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Effects of Irrigation Regimes on Fatty Acid Composition, Antioxidant and Antifungal Properties of Volatiles from Fruits of *Koroneiki* Cultivar Grown Under Tunisian Conditions

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Abstract: The olive tree is generally grown under rain-fed conditions. However, since the yield response to irrigation is great, even with low amounts of water, there is increasing interest in irrigated agriculture. The main goal of this study was, therefore, to investigate the effect of irrigation regimes on olive (*Olea europaea* L., cv. *Koroneiki*) obtained from an intensively-managed orchard in a semi-arid area with a Mediterranean climate in Tunisia. Different irrigation treatments 50% ETc, 75% ETc and 100% ETc were applied to the olive orchard. Accordingly, the effects of three irrigation regimes on volatile compounds, fatty acid composition and biological activities of *Koroneiki* cultivar were studied. The total profile of the volatile constituents of all samples revealed the predominance of 3-ethenylpyridine (from 14.9-19.6%), phenylethyl alcool (from 7.8-19.2%) and benzaldehyde (from 9.0 to 13.8%). During watering level treatments studied, the major fatty acids were oleic, palmitic and linoleic. Antioxidant activity of the fresh fruit volatiles cultivated at a watering level of 100% ETc was higher than that obtained under 50 and 75% Etc. The results of antifungal activity showed that the fruits volatiles of the three irrigation treatments had varying degrees of growth inhibition against the microorganisms tested.

Key words: Irrigation, olive cultivar, volatile compounds, fatty acids, antioxidant activity, antifungal activity

INTRODUCTION

Aromatic plants and their essential oils, mixtures of natural volatile compounds are gaining increasing interest because of their biological activities. These activities have been known for many thousands of years for their antibacterial, antifungal and antioxidant properties (Deans and Waterman, 1993). The aromatic plants are the source of natural antioxidants (Smelcerovic *et al*., 2006). Their main secondary metabolites, (poly)phenolic compounds can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation. They also have metal chelation properties (Rice-Evans *et al*., 1997). Thus, the volatile compounds have great application and demand in food, perfumery, cosmetics and pharmaceutical industries. In our search for commercially useful volatile compounds, *Olea europaea* L. was selected as one of the plants for study.

This plant is one of the main crops of the Mediterranean basin, which are now increasingly grown elsewhere. In the last decade, the worldwide area devoted to their cultivation has increased by 10% (FAOSTAT, 2007). But in areas with a Mediterranean climate, characterized by little or no rainfall during the most critical phenological stages of production, intensive olive growing is barely feasible without irrigation. Therefore, irrigation is a vital factor in improving both production and productivity (Moriana *et al*., 2003). In olive, irrigation can increase production (Samish and Spiegel, 1961; Lavee *et al*., 1990; Girona, 1996; Moriana *et al*., 2003) thereby affecting better the total oil production per tree. However, little and contradictory data revealed the amount of seasonal water, necessary to obtain quality-quantitatively good productions from different olive cultivars. Generally, the differences are influenced by varying degrees of cultivar adaptability to the pedoclimatic conditions and agronomic practices adopted in the field trials (Dettori *et al*., 1989; Patumi *et al*., 1999).

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In recent years, drip irrigation has become increasingly popular to reduce the amount of water and fertilizer that are applied to the crop, and also to reduce the amount of labor (Tan, 1995; Hanson *et al*., 1997; Yohannes and Tadesse, 1998). Then, drip irrigation is a vital factor, in areas where water is limited, in improving production and productivity, since these features make it potentially much more efficient than other irrigation methods. Therefore, a proper drip irrigation rate can affect both the minimum amount of water leached from the root zone and maintains a high soil matrix potential in the rhizosphere to reduce plant water stress. As a consequence, accurate information on yield responses in light of the amount of water applied by drip irrigation would be required to achieve the best drip irrigation management. However, it is very relevant to remark that any type of irrigation management employed in the olive orchard must take into account the ultimate effect on the volatile compounds and fatty acid composition, especially with regard to its content and profiles. Additionally, the chemical composition of olives depend on many agronomical factors, e.g., olive cultivar (Esti *et al*., 1998; Romani *et al*., 1999), pedoclimatic conditions (Vinha *et al*., 2005) and irrigation management (Patumi *et al*., 2002; Tovar *et al*., 2002). In fact, the studies on the effect of irrigation on the fatty acid composition of the olive fruit are contradictory (Gatto, 1989; Milella and Dettori, 1987; Lavee and Wodner, 1991).

Nevertheless, currently there is a lack of sound detailed information about the effect of different irrigation, on the major and minor composition of olive (Servili *et al*., 2007). Therefore, we evaluated how drip irrigation affects the volatiles produced by fruits of European olive variety *Koroneiki* cultivated in the north of Tunisia. For these reasons, the main goal of the present study is first to determine irrigation regime effects on the chemical composition of volatile fraction of European olive variety (cv. *Koroneiki*); secondly, to determine the fatty acid composition finally, to evidence the influence of the various drip irrigation rates on antioxidant and antifungal properties.

MATERIALS AND METHODS

Plant material and growth conditions: Unripe fruits were collected from *Koroneiki* olive (*Olea europaea* L.) cultivar planted in the experimental farm of Elkef in north- western of Tunisia (Latitude: 36'' 18' N; Longitude: 09'' 07' E; Altitude: 500 m above sea level) during the crop season 2008-2009. Elkef region is characterized by a mean annual rainfall of 450 mm, concentrated mainly from autumn to spring and an average evapotranspiration (ETc) of 1500 mm. The warmer months are July/August and the coldest are December/January with a mean annual temperature varied from 7.8-28.5°C. In this olive orchard (20 ha), water was delivered three times per week at a rate of 4 h j^{-1} using a localized irrigation system with four drip nozzles of 4 l h^{-1} each per tree (two per side), set in a line along the rows at a distance of 0.5 m around the trunk with a unit flow of 8 l h^{-1} (total flow per tree was 16 l h^{-1}). *Koroneiki* olive oil cultivar, was tested in a factorial combination with three irrigation levels [three plots of 36 m^2 (6 m x 6 m) (+ three trees per treatement, three plot of 3× 36 m^2) each were designed for each irrigation]: stressed (T1), moderated (T2) and well irrigation (T3) receiving a seasonal water irrigation amount equivalent to 50, 75 and 100% ETc (Dabbou *et al*., 2010). The calculation procedures used by this model are based on the Penman-Monteith- FAO method (Allen *et al*., 1998) with a single estimated crop coefficient (Kc = 0.6), a reference of evapotranspiration applied for each irrigation treatment (ETo) and a coverage coefficient (Kr = 0.5) (D'Andria *et al*., 2004) where ETc = Kr . Kc . ET0.

The soil was silty (18%) with alkaline pH (8.10) and consisted of 33% calcium carbonate, 1.20% organic matter, 0.65‰ N_2, 255 mg kg^{-1} K_2O, and 6 mg kg^{-1} P_2O_6. The experimental plot was grown intensively at a planting density of 286 plants ha^{-1} and a tree spacing of 6 m x 6 m with olive oil trees of 5-year-old after planting.

Extraction method: A 500 g of each sample of the fresh fruits was subjected to hydrodistillation for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The volatiles obtained after trapping in diethyl ether were dried over anhydrous sodium sulphate, evaporated and concentrated under a gentle stream of N_2 and stored at 4°C until use for further analysis. The percentage of volatile yield was calculated as the weight of volatile divided by the weight of fresh fruits.

Identification of the volatiles constituents: Volatile compounds analysis by GC was performed on a gas chromatograph HP-5890 series II equipped with dual Flame-ionization Detector (FID). HP-Wax and HP-5 capillary columns (30 m ×0.25 mm, 0.25 μm film thickness) were used. The oven temperature was kept at 60°C for 10 min followed by a 5°C min^{-1} ramp to 220°C. The carrier gas was nitrogen with a flow rate of 2 mL min^{-1}; split ratio was 1:30. Injector and detector temperatures were maintained at 250°C. The injected volume was 0.5 μL. The identification of the components was performed, for both columns, by comparison of their retention times with

those of pure authentic samples and by means of their Linear Retention Indices (LRI) relative to the series of n-hydrocarbons.

Gas chromatography-electron impact mass spectrometry (GC-EIMS) analyses were performed with a Varian CP 3800 gas chromatograph (Varain, Inc., Palo Alto, CA) equipped with a DB-5 capillary column (Agilent Technologies Hewlett-Packard, Waldbronn, Germany; 30 m× 0.25 mm; coating thickness×0.25 mm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperature at 250 and 240°C, respectively; oven temperature was programmed from 60-240 at 3°C min^{-1}; carrier gas, helium at 1 mL min^{-1}; splitless injector. Identification of the constituents was based on comparison of the retention times with those of the authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons, and on computer matching against commercial (NIST 98 [U.S. National Institute of Standards and Technology; ADAMS Adams, 1995) and homemade library mass spectra built from pure substances and components of known oils and MS literature data (Stenhagen *et al*., 1974; Massada, 1976; Jennings and Shibamoto, 1980; Swigar and Silvestein, 1981; Davies, 1990; Adams, 1995). Moreover, the molecular weights of all the identified substances were confirmed by gas chromatography-chemical ionization mass spectrometry (GC-CIMS), using methanol as the chemical ionization gas.

Fatty acid methylation and analysis: Triplicate sub-samples of 0.5 g were extracted using the modified method of Bligh and Dyer (1959). Thus, fruit samples were kept in boiling water for 10 min to inactivate lipase (Douce, 1964) and then ground manually using a mortar and pestle. A chloroform/methanol (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) mixture (1:1, v/v) was used for total lipid extraction. After washing with water and centrifugation at 3000×g for 10 min, the organic layer containing total lipids was recovered and dried under a nitrogen stream. Total Fatty Acids (TFA) were converted into their methyl esters using sodium methoxide solution (Sigma, Aldrich) according to the method described by Cecchi *et al*. (1985). Methyl heptadecanoate (C17:0) was used as an internal standard. Those fatty acids methyl esters (FAMEs) obtained were subsequently analyzed.

The fatty acid methyl esters were analyzed on a HP 5890 gas chromatograph (Agilent Palo Alto, CA, USA) equipped with a flame ionization detector (FID). The esters were separated on a RT-2560 capillary column (100 m length, 0.25 mm i.d., 0.20 mm film thickness). The oven temperature was kept at 170°C for 2 min, followed by a 3°C min^{-1} ramp to 240°C and finally held there for an additional 15 min period. Nitrogen was used as carrier gas at a flow rate of 1.2 mL min^{-1}. The injector and detector temperatures were maintained at 225°C. A comparison of the retention times of the FAMEs with those of co-injected authentic standards (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) was made to facilitate identification.

Evaluation of antiradical activity by dpph assay: The scavenging activities of the methanolic extracts were measured according to the method described by Blois, (1958). Volatiles Methanolic extracts (1 mL) at different concentrations were added to 1 mL of DPPH methanolic solution (0.004%). The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. Then the absorbance was measured at 517 nm against a blank by a spectrophotometer (Secommam, U-1789, France). Inhibition of free radical, DPPH, in percent (I%) was calculated according to formula:

$$\frac{I(\%) = (A_{blank} - A_{sample})}{A_{blank}} \times 100$$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate. Butylated hydroxytoluene (BHT) was used as positive control.

Evaluation of antiradical activity by abts assay: The $ABTS^+$ radical cation scavenging activity of each volatile fraction and ascorbic acid (control) was determined according to Yvonne *et al*., (2005). In brief, 5.0 mL of a 7.0 mM ABTS was reacted with 88.0 µL of a 140 mM potassium persulfate overnight in the dark to yield the $ABTS^+$ radical cation. Prior to use in the assay, the $ABTS^+$ radical cation was diluted with ethanol for an initial absorbance of about 0.700 (ratio of 1:88) at 734 nm, with 30°C. Free radical scavenging activity was assessed by mixing 1.0 mL diluted $ABTS^+$ radical cation with 10 µL of test sample and monitoring the change in absorbance at 0, 0.5 and 1 min and again 5 min intervals until a steady state was achieved. The antioxidant capacity of volatile fraction was expressed as IC_{50}, the concentration necessary for 50% reduction of $ABTS^+$.

Antifungal activity: Four fungal species were used for the antifungal testing, namely: *Candida glabrata* ATCC 90030, *Candida kreusei* ATCC 6258, *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 90028.

Micro-well dilution assay: Minimum Inhibitory Concentration (MIC) values were determined by micro-titre plate dilution method (Sahin *et al*., 2004). The inocula of the bacteria and yeasts were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The volatile fractions were first dissolved in 10% DMSO and then diluted to the highest concentration (10 mg mL^{-1}) to be tested, and then serial two fold dilutions were made in 10 mL sterile test tubes containing nutrient broth.

In brief, the 96-well plates were prepared by dispensing into each well 95 μL of nutrient broth and 5 μL of the inocula. An aliquot of 100 μL from the stock solutions of the volatiles initially prepared at the concentration of 10 m mL^{-1} was added into the first wells. Then, 100 μL from their serial dilution were transferred into six consecutive wells. The last well containing 195 μL of nutrient broth without compound and 5 μL of the inocula on each strip was used as negative control. The final volume in each well was 200 μL. The plate was covered with a sterile plate sealer and then incubated for 18 h at 37°C. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms, after incubation. Results were expressed in microgram per milliliter.

Statistical analysis: All parameters analyzed were carried out in triplicate. The results are reported as mean values of three repetitions and standard deviation. Data were subjected to statistical analysis using the SPSS programme, release 11.0 for Windows (SPSS, Chicago, IL, USA). The one-way analysis of variance (ANOVA) followed by Duncan multiple range test were employed and the differences between individual means were deemed to be significant at $p<0.05$.

RESULTS AND DISCUSSION

Volatiles yield and chemical composition: GC/MS analysis was conducted on the volatile fruits of the three irrigation treatments. The list of detected compounds with their relative percentages and retention indices are given in Table 1 in order of their elution on the column.

The volatile fractions correspond to the 5th year of cultivation practices, in which the volatile yield, vary between 0.25 and 0.33% for 100 and 50% ETc equivalent, respectively (Table 2). As reported by Sotomayor *et al*. (2004) in other fruit species *Thymus zygis* ssp., *gracilis*, increases in volatile yield are associated with the decrease in amount of water added. Moreover, a decrease in volatile yield is detected for the olive cultivar *Koroneiki* under watering levels higher than 50% ETc. It seems that variation in volatile yield can be attributed to some factors like conditions of cultivation especially irrigation (Arganosa *et al*., 1998). From this, it can be concluded that the cultivar *Koroneiki* grown under watering levels higher than 50% ETc decreased their volatile production quickly with time. Thus, statistically significant differences were observed among yields of the three irrigation treatments studied (Table 2). Nineteen components were obtained in the volatile fraction of *Koroneiki* cultivar grown under a 50% ETc watering level. The major constituents were 3-ethenylpyridine (19.2%),

Table 1: Watering level effect on *Olea europaea* L. cultivar (*Koroneiki*) volatile composition[a]

		Relative concentration (%)		
Volatile compounds	LRI[b]	50% Etc	75% Etc	100% Etc
(E,Z)-2,4-hexadienal	801	3.8	5.7	3.9
(E)-3-hexenol	855	3.4	3.2	6.3
1-hexanol	868	2	1.9	4.9
Benzaldehyde	957	11.1	9	13.8
3-ethenylpyridine	970	19.2	19.6	14.9
Phenol	977	0.5	0.1	0.4
(E,E)-2,4-heptadienal	1012	1.5	0.9	2.3
(E,Z)-2,4-heptadienal	1016	3.7	1.2	3.8
benzyl alcohol	1036	2.9	2.3	2.2
Phenylacetaldehyde	1045	5.5	2.9	3.7
(E)-2-octenal	1064	Tr[c]	Tr	0.7
cis-linalool oxide (furanoid)	1074	-	1.3	1.3
Ortho-guaiacol	1088	1.5	1.5	1.1
Linalool	1098	1.3	2.2	1.4
Nonanal	1103	0.9	0.9	0.9
phenylethyl alcool	1111	17	19.2	7.8
á –terpineol	1188	-	0.6	0.6
2-ethylbenzaldehyde	1221	1.5	1.2	0.7
Citronellol	1231	Tr	2	1.4
Geraniol	1254	Tr	1.4	0.7
Salicylic alcohol	1268	Tr	1.3	2.7
1-tridecene	1291	-	-	0.4
(E,Z)-2,4-decadienal	1295	2	1	2.2
Carvacrol	1298	Tr	0.5	1.1
4-vinylguaiacol	1316	Tr	0.8	0.1
(E,E)-2,4-decadienal	1318	4.3	2.2	5.9
(E)-2-undecenal	1349	2.6	2.3	2.1
(E)-b-damascenone	1384	3.3	2.4	2.2
Tetradecane	1400	-	-	0.8
Monoterpene hydrocarbons		Tr	2.5	2.5
Oxygenated monoterpenes		1.3	5.5	4.0
Sesquiterpene hydrocarbons		3.3	2.4	2.2
Non-terpene hydrocarbons		-	-	1.2
Oxygenated non terpenes derivatives		64.2	57.6	65.5
Nitrogen derivatives		19.2	19.6	14.9
Total identified volatile compounds		88	87.6	90.3

[a]Percentages obtained by flame ionization detector (FID) peak area normalization (HP-5 column), [b]Linear retention indices (DB-5 column), [c]Traces (Tr) <0.1%

Table 2: Watering level effect on European olive cultivar *Koroneiki* volatile fractions yields and radical-scavenging activities

Watering level (%Etc)	Volatile yield (%FW, w/w)	DPPH IC50 (μg mL^{-1})	ABTS^{+}IC50 (μg mL^{-1})
50	0.33±0.01^{a}	1599.70±28.59^{a}	15246.30±40.56^{b}
75	0.27±0.03^{ab}	547.50±7.50^{b}	17479±45^{a}
100	0.25±0.04^{c}	361.91±90.41^{c}	14431.68±58.56^{c}

Data are expressed by mean values ±SD of three independent experiments, Values in each column with different superscript letters present significant differences ($p<0.05$) between the different irrigation strategies for each parameter

phenylethyl alcool (17.0%) and benzaldehyde (11.0%). Twenty-seven components were identified in the volatile fraction for 75% ETc, were the principle components 3-ethenylpyridine (19.6%), phenylethyl alcool (19.2%) and benzaldehyde (9%). Twenty-nine components were characterized in the volatile fraction from plants treated 100% ETc with as major components 3-ethenylpyridine (14.9%), benzaldehyde (13.8%) and phenylethyl alcool (7.8%). Among the major common components, 3-ethenylpyridine was the component present in all the three samples (14.9-19.6%). Benzaldehyde and phenylethyl alcool were the other prominent components found in considerable quantity in all samples.

Table 1 contains the relative amounts of different compound families, found in the volatile fruits of the three irrigation treatments. Oxygenated non terpene derivatives (64.2-65.5%) comprised the most abundant class of the compounds detected in all studied samples and contained phenylethyl alcool (7.8-19.2%) and benzaldehyde (9.0-13.8%) as the main compounds. The nitrogen derivatives (19.2-19.6%) were the second main class of all volatile samples. Therefore, it is interesting to note, on one hand, that the sesquiterpenes hydrocarbons and oxygenated monoterpenes were present in minor percentages (1.3-5.5%) in all volatile of the three watering level treatments studied. On the other hand, all volatile samples were also poor in monoterpenes hydrocarbons and non-terpene hydrocarbons. However, it has been suggested that the accumulation of monoterpenes under watering levels higher than 50% ETc, has physiological and ecological role such as a photorespiration-like protection (Penuelas and Llusia, 2002). The secondary metabolite production is believed to be stimulated by the irrigation treatments. Like all secondary metabolites, the volatiles compounds are known to have several important functions such as protection against predators (microorganisms; fungi; insects; herbivores), against UV radiations but may also serve as secondary functions attracting the natural enemies of these herbivores, attraction of pollinators and dispersal of diasopres, inhibitors of germination and growth, etc. (Kessler and Baldwin, 2001).

Fig. 1 show the distribution of C6 alcohols compounds derived from the LOX pathway, expressed as (%), in olive cultivar *Koroneiki* samples according to the

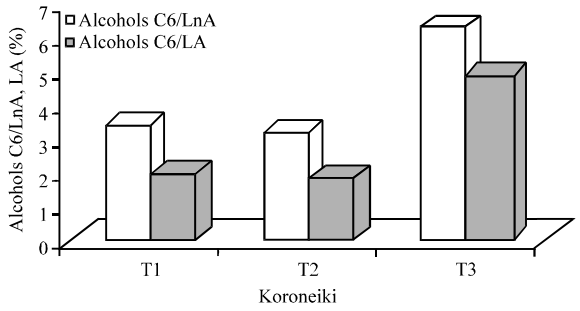

Fig. 1: Contents of C6 alcohols compounds derived from linolenic acid (LnA) and linoleic acid (LA) in olive fruit from the introduced cultivar as affected by irrigation levels. Alcohols C6/LnA represents (E)-hex-3-en-1-ol. Alcohols C6/LA represents the contents of hexan-1-ol

different irrigation levels studied. Those C6 alcohols compounds are derived from the cascade of enzymatic reactions starting with the formation, by lipoxygenase (LOX) action, of 13-hydroperoxides from linolenic and linoleic acid (Olias *et al*., 1993). The total volatile C6 alcohols compounds derived from linolenic acid ranged from 3.4-6.3% and from 2-4.9% in C6 alcohols compounds derived from linoleic acid. In fact, regarding the levels of C6 alcohols compounds such as (E)-hex-3-en-1-ol and hexan-1-ol derived from linolenic and linoleic acid, they decreased with 75% Etc and then increased down to 6.3 and 4.9%, respectively with 100% ETc. Consequently, specific trend with regard to the change in C6 alcohols compounds as a function of irrigation regime was observed. Nevertheless, the enzymatic activity is affected by raising the irrigation levels of olive trees. In addition, irrigation regime may also influence the production of volatiles (Baccouri *et al*., 2008). According to the data reported in Fig. 2, the %C6 compounds and total alcohols increased in the volatiles obtained from *Koroneiki* fruits with increased the irrigation levels. Both classes of compounds were affected by the irrigation in the sense that the increase in the water applied to the *Koroneiki* olive trees led to an increase in these volatiles. However, inconsistent changes in the content of the %C_6 aldehydes/C_6 were observed for Koroneiki fruits. Nevertheless, the trend of total aldehydes compounds and %C_6 alcohols/C_6 decreased with 75% Etc and then increased down to 40% and 72.25%, respectively with

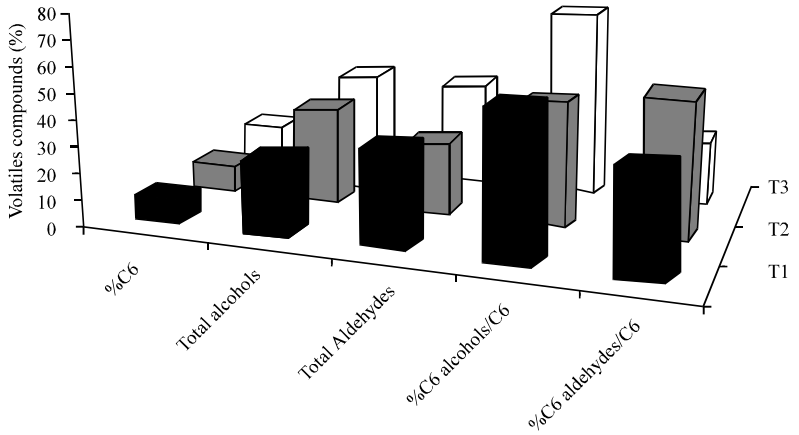

Fig. 2: Contents of $\%C_6$, total alcohols, total aldehydes, $\%C_6$ alcohols/C_6, % C_6 aldehydes /C_6 from the three introduced cultivars as affected by irrigation levels

Table 3: Variations in fatty acids composition (as a percent of TFA) of total lipids from fresh fruits of *Olea europaea* L. cultivar (*Koroneiki*) grown under three irrigation regimes

	Watering level		
Fatty acid	50% Etc	75% Etc	100% Etc
Palmitic acid	11.39± 0.32^{a,b}	11.78± 0.17^{a}	11.16±0.08^{b}
Margaric acid	0.18±0.07^{a}	0.17±0.00^{a}	0.20±0.11^{a}
Stearic acid	2.62±0.12^{a,b}	2.46±0.07^{b}	2.77±0.12^{a}
Arachidic acid	1.24±0.58^{a}	1.09±0.48^{a}	0.42±0.38^{a}
Behenic acid	0.50±0.73^{a}	0.21±0.19^{a}	0.29±0.07^{a}
Lignoceric acid	0.17±0.06^{a}	0.24±0.26^{a}	0.23±0.02^{a}
SFA	16.12±0.82^{a}	15.95±0.90^{a,b}	15.07±0.18^{b}
Palmitoleic acid	1.10±0.04^{b}	1.46±0.18^{c}	0.94±0.00^{a}
Margaroleic acid	0.22±0.02^{a}	0.38±0.28^{a}	0.25±0.04^{a}
Oleic acid	73.14±3.31^{a}	73.85±0.24^{a}	73.98±0.52^{a}
Gadoleic acid	1.33±0.86^{a}	0.81±0.31^{a}	0.55±0.42^{a}
Erucic acid	0.15±0.07^{b}	0.14±0.14^{b}	0.35±0.10^{a}
MUFA	75.94±0.55^{a}	76.65±0.30^{a}	76.07±0.03^{a}
Linoleic acid	6.37±0.95^{a}	6.17±0.33^{a}	7.12±0.33^{a}
Linolenic acid	1.48±0.87^{a}	1.13±0.12^{a}	1.66±0.47^{a}
PUFA	7.85±1.67^{a}	7.30±0.21^{a}	8.78±0.13^{a}
MUFA/PUFA	9.67±2.34^{a}	10.50±0.34^{a}	8.66±0.12^{a}
O/L ratio	11.48±2.31^{a}	11.96±0.68^{a}	10.39±0.56^{a}

Data are expressed by mean values±SD of three independent experiments. Values in each row with different superscript letters present significant differences ($p<0.05$) between the different irrigation strategies for each parameter. C16:0: Palmitic acid, C16:1: Palmitoleic acid, C17:0: Margaric acid, C17:1: Margaroleic acid, C18:0: Stearic acid, C18:1: Oleic acid, C18:2: Linoleic acid, C18:3: Linolenic acid, C20:0: Arachidic acid, C20:1: Gadoleic acid, C22:0: Behenic acid, C22:1: Erucic acid and C24:0: Lignoceric acid, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, O/L: Oleic/Linoleic ratio

100% ETc. Hence, these different amounts of total volatiles should be related to the agronomic conditions (the irrigation regime).

Fatty acid composition: Based on our experimental data, it was shown that major fatty acids for the three irrigation regimes were oleic and palmitic (Table 3). Furthermore, the differences were statistically significant among three watering level treatments studied of the *Koroneiki* cv., especially in palmitoleic acid ($p<0.05$). The percentage of Saturated Fatty Acids (SFA), mainly that of arachidic acid (C20:0) decreased from 1.24% under 50% ETc watering level to 0.42 for 100 % ETc (Table 3). In addition, we noted that drip irrigation decreased the SFA proportions. It was also mentioned that oleic acid levels increased to reach 73.14 at 50% ETc watering level and 73.98 under 100% ETc, respectively. Drip irrigation rates did not elicit significant changes in the oleic acid proportion. Current biochemical evidence indicates that, in olive and other plant species, the polyunsaturated fatty acids (C18:2 and C18:3) are produced by the consecutive desaturation of oleic acid. The major variation consisted in a decrease of the saturated fatty acids in favor of an increase in monounsaturated ones. Another important fatty acid is linoleic acid; its content was in the range of 6.37-7.12% for 50 and 100% ETc. Interest in the PUFA, as health-

promoting nutrients, has expanded dramatically in recent years. A rapidly growing literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Finley and Shahidi, 2001; Riemersma, 2001). The ratio of the oleic/linoleic acid (O/L) and monounsaturated/polyunsaturated fatty acids (MUFA/PUFA), decreased (from 11.48-10.39%) and (from 9.67-8.66%), respectively at the irrigation treatments, except a higher amount detected at 75% ETc (11.96%) and (10.5%), respectively. On the other hand, Unger (1982) found a positive correlation between oleic acid content and water use at the vegetative stage. Differences among three irrigation levels in both the ratio O/L and MUFA/PUFA are not statistically significant ($p<0.05$). In addition, we noted that the oleic/linoleic acid (O/L) and monounsaturated/polyunsaturated fatty acids (MUFA/PUFA), which are largely responsible for the sapidity and healthful effects of the Mediterranean diet.

Antioxidant activity: Antioxidant activity of the fresh fruits volatiles of European olive cultivar *Koroneiki* subjected to three irrigation treatments were determined by two different test systems namely DPPH and $ABTS^{+}$ radical-scavenging assays. All of the data are presented in Table 2.

Antioxidant activity by DPPH: The effects of antioxidants in the DPPH-radical-scavenging test reflect the hydrogen-donating capacity of a compound. When the radical form of DPPH is scavenged by an antioxidant, through the donation of hydrogen, to form a stable DPPH molecule, this leads to a color change from purple to yellow and a decrease in absorbance. Free radical scavenging properties of the fresh fruits volatiles are presented in Table 2. The antioxidant capacity of volatiles was expressed as IC_{50} (concentration of antioxidant required to quench 50% of the stable free radical), which was used to acquire the optimized extraction condition. The IC50 values for *Koroneiki* cv. grown under 50, 75 and 100% ETc were 1599.70, 547.50 and 361.91 μg mL^{-1}, respectively. The volatile fraction for 100% ETc showed higher scavenging ability on DPPH radicals than those for 50 and 75% ETc. These results suggest that an increase in the watering level applied favored the radical-scavenging activity of the volatile fractions. Thus, we reported increases in antioxidant activity are associated with the increase in the amount of water added. Our results are in agreement with those of Jordan *et al*. (2009) conducted on *Thymus zygis* ssp. *gracilis*. Moreover, DPPH scavenging abilities of the studied samples were lower than that of synthetic antioxidant BHT (IC_{50} = 181.20 μg mL^{-1}). These results were found to be statistically significant ($p< 0.05$).The DPPH-radical-scavenging activity of the volatiles compared to BHT decreased in the order of BHT>volatiles for 100% ETc>volatiles for 75% ETc>volatiles for 50% ETc.

Literature review shows that oxygenated monoterpenes are mainly responsible for the antioxidant potential of the plant oils which contain them (Baratta *et al*., 1998). Monoterpene hydrocarbons could also be taken into account for the antioxidative activity observed, but obviously, none has stronger than that of oxygenated monoterpenes. The presence of strongly activated methylene groups in these molecules is probably the reason for this behaviour (Ruberto and Baratta, 2000). According to Table 1, the amount of oxygenated monoterpenes and monoterpene hydrocarbons is higher in volatile fraction for 75 and 100% ETc than the other. The slight quantitative differences in the amounts of these compound families might also explain the differences of the antioxidant activities between the irrigation treatments studied.

Antioxidant activity by ABTS: The free radical scavenging capacity of volatile fractions from *Koroneiki* cultivar was evaluated by the ABTS assay. The radical-scavenging activities of the different volatile fractions, according to the watering levels applied, are shown in Table 2. In the present study, the weakest radical scavenging activity was exhibited by the volatile fraction obtained under 50% ETc (17479 μg mL^{-1}). Antioxidant activity of the fresh fruit volatiles cultivated at a watering level of 100% ETc was higher than that obtained under 50 and 75% ETc with an IC50 value of 14431.68 μg mL^{-1}. On the other hand, none of the samples showed activity as strong as the positive control ascorbic acid (7.45 μg mL^{-1}). Furthermore, regarding the watering-level effect on radical-scavenging activity, results were found statistically significant ($p<0.05$). As a major conclusion, from a watering level obtained with 100% ETc must be obtained volatile fractions from *Koroneiki* cultivar with high radical-scavenging activity.

Antifungal activity: The antifungal activities of the volatiles coming from fresh fruits of *Koroneiki* cultivar grown under three watering levels were assayed *in vitro* by a broth micro-dilution method against four phytopathogen strains and the results are reported in Table 4.

Minimal Inhibitory Concentration (MIC) values of the fresh fruits volatiles towards the four yeast strains were determined in μg mL^{-1}. The results of antifungal activity showed that the fruits volatiles of the three

Table 4: Antifungal activity of volatile fractions from fresh fruits of *Olea europaea* L. cultivar (*Koroneiki*) grown under three irrigation regimes

	MIC values (μg mL^{-1})			
Microorganisms	50% Etc	75% Etc	100% Etc	Amphotericin[c]
C. Kreusei ATCC6258	625	1250	n.a	0.05
C. parapsilosis ATCC 22019	312	2500	312	0.05
C.albicans ATCC90028	78	1250	312	0.05
C.glabrata ATCC90030	312	156	78	0.05

MIC: Minimum Inhibitory Concentration, Not active, [c]Control antifungal

Irrigation treatments had varying degrees of growth inhibition against the microorganisms tested. The strongest fungicidal activity was exhibited by the fresh fruits volatile cultivated at a watering level of 50% ETc against *C. albicans* (MIC values 78 μg mL^{-1}). The same sample demonstrated moderate activities against *C. kreusei*, *C. glabrata* and *C. parapsilosis* (Table 4). *C. glabrata* showed, on one hand, best susceptibility towards the volatile fraction for 75% ETc with a MIC value of 156 μg mL^{-1} followed by *C. kreusei*, *C. albicans* MIC 1250 μg mL^{-1} and *C. parapsilosis* MIC 2500 μg mL^{-1}. On the other hand, volatiles coming from fresh fruits cultivated under 100% ETc watering level were presented as the most active against *C. glabrata* with a MIC value of 78 μg mL^{-1}. Whereas, no antifungal activity against *C. kreusei* was detected by volatile fraction for 100% ETc.

Fruit volatiles of the three irrigation treatments are complex mixtures of volatile compounds (Table 1). In fact, Matasyoh *et al*. (2007) showed that the chemical compounds like linalool and α-terpineol had antifungal activity. In addition, it is also possible that the trace/minor components might be involved in some type of antifungal synergism with other active components of volatiles. These findings are in agreement with previous reports (Marino *et al*., 2001; Hutchings *et al*., 1996).

CONCLUSION

Regarding first the volatile fraction yield and quality, secondly, the fatty acid and volatile composition finally, the antioxidant and antifungal activities, a single watering level cannot be pre-selected in order to obtain the most satisfactory values for these parameters. On the one hand, high levels of water are required for achieving lower IC50 values; on the other hand, volatile fraction yield is favored by lower watering levels. Hence, the olive volatiles compounds described herein may be suggested as a potential source of natural antioxidant and antifungal for food industry.

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REFERENCES

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publisher, Carol Stream, IL., USA.

Allen, R.G., L.S. Pereira, D. Raes and M. Smith, 1998. Crop evapotranspiration-guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper No. 56, FAO, Rome, Italy. http//www.fao.org/docrep/X0490E/x0490e00.htm

Arganosa, G.C., F.W. Sosulski and A.E. Slikard, 1998. Seed yield and essential oil of Northern-grown coriander (*Coriandrum sativum* L.). J. Herbs Spices Med. Plants, 6: 23-32.

Baccouri, O., A. Bendini, L. Cerretani, M. Guerfel and B. Baccouri *et al*., 2008. Comparative study on volatile compounds from Tunisian and Sicilian monovarietal virgin olive oils. Food Chem., 111: 322-328.

Baratta, M.T., H.J.D. Dorman, S.G. Deans, D.M. Biondi and G. Ruberto, 1998. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. J. Essential Oil Res., 10: 618-627.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200.

Cecchi, G., S. Biasini and J. Castano, 1985. Methanolyse rapide des huiles en solvant medium. Revue Francaise des Corps Gras, 32: 163-164.

Clevenger, J.F., 1928. Apparatus for the determination of volatile oil. J. Am. Pharm. Assoc., 17: 345-349.

D'Andria, R., A. Lavini, G. Morelli, M. Patumi, S. Tiranziani, D. Calandrelli and F. Fragnito, 2004. Effects of water regimes on five pickling and double aptitude olive cultivars (*Olea europaea* L.). J. Hortic. Sci. Biotechnol., 79: 18-25.

Dabbou, S., H. Chehab, B. Faten, S. Dabbou and S. Esposto *et al*., 2010. Effect of three irrigation regimes on Arbequina olive oil produced under Tunisian growing conditions. Agric. Water Manage., 97: 763-768.

Davies, N.W., 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. J. Chromatogr. A, 503: 1-24.

Deans, S.G. and P.G. Waterman, 1993. Biological Activities of Volatile Oils. In: Volatile Oil Crops: Their Biology, Biochemistry and Production, Hay, R.K.M. and P.G. Waterman (Eds.). Longman, London, UK., pp: 113.

Dettori, S., M.R. Filigheddu and M. Schirra, 1989. Yield response of olive to different watering regimes. Irrigazione e Drenaggio, 4: 183-186.

Douce, R., 1964. Identification et dosage de quelques glycerophosphatides dans les souches normales et tumorales de scorsoneres cultivees *in vitro*. C. R. Acad. Sci. Paris, 259: 3066-3068.

Esti, M., L. Cinquanta and E. La Notte, 1998. Phenolic compounds in different olive varieties. J. Agric. Food Chem., 46: 32-35.

FAOSTAT, 2007. FAO statistical databases. Agriculture Data Collection (Primary Crops). http://faostat.fao.org/

Finley, J.W. and F. Shahidi, 2001. The Chemistry, Processing and Health Benefits of Highly Unsaturated Fatty Acids: An Overview. In: Omega-3 Fatty Acids, Chemistry, Nutrition and Health Effects, John, W.J. and F. Shahidi (Eds.). Vol. 1-13. American Chemical Society, Washington, DC., USA., pp: 258-279.

Gatto, L., 1989. Influence of the seasonal irrigation volumes on the yield of drip-irrigated oil olives (cv. Coratina). First results. LIrrigazione, 4: 187-190.

Girona, J., 1996. Water requirements of olive. Strategies for application oflimited amounts of water for irrigation in Arbequina. Fruticult. Prof., 81: 32-40.

Hanson, B.R., L.J. Schwankl, K.F. Schulbach and G.S. Pettygrove, 1997. A comparison of furrow, surface drip and subsurface drip irrigation on lettuce yield and applied water. Agric. Water Manage., 33: 139-157.

Hutchings, A., A.H. Scott, G. Lewis and A.B. Cunningham, 1996. Zulu Medicinal Plants, an Inventory. University of Natal Press, Pietermaritzburg.

Jennings, W. and T. Shibamoto, 1980. Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Column Gas Chromatography. Academic Press, New York.

Jordan, M.J., R.M. Martinez, C. Martinez, I. Monino and J.A. Sotomayor, 2009. Polyphenolic extract and essential oil quality of *Thymus zygis* ssp. *gracilis* shrubs cultivated under different watering levels. J. Ind. Crops Prod., 29: 145-153.

Kessler, A. and I.T. Baldwin, 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science, 291: 2141-2144.

Lavee, S., M. Nashef, M. Wodner and H. Harshemesh, 1990. The effect of complementary irrigation added to old olive trees (*Olea europaea* L.) cv. Souri on fruit characteristics, yield and oil production. Adv. Hortic. Sci., 4: 135-138.

Lavee, S. and M. Wodner, 1991. Factors affecting the nature of oil accumulation in fruit of olive (*Olea europaea* L.) cultivars. J. Hortic. Sci., 66: 583-591.

Marino, M., C. Bersani and G. Comi, 2001. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiacea* and *Compositae*. Int. J. Food Microb., 67: 187-195.

Massada, Y., 1976. Analysis of Essential Oil by Gas Chromatography and Spectrometry. John Wiley and Sons, New York.

Matasyoh, J.C., J.J. Kiplimo, N.M. Karubiu and T.P. Hailstorks, 2007. Chemical composition and antimicrobial activity of essential oil of *Tarchonanthus camphorates*. Food Chem., 101: 1183-1187.

Milella, A and S. Dettori, 1987. Regimes water optimal and partial to young trees from the table. Frutticoltura, 8: 65-69.

Moriana, A., F. Orgaz, M. Pastor and E. Fereres, 2003. Yield responses of a mature olive orchard to water deficits. J. Am. Soc. Hortic. Sci., 128: 425-431.

Olias, J.M., A.G. Perez, J.J. Rios and L.C. Sanz, 1993. Aroma of virgin olive oilbiogenesis of the green odor notes. J. Agric. Food Chem., 41: 2368-2373.

Patumi, M., R. D'Andria, G. Fontanazza, G. Morelli, P. Giorio and G. Sorrentino, 1999. Yield and oil quality of intensively trained trees of three cultivars of olive (*Olea europaea* L.) under different irrigation regimes. J. Hortic. Sci. Biotechnol., 74: 729-737.

Patumi, M., R. d'Andria, V. Marsilio, G. Fontanazza, G. Morelli and B. Lanza, 2002. Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L., cv. Kalamata) in different irrigation regimes. Food Chem., 77: 27-34.

Penuelas, J. and J. Llusia, 2002. Linking photorespiration, monoterpenes and thermotolerance in Quercus. New Phytol., 155: 227-237.

Rice-Evans, C.A., N. Miller and G. Paganga, 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci., 2: 152-159.

Riemersma, R.A., 2001. The Demise of the n-6 to n-3 fatty acid ratio A dossier. Eur. J. Lipid Sci. Technol., 103: 372-373.

Romani, A., N. Mulinacci, P. Pinelli, F.F. Vincieri and A. Cimato, 1999. Polyphenolic content in five tuscany cultivars of *Olea europaea* L. J. Agric. Food Chem., 47: 964-967.

Ruberto, G. and M.T. Baratta, 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem., 69: 167-174.

Sahin, F., M. Gulluce, D. Daferera, A. Sokmen, M. Sokmen *et al*., 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* sp. vulgare in the Eastern Anatolia region of Turkey. Food Contry, 15: 549-557.

Samish, R.M. and P. Spiegel, 1961. The use of irrigation in growing olives for oil production. Isr. J. Agric. Res., 11: 87-95.

Servili, M., S. Esposto, E. Lodolini, R. Selvaggini and A. Taticchi *et al*., 2007. Irrigation effects on quality, phenolic composition and selected volatiles of virgin olive oil cv. Leccino. J. Agric. Food Chem., 55: 6609-6618.

Smelcerovic, A., M. Spiteller and S. Zuehlke, 2006. Comparison of methods for the exhaustive extraction of hypericins, flavonoids and hyperforin from *Hypericum perforatum* L. J. Agric. Food Chem., 54: 2750-2753.

Sotomayor, J.A., R.M. Martinez, A.J. Garcia and M.J. Jordan, 2004. *Thymus zygis* Subsp. *gracilis*: Watering level effect on phytomass production and essential oil quality. J. Agric. Food Chem., 52: 5418-5424.

Stenhagen, E., S. Abrahamsson and F.W. McLafferty, 1974. Registry of Mass Spectral Data. John Wiley and Sons, New York, USA.

Swigar, A.A. and R.M. Silvestein, 1981. Monoterpenes. Aldrich Chemical Company, Milwaukee, WI., USA..

Tan, C.S., 1995. Effect of drip and sprinkler irrigation on yield and quality of five tomato cultivars in southwestern ontario. Can. J. Plant Sci., 75: 225-230.

Tovar, M.J., M.P. Romero, J. Girona and M.J. Motilva, 2002. L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L cv Arbequina) fruit grown under different irrigation regimes. J. Sci. Food Agric., 82: 892-898.

Unger, P.W., 1982. Time and frequency of irrigation effects of sunflower production and water use. Soil Sci. Soc. Am. J., 46: 1072-1076.

Vinha, A.F., F. Ferreres, B.M. Silva, P. Valentao and A. Goncalves *et al*., 2005. Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influence of cultivar and geographical origin. Food Chem., 89: 561-568.

Yohannes, F. and T. Tadesse, 1998. Effect of drip and furrow irrigation and plant spacing on yield of tomato at Dire Dawa, Ethiopia. Agric. Water Manage., 35: 201-207.

Yvonne, Y.V., D.E. Bone and M.F. Carrington, 2005. Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated *in vitro*. Food Chem., 91: 485-494.