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Research Article

Impact of water regimes on the chemical composition, antioxidant activity and quality of olive oil (*Olea europaea* L.) under semi-arid conditions in Tunisia

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Abstract

Water scarcity is the main challenge in irregular olive oil production in Tunisia. In olive orchards the use of deficit irrigation technique is the main method to optimize water saving and achieve sustainable olive production. The aim of this study was to assess four levels of drip irrigation (100% ETc, 60% ETc, 50% ETc and 40% ETc (evapotranspiration)) on 'Chemlali' and 'Koroneiki' olive oil varieties, in semi-arid conditions, for two consecutive crop years. The quality parameters, antioxidant compounds, and antioxidant properties against DPPH and ABTS cation radicals of oils, were evaluated. Moreover, the fatty acid composition of 'Chemlali' and 'Koroneiki' oils was determined through gas chromatographic analysis. The result showed that irrigation regimes had a moderate effect on the standard quality parameters (free fatty acids, peroxide value, K232 and K270) of virgin olive oil as well as on the composition of the fatty acids. The oil obtained from trees treated with 50% ETc irrigation strategy was characterized by a high content of oleic acid (approximately 61% for 'Chemlali' and 78% for 'Koroneiki'), a low level of palmitic acid, a high content of phenolic compounds (217.44 and 198.99 mg of eq catechin kg^{-1} of oil for 'Chemlali' and 'Koroneiki', respectively), O-diphenols, chlorophyll and carotenoids and high antioxidant properties for both olive cultivars. Therefore, this method is considered the best irrigation strategy to optimize water management and improve the quality, the antioxidant content and properties of oil.

Keywords: antioxidant compounds; fatty acids; irrigation treatments; olive oil; quality parameters

Introduction

Tunisia is the largest producer of olives and olive oil in the southern Mediterranean. Excluding the European Union, Tunisia is considered the leading producer of olive oil (Gharbi *et al.*, 2014) with production reaching approximatively 400.000 tons (COI, 2020). However, its productivity remains irregular. This

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irregularity is due to environmental factors, especially water scarcity and high temperatures. Water shortage is the primary challenge Tunisia faces, and limited water resources threaten the sustainability of agricultural activity, particularly the Tunisian olive growing.

Traditionally, the olive trees have been a rain-fed crop. However, irrigation has been essential for increasing production and achieving sustainable productivity (Ahumada-Orellana *et al.*, 2018). The resilience of the olive tree makes it possible to apply deficit irrigation techniques, allowing for high productivity with irrigation doses that do not fully satisfy the crop's water requirements. Numerous studies have demonstrated that the regulated deficit irrigation (RDI) in olive orchards can be used as a water saving management tool without negatively affecting oil yield and quality (Trentacoste *et al.*, 2015; Ahumada-Orellana *et al.*, 2018). As a result, Tunisia adopted intensification and irrigation practices, transforming its traditional olive groves into other more productive cultural models as part of a national strategy and a challenge to improve the productivity of olive trees and the quality of the oil.

Olive oil is a typical source of fats in the Mediterranean diet. His nutritional value is primarily attributed to its fatty acids composition, particularly oleic acid and its richness in natural antioxidants such as phenolic compounds, tocopherols, flavonoids, and carotenoids (El Yamani *et al.*, 2022) which protect against cellular damage induced by free radicals (De Faveri *et al.*, 2008). Olive oil yield is largely influenced by water-use efficiency. Interestingly, reduced irrigation regime may have an advantageous effect on olive oil properties. In addition, deficit irrigation strategies often have similar, or even better, olive oil quality compared to well-irrigated trees (Fernandes-Silva *et al.*, 2013; Garcia *et al.*, 2013). Moreover, Motilva *et al.* (2000) found that applying RDI strategies cv. Arbequina led to an increase in polyphenol concentration and oil oxidative stability coinciding with a higher content of phenol derivatives (Ahumada-Orellana *et al.*, 2018). These compounds are of great interest because they influence the quality, palatability and increase the shelf life of olive oil (Abaza *et al.*, 2005).

Despite the importance of the olive sector in Tunisia, olive oil production and quality are still affected by various biotic and abiotic factors, mainly climatic disturbances, particularly rainfall and irrigation water management. These disturbances have a detrimental impact on olive oil yield and quality. Concerns about its dependence on irrigation methods combined with predictions of declining in water supply prompt us to study the impact of irrigation dose on the quality parameters, and the antioxidant composition of olive oil, as reflected in its stability power.

Materials and Methods

Study site and experimental conditions

The experiment was conducted during the 2018 and 2019 crop seasons, in two olive oil varieties *Olea europaea* L.; a local variety cv. 'Chemlali' with 50-year-old and Greece introduced variety cv. 'Koroneiki' with 20-year-old, planted with a tree spacing of 6 m × 6 m, located at Menzel-Mhiri-Kairouan (35'44"N, 9.81'06"E), in the Center of Tunisia (41.33° N, 7.04° W; 240 m altitude) (Figure 1).

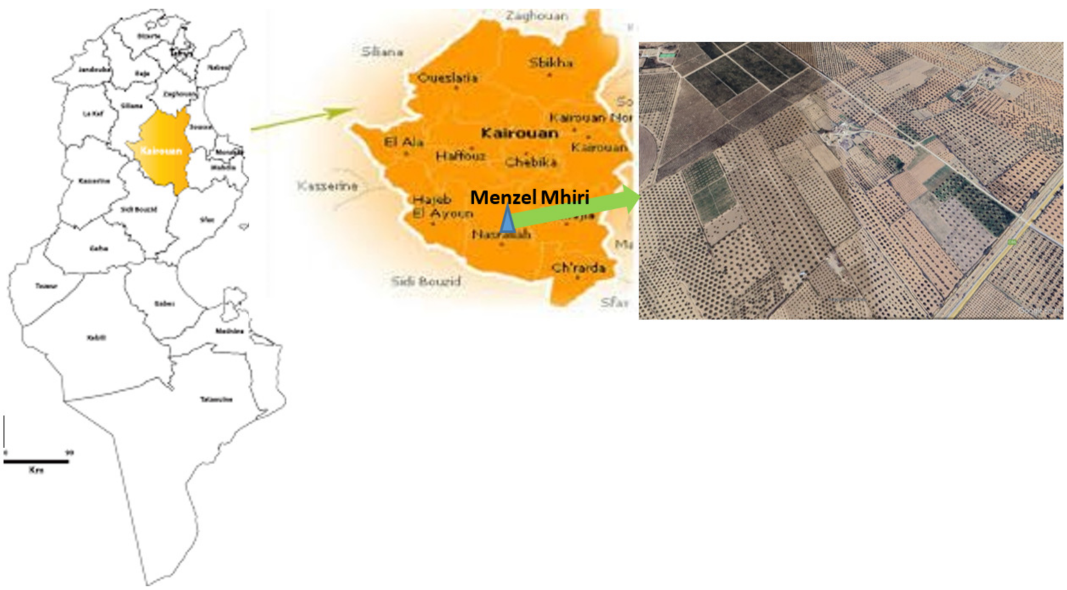


Figure 1. Description of geographical position of the investigated area of olive trees in center of Tunisia

Four irrigation treatments were applied: full irrigation by the farmer (T1) which received seasonal water equivalent to 100% of the estimated crop evapotranspiration (ETc), growing deficit irrigations that received a volume of water equivalent to 60% ETc (T2); 50% ETc (T3) and 40% of estimated ETc (T4) respectively. The water was supplied by drip irrigation with two pressure compensated drippers per tree, each with a flow rate of 4 L h^{-1} per tree.

The experimental plan consisted of four adjacent blocks each of these made of four rows with twenty-five olive trees, where only the six central trees were used for sampling. Relative humidity, temperature and precipitation of the studied area were estimated from a standard meteorological station adjacent to the experimental field during the 2018 and 2019 crop seasons (Figure 2).

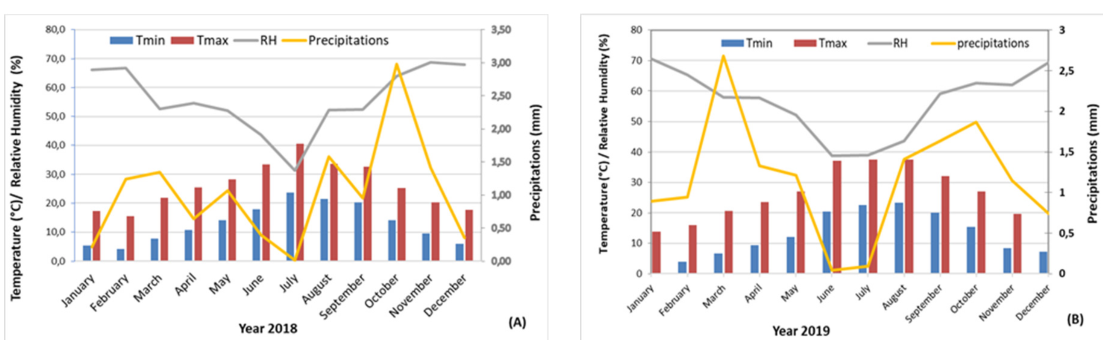


Figure 2. Monthly average of temperature, relative humidity and precipitations of Tunisian studied area during crop seasons 2018 (A) and 2019 (B) of olive tree 'Chemlali' and 'Koroneiki' (Tmax: Maximal Temperature; Tmin: Minimal Temperature and HR: Relative Humidity)

Olive water requirement

The water requirements for the irrigated plot were estimated based on crop evapotranspiration (ETc) calculated using the Penman-Monteith method (Allen *et al.*, 1998), and supplied through a drip irrigation system, with two online drippers (4 L $h^{-1)}$ per tree, each placed at 0.75 cm from the tree-trunk base. The irrigation water requirements were 315.1 mm, 275.5 mm, 174.8 mm and 118.0 mm during 2017-2018, for treatments T1-T4, respectively. During this campaign, the rain amounted to about 254 mm with an effective rain amount of about 178 mm. Thus, the total water input (Irrigation and effective rain) was 492.5 mm, 453.1 mm, 348.4 mm and 295.7 mm, for treatments T1, T2, T3 and T4, respectively.

During the 2018-2019 growing season, water requirements were 153.5 mm, 153.5 mm, 83.4 mm, and 43.4 mm, under treatments T1-T4, respectively. During this experimental year, T1 and T2 were equal. We also noted that the real rain amount was 427 mm and effective rain was 299 mm in 2019. The maturity index of the fruits collected from each treatment was determined for both crop seasons.

Olive oil extraction

The collected samples, around three kg of each treatment, were collected and used for olive oil extraction by the Oleodoseur. The paste underwent malaxation at 25 °C for 30 min and the oil was extracted using a two-phase decanter.

Olive oil analysis

The obtained quantity of oil was weighed and the oil percentage and oil production, for each treatment for the two studied years 2018 and 2019, were determined. For each treatment, different quality parameters and antioxidant compounds in olive oil were determined such as acidity, peroxide indices, K232, K270, phenols, O-diphenols, chlorophylls, carotenoids, and fatty acids.

Determination of quality indices

The free acidity (IOC, 2017a), peroxide value (IOC, 2017b) and UV spectrophotometric indices (K232, K270) (IOC, 2019) of oil (ISO, 2016) were determined.

The free acidity (FA) content was determined in triplicate by the titration method as follows: 5 g of oil were dissolved in 20 mL ethanol with phenolphthalein as an indicator. The mixture was titrated with 0.177 N NaOH solutions until the appearance of a faint pink color. The FFA content was expressed as a percentage of oleic acid.

The peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq O_2 kg^{-1}), was measured as follows: Five grams of olive oil was mixed with chloroform/acetic acid then the mixture was left to react in darkness in saturated potassium iodide. The iodine liberated by the peroxides was titrated with a standardized sodium thiosulphate solution using starch as an indicator.

K232 and K270 were calculated from absorption at 232 and 270 nm, respectively, using a UV spectrophotometer (SPECUVIS1; UV-Visible), with a 1 % solution of olive oil in cyclohexane and a path length of 1 cm.

Determination of pigments

The pigment contents (mg kg^{-1} of oil) were determined by a spectrophotometer; UV-Visible, following the method described by Minguez-Mosquera *et al.* (1991). A sample of 7.5 g oil was dissolved in 25 mL of cyclohexane. The absorbance of this solution was read at 670 and 470 nm for chlorophylls and carotenoids, respectively.

Determination of phenolic compounds

Total phenolic content

The determination of total phenols was carried out according to (Papoti and Tsimidou, 2009) with few modifications. One gramme of olive oil samples was dissolved in 5 mL of n-hexane and extracted three times with aqueous methanol (60/40, v/v, 5 mL). The total phenols content was determined spectrophotometrically following Folin and Ciocalteu method (1927). The Folin-Ciocalteu reagent was added to the diluted polar extract and the absorbance was measured at 725 nm using standard catechin (Sigma Aldrich, St. Louis, MO, USA). Values for total phenols content are given as mg equivalent catechin per kg of oil.

Total O-diphenols

The polar fraction, prepared for phenol extraction, was diluted with water, 0.1 M phosphate buffer (1 mL) and 5% sodium molybdate solution were added. Absorbance was measured after 15 min at 350 nm against a reagent blank. Caffeic acid was used as a standard to prepare a standard range within the concentration range of 10-50 μg mL^{-1}.

Fatty acid compositions

The determination of fatty acids composition was realized by the method described in the regulation EEC 2568/91 (EEC, 1991). The fatty acids were converted to fatty acid methyl esters (FAMEs) before analysis by Gas Chromatography (GC). The FAMEs were prepared by saponification/methylation with sodium methylate (NaOMe/MeOH) solution according to European Regulations. Chromatographic analysis was performed on a Hewlett-Packard model 4890D gas chromatograph equipped with a 30 m × 0.25 mm × 0.25 μm film thickness Fused Silica capillary column (Innowax) coupled to a flame ionization detector (column temperature 210 °C). Fatty acids were identified by comparing their retention times with those of standard compounds and were expressed as percentages.

Determination antioxidant activity of olive oils

DPPH method

The antioxidant activity of oil samples was determined by the procedure described by Turkmen *et al.* (2006). Different concentrations of oil (10 to 40 μg mL^{-1}) were tested with DPPH ethanolic solution in different tubes. A 0.50 ml of ethanolic solution of 2,2 –diphenyl-1-picrylhydrazyl radical (DPPH) (0.060 mM) was added to each tube containing the of oil sample and shaken vigorously and stand at room temperature for 30 min. The control was prepared with ethanol as above without any extract. The absorbance of the samples was measured at 517 nm. The DPPH radical scavenging activity was calculated as a percentage of the inhibition of DPPH according to the following equation:

$$DPPH\ [\%] = (A0-AS)/A0\times100$$

where A0 represent the absorbance of the blank solution and AS is the absorbance of the sample.

The IC_{50} values were deduced, representing the concentration of the sample required to scavenge 50% of DPPH radicals (Fujita *et al.*, 1998). Trolox was used as a standard.

ABTS method

The method was based on the oxidation of the ammonium salt of 2,2'-azinobis 3-ethylbenzothiazoline-6-sulphonic acid to radical cation $ABTS^{\bullet+}$. This method was carried out according to Re *et al.* (1999) with slight modifications. A mixture of commercial 39.2 mg $ABTS^{\bullet+}$ and 6.7 mg $K_2S_2O_8$ in 10 mL of deionized water was prepared. This solution was incubated in the dark at room temperature for 24 hours before use. The mixture of varying volumes of the olive oil was reacted with $ABTS^{\bullet+}$ solution and the absorbance are determined at 734 nm at several time intervals (5, 10, 15, 20 and 30 minutes) and each test is repeated three times.

Statistical analysis

Values are presented as the mean ± standard deviation of three measurements. All statistical values were calculated using the SPSS program, release 20.0 for Windows (SPSS, Chicago, IL, USA). Significant differences between the values of the studied parameters were determined at $p < 0.05$ according to the ANOVA (Tukey's multiple range test). Correlation analyses between all studied parameters were performed using Pearson's test. Finally, non-standardized principal component analysis (PCA) was performed on the whole data set (quality parameters, antioxidant compounds, fatty acids, and antioxidant activity tests according to DPPH and ABTS radicals) to create biplots for all parameters and treatments at a 95% confidence level. The PCA type was Pearson, the biplot type and the coefficient was automatic.

Results and Discussion

Effect of irrigation rate on the repining index

The maturation process of olives, from green to black, is complex and involves several physiologic and biochemical changes that depends on olive fruit maturity stages and agronomic factors such as irrigation. Values of the maturity indices during 2018 and 2019 of both varieties 'Chemlali' and 'Koroneiki' are shown in Table 1.

Table 1. The maturity index of olive fruits and the extinction coefficients (K232 and K270) of 'Chemlali' and 'Koroneiki' olive oil cultivated under different irrigation levels during the 2018 and 2019 crop seasons

Parameters/ Variety	Maturity index				K232				K270			
	Chemlali		Koroneiki		Chemlali		Koroneiki		Chemlali		Koroneiki	
Crop season/ Treatment	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
T1	2.9± 0.0d	4.2± 0.1b	1.9± 0.0 f	3.4± 0.3 c	2.2± 0.13a	2.98± 0.08A	1.13± 0.04b	1.90± 0.04BC	0.03± 0.00c	0.108± 0.01A	0.041± 0.009c	0.01± 0.00A
T2	3.0± 0.0c	4.2± 0.0b	1.6± 0.0g	2.5± 0.2 d	2.08± 0.08a	2.99± 0.15A	1.14± 0.04b	2.08± 0.15B	0.13± 0.01a	0.108± 0.00A	0.042± 0.001c	0.12± 0.01A
T3	4.4± 0.0a	4.8± 0.1 a	1.8± 0.0h	3.4± 0.1c	2.36± 0.07a	2.85± 0.07A	1.14± 0.07b	1.78± 0.11C	0.09± 0.00b	0.115± 0.00A	0.091± 0.001b	0.11± 0.00A
T4	4.2± 0.0b	5.1± 0.0a	1.9± 0.0e	3.5± 0.04 c	1.96± 0.11a	2.75± 0.06A	1.11± 0.11b	1.92± 0.96BC	0.08± 0.00b	0.096± 0.01A	0.100± 0.018b	0.096± 0.00A

Full irrigation by the farmer (T1:100% ETc) and different deficit irrigation treatments (T2: 60% ETc, T3: 50% and T4: 40% ETc. Values are means ± SD (n = 3); a - g: different letters in the same column were significantly different at $p < 0.05$ according to Tukey's test

The samples for different irrigation treatments were harvested, at the end of November 2018 and mid-December 2019. The results showed that the maturity index varied depending on variety, crop season and irrigation treatment. Values ranged from 1.60 ± 0.01 for 'Koroneiki' (T2) to 5.13 ± 0.04 for 'Chemlali' (T4). Water stress increased the maturity process. In both years and for the two varieties, the maturation indices were lower in fully irrigated trees T1, whereas the highest values were observed in the most deficit irrigations treatment (T4). These findings are consistent with the study of Fernandes-Silva *et al.* (2013) which showed that the maturation indices at the ripening data were lower in fully irrigated trees (3.07), whereas they had the highest values in rainfed trees (3.81). This can be explained by the higher crop load of the trees in the irrigated treatments, which delays fruit maturation. Additionally, several studies have shown that a low crop load hastens and shortens fruit ripening (Barone *et al.*, 1994).

Effect of irrigation status on oil quality parameters

Spectrophotometric indices K232 and K270

The evaluation of the oxidation level of oil was carried out by determining the extinction coefficients K232 and K270. These indices are a good indicator of oil peroxide content (Veillet, 2010). The results (Table 1) showed that the K232 varied between 1.11% (40% of ETc) to 2.99% (60% of ETc). The farmer and 60% ETc treatments, for 'Chemlali' oil cultivated in the 2019 crop season, showed the most important values of K232. The K270 parameter varied from 0.041 to 0.13. It is important to note that limits established for EVOO is 2.50 and 0.22 for K232 and K270, respectively.

The response of olive trees to different irrigation regimes demonstrated significant varietal differences, mainly between the farmer treatment and other deficit irrigation treatments. Irrigation scarcity seemed to influence oil oxidation and quality. Specifically, deficit irrigation of olive trees with 50%ETc and 40%ETc appeared beneficial in decreasing K232 and K270, thereby maintaining the high quality of oil (El Riachy *et al.*, 2017). Similar results were reported by (Gómez Rico *et al.*, 2007) who found a decrease in K232 and K270 with irrigation shortage. These authors explained this decrease from the interference of higher content of phenolic compounds, in rainfed conditions, which absorbs in the UV area in these analytical determinations.

Free acidity

The acidity of the oil is expressed as a percentage of oleic acid. The values of the free acidity (FA) of oil samples were ranged from 0.19% (farmer) to 0.44% (40% ETc) (Figure 3). Hence, all values were below 0.8% the upper limit established by the IOC (2017a) for extra virgin olive oil (EVOO). Also, water stress decreased the FA of oils from 'Chemlali' and 'Koroneiki' during the 2018 and 2019 crop seasons. The irrigation treatment of 50% ETc, for both varieties and the two crop seasons, is considered the best behavior. This was also observed by Fernandez *et al.* (2013) in Cobrancosa virgin olive from different irrigation regimes.

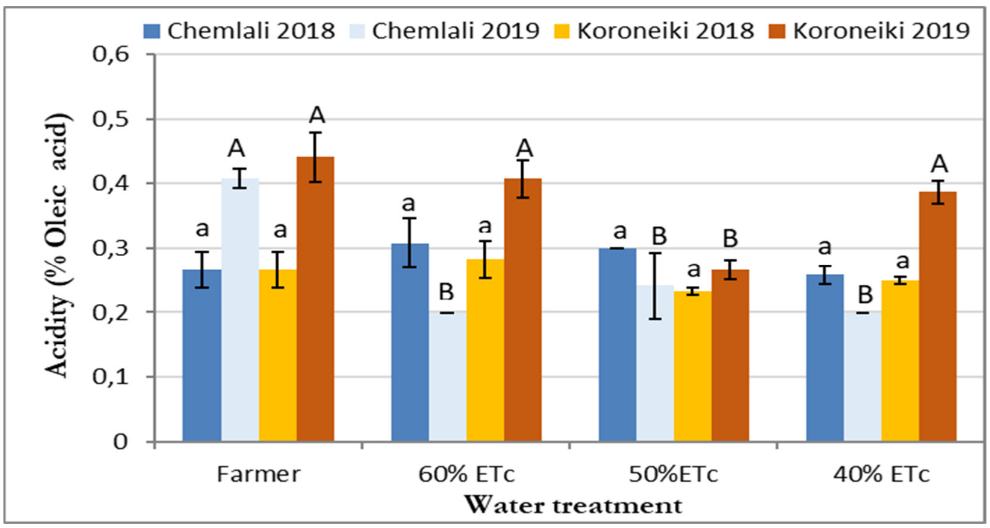


Figure 3. Olive oil acidity of 'Chemlali' and 'Koroneiki' under different water regimes (farmer, 60% ETc, 50% ETc and 40% ETc) during the 2018 and 2019 crop seasons

Full irrigation by the farmer that received a seasonal water equivalent to 100% of estimated crop evapotranspiration (ETc); the growing deficit irrigations that received a volume of water equivalent to 60%; 50% and 40% of estimated ETc respectively. Different letters have significantly different values ($p < 0.05$). For each crop season and for both cultivars, means with different letters were significantly different at $p < 0.05$

Peroxide value

The peroxide value reflects the initial oxidation state of the oil. For the two crop seasons, 2018 and 2019, a difference was observed in the peroxide indices of 'Chemlali' and 'Koroneiki' oils corresponding to the different irrigation treatments (Figure 4).

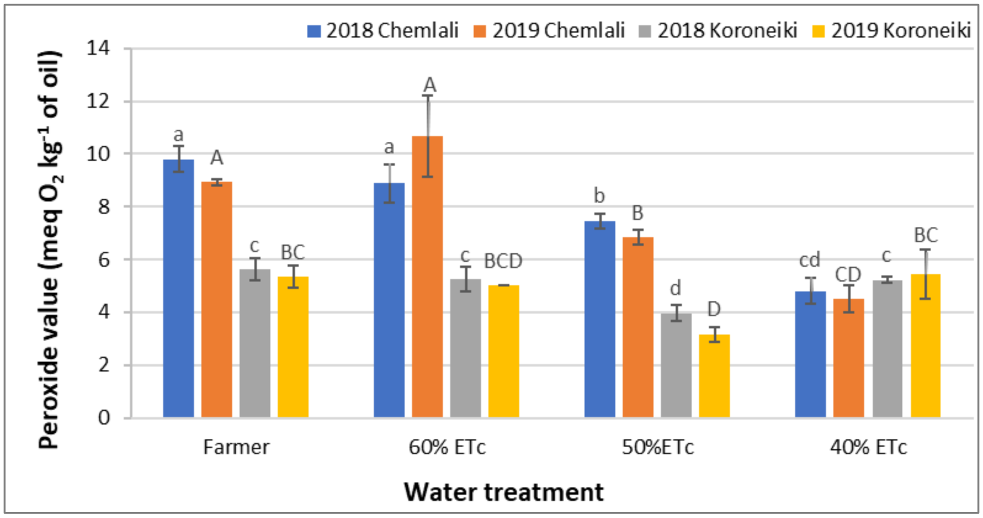


Figure 4. Olive oil peroxide value of 'Chemlali' and 'Koroneiki' under different water regimes (farmer, 60% ETc, 50% ETc and 40% ETc) during the 2018 and 2019 crop seasons

Full irrigation by the farmer that received a seasonal water equivalent to 100% of estimated crop evapotranspiration (ETc); the growing deficit irrigations that received a volume of water equivalent to 60%; 50% and 40% of estimated ETc respectively. Different letters have significantly different values ($p < 0.05$). For each crop season and for both cultivars, means with different letters were significantly different at $p < 0.05$

This index gradually decreased for all varieties with the reduction of irrigation levels, the values reached are significantly lower than the commercial standard (20 meq O_2 kg^{-1}) of olive oil of the extra virgin category (IOC, 2019). These results agree with previous studies (Fernandez *et al.*, 2013; El Riachy *et al.*, 2017) that noted a tendency to increase peroxide value with irrigation level (the peroxide value can be up to 50% higher in watered treatments compared to rainfed). However, this parameter did not show a clear response to maturity index. The lowest values of peroxide were recorded for the irrigation at 50% ETc and 40% ETc, for both varieties across 2018 and 2019 crop seasons, which could be the most recommended.

Effect of irrigation status on pigment content

The results showed a richness in chlorophyll pigments for both varieties during the two crop seasons (Table 2).

Table 2. Carotenoids and chlorophylls content of 'Chemlali' and 'Koroneiki' olive oils for farmer and different deficit irrigation treatments (60% ETc, 50% and 40% ETc) during 2018 and 2019 growing seasons

Variety	Carotenoids (mg kg^{-1} of oil)				Chlorophylls (mg kg^{-1} of oil)			
	Chemlali		Koroneiki		Chemlali		Koroneiki	
Crop season/ Treatment	2018	2019	2018	2019	2018	2019	2018	2019
T1	0.92±0.04 de	2.28±0.20 AB	2.18±0.28 Bc	1.56±0.29 CD	1.03±0.01 C	2.10±0.05 BC	4.07±0.61 b	4.49±0.40 A
T2	0,79±0,03 e	2.33±0.30 AB	2.26±0.53 Bc	1.06±0.02 D	1.07±0.03 C	2.25±0.53 BC	5.14±1.10 ab	3.08±0.55 ABC
T3	1.67±0. 19 cd	2.49±0.05 A	3.18±0.52 a	1.51±0.4 CD	1.53±0.4 C	3.70±0.27 AB	6.63±0.49 a	2.56±1.06 BC
T4	1.45±0. 17 de	1.80±0.10 BC	2.74±0.01 ab	1.46±0.05 CD	1.98±0.15 C	2.03±0.53 C	5.70±0.45\a b	3.40±0.66 ABC

Full irrigation by the farmer (T1:100% ETc) and different deficit irrigation treatments (T2: 60% ETc, T3: 50% and T4: 40% ETc. Values are means ± SD (n = 3). Means in the same column for each crop season, with different letters showed significant differences between treatments at $p < 0.05$ according to Tukey's test

The chlorophyll concentration in the 'Chemlali' and 'Koroneiki' oil varieties increased with decreasing irrigation treatment from the farmer (100% ETc) to the irrigation regime of 40% ETc. This concentration varied between 6.63 mg kg^{-1} (for 50% ETc) and 1.03 mg kg^{-1} (for farmer); it was six times higher in T3 than in the farmer. These findings were higher than those reported by Faghim *et al.* (2021) (1.03 ppm and 3.47 ppm) and in concordance with those of Baccouri *et al.* (2008). The effect of irrigation treatment on chlorophyll was highly significant. The level of chlorophylls increased with water stress and reached an optimal level at 50% ETc. Conversely, El Riachy *et al.* (2017) showed that the content of total chlorophyll in oils of the 'Edlbi' variety decreased with irrigation.

For all treatments, 'Koroneiki' olive oil exhibited the higher chlorophyll content (from 2.56 to 6.63 mg kg^{-1}) than 'Chemlali' (from 1.03 to 3.70 mg kg^{-1}) for the 2018 and 2019 crop seasons. This difference can be attributed to the maturity index of 'Koroneiki' (1.6 to 3.48) which is lower than those of 'Chemlali' (2.93 to 5.13). Under the same treatment, the result showed that the chlorophyll content of olive oil depends on the variety and the ripening of the fruit. These findings align with previous studies reporting that chlorophyll concentration depends on the cultivar, the degree of fruit ripening, and growing conditions (Bchir Ben Hassine *et al.*, 2021; Omri *et al.*, 2021; Tekaya *et al.*, 2022).

The reduction in irrigation treatment had an important effect on carotenoid pigments which increased, during crop season 2018, from 0.92 mg kg^{-1} (farmer) to 1.97 mg kg^{-1} (50% ETc) for 'Chemlali' and from 2.18 mg kg^{-1} (farmer) to 3.18 mg kg^{-1} (50% ETc) for 'Koroneiki'. In contrast, Gómez-Rico *et al.* (2007) reported that pigments were not influenced by irrigation, in concordance with results obtained by El Riachy *et al.* (2017). However, our findings indicate that water stress significantly improves the chlorophylls and carotenoids pigment reaching an optimal level at 50% ETc. These pigments, with their antioxidant properties, help prevent the human body from many diseases. Chlorophylls and carotenoids are responsible for the production of fruity oil with an attractive green color, which is a desirable criterion for sensorial analysis and acceptability for consumer use (Tekaya *et al.*, 2013; El Riachy *et al.*, 2017; Sicari *et al.*, 2021).

Effect of irrigation status on total phenolic (TP) content

The oils' phenolic content was influenced by irrigation regimes, studied cultivar and crop year (Table 3). For both varieties, 'Chemlali' and 'Koroneiki', olive oils showed the highest average of total phenolic content in 2018; while the lowest means were recorded in the 2019 crop season. Total phenolic content of the oils increased with water restraint; in fact, at 50% ETc and 40% ETc, total phenols exhibited the highest levels. Our findings were consistent with many researchers (Motilva *et al.*, 2000; Gómez-Rico *et al.*, 2007; Baccouri *et al.*, 2008; El Riachy *et al.*, 2017; El Yamani *et al.*, 2020; Conde-Innamorato *et al.*, 2022) that demonstrated higher levels of irrigation reduced the total phenol content in olive oils, with the highest concentrations were recorded in deficit-irrigated trees.

Differences in phenol content in oils, for both varieties and crop seasons, could be a consequence of the different levels of water stress in olives from farmer to different irrigation regimes. These conditions involved changes in the activity of enzymes responsible for phenolic compound synthesis, such as L-phenylalanine ammonia-lyase, whose activity is greater under higher water stress conditions (Gómez-Rico *et al.*, 2007; Patumi *et al.*, 1999; Tovar *et al.*, 2002); and its activity decreases as the amount of water applied increase (Patumi *et al.*, 1999; Tovar *et al.*, 2002).

It is well known that the phenolic compounds level in oil, due to its higher antioxidant properties, protected olive oil from oxidation, influences its sensory bitterness properties and ameliorate their health values. Bitterness is recognized as a positive sensory characteristic of olive oil and is frequently attributed to the presence of phenolic derivatives. Thus, the phenol oil richness of the variety, increase olive oil's shelf-life and quality (Tekaya *et al.*, 2022).

Effect of irrigation status on O-diphenols content

The result showed that the O-diphenols content was lower in oils obtained from fully irrigated trees by the farmer compared to those from the treatment with deficit irrigated at 60% ETc, 50%ETc and 40%ETc (Table 3). For both crop seasons 2018 and 2019 and for 'Chemlali' and 'Koroneiki' varieties, the concentration of O-diphenol olive oil increased with water stress. Thus, the O-diphenol oil contents from deficit irrigated trees at 50% ETc and 40%ETc were the highest. Previous similar researches reported that the contents of O-diphenol oil obtained from fully irrigated trees were lower than those originating from deficit-irrigated ones for Arbequina (Tovar *et al.*, 2001; Garcia *et al.*, 2013) and Frantoio (Caruso *et al.*, 2014) varieties.

Table 3. Total phenols and O-diphenol contents of 'Chemlali' and 'Koroneiki' olive oil under different water regimes (farmer (T1), 60%ETc (T2), 50% ETc (T3) and 40% ETc (T4)) during 2018 and 2019 crop seasons

Variety	Total phenols (mg eq catechin kg^{-1} of oil)				O-diphenols content (mg eq caffeic acid kg^{-1} of oil)			
	Chemlali		Koroneiki		Chemlali		Koroneiki	
Crop season/ Treatment	2018	2019	2018	2019	2018	2019	2018	2019
T1	169.36±9.05 C	100.76±4.53 B	179.97±11.3 bc	142.66±6.3 2A	57.73±0.52 Cd	40.38±0.52 F	55.07±0.43 d	49.14±0.43 DE
T2	202.47±7.6 Ab	98.15±0.67 B	198.99±7.38 ab	147.10±4.9 4A	58.97±1.43 Bc	42.3±1.44 EF	56.82±0.7 cd	57.91±0.71 B
T3	217.44±16.05 Ab	103.04±0.8 B	206.6±4.8 ab	143.46±3.9 2A	59.05±0.35 Bc	54.99±0.35 CD	65.41±1.94 a	64.92±1.94 B
T4	220.85±0.13 A	121.59±0.33 AB	202.78±7.6 ab	140.21±9.5 4A	55.07±1.55 D	60.83±0.66 BC	61.67±0.88 a	75.44±0.88 A

Full irrigation by the farmer (T1:100% ETc) and different deficit irrigation treatments (T2: 60% ETc, T3: 50% and T4: 40%ETc). Values are means ± SD (n = 3); At each crop season and for both varieties, different letters are statistically significant according to the Tukey test at $p < 0.05$

O-diphenol contents were affected by the deficit-irrigation strategy, with increasing values as water applied decreased. Deficit irrigation at 50%ETc and 40%ETc proved to be the most efficient treatment for obtaining a higher level of antioxidants in olive oil, which help preserve the olive oil's quality.

Effect of irrigation status on oil fatty acid composition

In previous research, the composition of fatty acids constitutes a criterion for oil classification (Faghim *et al.*, 2021; Motilva *et al.*, 2001). In this study, the oil composition in fatty acids showed differences between irrigation treatments (Table 4). Additionally, the response of olive trees to irrigation regimes varied significantly between varieties. Palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2) are considered the main fatty acids in 'Chemlali' and 'Koroneiki', which are affected by irrigation treatment. Although, 'Chemlali' variety noted the higher percentages of C16:0, C16:1 and C18:2; while 'Koroneiki' presented a richness in C16:0 and C18:1. These results align with those obtained with those obtained by Dabbou *et al.* (2010) for the Arbequina variety, which also showed that irrigation positively affected the palmitic acid level in oil. The oleic acid percentage, the main monounsaturated fatty acid, decreased under full irrigation treatment from 61.3 ± 0.16 in oil of 50% ETc (T3) to 58.30 ± 3.05 for Farmer (T1=100% ETc) for the 'Chemlali' variety. Meanwhile, the 'Koroneiki' virgin olive oils showed a clear variation of C18: 1 relative to the irrigation level between 78.22 ± 0.48 for 50% ETc (T3) to 74.12 ± 7.70 from trees submitted to 60% ETc (T2). Furthermore, the second most important unsaturated fatty acid, linoleic acid (C18:2) showed a great variation between 'Chemlali' (from 19.18 ± 0.14 to 16.15 ± 0.37) and 'Koroneiki' (from 5.20 ± 0.31 to 9.14 ± 0.17) varieties and was inversely proportional to the content of the oleic / linoleic acid ratio. In both seasons, the level of linoleic acid (C18:2) increased with the total irrigated treatment in 'Chemlali'. Whereas, this fatty acid decreased under full irrigation (100% ETc) in 'Koroneiki' cultivar. This variability could be caused by different varietal capacities of oleate desaturase enzymes (Hernández et al., 2021) that convert oleic acid (C18:1) into linoleic acid (C18:2) during the fruit maturation process (Boulfane *et al.*, 2015).

However, for both 2018 and 2019 crop years, the level of palmitic acid C16:0, the main saturated fatty acid, increased proportionally with the water request when 100% ETc was applied to olive trees. The same result was found by Dabbou *et al.* (2010) at 100% ETc irrigated trees. Furthermore, the sums and ratios of studied fatty acids revealed significant differences between varieties with 'Chemlali' exhibiting higher levels of SFA and PUFA and 'Koroneiki' higher MUFA, C18:1/C18:2, USFA/SFA and MUFA/PUFA ratios.

Table 4. The fatty acids content (%) of 'Chemlali' and 'Koroneiki' varieties under different irrigation treatments (Farmer, 60%ETc, 50% ETc and 40%ETc) during the 2018-2019 crop seasons

Year/ Variety	2018								2019							
	Chemlali				Koroneiki				Chemlali				Koroneiki			
Treatment	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**
Palmitic C16:0	18.31±0.81 a	17.83±0.59a	**17.26 ±0.85 a**	17.60 ±0.13 a	12.32 ±0.19 b	13.64 ±2.61 b	**12.34 ±0.27 b**	**12.07 ±0.21 b**	17.83 ±0.28 a	18.25 ±0.09 a	**17.04 ±0.08 a**	17.70 ±0.39 a	11.86 ±0.14 b	12.60 ±0.19 b	**12.32 ±0.12 b**	12.25 ±0.12 b
Palmitoleic C16:1	2.56± 0.04a	2.37 ± 0.20a	2.21± 0.05a	2.05± 0.16a	0.80± 0.11b	1.00± 0.60b	0.67± 0.07b	0.69± 0.01b	1.97± 0.09a	2.17± 0.11a	1.98± 0.18a	2.15± 0.22a	0.97± 0.06b	1.05± 0.01b	0.74± 0.04b	0.93± 0.06b
Margaric C17:0	0.01± 0.01a	0.01 ± 0.001a	0.01± 0.00a	0.01± 0.00a	0.01± 0.00a	0.01± 0.00a	0.01± 0.00a	0.01± 0.00a	0.01± 0.00 a	0.01± 0.00 a	0.01± 0.00 a	0.02± 0.01 a	0.01± 0.00 a	0.01± 0.00 a	0.01± 0.00 a	0.02± 0.0 a
Margaroleic C17:1	0.06± 0.00 abcd	0.07 ±0.01 ab	0.06± 0.01 abcd	0.07± 0.01 abc	0.08± 0.00 a	0.06± 0.0 abcd	0.07± 0.03 abc	0.06± 0.00a bcd	0.05± 0.01b cd	0.04± 0.00c d	0.04± 0.00 e	0.05± 0.01b cd	0.03± 0.01 e	0.04± 0.01c d	0.04± 0.01 e	0.04± 0.00c d
Stearic C18:0	2.14± 0.03 bcd	2.15 ±0.12bcd	2.08± 0.16c d	1.94± 0.03d	2.47± 0.08a bc	2.55± 0.04a b	2.24± 0.33a bc	2.48± 0.18a bc	2.30± 0.06a bcd	2.06± 0.11c d	2.42± 0.18a bc	2.31± 0.38a bcd	2.56± 0.09a b	2.67± 0.04 a	2.55± 0.05a b	2.50± 0.36a bc
Oleic C18:1	58.30± 3.05 b	59.84±1.67b	**61.21 ±0.98 b**	58.46 ±0.09 b	77.99 ±0.52 a	74.12 ±7.70 a	**78.22 ±0.48 a**	**78.57 ±0.59 a**	57.74 ±0.19 b	57.10 ±0.28 b	**61.63 ±0.16 b**	58.64 ±0.19 b	76.98 ±0.25 a	76.49 ±0.52 a	74.50 ±0.24 a	74.76 ±0.25 a
Linoleic C18:2	17.73± 2.15 abc	16.85±1.02bc	**16.22 ±1.34 c**	18.91 ±0.79 ab	5.18± 0.34 f	5.45± 0.22 f	**5.39± 0.03 f**	**5.20± 0.31 f**	19.18 ±0.14 a	19.49 ±0.07 a	**16.15 ±0.37 c**	18.25 ±0.20 abc	6.72± 0.07 ef	6.39± 0.17 f	9.14± 0.17 d	8.76± 0.02 de
Linolenic C18:3	0.56± 0.11 ab	0.54 ± 0.05 ab	0.55± 0.12 ab	0.60± 0.01 ab	0.61± 0.07 ab	0.40± 0.16 b	0.55± 0.08 ab	0.50± 0.11 ab	0.64± 0.05 a	0.62± 0.01 ab	0.47± 0.016a b	0.60± 0.01 ab	0.49± 0.03 ab	0.50± 0.03 ab	0.45± 0.02 ab	0.48± 0.01 ab
Arachidic C20:0	0.19± 0.03 bc	0.22 ±0.02 abc	0.25± 0.06 abc	0.22± 0.00 abc	0.34± 0.08 a	0.26± 0.10 abc	0.29± 0.08 ab	0.25± 0.08 abc	0.18± 0.01 bc	0.18± 0.01 bc	0.17± 0.01 bc	0.20± 0.01 abc	0.24± 0.03 abc	0.15± 0.03 c	0.16± 0.02 bc	0.17± 0.01 bc
Gondoïc C20:1	0.12±0.01 cdefg	0.13 ±0.00 cdef	0.14± 0.02 bcd	0.14± 0.01 bcde	0.20± 0.00 a	0.17± 0.01 abc	0.21± 0.03 a	0.18± 0.04 ab	0.08± 0.01 gh	0.08± 0.03 gh	0.08± 0.01 h	0.07± 0.02 h	0.14± 0.01 bcde	0.10± 0.01 defgh	0.09± 0.01e fgh	0.10± 0.15 defgh
Saturated fatty acids (SFA)	20.66± 0.76 a	20.21±0.45a	19.60 ±0.67 a	19.77 ±0.90 a	15.14 ±0.29 b	16.46 ±2.68 b	14.88 ±0.63 b	14.80 ±0.42 b	20.33 ±0.30 a	20.51 ±0.13 a	19.65 ±0.32 a	20.23 ±0.28 a	14.67 ±0.19 b	15.43 ±0.26 b	15.05 ±0.17 b	14.94 ±0.40 b
MUFA	61.04± 3.02b	62.41±1.46b	63.62 ±2.02 b	60.72 ±0.98 b	79.07 ±0.57 a	75.35 ±7.10 a	79.17 ±0.28 a	79.50 ±0.55 a	59.84 ±0.28 b	59.39 ±0.22 b	63.72 ±0.35 b	60.92 ±0.48 b	78.12 ±0.18 a	77.68 ±0.43 a	75.36 ±0.21 a	75.82 ±0.40 a
PUFA	18.29± 2.26 abc	17.39±1.02bc	**16.78 ±1.36 c**	19.51 ±0.77 ab	5.79± 0.32 f	5.86± 0.38 f	5.95± 0.45 f	5.70± 0.33 f	19.83 ±0.1 A	20.10 ±0.09 a	**16.63 ±0.01 c**	18.85 ±0.20 abc	7.21± 0.09 ef	6.89± 0.19 f	9.59± 0.16 d	9.24± 0.25 de
USFA	79,34± 5.28 e	79,79±2.47 de	80,40 ±3.37 abcde	80,227±1.62 abcde	84,86 ±0.88 abc	81,21 ±7.47 abcde	85,12 ±0.73 a	85,20 ±0.88 a	79,67 ±0.37 bcde	79,49 ±0.30 de	80,35 ±0.66 abcde	79,77 ±0.48 bcde	85,33 ±0.28 a	84,57 ±0.62 abcd	84,95 ±0.36 ab	85,06 ±0.64 a
Oleic /Linoleic	3.33±0.54 e	3.56 ±0.30 e	3.80± 0.46 e	3.10± 0.15 e	15.10 ±1.04 a	13.64 ±1.91 ab	14.58 ±1.32 a	15.15 ±0.94 a	3.01± 0.02 e	2.93± 0.02 e	3.82± 0.01 e	3.21± 0.02 e	11.46 ±0.13 c	11.97 ±0.38 bc	8.16± 0.18d	8.54± 0.28d
MUFA /PUFA	3.38± 0.55 e	3.60 ±0.28 e	3.81± 0.42 e	3.12± 0.17 e	13.68 ±0.82 a	12.95 ±1.98 ab	13.37 ±1.03 a	13.99 ±0.91 a	3.02± 0.02 e	2.95± 0.02 e	3.83± 0.01 e	3.23± 0.02 e	10.84 ±0.15 d	11.28 ±0.37 bc	7.86± 0.15 d	8.21± 0.27 d
USFA/SFA	3.84±0.17b	3.95 ±0.10b	4.10± 0.17b	4.05± 0.09b	5.60± 0.12a	4.93± 0.12b	5.71± 0.28a	5.75± 0.19a	3.911 ±0.07 b	3.87± 0.03b	4.09± 0.03b	3.94± 0.11b	5.81± 0.08a	5.48± 0.10a	5.60± 0.02a	5.69± 0.06a

Values are means ± SD (n = 3). Means in the same row with different letters, for both crop season and varieties, showed significant differences between treatments ($P < 0.05$) according to Tukey's test

USFA: Unsaturated fatty acids**;** **MUFA:** Mono-unsaturated fatty acids; **PUFA**: Polyunsaturated fatty acids**;** **SFA:** Saturated fatty acids

The irrigation regimes mainly affected the fatty acid composition of the oils (Table 3). In both varieties, C16:0, C16:1, C18:1, C18:2, SFA, MUFA, PUFA, C18: 1/ C18: 2, and MUFA/PUFA were the most affected. Besides, C18:0, C17:0 and C17:1 presented inconsistent differences between varieties. Palmitic acid showed a higher value in the oil from the fruits of full-irrigated trees by the farmer.

Nonetheless, oils from both cultivars exhibited higher levels of oleic acid and mono-unsaturated fatty acids under irrigation stress regimes, which is consistent with prior investigations (Stefanoudaki *et al.*, 2001; El yamani *et al.*, 2020). Higher percentage of MUSFA and oleic acid are considered a key quality characteristic of olive oil (El Riachy *et al.*, 2017). Furthermore, the ratio of USFA/SFA was influenced by different irrigation regimes with the highest values in the oils of trees watered with 50% ETc and 40% ETc. The results of this study agree with those obtained by Stefanoudaki *et al.* (2001) and El Riachy *et al.* (2017) who reported higher ratio of unsaturated /saturated fatty acids to the oils from water restriction trees.

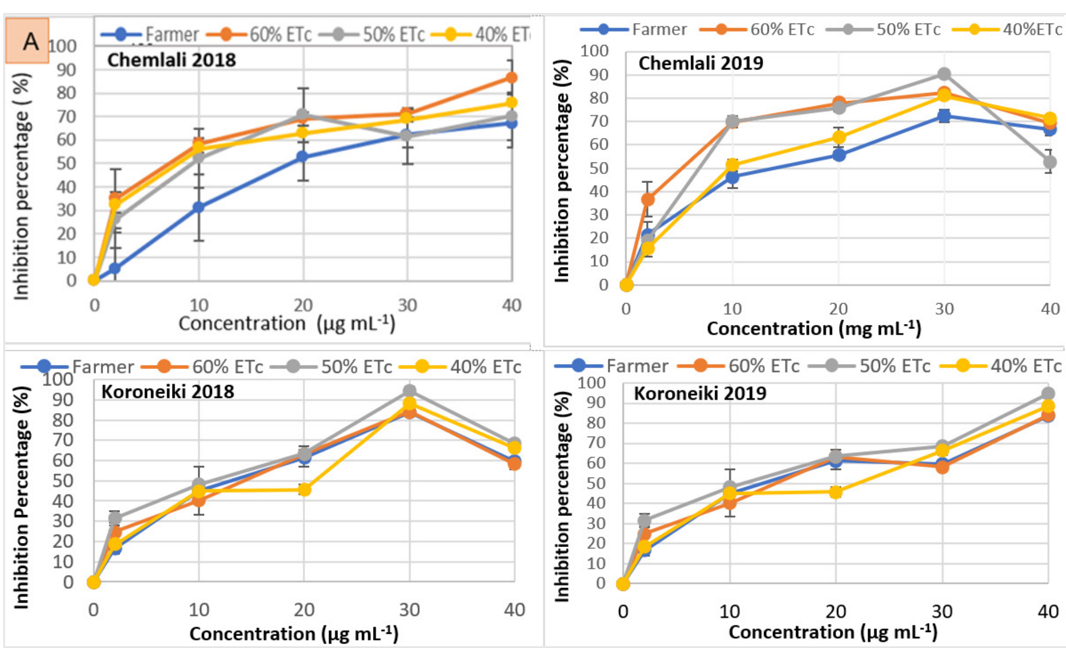
Thus, the restoration of the 50% ETc treatment proved to be the most effective irrigation regime for increasing the oleic acid content and reducing the palmitic acid of the oils from both cultivars, thereby achieving a good oil content. These results are in accordance with those of Sánchez-Rodriguez *et al.* (2019), who showed that a moderate irrigation strategy (or at 'moderate' stress levels) improved phenols content and slightly enriched the fatty acid profile by increasing oleic acid and simultaneously decreasing saturated fatty acids.

Impact of irrigation status on antioxidant activity of olive oil

Several factors affect the antioxidant activity of olive oils including the fruit ripening index, cultivar, climatic conditions, seasonal changes and olive growing conditions Sevim *et al.* (2019). In this study, the antioxidant properties of oils were determined by two methods DPPH and $ABTS^{\bullet+}$ radicals, both commonly used to evaluate the antioxidant power of olive oil, as they provide a good prediction of its sensitivity to oxidative degradation.

<u>DPPH method</u>

The antioxidant activity of oils from both varieties, during the first and second year, were determined between the irrigation treatments towards the DPPH test (Figure 5).



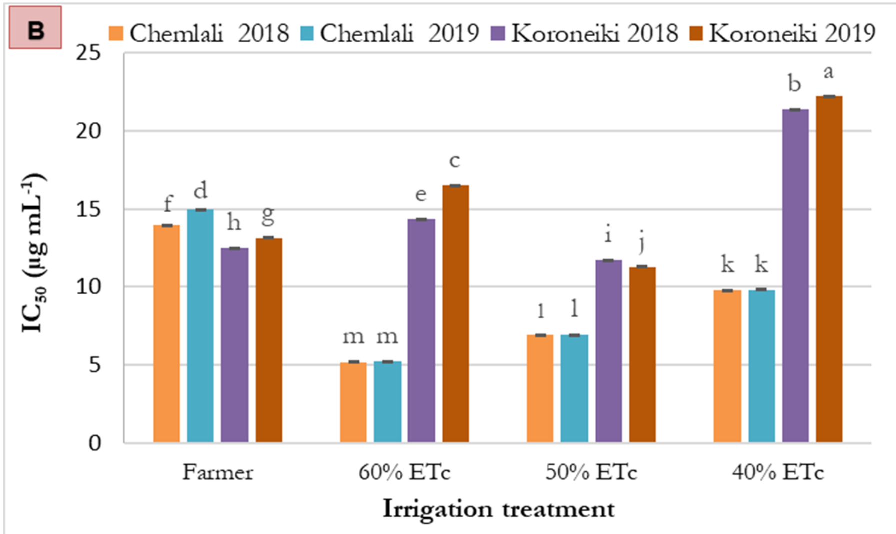


Figure 5. Antioxidant activity (A) and the IC50 (B) of olive oil of 'Chemlali' and 'Koroneiki', towards DPPH radical, under different irrigation doses and during 2018 and 2019 crop seasons
IC50: 50% of free radicals' inhibitory concentration. Values are means ± SD (n = 3). Means with different letters showed significant differences between treatments ($P < 0.05$) according to Tukey's test

The result showed that irrigation treatment affects the antioxidant property of oils for both varieties. The DPPH radical scavenging activity of oils increased as the irrigation level decreased, with the highest activity observed in the 60% ETc and 50% ETc water regimes. In fact, in the 2018 crop season, the antioxidant activity of the oils, for both varieties, was around 80% at the concentration of 40 µg mL^{-1}. Whereas, in the 2019 crop season, the highest antioxidant property was observed at a concentration of 30 µg mL^{-1} for all irrigation regimes.

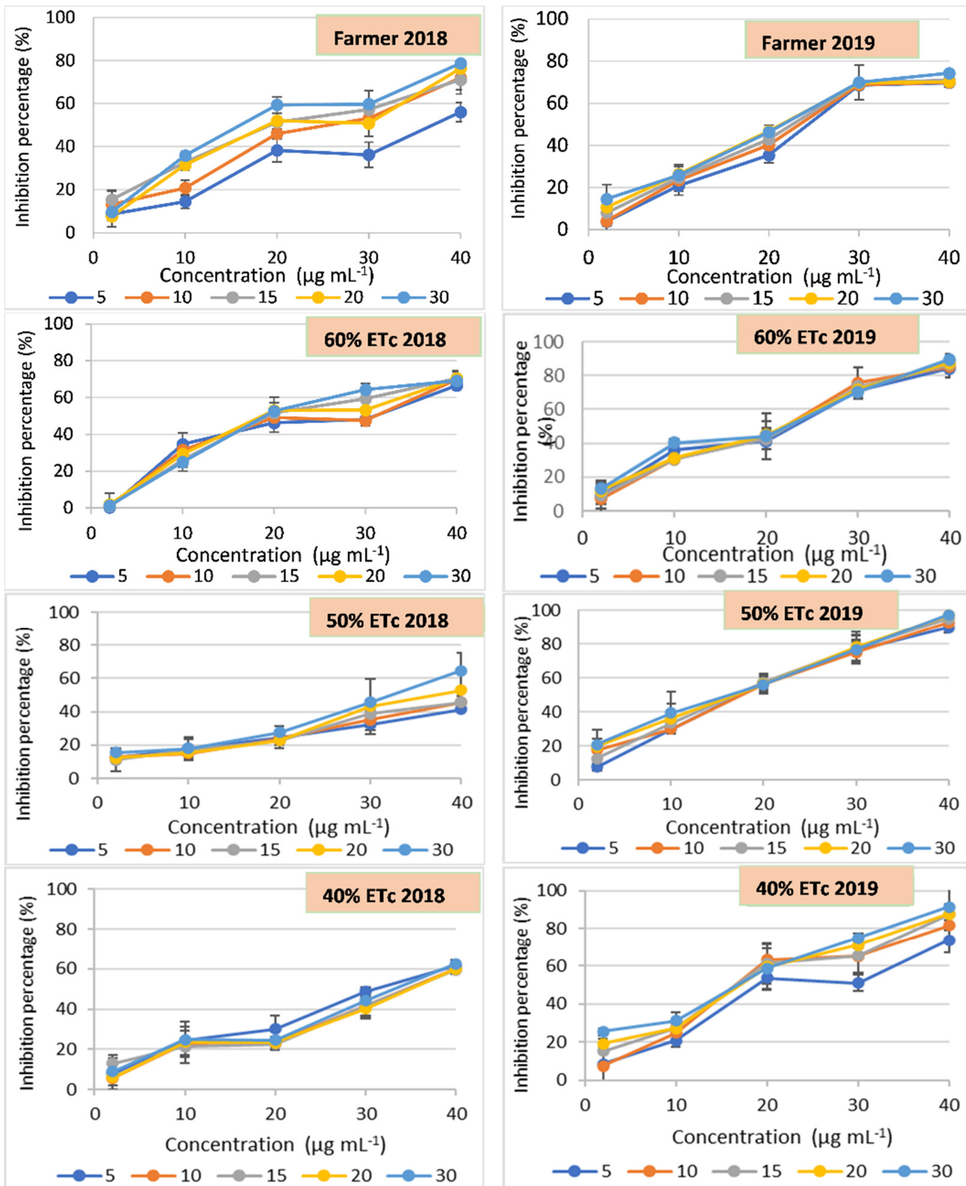
The powerful capacity to scavenge DPPH radicals by olive oil extracts (for both varieties) could attributed to the richness of oils in phytochemical and the ability of polyphenols to protect against oxidants. Furthermore, we can note that water stress may increase the synthesis of antioxidants compounds in oils aiding the olive tree's defense against oxidative stress. Several authors have reported that antiradical activity is directly related to the content of total phenolic compounds (Pourmorad *et al.*, 2006; Bouaziz *et al.*, 2008). A high correlation between antioxidant property and total phenol, Ortho-diphenols, carotenoids, and tocopherol contents has also been demonstrated by Deflaoui (2009), Kouka *et al.* (2019) and Fratianni *et al.* (2019).

The IC_{50}value defined as the amount of antioxidant activity needed to scavenge 50% of DPPH radicals. A lower IC_{50} value indicates a higher antioxidant property (Yorulmaz and Konuskan, 2017). As shown in Figure 5, moderate water restriction, at 40% ETc and 50% ETc, increased the effective antioxidant activity of oils for both varieties. Furthermore, a moderate water restriction (50% ETc) promotes olive trees, enhancing the synthesis and accumulation of polyphenols and antioxidant compounds in olive oils, which enhances their antioxidant potential at lower concentrations. In fact, 11 µg mL^{-1} of 'Koroneiki' oil could scavenge 50% of DPPH free radicals; when only 5 µg mL^{-1} of 'Chemlali' olive oil could detoxify 50% of DPPH radicals. These findings highlight powerful antioxidant properties over 'Carrasquena', 'Arbequina' and 'Corniche' oils studied by Franco *et al.* (2014); than the IC_{50} of Italian varieties 'Coratina' and 'Maiatica' about 31.9 and 53.4 µL of oil, respectively (Condelli *et al.*, 2015).

These results are consistent with previous studies (Sevim *et al.*, 2019; Gorinstein *et al.*, 2003), which showed that the antioxidant activity is affected by antioxidants (polyphenols, tocopherol, carotenoids, chlorophylls) of olive oils. These researchers also reported that DPPH radical scavenging activity of olive oils increased significantly as the irrigation level decreased. Furthermore, the 'Chemlali' local variety oil has a stronger antioxidant activity compared to the introduced variety, 'Koroneiki', under all irrigation treatments.

<u>ABTS method</u>

The evolution of the antioxidant activity of oils from both varieties, 'Chemlali' and 'Koroneiki', towards ABTS$^{•+}$cation radicals, is illustrated in Figure 6 (A and B).

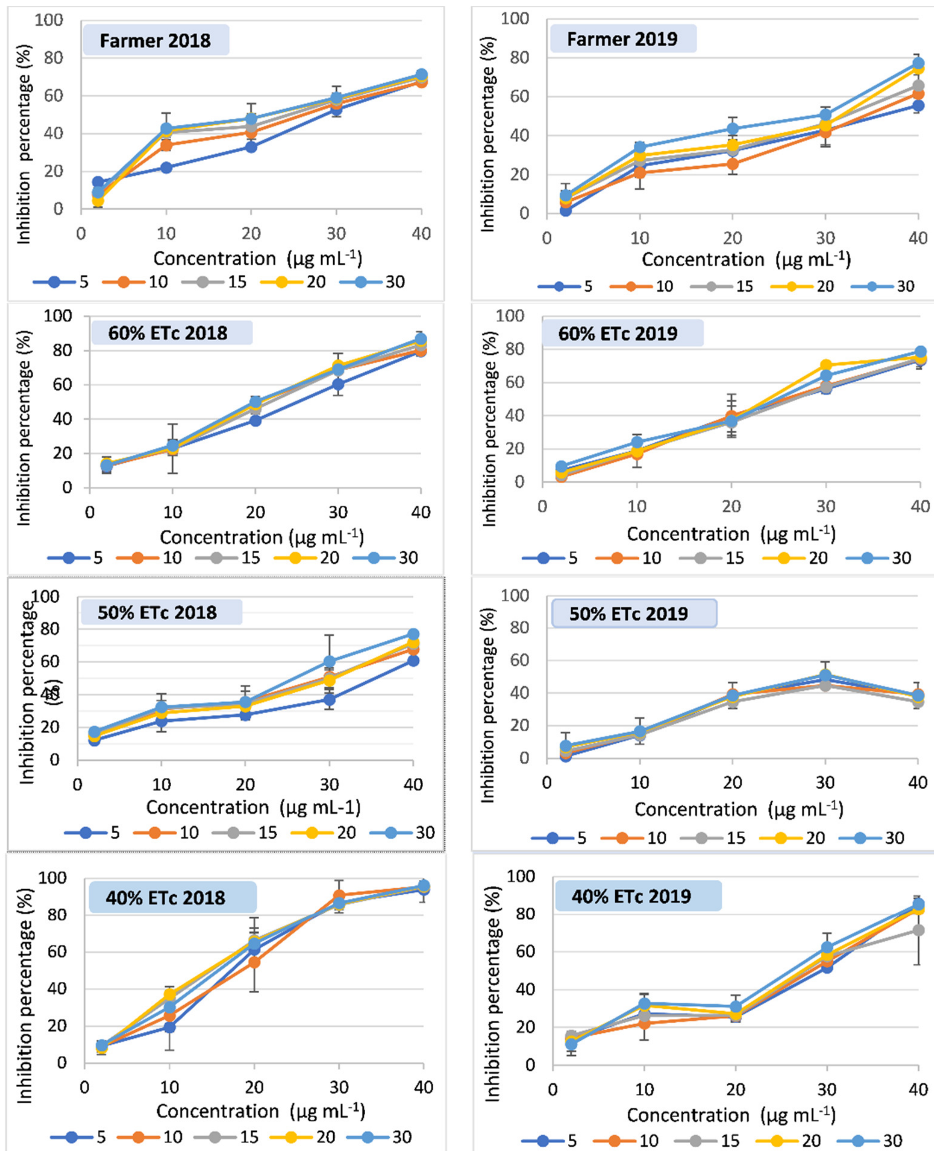


(A)

Figure 6 (A). Antioxidant activity of 'Chemlali' (A) and 'Koroneiki' (B) olive oils towards ABTS$^{•+}$ cation radical, according to different irrigation treatments and during 2018 and 2019 crop seasons

Values are means ± SD (n = 3). The antioxidant activity of different concentration of olive oils were tested at different incubation times: 5; 10; 15; 20 and 30 min

For each variety, the result showed the antioxidant property of oils, increased with longer contact times, higher oil concentrations, and under reduced irrigation treatments. As illustrated in Figure 6B, irrigation treatment of 40% ETc and 50% ETc corresponded to the maximum activities reaching 100% inhibition of ABTS$^{•+}$ radicals. It was determined that ABTS$^{•+}$ radical scavenging activity (RSA) of the oils increased significantly as the irrigation level decreased (Baccouri *et al.*, 2007). For the 'Chemlali' variety, the antioxidant activity of oil increased with oil concentrations, incubation time, and water treatment. The highest oil activity was reached at a concentration of 40 μg mL^{-1} under a 50% ETc and 40% ETc water treatmtnt, especially during the 2019 growing season, which reached 100% inhibition. These results are consistent with those of Baccouri *et al.* (2007) who also noted that irrigation influenced the oil $ABTS^{\bullet+}$ radical scavenging activity in cv. 'Chemlali'.



(B)

Figure 6(B). Antioxidant activity of Koroneiki (B) olive oils towards $ABTS^{\bullet+}$ cation radical. Values are means ± SD (n = 3)

However, the 'Koroneiki' variety, during both crop seasons, showed the highest antioxidant activity at 40% ETc irrigation treatment. It appears that the olive oil extract at a concentration of 40 μg mL^{-1} was able to scavenge more than 90% of ABTS free radicals. These results were in accordance with those of De Falco *et al.*

(2021) which showed that irrigation has a significant impact on metabolite content, antioxidant activity and phenols content with higher values found under 50% water supply compared to full irrigation by the farmer.

Principal component analysis (PCA)

Principal Component Analysis (PCA) was applied to group and separate the analyzed variables in the oil obtained after different irrigation treatments of 'Chemlali' and 'Koroneiki' olive trees. The performed PCA was applied, for both varieties, to the maturity index of the fruits, the quality parameters (Free acidity, K232, K270, peroxide index), total phenols, O-diphenols, pigments such as chlorophylls and carotenoids, fatty acids composition and the antioxidant activity explained by IC50 against DPPH and TEAC of different oils (Figure 7).

The Principal Component Analysis (PCA) of the 'Chemlali' variety is illustrated in Figure 7 (I (A and B)). The variance explained by the two first components is 44.09% and 23.23% for the first and the second, respectively accounting for a total of 67.32% of the variability.

The first axis (Axe 1), representing about 44.09% of the total variance, was positively associated with oleic acid (C18:1), monounsaturated fatty acid (MUFS), unsaturated fatty acid, the ratio of unsaturated fatty acid (USFA)/saturated fatty acid (SFA). These unsaturated fatty acids were negatively related to IC_{50}, which contributes to the high antioxidant activity of oil. The richness of oil in monounsaturated and unsaturated fatty acids increases the nutritional quality and stability of oil (Ayton, 2006).

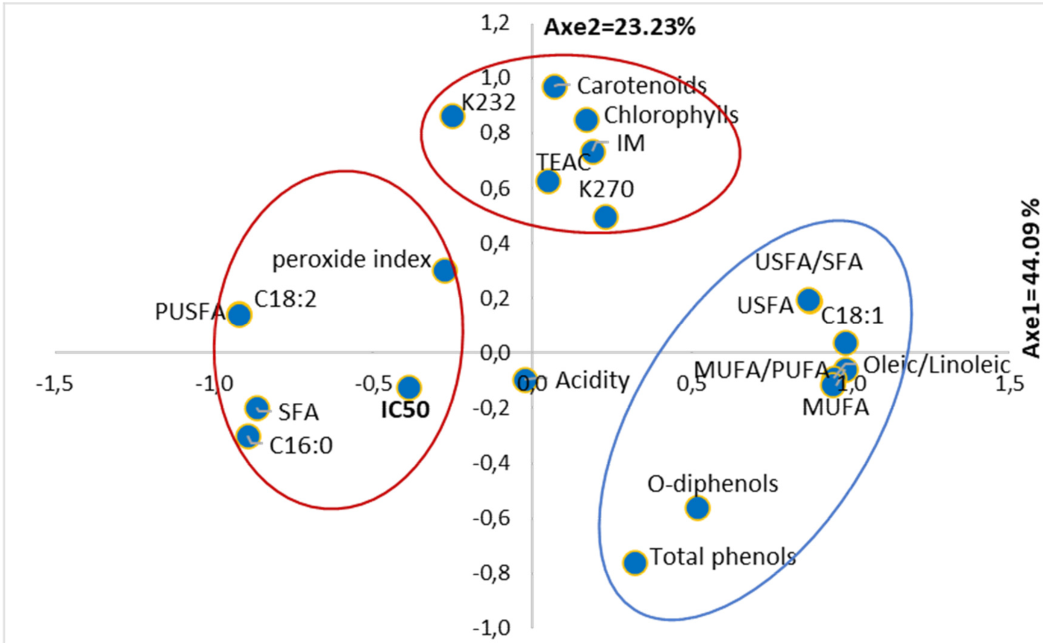
Axis 1 was also correlated with the most antioxidant compounds such as carotenoids, chlorophylls, phenols and O-diphenols which were negatively related to IC50 (Figure 7 (IA)). This correlation indicated a high antioxidant activity of 'Chemlali' oil due to its content of these antioxidants. These compounds, which contribute to the pungency and bitterness of the oil, improved the quality and shelf-life stability of the oil (by the oil against oxidation) and its nutritional values (Ayton, 2006). On the opposite side, another group of highly correlated variables was characterized by saturated fatty acids (SFA) and palmitic acid (C16:0) which positively correlated with IC_{50} reflecting the low antioxidant activity of oil.

The second axis (Axe 2), representing 23.23%, was positively correlated with chlorophylls, carotenoids, K270, TEAC and maturity index (MI). Furthermore, the antioxidant activity represented by IC_{50} was highly and inversely correlated with these antioxidants. The maturity index largely influenced the carotenoids and chlorophyll content in 'Chemlali' oils.

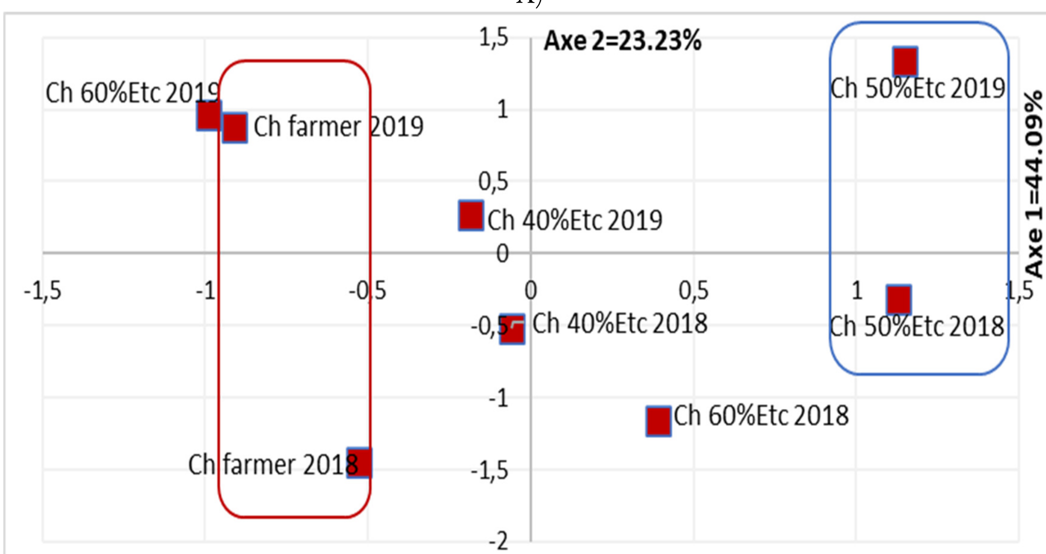
The biplot plan showed a clear differentiation between the oils obtained from the olive orchards irrigated by the farmer and those obtained after different irrigation treatments (Figure 7 (IB). The projection of the different irrigation treatments in the plan generated by the two axes showed that the 50% ETc treatment was distinct from the others. 'Chemlali' oil treated with 50% ETc for the 2018 and 2019 crop seasons was plotted in the upper right quadrant. According to this distribution, the oil from 'Chemlali' olive trees irrigated with 50%ETc is characterized by a high percentage of MUFA, high content of oleic acid C18:1, high content of phenols, O-diphenols, chlorophyll and carotenoids and antioxidant properties.

The richness of the oil in monounsaturated fatty acid, especially oleic acid, and the antioxidant compounds can enhance oil's shelf life and stability (Fernandez *et al.*, 2013). Research of García-Tejero and Duran (2018) also showed that irrigation decreases photosynthetic pigments, polyphenol contents and favors the unsaturation of fatty acids in oils.

This outcome suggests that water management, through irrigation of the olive trees with 50% ETc under semi-arid climatic conditions, during the two crop seasons increased the quality of 'Chemlali' oil.



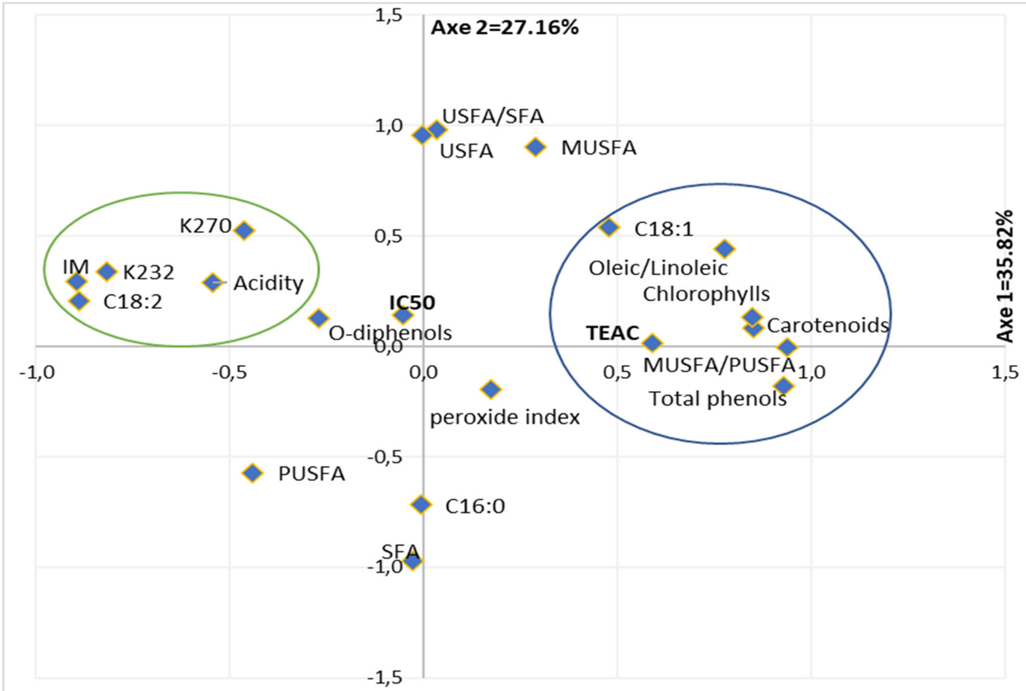
A)



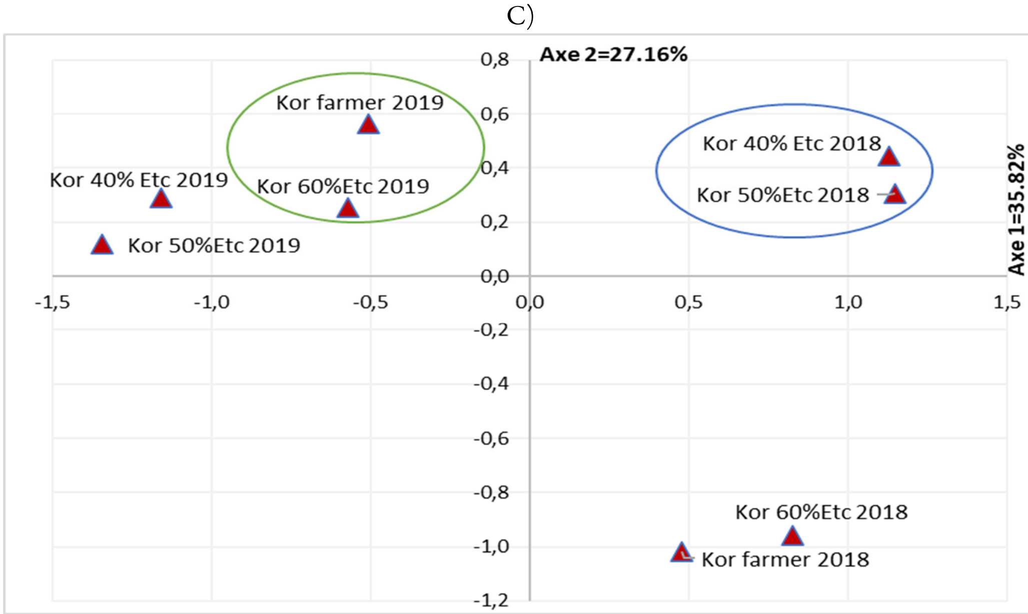
B)

(I) 'Chemlali'

Figure 7. IA-IB Principal Component Analysis of 'Chemlali' (I) and 'Koroneiki' (II) olive oil subjected to different irrigation treatments (A and C). Groups of individuals contributing to axes 1 and 2 (B and D) (A and C). SFA: Saturated fatty acid; C16:0: Palmitic acid; C18:0: Stearic acid; C18:1: Oleic acid; C18:2: Linoleic acid; MUFA: Monounsaturated fatty acid; PUFA: Polyinsaturated fatty acid; MUFA/PUFA: Monounsaturated fatty acid/Polyinsaturated fatty acid ratio; USFA/SFA: Unsaturated fatty acid/ Saturated fatty acid ratio; Oleic/ Linoleic: Oleic acid/ linoleic acid ratio; IC50: Inhibition percentage of 50% of free radicals; TEAC: Trolox equivalent antioxidant capacity. (B and D); MI: maturity index. Ch: 'Chemlali' variety; Kor: 'Koroneiki' variety, crop evapotranspiration (Etc).



C)



D)

(II) 'Koroneiki'

Figure 7. IIC-IIC Principal Component Analysis of 'Chemlali' (I) and 'Koroneiki' (II) olive oil subjected to different irrigation treatments (A and C). Groups of individuals contributing to axes 1 and 2 (B and D) (A and C). SFA: Saturated fatty acid; C16:0: Palmitic acid; C18:0: Stearic acid; C18:1: Oleic acid; C18:2: Linoleic acid; MUFA: Monounsaturated fatty acid; PUFA: Polyinsaturated fatty acid; MUFA/PUFA: Monounsaturated fatty acid/Polyinsaturated fatty acid ratio; USFA/SFA: Unsaturated fatty acid/ Saturated fatty acid ratio; Oleic/ Linoleic: Oleic acid/ linoleic acid ratio; IC50: Inhibition percentage of 50% of free radicals; TEAC: Trolox equivalent antioxidant capacity. (B and D); MI: maturity index. Ch: 'Chemlali' variety; Kor: 'Koroneiki' variety, crop evapotranspiration (Etc).

As for the introduced variety 'Koroneiki', all the collected data were subjected to principal component analysis (PCA) to better understand the changes in the pigments, total phenols, quality parameters and fatty acids of the studied oils under the four irrigation treatments. The average values of these compounds were used to build the PCA. The two first principal components (Axe 1 and Axe 2) explained 62.98% of the variance; with Axe 1 contributing 35.82% and Axe 2 contributing 27.16% (Figure 7 (II C)). The biplot plan illustrated the projection of different irrigation treatments in the plan generated by the two axes (Figure 7 (II D)).

The unsaturated and monounsaturated fatty acids, the oleic acid, the MUFA/PUFA ratio, the chlorophylls, the carotenoids, the TEAC (Trolox equivalent antioxidant capacity), and the total phenolic content of oils were highly and positively correlated with Axe 1. Moreover, the antioxidant activity represented by IC_{50} was highly and negatively correlated with total phenols, pigments, and fatty acids. This indicates that this group has powerful antioxidant.

Data from the 2018 harvest indicated that optimal oil richness in antioxidant compounds and mono-unsaturated fatty acids was achieved over a wide range of irrigation treatments of 40% ETc and 50% ETc. For this reason, oil quality must also be considered when optimizing water usage (Grattan *et al.*, 2006).

According to PCA, the response of the local 'Chemlali' and the introduced 'Koroneiki' cultivars to different irrigation treatments was different. The oil content in total phenols, O-diphenols, pigments, oleic acid, the unsaturated fatty acid/saturated fatty acid ratio and the oleic acid/ linoleic acid ratio were most prominent under the irrigation treatment of 50% ETc for 'Chemlali', and under the treatment of 50% ETc and 40% ETc for the 'Koroneiki' cultivar. However, increasing irrigation delayed changes in the fruit's skin color and altered several oil parameters. Irrigation also decreased photosynthetic pigments, reduced the total phenol content, and increased the saturated and polyunsaturated fatty acids of oils. These findings agreed with those of El Yamani *et al.* (2020) and Fernández *et al.* (2018), who showed that irrigation favoured the decrease in polyphenol content, the decline in photosynthetic pigments, and the increase in fatty acid unsaturation. In addition, Fernandez *et al.* (2013) showed that Changes in the ratio of unsaturated/saturated fatty acids affect the organoleptic properties of olive oil, with the high content of saturated fatty acids declined the quality and the stability of the oil.

The changes in phenolic compounds and pigments in the extracted oils varied mainly according to the variety, the crop season, and especially the water applied. The PCA results proved that appropriate irrigation can optimize the quality and phenolic concentrations of virgin olive oil. Consequently, the irrigation level of 50% of ETc seemed to be sufficient to achieve a higher content of antioxidant compounds. These results could be useful and of noted importance for farmers, who are encouraged to reduce the irrigation levels to improve the quality and stability of olive oil through enriching with antioxidant compounds. Further studies are needed to assess the effects of different irrigation levels in terms of productivity and oil quantity and quality at additional crop seasons and at different ripening degrees.

Conclusions

In Tunisia, around 95% of the olive fields are not irrigated. Most olive trees are cultivated under semi-arid and arid conditions characterized by water scarcity. To ensure the sustainability of olive production, adopting modern irrigation technique is required. It is very important to gain habits towards the producers due to the irrigation novelty of olive cultivation. A good determination of agricultural irrigation strategies is essential to obtain regular crops, high yields, and high-quality olive oil every year in olive growing. For this reason, our study was carried out under four irrigation treatments such as 100% ETc, 60% ETc, 50% ETc and 40% ETc.

Our research showed that olive oil quality parameters improved under water stress. In fact, in both crop seasons, deficit irrigation of olive trees with 50% ETc and 40% ETc appeared to be beneficial in decreasing acidity, peroxide index, K232 and K270, while maintaining high oil quality. Moreover, moderate water restriction promoted olive trees to provide the synthesis and the accumulation of polyphenols and bioactive compounds in olive oils enhancing their antioxidant potential in lower concentrations. Overall, these results suggest that the oil obtained from olive tree treated with irrigation of 50% ETc is characterized by a higher content of total phenols, O-diphenols, chlorophylls and carotenoids, along with superior antioxidant property for both 'Chemlali' and 'Koroneiki' cultivars.

Additionally, the irrigation treatments had a significant impact on the main fatty acids. During both crop seasons and for both varieties, the deficiency of irrigation, especially the 50% ETc treatment of the olive trees, resulted in higher content of oleic acid and lower level of palmitic acid. Irrigation increased the saturated and polyunsaturated fatty acids in the oils.

The principal component analysis (PCA) showed that the varietal response to various irrigation treatments was observed. The oil content in total phenols, O-diphenols, pigments, oleic acid, the unsaturated fatty acid/saturated fatty acid ratio, and the oleic acid/ linoleic acid ratio were the most important at the irrigation treatment of 50% ETc for 'Chemlali' and the treatment of 50 % ETc and 40% ETc for 'Koroneiki' cultivar. Using 50% ETc irrigation is considered the best treatment optimizing water management, improving oil quality and stability, oil antioxidant content and properties, and could increase oil productivity.

These interesting preliminary results warrant further investigated in order to draw solid conclusions that can help in choosing the irrigation strategy for olive crop with the best compromise between water use efficiency, oil productivity and quality.

Authors' Contributions

Conceptualization: SBMG; AB; DS; Data curation: SBMG and DS; Formal analysis; Funding acquisition: AB; Methodology SBMG and DS; Supervision MB and DM; Writing and editing the original draft: SBMG. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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