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Full Length Research Paper

The effect of different irrigation levels on the oleuropein contents of olive tree (*Olea europaea* L. cv. Memecik) in the western coastal region of Turkey

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In this study, the effect of different irrigation levels on oleuropein content of olive (*Olea europaea* L. cv. Memecik) was researched in the Aegean Region during the crop season of 2007. Six different irrigation water levels of drip irrigation were tested using class A pan evaporation technique [I_0 (kpc:0), $I_{0.25}$ (kpc:0.25), $I_{0.50}$ (kpc:0.50), $I_{0.75}$ (kpc:0.75), $I_{1.00}$ (kpc:1.00) and $I_{1.25}$ (kpc:1.25)]. Oleuropein contents of olive leaves were detected by high performance liquid chromatography (HPLC). Olive leaves showed considerable contents of oleuropein (16.25 ± 0.25%) collected from waterless regime applied trees during summer period. In the same regime, contents of oleuropein were decreased depending upon the increasing irrigation regimes: 0.25 (10.28 ± 0.13%); 0.50 (2.19 ± 0.12%); 0.75 (1.43 ± 0.03%); 1.00 (0.09 ± 0.05%), although in winter period, contents of oleuropein were approximately stable as 0.55 ± 0.01% in waterless regime (rain-fed olives), equal in 0.25 (0.25 ± 0.04%) and 0.50 (0.25 ± 0.01%), in 0.75 (0.21 ± 0.01%), 1.00 (0.16 ± 0.02%) and (0.14 ± 0.03%) in 1.25 irrigation regimes. Contents of oleuropein was observed to be increased inversely proportional with increased irrigation regimes in autumn period. The oleuropein values were 1.21 ± 0.01% in waterless, 0.76 ± 0.01% in 0.25 irrigation, 0.45 ± 0.03% in 0.50 irrigation, 0.44 ± 0.03% in 0.75 irrigation, 0.33 ± 0.01% in 1.00 irrigation and 0.25 ± 0.02% in 1.25 irrigation regimes. No important increase or decrease between contents of oleuropein depending upon the gradually increased irrigation regimes was observe in spring period [in waterless regime (0.51 ± 0.05%), 0.25 irrigation (0.39 ± 0.04%); 0.50 irrigation (0.38 ± 0.14%); 0.75 irrigation (0.11 ± 0.01%); 1.00 irrigation (0.55 ± 0.04%); and 1.25 irrigation (0.79 ± 0.06%)]. Statistically important increases in oleuropein contents were in inverse proportion with the exponential irrigation regimes.

Key words: *Olea europaea* L., olive leaves, oleuropein, irrigation, seasonal variation.

INTRODUCTION

The olive (*Olea europaea* L.) originated from Upper Mesopotamia, which includes Mardin, Hatay, west sides of Suriye and Filistin named South East Anatolia (Rallo et al., 1997). Olive tree belongs to the Oleaceae family which contains 20 to 29 genera according to the classification system (Morettini, 1972). The genus *Olea* has a lot of species and subspecies that are mostly brushes. Among them, *O. europaea* is the only species having eatable fruit. Turkey is one of the leading olive growing countries in the world and olive is a special and strategic crop with its added value to the national economy. Turkey has the 4th place in the chart of olive production and the 6th place in the olive oil production in the world, with 148,750 and 82,500 ton, respectively. Some standard Turkish cultivars of olive tree nominated as Ayvalik, Domat, Gemlik, Halhali, Kilis Yaglik, Manzanilla, Memecik, Nizip, Yaglik and Tavsan Yuregi,

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had been identified by morphological characteristics and in this research, Memecik cultivar, which originated from Mugla city contains 45% of West Anatolia olive trees and 50% of them in Turkey are used as the investigation species (Kaya, 2010).

Olive tree is one of the important fruit-trees and food products of Mediterranean countries (Amiot et al., 1986a) and is known with its high biophenol contents (Amiot et al., 1986b). Oleuropein is one of the main biophenols of olive fruit-trees and it is responsible for the bitter taste of olive tree, gives bitterness and astringent to olive oil with its metabolites (Mateos et al., 2004). Although, most of the phenolic compounds of olive oils are made from derivatives of secoiridoids, olive trees contain phenyl alkaloids and flavonoids at the same time (Ranalli et al., 2000). Oleuropein, a bitter-tasting secoiridoid glycoside present in olive leaves, is suggested to support hypotensive activity (Shuichi, 2004) and have preserving activity against lung cancer, colorectal, pharyngeal and esophagus cancer (Uccella and Saija, 2001). Oleuropein at the same time is known with antioxidant and antimicrobial activities (Shuichi, 2004). due to the fact that olive tree leaves are rich in antioxidant phenols, mixing them in portion of 2 to 3% with excessively matured fruits provide either permanent fragrance or resistance to oxidation (Ranalli et al., 2003, 2004); this affects its shelf life because it retard oxidation (Keceli and Gordon, 2001; Okogeri and Tasioula-Margari, 2002) and its sensorial properties; color, astringency, bitterness and flavor (Okogeri and Tasioula-Margari, 2002; Angerosa et al., 2000; Mateos et al., 2004; Ranalli et al., 2000; Servili and Montedoro, 2002).

Studies with polyphenols help ecophysiology by determining the response mechanisms to environmental conditions. Ecological and cultural variations must permit optimization of cultural conditions for products of olive tree leaves. Oleuropein provides recognition of varieties and causes the evaluation of their homogeneities (Savournin et al., 2001). Olive tree is known as a xerofitic plant because of its adaptation to dry and semi-arid bio-climatic areas such as the Mediterranean areas and for this reason, irrigation occupies the most important place in quantitative ecoanatomical researches. Since the emergence of agriculture which has constituted a ‘revolution’ in the development of societies and civilizations, irrigation is an innovation allowing the practice of an intensive and productive agriculture. Climatic, demographic and economic factors are some of the many factors which lead to the expansion of irrigation, and the establishment of an intensive agriculture (Terral and Durand, 2006). The contents of olive trees grown in dry and semi-arid bio-climatic Mediterranean areas are known to be affected by climatic and anthropogenic activities.

While olive oil contains mono saturated oil acids like as oleuropein, hydroxytyrosol and tyrosol at high ratio between the third phenolic compounds including flavonoids, squalene, beta-carotene and alfa-tocopherol (Stark and Madar, 2002). In the leaves of *O. europaea* oleuropein and its decomposition, product of hydroxytyrosol is the most densely found phenolic compound (De Leonardis et al., 2008). In this study, we detected the effects of different irrigation regimes to the oleuropein content of olive (*O. europaea* L. cv. Memecik) leaves.

MATERIALS AND METHODS

Olive plantation and irrigation treatment

The study was performed on 18 year old olive trees (*O. europaea* L. cv. Memecik which is the dominant cultivar and economically most important variety in the Aegean region, with about 75% of olive trees in the region being of this variety) in Olive Research Institute in Izmir, Turkey during the 2007/2008 and 2008/2009 olive crop seasons. The plot was situated on flat land on a medium clay-loam soil. The available water capacity is 94 mm for 90 cm of the crop root depth. The climate is semi arid Mediterranean with hot dry summers; annual evaporation and rainfall were 1519 and 487 mm, respectively for 2007, and 1676 and 427 mm, respectively for 2008.

Irrigation

In this study, the effect of different irrigation levels on olive (*O. europaea* L. cv. Memecik) oil quality parameters was researched in the Aegean Region during the crop seasons. Six different irrigation water levels of drip irrigation were tested using Class A pan evaporation technique [I_0 (kpc:0), $I_{0.25}$ (kpc:0.25), $I_{0.50}$ (kpc:0.50), $I_{0.75}$ (kpc:0.75), $I_{1.00}$ (kpc:1.00) and $I_{1.25}$ (kpc:1.25)]. Irrigation treatments were randomly applied with replicates, each having four trees. The first irrigation was realized when the amount of available water in the soil dropped to 50%. Irrigation was performed every five days from June to September. Olive harvesting was done manually for four seasons in autumn, winter, spring, summer.

Extraction

Powdered olive leaves were extracted with Methanol three times for 10 min. using ultrasound-assisted extraction. Plant material were extracted by dissolving in tertiary butanol, intensified with a rotary evaporator, and then lyophilized.

Establishment of high performance liquid chromatography (HPLC) method

HPLC analysis was performed on a Thermo Surveyor PDA (ThermoFisher Scientific, Massachusetts, USA) plus photo diode array apparatus using and Cosmosil C18-Ms 250X4, 6X5 µM (Nacalai Tesque, Kyoto, Japan) column. The plant extract and oleuropein standard samples were prepared at 1 mg/ml concentration and used to optimize HPLC methods. After a few run OLEM8 (Table 1) was developed to perform the analysis. The mobile phase comprised 0.01% *trifluoroacetic acid* (TFA) in water (A), 0.01% TFA in acetonitrile (B) and methanol (C). Isocritic elution was performed with 78A/22B without changing the solvent composition between 0 and 25 min., followed by a washing step 100°C between 25 and 28 min. and equilibration period with 78A/22B between 28 and 40 min. Detection wavelength, flow rate and column temperature were set to 240 nm, 1.25 ml min.$^{-1}$ and

Table 1. Calibration points for oleuropein.

S/N	Code	CON (µg/mL)
1	OC1	2000
2	OC2	6.666.666.667
3	OC3	2.222.222.222
4	OC4	7.407.407.407
5	OC5	2.469.135.802
6	OC6	8.230.452.675

Table 2. HPLC method for Oleuropein.

Time (min)	A	B	C	Flow rate (mL/min)
0.01	78	22	0	1.25
25	78	22	0	1.25
25.01	0	0	100	1.25
28	0	0	100	1.25
28.01	78	22	0	1.25
40	78	22	0	1.25

A, 0.01% TFA - water; B, 0.01% TFA - acetonitrile; C, methanol. Flow rate, 1.25 ml/min

30°C respectively. Also, gas pressure was set to 30 psi, and drift tube was set to 100°C for evaporative light-scattering detection (ELSD) analysis. For all solutions (samples and standards), 10 µL were injected.

Calibration

Calibration curves were established by dissolving 10 mg oleuropein with 5 ml HPLC grade methanol (Figure 1) and six level calibration curves were prepared by serially diluting this stock solution three fold with methanol (Table 2). Detector response was linear within this range and R^2 was 0.992885.

Statistical analysis

Analyses were done on a completely randomized design. All data obtained were subjected to one way analyses of variance (ANOVA) and the mean differences were compared by lowest standard deviation (L.S.D.) test. The experiments were repeated twice with four replicates for each (n=6) and comparisons with $P< 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

An accurate high performance liquid chromatographic method was developed for the leaf extracts samples of olive leaves taken from one major dominant cultivar of Aegean region, *O. europaea* L. cv Memecik which was the most economical important variety, being about 75% of olive trees in this region (Figure 2). The impact of the different irrigation factors on the contents of oleuropein in olive leaves was evaluated by the collecting period (spring, autumn, winter and summer). When waterless irrigation regime was applied, the highest oleuropein content was determined as 16.25 ± 0.25% in summer (Figures 3 to 5). The oleuropein contents of olive leaves gathered from spring period under six different irrigation regimes did not show any statistically important differences [in waterless regime (0.51 ± 0.05%), and in 1.25 irrigation regime (0.79 ± 0.06 %)] (Figures 6 to 8). When oleuropein contents of autumn period under waterless irrigation regime were compared with the spring periods, similar differences were seen to be valid for autumn period (1.21 ± 0.01% in waterless regime) (Figures 9 to 11).

Secondary metabolite production has been decreased because of the saturation of soil to the water, so there has not been any circumstance to force the metabolism of plant. Oleuropein contents were adequately stable in winter periods (0.55 ± 0.01% in waterless regime) (Figures 12 to 14), but in summer periods, oleuropein contents increased statistically important adversely with the increased irrigation regimes. In the high irrigation regimes of summer period, plants oleuropein contents were high when compared with the other seasonal oleuropein values, but they were low between the irrigation regimes of summer period. Water availability therefore has a considerable effect on phenolic composition of olive fruit. This effect may be explained by modification of activity of enzymes responsible for phenolic synthesis, as L-phenylalanine ammonia-lyase,

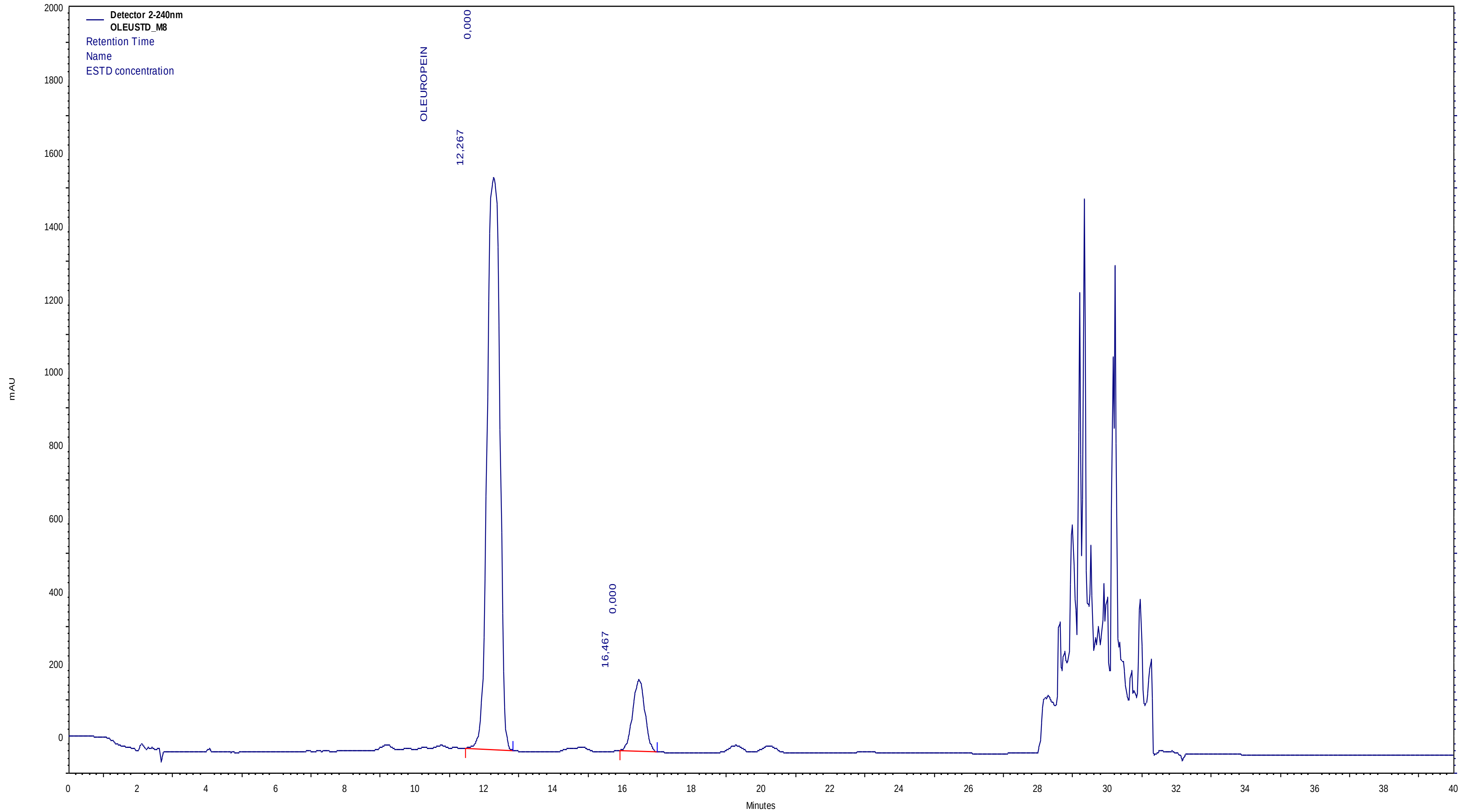

Figure 1. HPLC 240nm profile of oleuropein standard.

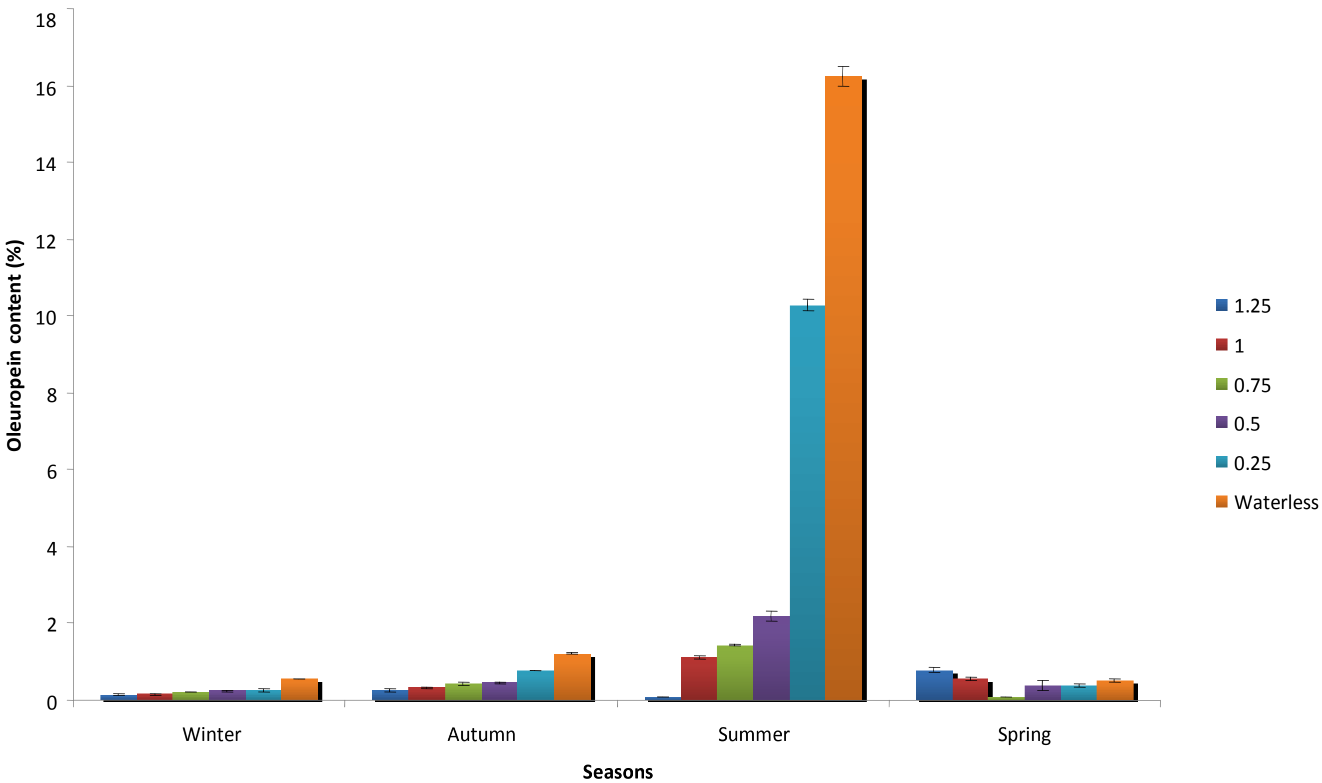

Figure 2. Changes of oleuropein contents with different irrigation regimes (waterless, 0.25, 0.50, 0.75, 1.00, 1.25) in four seasons (winter, autumn, summer, spring) in the olive tree (*Olea europea* L. cv. Memecik) leaves.

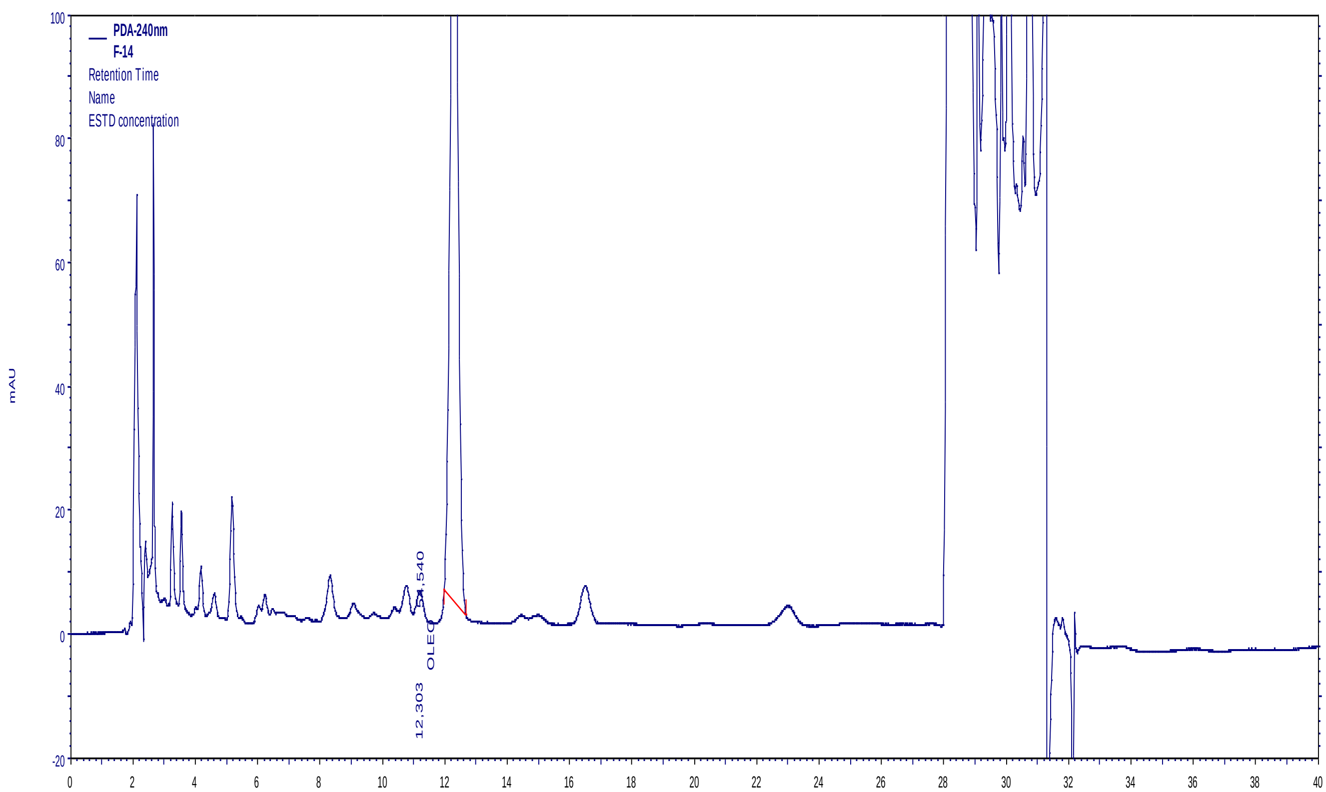

Figure 3. Oleuropein content in waterless regime in summer period.

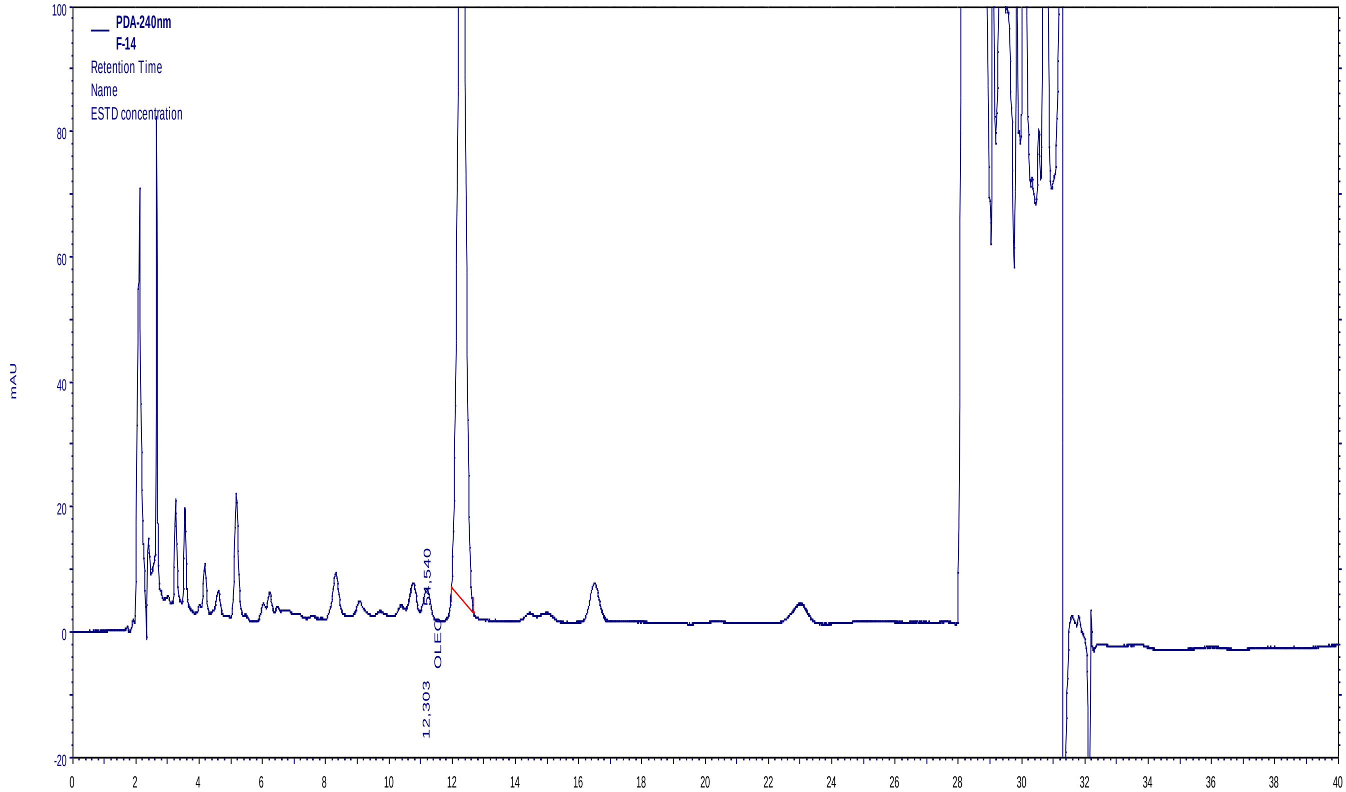

Figure 3. Oleuropein content in waterless regime in summer period.

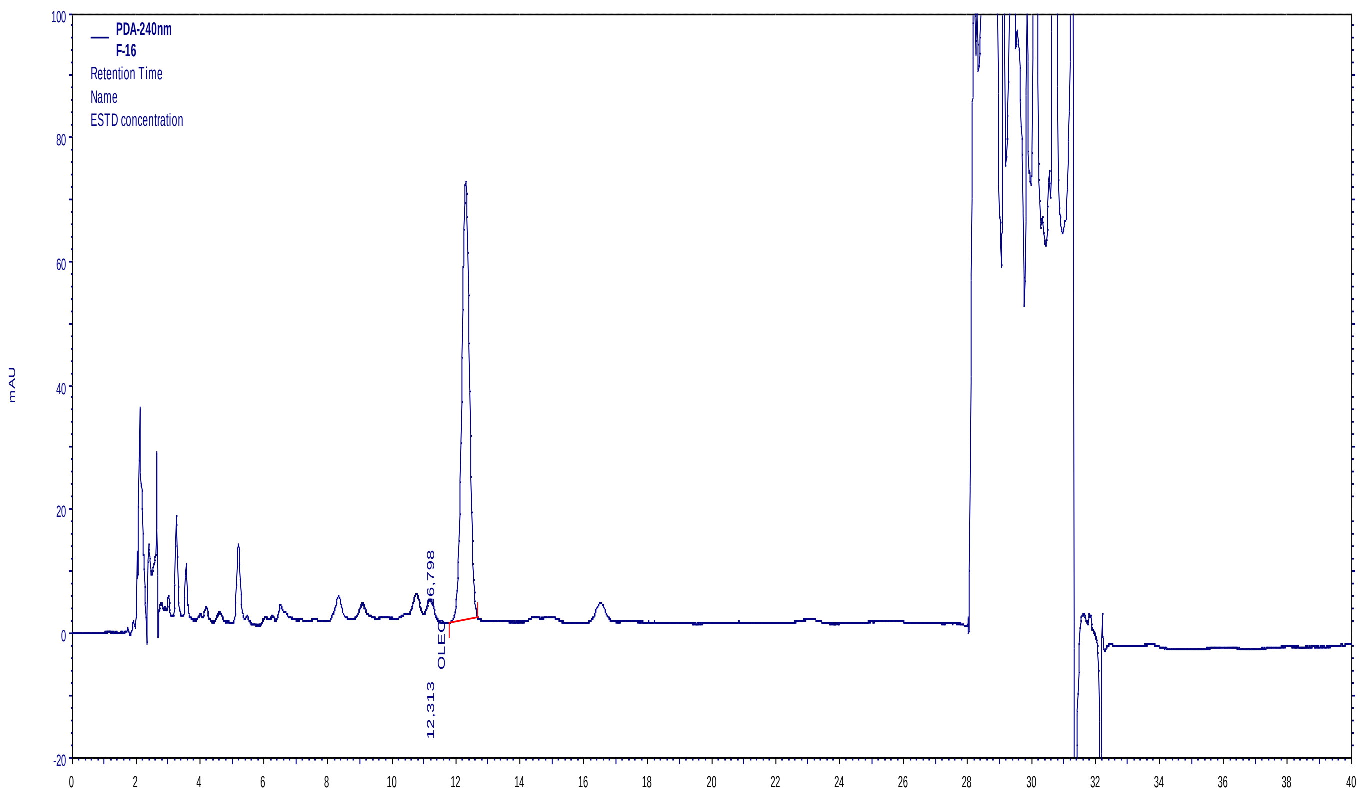

Figure 4. Oleuropein content in 0.25 regime in summer period.

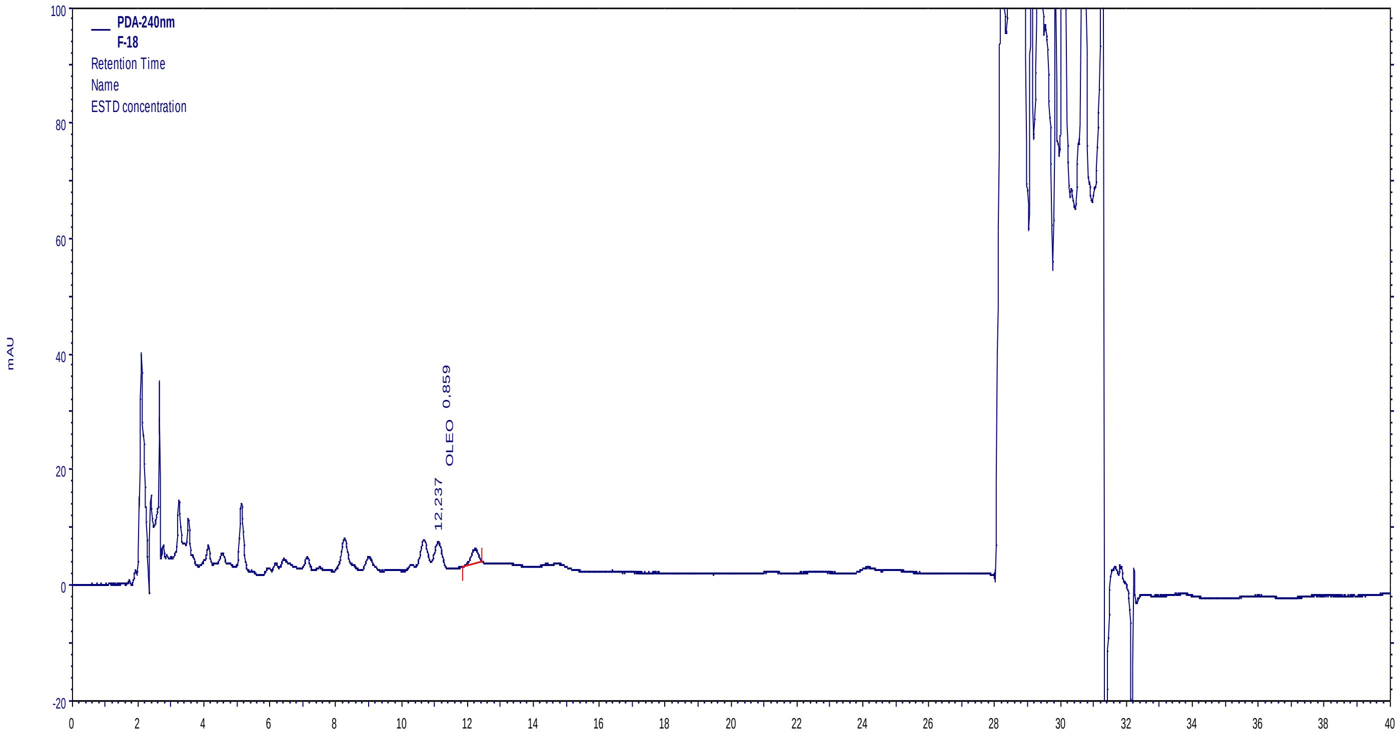

Figure 5. Oleuropein content in 1.25 regimes in summer period.

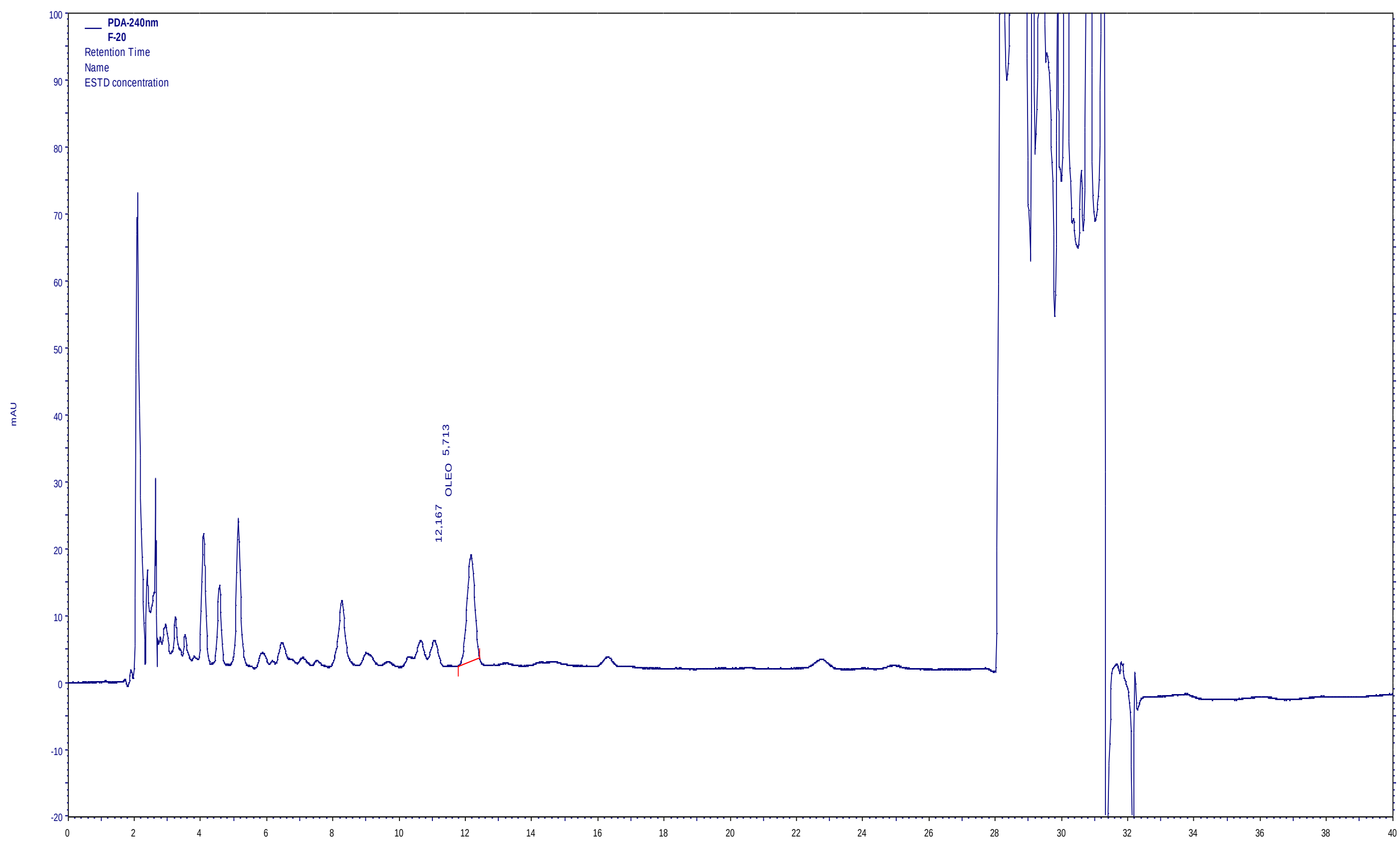

Figure 6. Oleuropein content in waterless regime in spring period.

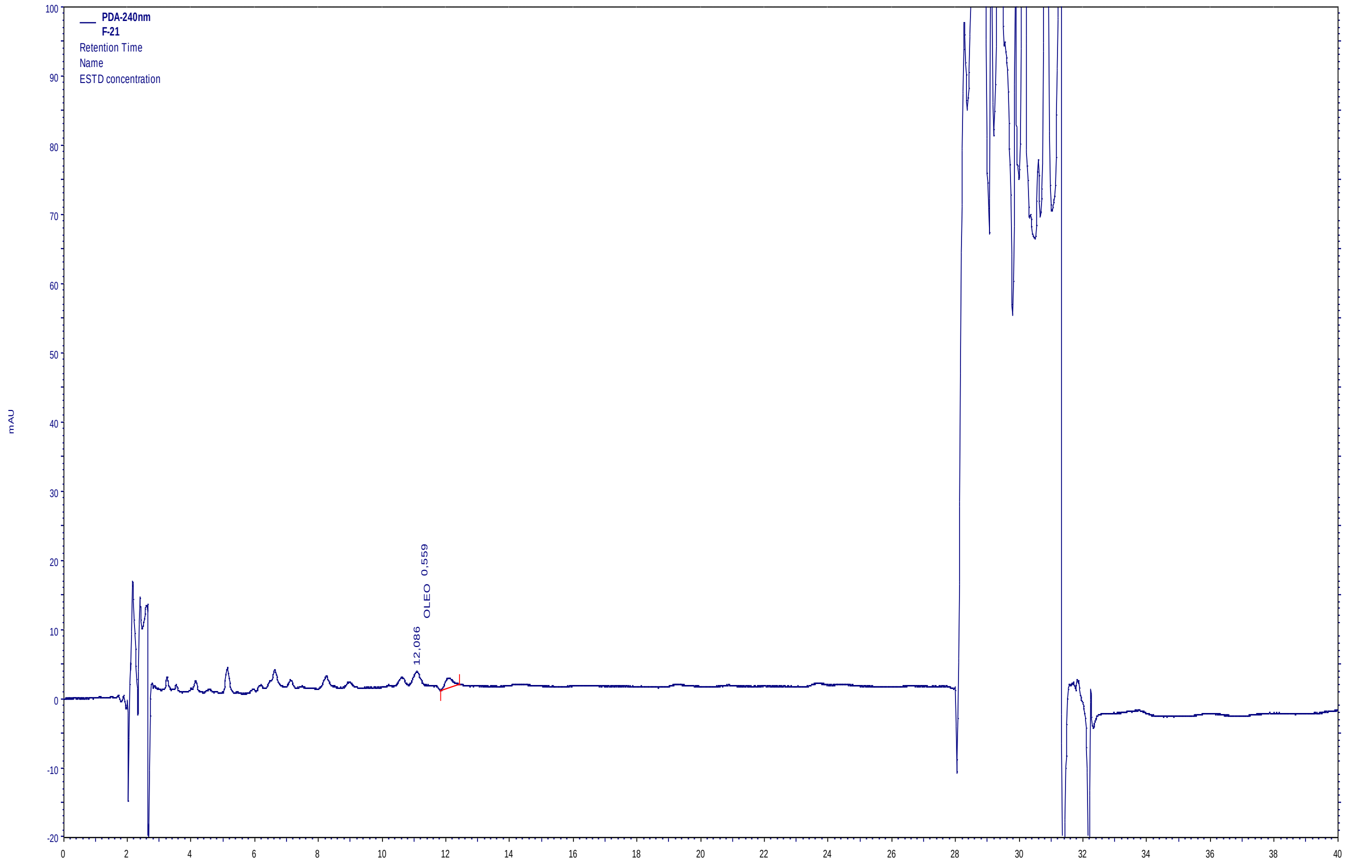

Figure 7. Oleuropein content in 1.25 regimes in spring period.

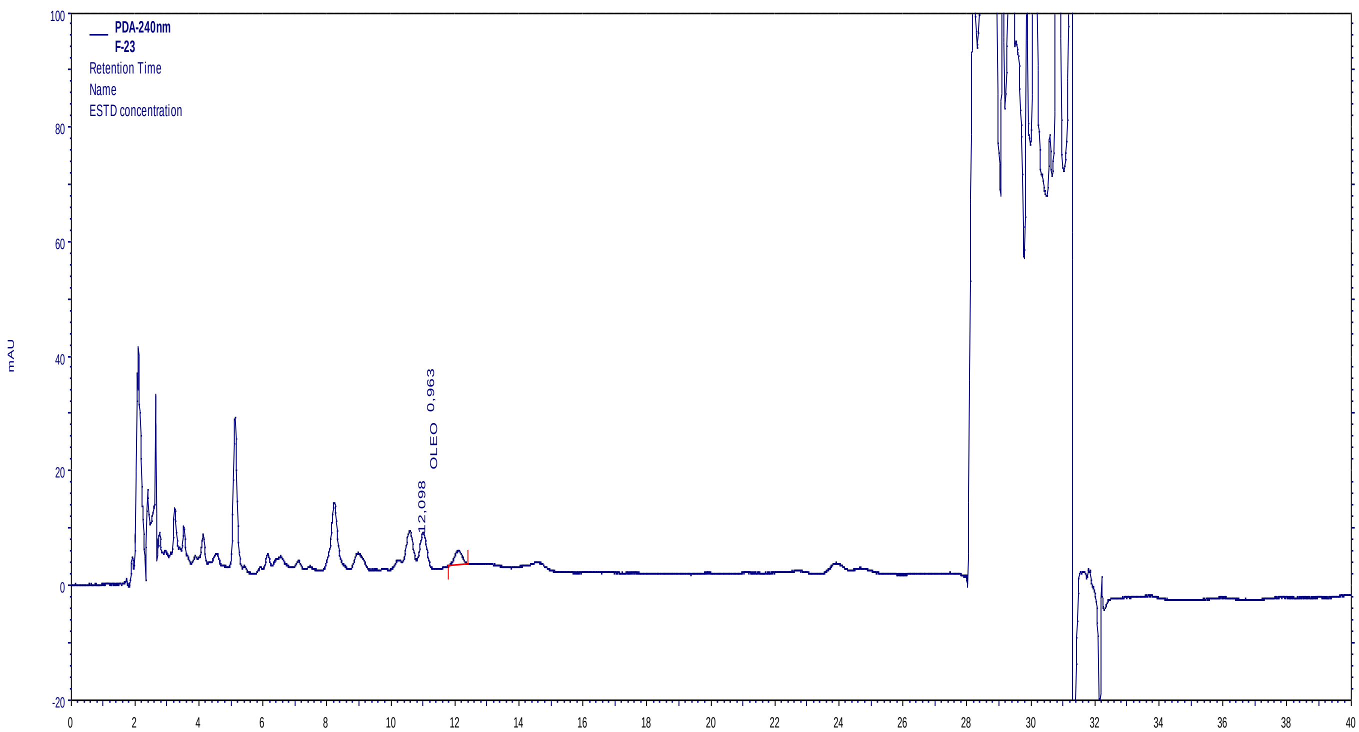

Figure 8. Oleuropein content in 0.5 regime in spring period.

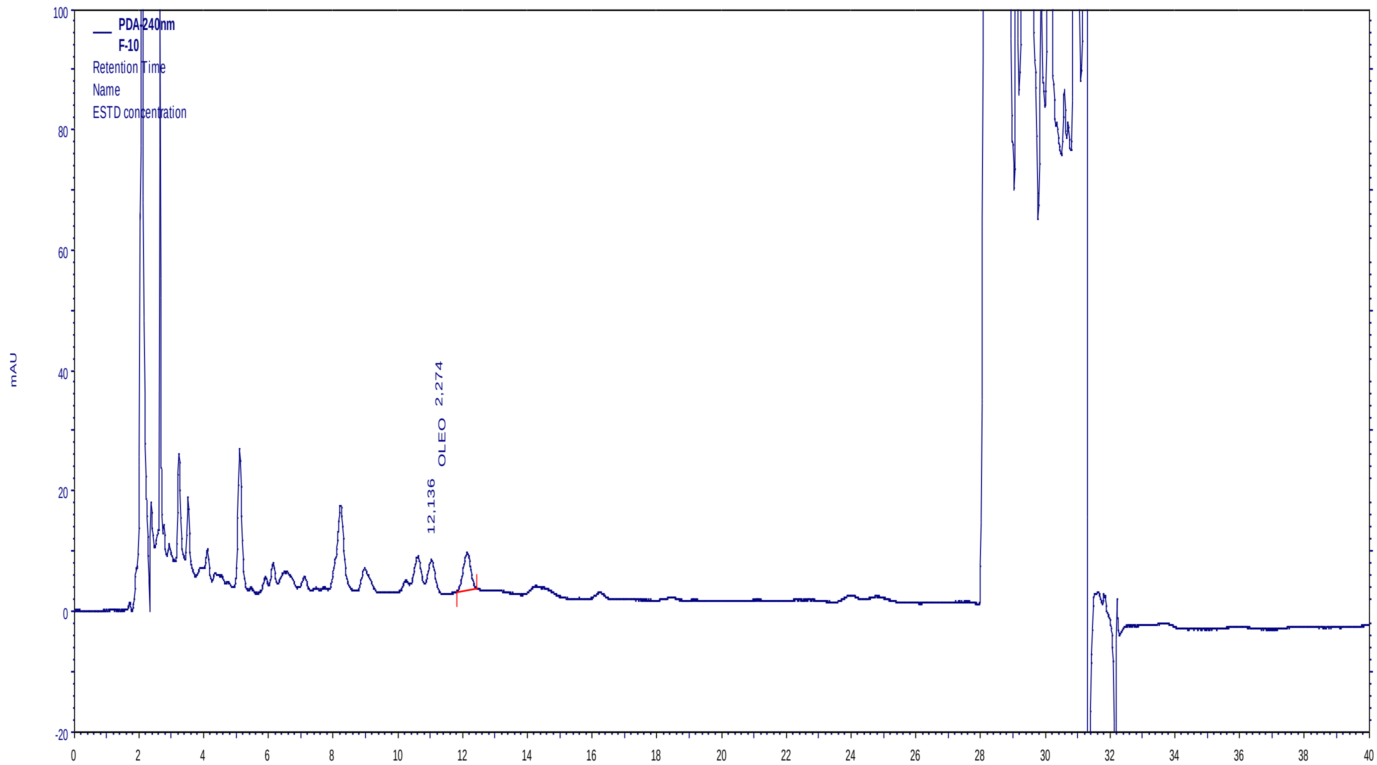

Figure 9. Oleuropein content in 1.25 regimes in autumn period.

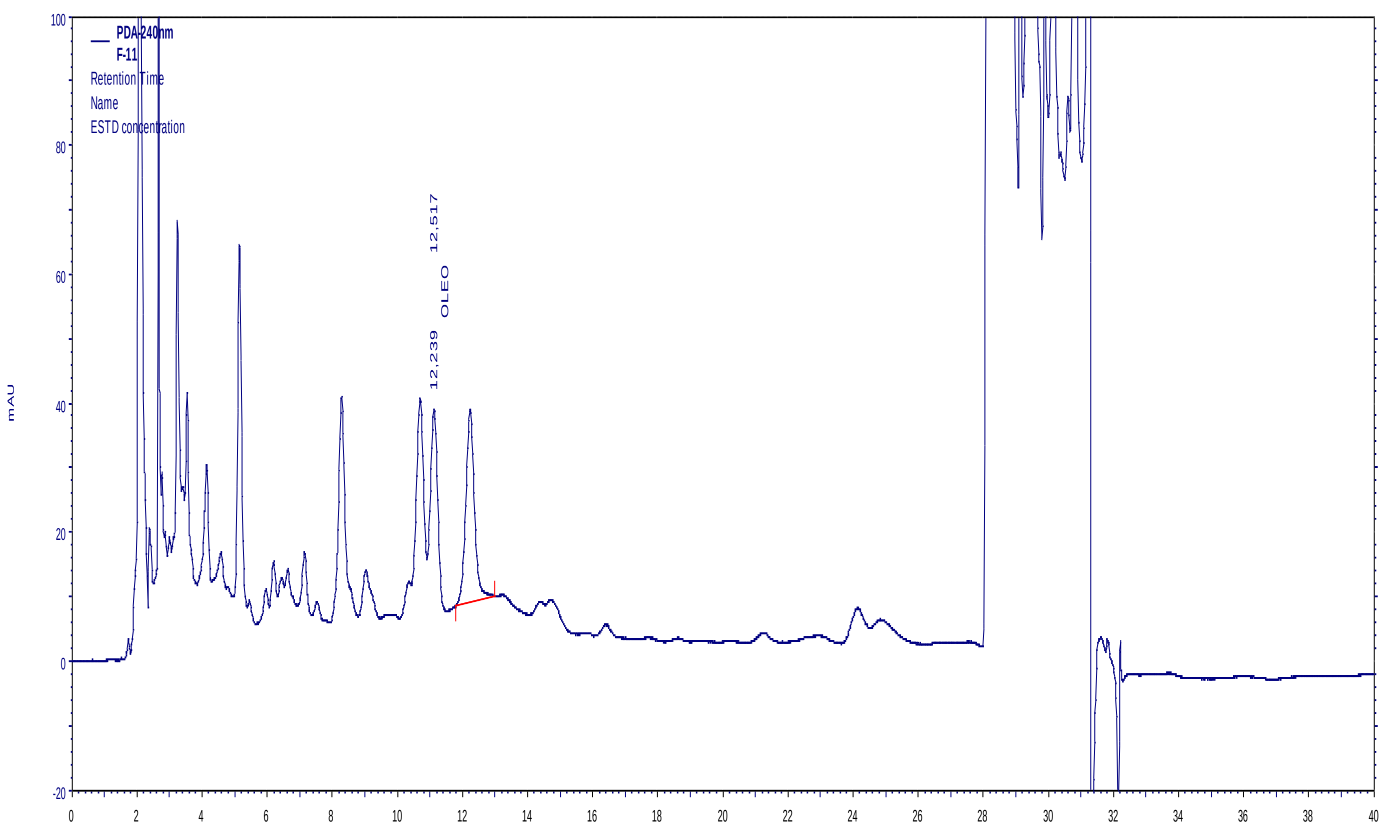

Figure 10. Oleuropein content in waterless regime in autumn period.

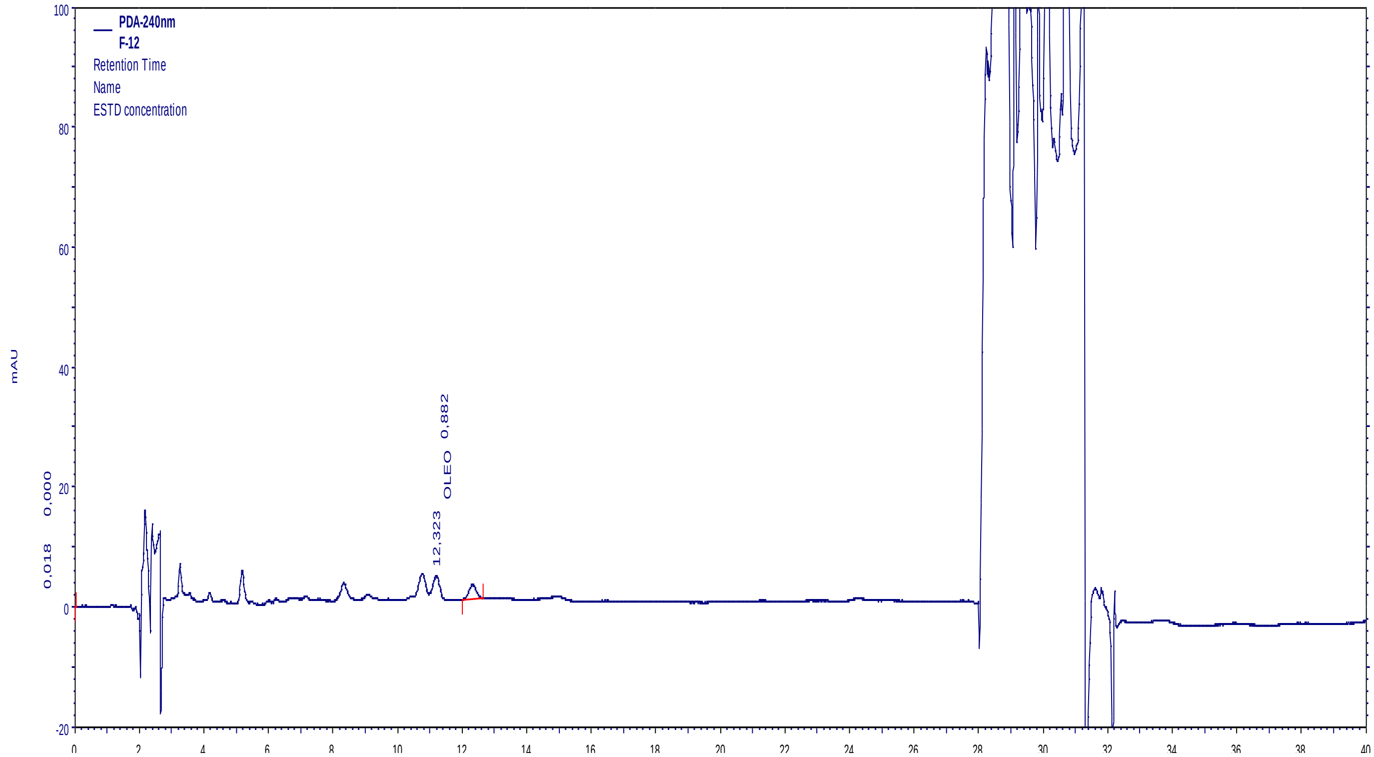

Figure 11. Oleuropein content in 0.25 regime in autumn period.

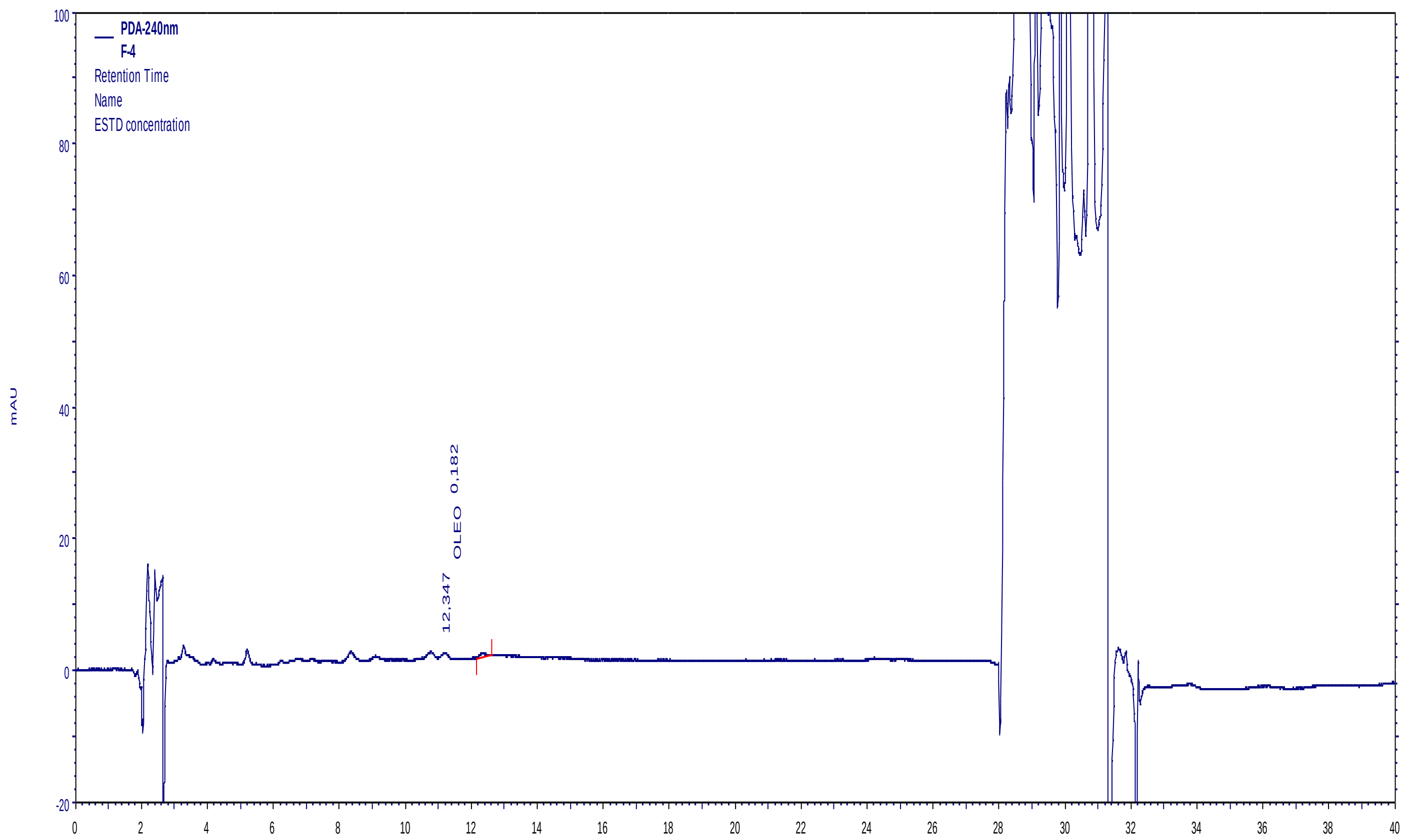

Figure 12. Oleuropein content in 1.25 regime in winter period.

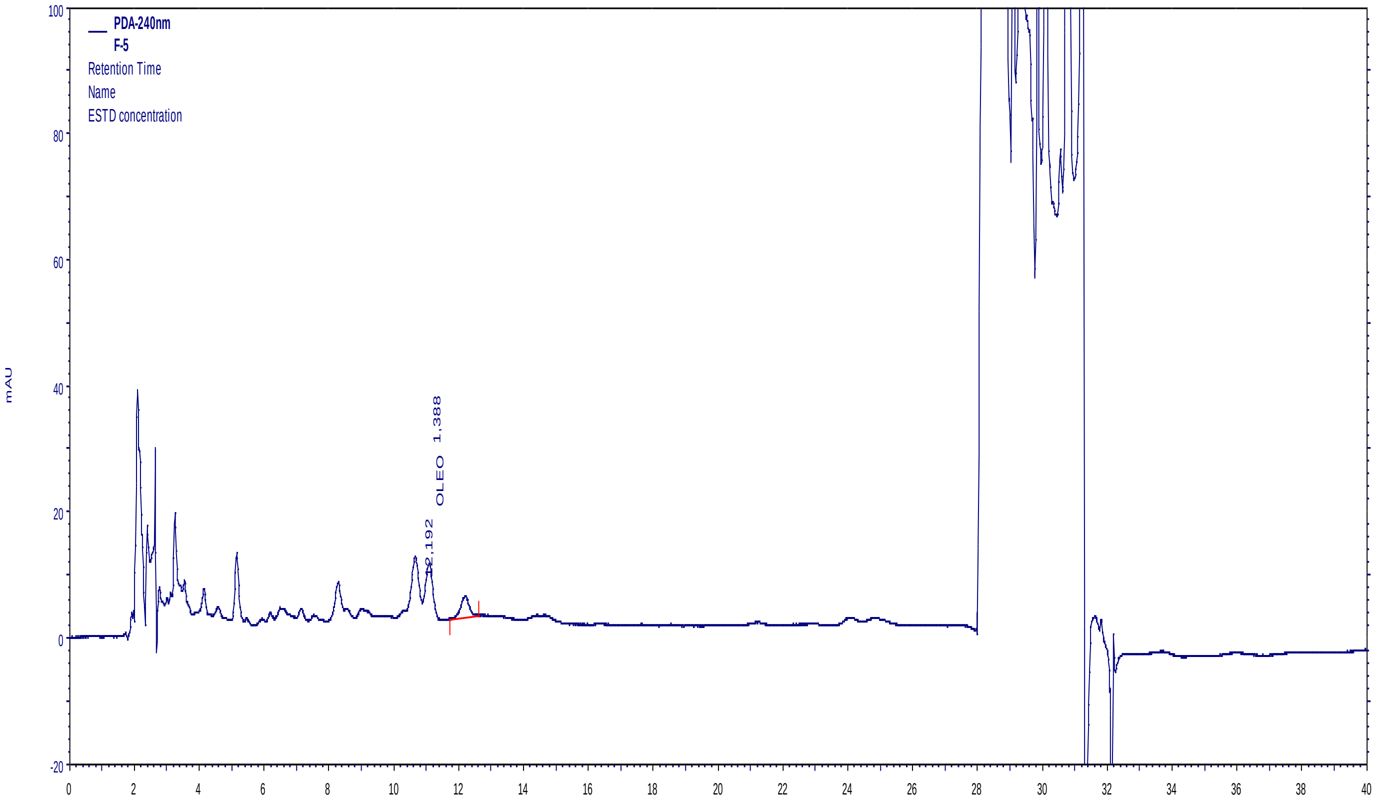

Figure 13. Oleuropein content in 0.25 regime in winter period.

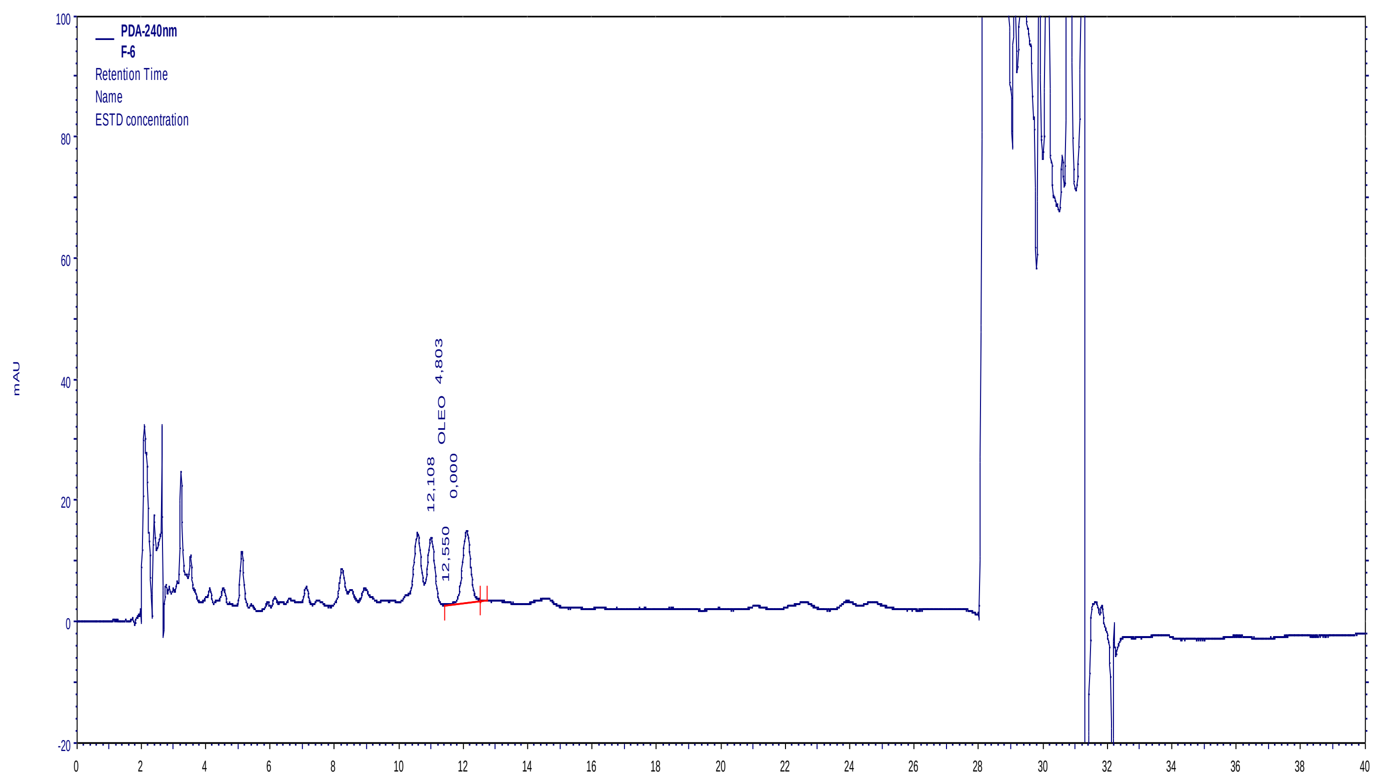

Figure 14. Oleuropein content in waterless regime in winter period.

the activity of which increases with water stress (Servili et al., 2007; Morello´et al., 2005; Gómez-Rico et al., 2007). Major results support that increased amount of water produces oils with lower phenolic content (Gómez-Rico et al., 2007; Patumi et al., 2002). Nevertheless, some authors reported no effect or even an increase in phenolic compounds, particularly during the first stages of fruit ripening in irrigated trees compared to rain fed ones. This effect may be related to genetic or other agronomic aspects (Tovar et al., 2001; Motilva et al., 1999). Meanwhile, relatively recent research (Artajo et al., 2006) supports that the concentration of secoiridoids increases with water stress. Servili et al. (2007) also reported an increase in aglycon derivatives of oleuropein and a decrease in tyrosol in water-stressed trees.

In an original study performed without taking into consideration the seasonal and irrigational differences, the oleuropein contents of the leaves and branches of natural *O. europaea* L. *sylvestris* and cultured (*O. europaea* L. var *europea*) olive trees were assigned by reversed phase HPLC method in C18 column quantitatively. Oleuropein content in *O. europaea* L. var. *europaea* was 3.506% in leaves and 1.438% in branches in Balikesir-Edremit gathered plants, 4.020% in leaves and 1.097% in branches of samples gathered from Konya, and 5.197% in leaves and 1.462% in branches of *O. europaea* L. var. *sylvestris* plant from Osmaniye (Altinyay and Altun, 2006). Great diversities in oleuropein content of leaves of seven Italian cultivar according to colour, age, genetic differences and gathering period (spring, autumn) were identified quantitatively. Dark green leaves of development period were rich in oleuropein; the oleuropein content was rather lowest in the falling period of the yellowish leaves. This indicates that oleuropein decomposed gradually in the leaves with the increasement in the age because of the key role of beta glycosidase enzyme (Briante et al., 2000, 2004; Moracci et al., 1995; Briante et al., 2001, 2002). Oleuropein compound was also affected from the gathering period of olive leaves. There were not any differences between the homolog varieties in the same colour and age gathered March-October months, but this evaluation was realized without notifying the culture condition and analytical method. Leaf samples gathered in October showed lower oleuropein content than those gathered in March. Oleuropein glycoside decomposed highly in autumn periods and additionally young green leaf production decreased according to spring (Ranalli et al., 2006). But in our study only the green leaves of plant were gathered and with the application of different irrigation regimes oleuropein contents were found low (1.21 ± 0.01%) in waterless regime in autumn and this content decreased adversely with an increasement in irrigation. Moreover, oleuropein content was 0.25 ± 0.02% in 1.25 irrigation regimes in autumn, while this value showed an increase as 0.79 ± 0.06% between the values of different irrigation regimes of spring period.

An additional major advancement is the introduction of irrigation into traditionally rain-fed olive oil orchards (Moriana et al., 2003). Irrigation has been shown to significantly increase tree- and orchard-scale oil yield (Gómez-Rico et al., 2006; Patumi et al., 1999, 2002; Tovar et al., 2002a). Several studies have demonstrated the effects of irrigation level on oil quality and environmental conditions of southern European characteristic cultivars (Moriana et al., 2003; Artajo et al., 2006; G´omez-Rico et al., 2007; Moriana et al., 2007). On the other hand, polyphenols and consequently the bitter index and oxidative stability decreased with increasing irrigation levels (Tovar et al., 2002a). A similar negative effect of irrigation level on polyphenol content, and consequently on bitterness taste was reported for ‘Cornicabra’ in Spain11 and for ‘Kalamata’ and other varieties in Italy (Patumi et al., 2002; Tovar et al., 2002b). These studies did not find other criteria of oil quality to be affected by additional irrigation. The olive oil content decreased when the water application was increased but rose during ripening. Moreover, phenol contents increased according to the fruit ripening, whereas no clear cut differences or consistent effects was observed by irrigation. Consequently, a restitution of 75% of crop evapotranspiration (ETc.) was sufficient to achieve good minor compounds; however, higher water volumes (100% etc.) gave little additional α-tocopherol and phenols increases (Dabbou et al., 2011).

To investigate the effect of irrigation level on the quality of virgin olive oil (VOO) produced from cv. Souri, six regimes, ranging from deficit to excess, were applied to trees in a mature and traditional orchard as it was converted to irrigation. Data were collected from two consecutive growing seasons. Increased irrigation quantity increased the free acidity level of the oil and decreased the total phenol content of VOO, while the peroxide level of the oil was not affected. Oil of hand-picked fruits had lower free acidity, higher polyphenol content and slightly lower peroxide level compared to the oil of mechanically harvested olives (Dag et al., 2008). Furthermore, 30 years old trees (irrigated with rain water) were used in Italian cultivars. Seven species at maturation period were taken between the months August to November (Ortega Garcia and Juan, 2010). Decrease in oleuropein concentrations were observed during the maturation period (Ortega Garcia et al., 2008). Hydroxytyrosine and tyrosine contents were found to increase from mid September to October in stems and concentration of these three compounds were higher in stems than in roots. Meanwhile, oleuropein concentration increased in mid September towards the late November, but in roots oleuropein and hyrdroxytyrosine concentrations highly decreased during the maturation (Ortega Garcia and Juan, 2010). Fruit dry weight increased from September to October. Because of the greater synthesis of phenols in stages prior to variation the stage at which fruits being to change their pigmentation. Oleuropein concentration increased from samples of September to November (Ortega Garcia and Juan, 2010).

While the oleuropein contents of four Tunus cultivars (grown under linear irrigation in the Sfax institute of Tunus) were high in the beginning of maturation stage, it decreased in harvest period. The main phenolic compounds were identified as hydroxytyrosol, and oleuropein by HPLC and liquid chromatography–mass spectrometry (LC-MS / MS), rutin and luteolin-7-glycoside were the flavonoids. Investigation of Tunisian olive drupes revealed considerable quantitative differences in oleuropein contents among the different cultivars: in the first stage of maturation, the amount of oleuropein ranged from 3.02 g kg^{-1} for Chemchali cv. to 4.02 g kg^{-1} for Chetoui cv., whereas, in the last stage of maturation, the oleuropein content declined significantly and varied from 1.33 to 2.50 g kg^{-1} in Chetoui and Chemchali cultivars, respectively. In all the analysed cultivars, the maximum oleuropein content was above 3.34 g kg^{-1} and Chetoui cultivar had the highest concentration reaching 5.86 g kg^{-1} (Bouaziz et al., 2010). While hydroxytyrosol content increased in harvest time, oleuropein decreased. This difference could be explained by an increase in the activity of the hydrolytic enzymes, particularly in glycosidases which catalyze the hydrolysis of the oleuropein and secoiridoid derivatives with the production of oleuropein aglycone and hydroxytyrosol (Amiot et al., 1986; Briante et al., 2004; Selvaggini et al., 2004). All studied varieties had a remarkable antioxidant activity particularly in the last harvest (in black olive) (Montedoro et al., 1992; El Riachy et al., 2011).

According to the quality studies mentioned above, irrigation is the main parameter of the virgin olive quality. However, in our study, irrigation was used as a stress factor, which affected the oleuropein content. So far, considerable results were obtained to enhance the scarcity of reports of such investigation in literature. When waterless irrigation regime was applied, the highest oleuropein content was observed (16.25 ± 0.25%) in the leaves of *O. europaea* L. cv Memecik gathered from the summer season. Evaluating the four seasonal results related with irrigation regime will be helpful in industrial recovery of oleuropein from olive leaves in the future.

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