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The Effect of Salinity and Irrigation Regimes on the Level of Fatty Acids in Olive Flesh Oil

Ali Dindarlou[1], Ali A. Ghaemi[1], Mohammad T. Golmakani[2] & Fatemeh Razzaghi[1]

[1] Water Engineering Department, College of Agriculture, Shiraz University, Shiraz, Iran

[2] Department of Food Science, College of Agriculture, Shiraz University, Shiraz, Iran

Correspondence: Ali A. Ghaemi, Water Engineering Department, College of Agriculture, Shiraz University, Shiraz, Iran. Tel: 98-91-7111-5795. E-mail: ghaemi@shirazu.ac.ir/ghaemiali@yahoo.com

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Abstract

Olive trees have the capability of growing under semi-arid regions, where drought and salinity are the major concerns. Two years field experiments were carried out to investigate the interaction effects of natural saline well water and irrigation levels on the quantity and quality of fatty acids in the olive flesh fruits (“Roghani” cultivar). A factorial layout within a randomized complete blocks design with three replications of five irrigation levels (I_1 to I_5 as 0.25, 0.5, 0.75, 1 and 1.25 ET_c) and three saline water levels (S_1 to S_3 as 100%WW, 50%WW+50%FW and 100%FW) were considered. The fresh and brackish irrigation water were withdrawn from two different natural wells (fresh water (FW) and saline water wells (WW)). *Results revealed that* ***increasing salinity and decreasing irrigation water levels caused significant increment in the ratio of unsaturated fatty acids, palmitic acid to the percentage of oil and oil percentage in olive flesh fruit***. It is found that as water salinity increased from lowest to the highest level, the oleic acid trends to its highest value of 23.68% in I_1S_1. Mean values of palmitic acid in 2013 were 27.52% and decreased to 19% in 2014. *It is concluded that highest percentage of oleic, linoleic, linolenic and palmitic acids obtained under high saline and less applied irrigation treatment (S_1I_2) yielding to improve the olive oil quality.*

Keywords: fatty acids, interaction, olive, palmitic, salinity, water stress

1. Introduction

Limited research is available on olive oil quality under saline water irrigation, especially in areas with poor quality and quantity of groundwater resources. Generally, because of the shortage in rain and the growing demand for groundwater in most parts of Iran, the practical concern over drought is escalating and is even more prominent in southern areas of the country, such as the Fars province. In this region, groundwater salinity has become a major problem due to the high rates of water exploitation from wells. Local farmers have sought to plant cultivars that are tolerant to salinity, such as olive and pistachio. Disappointingly, however, there is no scientific method in practice for the selection of suitable plant varieties. More research is needed find out for different plant varieties the maxima and minima limits of irrigation using different quantities and qualities of groundwater. Little research has so far studied the interaction between salinity of irrigation water and deficit irrigation on tree fruits, even though numerous studies have evaluated the olive yield in general (Melgar et al., 2012) and also their combined effects on other crop productions (Amer, 2010; Shabani, Sepaskhah, & Kamkar-Haghighi, 2013; Azizian & Sepaskhah, 2014). Salinity and water stress both cause reductions in the osmotic and matric potential of water in the soil and reduce the uptake of water by the root. Therefore, these factors decrease as a result of evapotranspiration during the intervals between the two consecutive irrigations (Kramer & Boyer. 1995). Soil salinity intensified over time since the saline water added to the soil’s salt concentration. However, a mean value of salinity is defined for the soil in the growing season (Hoffman, Rhodes, Letey, & Sheng, 1990). Growing conditions including crop variety and the sensitivity of crops at different growth stages, climatic and soil factors, agronomic and irrigation management are important factors that affect the plant’s actual response to salinity (Chartzoulakis, 2005). Nevertheless, the response of perennial and evergreen trees to salinity over time is very complex. Plant growth and yield can be affected by the salinity that has been accumulated in the soil from previous years to the present.

The domestic consumption of olive oil is very low in comparison to other edible oils (Xiong, Matthews, &

Sumner, 2014). Nonetheless, it has more medicinal and antioxidant properties than the other oils. The global olive oil production has been 3.1 million tons (MT) in 2012 and has improved by 20 percent from 2000 to 2012 (Munoz & Moya, 2015). By adding up the harvests in 2013 and 2014, Spain, Italy and Greece produced 1.5, 0.5 and 0.23 MT respectively, as the three biggest producers (International Olive Council [IOC], 2014). The average consumption of olive oil compared to other vegetable oils is 2.25% (Dhifi, Hamrouni, Ayachi, Chahed, Saidani, & Marzouk, 2004), while consumption of palm (30%), canola (15%) and sunflower (9%) oils are very high (Rosillo-Calle, 2009). Because of medicinal and health-related concerns, the demand for olive oil is increasing (Luaces, Perez, & Sanz, 2003). Therefore, in this context, improving the quality of olive oil remains to be important (Mendez & Flaqué, 2007). Olive oil quality depends on its components (or fatty acids) such as oleic, linoleic and palmitic acids that comprise the highest chemical fraction of the olive oil.

Fatty acids quality influences by environmental criteria (soil and climate), irrigation and fertilization. The harvesting time and the method used, thereof, is also of significant importance. Fruit ripeness and the method whereby oil extraction can be enacted are similarly influential on oil quality (Aparicio & Iuna, 2002). Among the aforementioned factors, irrigation is very important. Fatty acids, polyphenols and the bitterness of olive oil, all depend on the adequate and suitable supply of water. Research results indicate that there can be a strong relation between the adaptations of the issue deficit irrigation to dry conditions (Tognetti, Morales-Sillero, d'Andria, Fernandez, Lavini, Sebastiani, & Troncoso, 2008). Water deficit treatments reduce crop growth but do not affect olive fruit yield (Iniesta, Testi, Orgaz, & Villalobos, 2009). Salinity tolerance in olive trees is associated to salt excretion mechanisms operating in the root (Melgar et al., 2012). High salinity reduces olive yield (Ben-Ahmed, Ben Rouina, Sensoy, & Boukhriss, 2009), the fruit size (Ben-Gal, 2011) and also pollen viability but does not reduce the fruit oil content (Ben-Ahmed et al., 2009), despite the fact that there can be concurrent increments in the fruit's percentage of dry weight, oil and oil per fruit (Ben-Gal, 2011). Furthermore, salinity can increase the linoleic–linolenic acid ratio, while at the same time it can reduce the oleic–linolenic acid ratio (Cresti, Ciampolini, Tattini, & Cimato, 1994) and increase the total phenol concentration in olive oil (Ben-Ahmed et al., 2009). By increasing salt concentration in the irrigation water, Palmitic acid (Zarrouk, Marzouk, Ben, & Cherif, 1996) and Oleic acid slightly increased and linoleic acid decreased in the total fatty acid composition in Egypt (El Agaimy, Neff, Elsayed, & Awatif, 1994). Salinity stress can influence the phenolic component in olive roots and leaves. More specifically, the phenol and oleuropein were observed to increase in the leaves, but the hydroxytyrosol concentration remained unchanged under saline condition (Petridis, Ioannis, Georgios, & Chrisoula, 2012). Applying a high irrigation frequency by micro irrigation could maintain high humidity in the soil and thus avoid the build-up of a harmful salt concentration around the root zone. The objectives of this study are to investigate the interactions effects of saline water (natural well water) and deficit irrigation, on fatty acids components of the olive oil ("Roghani" cultivar), the percentage of olive oil and the ratio of fatty acids to olive flesh oil.

2. Materials and Methods

2.1 Area Descriptions

The study area was located in the central part of Marv-Dasht city (near the ancient Persepolis tourist sites), where groundwater resources have been heavily exploited by farmers causing severe shortages in agricultural water resources. This research was conducted on 7-year-old olives of the "Roghani" cultivar in an orchard, with the spacing of 5.5×5.5 m between the trees, and an average population density of 330 trees ha^{-1}. The duration of the experiment was spanned from January to October 2013 and 2014. The physical parameters of the sandy soil texture are available in Table 1.

2.2 Methods and Techniques

The experimental design was factorial in complete randomized completed blocks consisting of three replications per treatment (irrigation and saline water levels) to irrigate the olive trees. Each replication consisted of three rows and fifteen trees in five columns which comprised of all the combinations of salinity and irrigation levels of treatments (Figure 1). The irrigation treatments were 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). There were three different levels of salinity water which consisted of S_1 (the saline water from the well: WW), S_2 (a combination of half-saline well-water [WW] and half-low saline well water, otherwise known as fresh water [FW]) and S_3 (low-saline, fresh well-water [FW]). In this research, water from wells (S_1 with EC = 2.2-7.7 dS m^{-1} varies with time), low-saline fresh well-water (S_3 with EC = 0.4-0.85 dS m^{-1}) and a half-half combination of the S_1 and S_3 were used under natural conditions. Because of the high ambient temperature, especially in summer, the water consumption by plants is expected to increase from January to September each year.

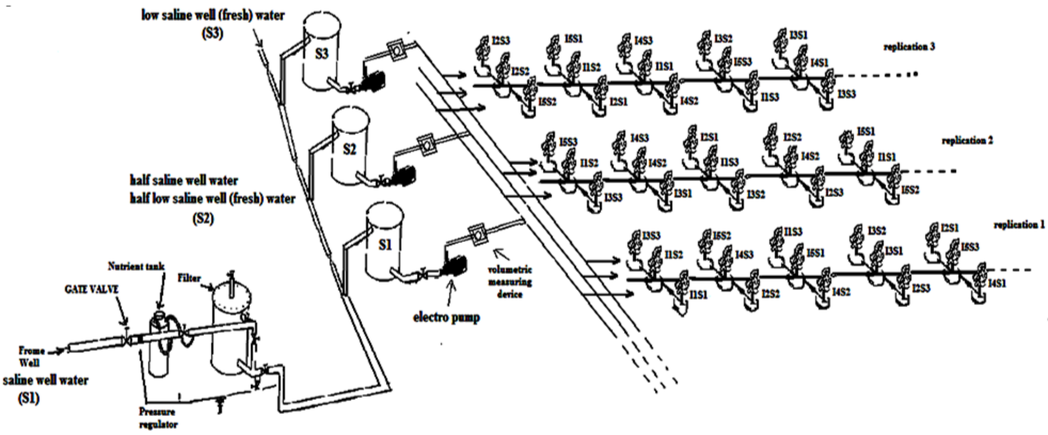

Figure 1. Experimental field layout with three replications

Table 1. Physical properties of the sandy soil texture in the site of experiment

Depth (cm)	Texture	ρ_b (g.cm^3)	Clay (%)	Silt (%)	Sand (%)	FC (%)	PWP (%)
0-30	sand	1.42	4.28	10	85.72	18	8.5
30-70	sand	1.47	5.28	8	86.72	16.5	7
70-140	sand	1.53	7.28	6	86.72	14	5.5

A modified adaptation of the FAO-Penman-Monteith equation (Razzaghi & Sepaskhah, 2012) was applied to calculate the reference crop evapotranspiration (ET_o) in mm day^{-1}. The evapotranspiration of olive (ET_c) in mm day^{-1} was estimated by equation 1 as follows.

$$ET_c = (1000(S_r \times S_p) \times P_s)\ ET_o \times K_c \times P_w \quad (1)$$

Where S_r and S_p are the row and plant spacing (m), P_s is the level of shading, P_w is the percentage of wetted soil area and K_c is the modified crop coefficient of the olive (Fereres, Villalobos, Orgaz, & Testi, 2011). Irrigation water requirement (IWR) was considered as ET_c plus the leaching fraction (10% amount of ET_c)

Before the experiment began, all of the trees were irrigated with the mild-saline well-water (the electrical conductivity [EC] of which measured 2-2.5 dS m^{-1}) using a micro irrigation system with an inlet pressure of one atmosphere. The irrigation was scheduled to perform daily and was set to fulfill the irrigation water requirement. Irrigation water was applied to each tree by a lateral loop pattern arrangement of 8 emitters (4 L hr^{-1}), at a distance of 0.8 meter from the tree trunk. Olive trees were irrigated every day and usually at night. Furthermore, to control the operating pressure of drippers and to monitor the irrigation duration, three electro pumps and three timers were installed. The amount of irrigation water was also measured by a volumetric measuring device. The I_4 irrigation level was considered as the control.

Farmers usually use water wells to irrigate their plants in the study area. In this study, the two natural wells one with high and the other with low salinity which are 150 meters apart were the main source of irrigation water (S_1 with EC = 2.2 - 7.7 dS m^{-1} varies with time), low-saline fresh well-water (S_3 with EC = 0.4 - 0.85 dS m^{-1}) and a half-half combination of the S_1 and S_3. Because of the high ambient temperature, especially in summer, the water consumption by plants is expected to increase from January to September each year.

Proportional to the changes in the exploitation of groundwater, the electrical conductivity (EC) of irrigation water in wells (S_1) was increased gradually. However, the EC variation in 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, as shown in Figure 2 in 2013 and 2014.

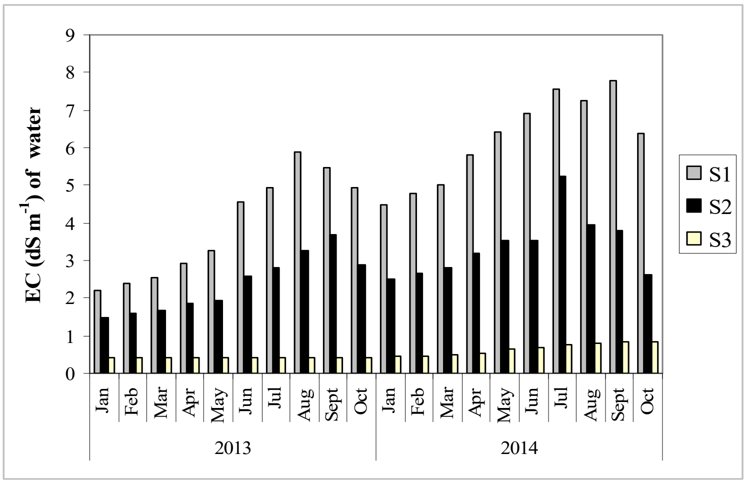

Figure 2. Changes in the EC (dS m^{-1}) of the three types of saline waters used for irrigation (S_1, S_2 and S_3) during two growing periods 2013-2014

Soil salinity was monitored during the growing season of different depths of the root zone. Before 2013, the EC of S_1 (the saline well-water) had been favorably low in the studied region (between 2-3 dS m^{-1}) which had no significant impact on olivsecutive growth and yield (Chartzoulakis, 2005). Based on the hot conditions and semi-arid climate of the studied region, olive fruits were harvested in end-November (29 and 30), 2013 and 2014 (Dag, Harlev, Lavee, Zipori, & Kerem, 2013), when 70 percent of the olive fruits had acquired a dark purplish color. After removing the seeds of fruits, the weight was measured and the fruits were dehumidified by lyophilizing, whereupon their weight was measured again. The olive flesh was stored at low temperatures (< 4 °C) until chemical extractions were due. Methyl tridecanoate was utilized to measure the percentage of olive oil by gas chromatography (GC) instead of the soxhlet method. To evaluate the interaction effect of salinity and irrigation levels on fatty acids of the olive fruit, the fatty acids components were measured by a GC device. A method by Golmakani, Rezaei, Mazidi, & Razavi (2012) was applied to prepare the extracts for GC analysis.

The extraction of methanolic was accomplished according to the Golmakani et al. (2012) method. Accordingly, the lyophilized olive flesh was grinded in an electric grinder, resulting in olive powder. Then 0.5 gram of each homogenized powder was mixed into one ml of methyl tridecanoate solution (25 mg) and 9 ml methyl acetyl chloride. The prepared sample was transferred to an oven (80 °C) and was treated there for one hour. After cooling, 10 ml of distilled water was added and the prepared sample was shaken for 2-3 min. After adding 1 ml of ultrapure N-hexane (containing 0.01% TBHQ antioxidant) and shaking again for a few seconds, the prepared samples were centrifuged (4000 rpm) for 10 min. Then, the supernatant was removed and was poured into dark containers to be placed in a refrigerator (4 °C) until further analysis. The fatty acids content was determinedin the prepared samples using GC device. Finally, GC data was incorporated into the Chromatography data handling system software, and the percentage of fatty acids was determined. The two-way ANOVA analysis was applied to investigate the effects of the three salinity levels and the five irrigation levels and also their interactions with each other. The Duncan's multiple range tests and a comparison between the mean values were employed to determine the significant differences between the measured parameters ($P \leq 0.05$). All statistical analyses were performed by SAS (Version 9.3 for Windows).

3. Results

Table 2 shows the combined statistical analysis (Duncan test, $p \leq 0.05$) that indicate the significant impacts of salinity and irrigation treatments on palmitic, stearic, oleic, linoleic and linolenic acids of the olive fruit flesh and their interactions in the two consecutive years 2013 and 2014. According to table 2, amount of palmitic acid and stearic acid had significant differences in 2013 and 2014 (according to $P \leq 0.05$ level of significance, the statistical analysis was more than 5 percent). Table 3 shows an increment of 8.52% in the average rate of palmitic acid in 2013 (27.52%) compared to 2014 (19%). Also, there was a 4.79% reduction in stearic acid in 2013 (1.8%) compared to 2014 (6.59%). There were no significant differences at $P \leq 0.05$ level among the average values of the rest of fatty acids (oleic, linoleic and linolenic acid) in 2013 and 2014 (Table 2, 3). The variations of palmitic, stearic, oleic, linoleic and linolenic acids in olive fruits show similarity trends as they vary due to the interaction effects of irrigations and salinity levels. Table 4 shows two-way ANOVA analysis of salinity and irrigation levels in two successive years, 2013 and 2014.

Table 2. Combined analysis (Duncan test, p≤ 0.05) between the years 2013 - 2014, salinity and irrigation treatments on fatty acids (palmitic, stearic, oleic, linoleic and linolenic)

	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Yr*	0.001	0.019	0.6555	0.7281	0.8432
Rep**(Yr)	<0.0001	0.003	0.0003	0.0003	0.0008
Salt	<0.0001	<0.0001	0.1632	0.6080	<0.0001
Irrig Ter ***.	<0.0001	<0.0001	0.6831	0.2143	<0.0001
Salt× Irrig Ter	<0.0001	<0.0001	0.0233	0.0034	<0.0001
Yr×Salt	<0.0001	<0.0001	0.0281	0.3926	<0.0001
Yr×Def.Irri	<0.0001	<0.0001	0.5756	0.7310	<0.0001
Yr×Salt×Def.Irri	<0.0001	<0.0001	0.0313	0.0368	<0.0001

Note. *:Year, **:Replication, ***: Irrigation treatments

Table 3. Average fatty acids (percentage) in 2013 and 2014. Numbers followed by the same letters (column and row) do not differ significantly (Duncan test, p ≤ 0.05)

	Fatty acids (%)				
Year	Palmitic	Stearic	Oleic	Linoleic	Linolenic
2013	27.52 a	1.80 b	46.07 a	24.02 a	1.40 a
2014	19.00 b	6.59 a	49.85 a	22.60 a	1.35 a
Salinity level					
S_1	23.70 a	2.66 c	49.27 a	23.16 a	1.39 b
S_2	23.10 b	5.25 a	47.05 a	23.12 b	16.64 a
S_3	23.00 b	4.66 b	47.54 a	23.65 a	1.11 c

Table 4. The two-way ANOVA analysis of salinity and irrigation levels and the mean values (μg ml^{-1}) for palmitic, stearic, oleic, linoleic and linolenic acids of olive fruit in the two consecutive years 2013 and 2014. Numbers followed by the same letters (column and row) do not differ significantly (Duncan test, p < 0.05)

Fatty acid	Year	Irrigation levels	Salinity levels						mean
			S_1		S_2		S_3		
				S.D		S.D		S.D	
Palmitic*(μg ml^{-1})	2013	I_1	28.70 c	0.29	27.13 gh	0.28	28.03 def	0.29	27.95 d
		I_2	28.27 cde	0.29	28.61 c	0.29	28.39 cd	0.20	28.42 b
		I_3	28.25 cde	0.29	26.95 h	0.27	30.35 a	0.31	28.51 a
		I_4	29.69 b	0.30	25.76 i	0.26	27.3 gh	0.28	27.59 e
		I_5	27.56 fg	0.28	27.86 ef	0.28	29.26 b	0.30	28.22 c
		Mean	28.49 b		27.26 c		28.67 a		
	2014	I_1	18.74 e	0.19	18.66 e	0.19	17.82 g	0.18	18.4 e
		I_2	18.22 f	0.18	19.55 b	0.20	20.00 a	0.21	19.26 b
		I_3	18.69 e	0.19	17.99 fg	0.19	18.69 e	0.19	18.45 d
		I_4	19.17 cd	0.19	18.99 de	0.20	19.31 bcd	0.20	19.16 c
		I_5	19.66 b	0.20	19.46 bc	0.20	20.18 a	0.20	19.76 a
		Mean	18.89 c		18.93 b		19.2 a		
Stearic* (μg ml^{-1})	2013	I_1	0.89 i	0.01	0.80 g	0.01	1.14 e	0.02	0.94 e
		I_2	1.13 e	0.02	1.11 f	0.01	1.31 c	0.01	1.18 b
		I_3	1.01 g	0.01	0.95 h	0.01	1.02 g	0.01	0.99 d
		I_4	1.29 d	0.02	1.39 b	0.02	0.50 i	0.01	1.06 c
		I_5	0.70 k	0.01	1.87 a	0.02	1.15 e	0.01	1.24 a
		Mean	1.00 c		1.22 a		1.02 b		
	2014	I_1	1.39 hi	0.02	1.37 hj	0.02	1.37 ij	0.02	1.38 e
		I_2	1.28 k	0.01	1.48 f	0.02	1.67 b	0.02	1.48 c
		I_3	1.35 j	0.02	1.41 gh	0.01	1.53 e	0.02	1.43 d

		I_4	1.42 f	0.02	1.63 d	0.02	1.64 cd	0.02	1.56 b
		I_5	1.77 a	0.02	1.50 e	0.02	1.66 bc	0.02	1.65 a
		mean	1.44 c		1.48 b		1.57 a		
Oleic* (μg ml^{-1})	2013	I_1	46.74 a	5.15	46.92 a	5.17	47.08 a	5.19	46.91 a
		I_2	45.35 a	5.00	43.62 a	4.81	42.12 a	4.65	43.46 c
		I_3	46.64 a	5.15	46.76 a	5.16	45.49 a	5.02	46.3 b
		I_4	42.79 a	4.72	48.62 a	5.36	49.38 a	5.45	46.93 a
		I_5	49.41 a	5.45	49.41 a	5.45	40.69 a	4.49	46.5 b
		mean	46.18 b		47.06 a		44.92 c		
	2014	I_1	4.97 de	0.55	16.06 a	1.77	3.59 ef	0.40	8.2 a
		I_2	4.25 def	0.47	4.09 ef	0.45	9.56 c	1.06	5.97 c
		I_3	3.27 f	0.36	3.35 f	0.37	11.96 b	1.32	6.19 c
		I_4	5.71 d	0.63	11.98 b	1.32	2.74 f	0.30	6.81 b
		I_5	3.38 f	0.38	10.91 bc	1.20	2.97 f	0.33	5.75 c
		mean	4.31 c		9.28 a		6.16 b		
Linoleic* (μg ml^{-1})	2013	I_1	22.86 bc	2.52	24.21 bc	2.67	23.09 bc	2.55	23.39 c
		I_2	26.13 ab	2.89	26.07 ab	2.87	26.97ab	2.74	26.39 a
		I_3	23.50 bc	2.59	24.15 bc	2.66	22.73 bc	2.51	23.45 c
		I_4	24.87 abc	2.74	23.00 bc	2.54	23.66 bc	2.61	23.84 b
		I_5	21.79 bc	2.41	19.80 c	2.18	29.63 a	3.27	23.74 b
		mean	23.83 b		23.44 c		25.21 a		
	2014	I_1	53.29 ab	5.88	40.51 d	4.47	52.73 abc	5.82	48.84 c
		I_2	51.83 abc	5.72	50.85 abcd	5.61	46.53 abcd	5.13	49.74 b
		I_3	52.26 abc	5.76	56.57 a	6.24	46.64 abcd	5.14	51.82 a
		I_4	51.94 abc	5.73	42.12 cd	4.65	53.72 ab	5.93	49.26 bc
		I_5	52.52 abc	5.79	45.16 bcd	4.98	51.03 abc	5.63	49.57 b
		mean	52.37 a		47.04 c		50.13 b		
Linolenic* (μg ml^{-1})	2013	I_1	1.71 cde	0.19	1.84 bcd	0.21	1.57 def	0.17	1.71 a
		I_2	0.02 g	0.00	1.50 ef	0.17	2.29 a	0.15	1.27 c
		I_3	1.52 def	0.17	2.1 ab	0.23	1.33 f	0.15	1.65 a
		I_4	2.27 a	0.25	2.14 ab	0.24	0.03 g	0.00	1.49 b
		I_5	1.45 ef	0.16	1.99 abc	0.22	0.17 g	0.02	1.2 c
		mean	1.39 b		1.91 a		1.78 c		
	2014	I_1	21.08 a	2.33	22.91 a	2.53	24.08 a	2.65	22.69 b
		I_2	23.91 a	2.64	23.54 a	2.60	21.76 a	2.40	23.07 a
		I_3	23.97 a	2.64	20.30 a	2.24	20.87 a	2.30	21.71 c
		I_4	21.36 a	2.36	24.79 a	2.74	22.24 a	2.46	22.8 b
		I_5	22.17 a	2.45	22.44 a	2.48	23.65 a	2.61	22.75 b
		mean	22.5 b		22.8 a		22.52 b		

Note.* 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

3.1 Fatty Acids

The interactions effects of irrigation levels and salinity stress on fatty acids (%) of olive fruits indicated significant differences ($P \leq 0.05$, $R^2=0.99$) in the two consecutive years (Table 4). Across all irrigation levels, the significant highest and lowest mean values of palmitic acid were observed in the S_3 and the S_2 salinity levels, respectively, (R^2 = 0.99) in 2013. However, the significant highest and lowest of the mean values in 2014 were observed in the S_3 and S_1, respectively (R^2 = 0.99). The significant highest and the lowest of mean stearic acid across irrigation levels were observed in the S_2 and S_1 in 2013 (R^2 = 0.99) and in the S_3 and S_1 in 2014 (R^2 = 0.99), respectively. In 2013 and 2014, the significant highest mean values of oleic acid were observed in S_2, linoleic acid in S_3 and S_1 (respectively) linolenic acid in S_2 (in the two successive years). The significant lowest mean values of oleic acid were observed in S_3 and S_1, linoleic acid in S_2 and linolenic acid in S_3 and S_1, respectively.

Across all salinity levels, the significant highest and the lowest mean values of palmitic acid were observed in I_3 and I_4 in 2013 and in I_5 and I_1 in 2014, respectively. The significant highest and lowest mean values of stearic acid were achieved by the I_5 and I_1, respectively, in both 2013 and 2014. The significant highest and lowest values of oleic acid were achieved by the I_4 and I_2 in 2013 and by the I_1 and I_5 in 2014, respectively. Linoleic acid had its significant highest and lowest values in I_2 and I_1 in 2013 and in I_3 and I_1 in 2014, respectively. Linolenic acid, however, significant highest and lowest values, respectively by the I_1 and I_5 had the in 2013 and I_2 and I_3 in 2014.

The interaction effects of salinity and irrigation level on fatty acids in olive fruit flesh is shown in Table 4. The highest and lowest percentages of palmitic acid were observed in I_3S_3 (30.35%) and I_4S_2 (25.76%), respectively in 2013 and in I_2S_3 (20%) and I_1S_3 (17.83%) in 2014. Similarly, the highest and lowest percentages for stearic acid were observed in I_5S_2 (1.87%) and I_4S_3 (0.5%), respectively in 2013 and in I_5S_1 (1.77%) and I_2S_1 (1.28%) in 2014. The highest and lowest percentages for oleic acid were achieved in I_5S_1 (49.41%) and I_5S_3 (40.69%) respectively in 2013 and in I_1S_2 (16.06%) and I_4S_3 (2.74%) in 2014. The highest and lowest percentages for linoleic acid were observed in I_5S_3 (29.63%) and I_5S_2 (19.8%), respectively in 2013 and in I_3S_2 (56.57%) and I_1S_2 (40.51%) in 2014. Finally, the highest and lowest of percentages for linolenic acid were observed in I_2S_3 (2.29%) and I_2S_1 (0.02%), respectively in 2013 and were in I_4S_2 (24.79%) and I_3S_2 (20.30%) in 2014.

3.2 Saturated and Unsaturated Fatty Acids

More unsaturated fatty acids in the olive fruit is considered as an advantage. To investigate this exclusivity, the ratio of unsaturated fatty acids (oleic, linoleic and linolenic acids) to saturated fatty acids (palmitic and stearic acids) was calculated (Table 5). The more the ratio of unsaturated to saturated fatty acids, the higher the quality of the olive fruit. The highest and the lowest ratio of unsaturated to saturated fatty acids were observed in I_4S_2 (2.72) and I_3S_3 (2.22) in 2013, and in I_3S_2 (3.35) and I_1S_2 (1.82) in 2014.

Table 5. Fatty acids and the ratio of unsaturated (oleic, linoleic and linolenic acids) to saturated fatty acids (palmitic and stearic acids) in olive flesh in 2013 and 2014

Years	Salinity Levels	Irrigation Levels	Fatty Acids (%)		Unsaturated to Saturated fatty acids ratio
			Unsaturated	Saturated	
2013	S_1	I_1	70.69	29.31	2.41
		I_2	70.88	29.12	2.43
		I_3	71.03	28.97	2.45
		I_4	69.32	30.68	2.26
		I_5	72.01	27.99	2.57
	S_2	I_1	72.33	27.67	2.61
		I_2	70.57	29.43	2.4
		I_3	72.38	27.62	2.62
		I_4	73.11	26.89	2.72
		I_5	70.55	29.45	2.40
	S_3	I_1	71.11	28.89	2.46
		I_2	70.59	29.41	2.4
		I_3	68.94	31.06	2.22
		I_4	72.43	27.57	2.63
		I_5	69.88	30.12	2.32
2014	S_1	I_1	74.87	25.13	2.98
		I_2	75.19	24.81	3.03
		I_3	76.38	23.62	3.23
		I_4	73.87	26.13	2.83
		I_5	75.02	24.98	3.00
	S_2	I_1	64.49	35.51	1.82
		I_2	74.68	25.32	2.95
		I_3	77.01	22.99	3.35
		I_4	68.32	31.68	2.16
		I_5	68.96	31.04	2.22
	S_3	I_1	76.48	23.52	3.25
		I_2	69.20	30.80	2.25
		I_3	68.47	31.53	2.17
		I_4	76.57	23.43	3.27

I_5	75.13	24.87	3.02

3.3 Saturated and Unsaturated Fatty Acids Component in Oil Percentage

Investigating the direct impact of salinity and irrigation level on fatty acids did not yielded consistent results. Therefore, it is suggested to evaluate the oil percentage in olive flesh in order to better understand the effects of interactions between salinity and irrigation level affecting the fatty acids in olive fruits. While this study shows that the percentage of oil in olive flesh could remain low, the ratio of unsaturated to saturated fatty acids can be high. On the other hand, the percentage of oil in the olive flesh could be high, but the ratio of unsaturated to saturated fatty acids can be low. The variation in such possibilities is a result of the different treatments. Therefore, it is essential to investigate the effects of various treatments on the ratio of fatty acids in the oil of olive fruits.

Oil percentage was measured by the GC device. The interaction effects of salinity and irrigation level were investigated along with their effects on the oil percentage in olive flesh (Table 6).

Table 6. The interaction effect of salinity and irrigation level: their effects on the percentage of oil in olive flesh (%) in 2013 and 2014

Year	Salinity levels	Irrigation levels				
		I_1	I_2	I_3	I_4	I_5
2013	S_1	50.67	48.90	46.29	42.00	40.42
	S_2	26.59	35.48	27.01	37.26	32.27
	S_3	32.55	32.41	32.47	32.08	34.55
2014	S_1	26.39	78.30	40.97	26.44	11.87
	S_2	33.85	11.51	39.62	31.15	25.23
	S_3	13.16	53.16	37.67	40.44	62.68

Significant differences ($P \leq 0.05$) between the ratios of fatty acids to the percentage of oil in the olive flesh are shown in Table 7 for both 2013 and 2014 years. Across all irrigation levels, the highest mean values of palmitic (R^2=0.99), oleic (R^2= 0.82), linoleic (R^2= 0.84) and linolenic (R^2=0.99) acids to the percentage of olive flesh oil were observed in S_1 in 2013 and S_3 in 2014, respectively. The lowest of palmitic acid (R^2 = 0.96) and linoleic acid (R^2 = 0.84) to the percentage of olive flesh oil were observed in S_2 in both years. The lowest of oleic and linolenic acid to the percentage of olive flesh oil were observed in S_3 (R^2 = 0.99) in 2013 and in S_2 in 2014.The highest and the lowest mean values of stearic acid to the percentage of olive flesh oil were observed in S_2 in 2013 (R^2 = 0.99) and in S_1 in 2014 (R^2 = 0.96), respectively.

Furthermore, the highest oil percentages occurred in S_1 and S_3 during 2013 and 2014 (R^2 = 0.81 and R^2 = 0.99), respectively, and the lowest oil percentages occurred in S_2 during both years (R^2 = 0.81 and R^2 = 0.99), respectively. In general results in 2013 show the similarities with the results reported by Ben-Ahmed et al. (2009). Across all salinity levels, the highest and the lowest mean values of palmitic acid to the percentage of oil in the olive flesh occurred, respectively, in I_1 and I_2 in 2013 and in I_2 and I_4 in 2014. The highest and lowest of mean values for stearic acid, put against the percentage of oil in the olive flesh, occurred respectively in I_2 and I_1 in 2013 and via I_2 and I_5 in 2014, respectively. The same case for oleic acid was achieved, respectively, in I_1 and I_3 in 2013 and in I_2 and I_1 in 2014, while for linoleic acid, it was via I_2 and I_5 in 2013 and via I_2 and I_1 in 2014. Regarding linolenic acid, the respective claim occurred via I_1 and I_2 in 2013 and via I_2 and I_1 in 2014. Furthermore, the highest and the lowest oil percentage occurred respectively via I_2 and I_3 in 2013 and via I_2 and I_1 in 2014**.**

The effect of interaction between salinity and water stress on the ratio of fatty acids to the percentage of oil in the olive flesh indicates that the highest and the lowest mean values of palmitic acid to olive flesh oil percentage, when regarded as a ratio, occurred respectively via I_1S_1 (14.54) and I_2S_3 (6.61) in 2013 and via I_2S_1 (14.45) and I_2S_2 (2.28) in 2014. Similarly, the same case for stearic acid occurred respectively via I_2S_3 (3.89) and I_4S_3 (0.16) in 2013 and via I_1S_2 (5.52) and I_5S_1 (0.41) in 2014. For oleic acid, it was via I_1S_1 (23.68) and I_1S_2 (12.48) in 2013 and via I_2S_1 (41.1) and I_2S_2 (5.94) in 2014. In the case of linoleic acid, it occurred via I_2S_1 (12.78) and I_5S_2 (6.39) in 2013 and via I_2S_1 (18.96) and I_5S_1 (2.68) in 2014. Linolenic acid had it respectively via I_4S_1 (0.95) and I_4S_3 (0.01) in 2013 and via I_2S_1 (1.11) and I_5S_1 (0.17) in 2014. Generally, the highest and lowest percentages of oil happened to occur via I_1S_1 (51.12) and I_1S_2 (26.83) in 2013 and via I_2S_1 (78.99%) and I_2S_2 (11.61%) in 2014

(Table 7).

Table 7. The two-way ANOVA analysis of salinity and irrigation levels, displaying the ratio of mean values of palmitic, stearic, oleic, linoleic and linolenic acids to the percentage of oil in the olive flesh (%). Data present the two years 2013 and 2014

Fatty acid	Year	Irrigation levels	Salinity levels						mean
			S_1		S_2		S_3		
				S.D		S.D		S.D	
Palmitic* (%)	2013	I_1	14.54 a	0.15	7.22 k	0.08	9.12 i	0.09	10.29 ^{a}
		I_2	13.82 b	0.14	10.15 f	0.10	6.16 l	0.07	10.04 ^{c}
		I_3	13.08 c	0.14	7.28 k	0.08	9.85 g	0.10	10.06 ^{b}
		I_4	12.47 d	0.13	9.60 h	0.10	8.77 j	0.09	10.28 ^{a}
		I_5	11.14 e	0.11	8.99 i	0.09	10.11 f	0.10	10.08 ^{b}
		mean	13.01 ^{a}		8.64 ^{c}		8.8 ^{b}		
	2014	I_1	5.01 ij	0.06	6.40 g	0.07	2.38 k	0.03	4.6 ^{e}
		I_2	14.45 a	0.15	2.28 k	0.03	10.81 c	0.11	9.18 ^{a}
		I_3	7.76 e	0.08	7.23 f	0.08	7.15 f	0.07	7.38 ^{b}
		I_4	5.14 i	0.05	6.01 h	0.06	7.94 d	0.08	6.36 ^{d}
		I_5	2.38 k	0.03	4.99 j	0.06	12.86 b	0.13	6.74 ^{c}
		mean	6.95 ^{b}		5.38 ^{c}		8.23 ^{a}		
Stearic* (%)	2013	I_1	0.45 e	0.01	0.21 k	0.01	0.37 g	0.01	0.34 ^{d}
		I_2	0.55 c	0.01	0.39 f	0.01	3.89 a	0.04	1.61 ^{a}
		I_3	0.47 e	0.01	0.26 j	0.01	0.33 h	0.00	0.35 ^{d}
		I_4	0.54 c	0.01	0.52 d	0.01	0.16 l	0.00	0.4 ^{c}
		I_5	0.28 i	0.01	0.60 b	0.01	0.40 f	0.01	0.42 ^{b}
		mean	0.46 ^{b}		0.40 ^{c}		1.03 ^{a}		
	2014	I_1	1.33 i	0.02	5.52 a	0.06	0.48 k	0.01	2.44 ^{b}
		I_2	3.38 e	0.04	0.48 k	0.01	5.17 b	0.05	3.00 ^{a}
		I_3	1.36 i	0.01	1.35 i	0.02	4.58 c	0.05	2.43 ^{b}
		I_4	1.53 h	0.02	3.80 d	0.04	1.13 j	0.01	2.15 ^{c}
		I_5	0.41 l	0.00	2.80 f	0.03	1.89 g		1.70 ^{d}
		mean	1.60 ^{c}		2.79 ^{a}		2.65 ^{b}		
Oleic* (%)	2013	I_1	23.68 a	2.61	12.48 e	1.38	15.32 de	1.69	17.16 ^{a}
		I_2	22.17 ab	2.45	15.47 de	1.71	13.65 e	1.51	17.1 ^{a}
		I_3	21.59 ab	2.38	12.63 e	1.39	14.77 de	1.63	16.33 ^{b}
		I_4	17.97 cd	1.98	18.12 cd	2.00	15.84 de	1.75	17.31 ^{a}
		I_5	19.97 bc	2.20	15.94 de	1.76	14.06 e	1.55	16.65 ^{b}
		mean	21.08 ^{a}		14.92 ^{b}		14.72 ^{b}		
	2014	I_1	14.26 de	1.57	13.90 de	1.54	7.03 f	0.78	11.73 ^{d}
		I_2	41.10 a	4.53	5.94 f	0.66	25.15 c	2.77	24.06 ^{a}
		I_3	21.70 c	2.40	22.73 c	2.51	17.84 d	1.97	20.25 ^{b}
		I_4	13.93 de	1.54	13.34 e	1.47	22.09 c	2.44	16.45 ^{c}
		I_5	6.35 f	0.70	11.57 e	1.28	32.52 b	3.59	16.81 ^{c}
		mean	19.47 ^{b}		13.5 ^{c}		20.93 ^{a}		
Linoleic* (%)	2013	I_1	11.58 ab	1.28	6.44 g	0.71	7.52 fg	0.83	8.51 ^{c}
		I_2	12.78 a	1.41	9.25 cdef	1.02	8.03 fg	0.89	10.02 ^{a}
		I_3	10.88 bc	1.20	6.52 g	0.72	7.38 fg	0.81	8.26 ^{d}
		I_4	10.44 bcd	1.15	8.57 ef	0.95	7.59 fg	0.84	8.86 ^{b}
		I_5	8.81 def	0.98	6.39 g	0.70	10.24 bcde	1.13	8.48 ^{c}
		mean	10.89 ^{a}		7.43 ^{c}		8.15 ^{b}		
	2014	I_1	5.64 f	0.62	7.86 e	0.87	3.21 g	0.36	5.57 ^{d}
		I_2	18.96 a	2.09	2.74 g	0.31	11.76 c	1.30	11.15 ^{a}
		I_3	9.95 d	1.10	8.15 e	0.90	7.98 e	0.88	8.7 ^{b}

		I_4	5.73 f	0.63	7.85 e	0.87	9.14 de	1.01	7.57^{c}
		I_5	2.68 g	0.30	5.75 f	0.63	15.07 b	1.66	7.83^{c}
		mean	8.60^{b}		6.47^{c}		9.43^{a}		
Linolenic* (%)	2013	I_1	0.87 ab	0.10	0.49 fg	0.05	0.51 fg	0.06	0.62^{a}
		I_2	0.01 h	0.00	0.53 efg	0.06	0.45 g	0.05	0.33^{d}
		I_3	0.70 cd	0.08	0.57 ef	0.06	0.43 g	0.05	0.57^{b}
		I_4	0.95 a	0.11	0.80 bc	0.09	0.01 h	0.00	0.59^{b}
		I_5	0.59 ef	0.07	0.63 de	0.07	0.06 h	0.01	0.42^{c}
		mean	0.62^{a}		0.60^{a}		0.29^{c}		
	2014	I_1	0.38 fgh	0.04	0.48 def	0.06	0.18 i	0.02	0.34^{e}
		I_2	1.11 a	0.13	0.18 i	0.02	0.75 c	0.08	0.68^{a}
		I_3	0.56 d	0.07	0.51 de	0.06	0.46 defg	0.05	0.51^{b}
		I_4	0.35 h	0.04	0.44 efgh	0.05	0.51 de	0.06	0.43^{d}
		I_5	0.17 i	0.02	0.36 gh	0.04	0.90 b	0.10	0.48^{c}
		mean	0.51^{b}		0.39^{c}		0.53^{a}		
Olive flesh oil percentage	2013	I_1	51.12 a	5.64	26.83 g	2.96	32.84 efg	3.62	36.93^{b}
		I_2	49.33 ab	5.44	35.79 de	3.95	32.70 efg	3.61	39.27^{a}
		I_3	46.70 abc	5.15	27.24 fg	3.01	32.76 efg	3.61	35.57^{c}
		I_4	42.37 bcd	4.67	37.59 de	4.14	32.36 efg	3.57	37.44^{b}
		I_5	40.77 cd	4.50	32.55 efg	3.59	34.85 def	3.84	36.06^{c}
		mean	46.06^{a}		32.00^{c}		33.10^{b}		
	2014	I_1	26.62 fg	2.94	34.15 def	3.77	13.27 h	1.46	24.68^{d}
		I_2	78.99 a	8.71	11.61 h	1.28	53.63 c	5.91	48.08^{a}
		I_3	41.33 d	4.56	39.97 d	4.41	38.00 de	4.19	39.76^{b}
		I_4	26.67 fg	2.94	31.42 efg	3.47	40.80 d	4.50	32.96^{c}
		I_5	11.98 h	1.32	25.45 g	2.81	63.23 b	6.97	33.55^{c}
		mean	37.11^{b}		28.52^{c}		^{a}		

Note. * 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

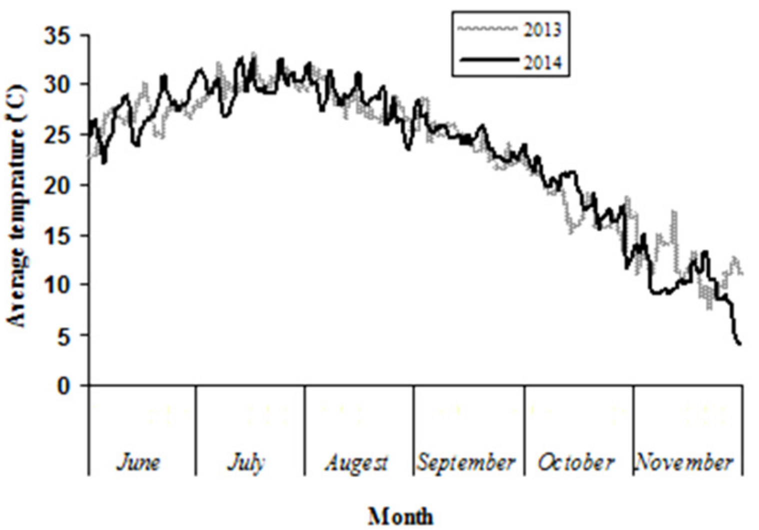

Figure 3. Daily average temptature varitions (oC) in different months during growing season in two consecutive years, 2013 and 2014

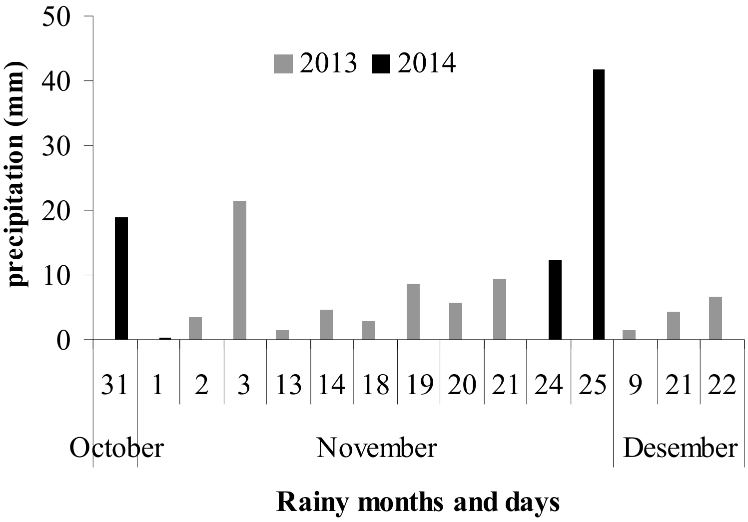

Figure 4. Average precipitation (mm) in different rainy months and days in 2013 and 2014

4. Discussion

In general, the variations of weather parameters between the years of 2013 and 2014 resulted an increment about 8.52% of mean values of palmitic acid in 2013 compare to the mean value in 2014. Although the mean value of stearic acid (1.8%) in 2013 show a reduction of 4.79% in comparison to the mean value (6.59%) in 2014. According to the variations in temperature and rain (Figure 3 and Figure 4) and regarding the results obtained for saturated fatty acids during two consecutive years, it can be conclude that saturated fatty acids variations are influenced by the weather variations parameters such as temperature (Figure 3) (lower temperatures reduced palmitic acid content) and precipitation (Figure 4) (there are similarities to the results reported by Beltran, Rio, Sanchez, & Martinez, 2004; Esmaeili, shaykhmoradi, & Naseri, 2012) and there also found an inverse relationship between palmitic and stearic acids. Increasing in electrical conductivity (EC) of irrigation water from 2.2 dS m^{-1} to 7.7 dS m^{-1} resulted in about 1.74 folds increase in oleic acid in 2014 at I_2S_1 treatment compare to 2013 at I_1S_1 treatment. This can be explained by the fact that increasing temperature (Figure 3) is consistent with salinity and water stresses that resulted in about 1.74 fold increase in oleic acid content in 2014.

Table 5 indicates that the maximum values of saturated fatty acids were observed at low salinity levels in 2013 (31.06% at S_3) and 2014 (35.51% at S_2). These were consistent with earlier results by Melgar et al. (2012). The ratio of unsaturated to saturated fatty acids reached its highest via the S_2 salinity level in 2013 (2.72) and also in 2014 (3.35). ***The highest olive lyophilized flesh oil percentage occurred via S_1 along with low deficit irrigation levels***, thereby causing the oil percentage to reach 51.12% in 2013 and 78.99% in 2014. The lowest of that percentage occurred via S_2 which led to 26.83% in 2013 and 11.61% in 2014, considering the application of low deficit irrigation levels during both years. Results revealed that based on average values of olives quality parameters, the optimum olive fruit flesh oil percentage performance was observed in I_1S_1 (about 37% more than average in 2013) and at I_2S_1 (more than double in 2014). The optimum palmitic performance was observed in I_1S_1 (about 43% more than average in 2013) and at I_2S_1 (more than double in 2014). The optimum oleic performance was observed in I_1S_1 (about 40% more than average in 2013) and at I_2S_1 (more than double in 2014). The optimum linoleic performance was observed in I_2S_1 (about 50% more than average in two years) and the optimum linolenic performance was observed in $I4S_1$ (about 87% more than average in 2013) and at I_2S_1 (more than two folds of average in 2014). On the other hand, the optimum stearic performance was observed more than double of average value in I_2S_3 (in 2013) and in I_1S_2 (in 2014). Across all salinity levels, the highest stearic, oleic, linoleic and linolenic contents and oil percentage were observed in 2014, but palmitic was observed in 2013 in two successive years of experiment. The values of olive flesh fruit quality parameters was intended in the olive oil percentage (Table 7), on the other hand, the average percentage of olive fruit oil in 2013 was about 2.3% higher than 2014 (this can be due to 52 mm rainfall per two days (24 and 25 November) in 2014 which is exactly one week before harvesting the olive fruit (November 30th). Regardless of different levels of irrigation, the maximum average palmitic, oleic, linoleic and linolenic were observed at S_1 in 2013 and S_3 in 2014. In general maximized interaction of these parameters has been observed at S_1 in two consecutive years. Low volume of irrigation water with high salinity level (I_1S_1) transfers less salt to the plant root zone compare to the high volume of irrigation water with the same percentage of salt content (I_5S_1) resulting a reduction in osmotic pressure and yielding more water uptake by plant root to improve the olive fruit quality. Qasim, et al (2013) reported that increasing plant salinity and water stress stimulates the P5CS gene in olive which causing the protein degradation and synthesis of proline and thereby increase its concentration in the plant. Proline moderates the negative effects of osmotic stress on plant, has a positive effect on olive quality enzymes and increased oleic acid and linoleic acid production. Generating these acids (which are good acids and polyunsaturated oils), reduces the amount of oil saturated acid (stearic acid), resulting in improving the oil quality (Qasim et al., 2013).

5. Conclusions

Different Salinity and water stress levels resulted significant impact on fatty acids composition and quality of olive fruit flesh oil. ***In generally, increasing salinity (S_1) and decreasing irrigation water levels (I_1 or I_2) caused significant increment in the ratio of unsaturated fatty acids, palmitic acid to the percentage of oil, and oil percentage in olive fruit flesh in two successive years 2013 and 2014. Stearic acid was significantly decreased in*** S_1***, although it was increased at lower irrigation water levels (I_2) in two years.*** These changes were sinusoidal trend almost for all quality parameters in olive fruit flesh. ***The highest olive lyophilized flesh oil percentage occurred in S_1 along with low deficit irrigation levels***. It is concluded*, across all salinity levels,* **the highest olive quality** *which is related to fatty acids and oil percentage occurred in I_2 in two consecutive years*. However, the lowest of these values occurred in S_2 and S_3 under various deficit irrigation levels. ***In general to obtain the highest palmitic and unsaturated fatty acids, grower can apply the I_2 irrigation level combined with S_1 salinity level.*** In most southern parts of Iran (study area which is located in arid and semi-arid region), ground water quality is dropping and farmers have to use saline water for irrigation. In the study area, drought phenomenon and lack of enough precipitation, caused declining the groundwater level, therefore the water crisis is being more severe. It is recommended that the growers irrigate their olive trees with salty groundwater and apply deficit irrigation strategies as S1I2. It is also recommended that further researches should be carried out on genetic responses of olive to water and salinity stresses.

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