity in 2013 and 2014.

Changes in the relative radical scavenging activity (RRSA) in olive fruits

Total RRSA [%] of olive fruits ("Roghani" cultivar) are expressed as the concentration of antioxidant which is needed to cause 50 percent loss in DPPHº activity (IC_{50}, μg ml^{-1}). This is evaluated with a standard deviation and analyzed for significant differences ($P \leq 0.05$; $R^2 = 0.99$) in 2013 and 2014. Lower IC_{50} and higher RRSA values represent higher antioxidant activity. Across all irrigation levels, the lowest and the highest RRSA were in salinity levels of S_3 and S_2 respectively with significant differences among the two values ($R^2 = 0.99$). In two successive years 2013 and 2014, across all salinity levels, the highest and the lowest RRSA were in I_5 and I_1 respectively ($R^2 = 0.99$) in 2013 and in I_2 and I_4 respectively ($R^2 = 0.99$) in 2014. Evaluation of the interactions effects between salinity and irrigation levels treatments indicated that the lowest RRSA was observed in the I_3 irrigation level combined with the S_2 salinity level in 2013 and 2014. Low RRSA was also observed in the I_1 and I_2 irrigation levels combined with the S_1 salinity level and in the I_1 irrigation level with the S_2 salinity level for two successive years. The highest RRSA was observed in the interaction of I_5 with S_3 and then in the I_3 with the S_1 salinity level in 2013. In the other hand in 2014, the highest RRSA was observed in the interaction of I_3 irrigation level with the S_1 and then in the I_2 with the S_3 salinity level. Each salinity level resulted in a significantly different outcome when combined with each irrigation level. More detail of above aforementioned are summarize in (Table 4). The highest and lowest TPC, TFC and RRSA at different salinity levels and different percentages of water are provided in (Table 4) in two consecutive years. It is shown that the highest and lowest values of these parameters were obtained in I_3 at invariant salinity levels in two successive years.

Table 4. The lowest and the highest TPC, TFC and RRSA obtained through irrigation levels by different salinity levels

Irrigation level	Salinity level											
	TPC*				TFC**				RRSA (%)			
	The lowest		The highest		The lowest		The highest		The lowest		The highest	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
I_1	S_2	S_2	S_3	S_1	S_1	S_3	S_2	S_1	S_2	S_1	S_3	S_3
I_2	S_1	S_3	S_3	S_2	S_1	S_3	S_2	S_1	S_1	S_1	S_2	S_3
I_3	S_3	S_3	S_2	S_2	S_3	S_3	S_1	S_1	S_2	S_2	S_1	S_1
I_4	S_3	S_3	S_1	S_1	S_2	S_2	S_1	S_1	S_2	S_1	S_3	S_3
I_5	S_2	S_2	S_1	S_1	S_1	S_2	S_2	S_1	S_1 or S_2	S_2	S_3	S_3
Means	S_3	S_2	S_1	S_1	S_1	S_3	S_2	S_1 or S_2	S_2	S_2	S_3	S_3

Note: *µg of Gallic acid equivalents g^{-1} of sample; ** µg of quercetin equivalents g^{-1} of sample

In our study in spite of the sinusoidal pattern in the TFC changes, the highest TFC was observed in the I_4 irrigation level in two consecutive years 2013 and 2014. In 2013, the lowest and the highest TPC were occurred in the interaction of I_1 with the S_2 and in the I_5 and I_4 with the S_1 salinity levels, respectively. The lowest and the highest RRSA were in the I_3 combined with the S_2 and in the I_5 combined with the S_3 salinity level, respectively. In 2014, the lowest and the highest TPC were occurred in the interaction of I_3 with the S_3 and in the I_4 with the S_1 salinity levels, respectively. The lowest and the highest RRSA were in the I_3 combined with the S_2 and in the I_3 combined with the S_1 salinity level, respectively. The highest TFC were in the I_4 when combined with the S_1 salinity levels. Olive fruits have important antioxidant properties that undergo changes due to the regional climate, maturity of fruits, cultivar and especially the quantity and quality of irrigation water [51].

CONCLUSIONS

Changes in the quality and quantity of irrigation water will have different effects on the olive fruit production quality parameters. As our findings, increase in salinity caused a significant decrement in antioxidant properties and significant build-up in the flavonoid and phenol contents in olive flesh fruit. These changes process were sinusoidal trend for antioxidant and flavonoid but was linear for phenol. According to the highest level of water salinity (S_1) and regarding the average values of olives quality parameters in two consecutive years, results revealed that the optimum performances were observed in I_1 for antioxidant (about 22% less than average), flavonoids in I_4 (about 35% more than average) and phenol in I_5 (about 57 percent more than the average). It is concluded that increasing irrigation water significantly improves the performance of phenol (15.7% in I_5) and flavonoid (19% in I_4) and reduce of antioxidant performance (18 percent in I_4). The linear trend obtained for average values of phenol but the variation of these changes were sinusoidal trend for antioxidant and flavonoid. Under severe water stress conditions (such as I_1 and I_2), the optimal performance of phenol was 56 percent more than the average values obtained from all salinity levels (at S_3 in 2013 and S_2 in 2014) and flavonoids 22% more than the average value (at S_2 in 2013 and S_1 in 2014) and antioxidants with 25 percent less than the average values in S_2 during the two successive years. Regarding the olive varieties, amount of irrigation water should be applied as different percentages of ETc. In general with respect to different water salinity and irrigation water levels for olives production in an arid and semi-arid region, to obtain the highest phenol, grower can apply the I_4 or I_5 irrigation levels combined with S_1 salinity level. It is also recommended to apply the I_3 irrigation level combined with S_1 salinity level in order to get the highest antioxidant. The highest flavonoid content is achieved by the application of the I_4 irrigation level combined with S_1.

COMPETING INTERESTS

The authors have declared that no competing interest exists.

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