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VARIATIONS IN POLYPHENOL CONTENTS AND RELATIVE WATER CONTENT IN OLIVE (*OLEA EUROPAEA* L.) LEAVES IN RESPONSE TO DROUGHT STRESS

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Abstract

Secondary metabolites or their precursors are influenced by biotic and abiotic environmental factors in addition to the different plant genotypes or cultivars of the species, agricultural practices and post-harvest processes. The present study was designed to examine the variations of polyphenol contents of olive leaves according to the irrigation and non-irrigation during months of the year. Furthermore, two cultivars of olive trees were compared for their response to the conditions. Also, young and old trees of Kilis Yaglik were compared for their content. Along with the present study, total phenolic content and flavonoid content were higher in Kilis Yaglik and irrigated conditions but higher relative water content was observed in Gemlik. Furthermore, higher phenolic content and relative water content were determined in young trees of Kilis Yaglik whereas old trees exhibited higher flavonoid content. Considering the monthly changes of contents, higher phenolic content was found in months of spring whereas higher content of flavonoids was observed in months.

***Key words**: Olea europaea L., water related content, leaf content, polyphenol, drought*

INTRODUCTION

Olive (*Olea europaea*; *Oleaceae* family) is represented with many genera distributing in Upper Mesopotamia including the South-eastern Anatolian Region and South Asia (Owen et al., 2000). Approximately 97% of world olive tree and olive production belongs to Mediterranean countries (Menduh, 2015). Ripe fruits and edible oil extracted from fruits are of great interests for Mediterranean cousins. In addition to the edible consumption, the uses of olive oil and the leaves for alternative and complementary medicine system are also common. The studies reported the therapeutic activities of olive leaves such as lowering blood pressure, enhancing immune system, and antibacterial, antifungal and anti-inflammatory. Ryan et al. (2001) and Ferreira et al. (2007) indicated the importance of olive for their active polyphenol contents. The olive leaf extracts are characterized with oleuropein, rutin, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside (Benavente-Garcia et al., 2000; Savournin et al., 2001). We should note that the content and composition of those metabolites are not constant. The metabolites are stress-driven or exhibit variations in response to the any alterations of living conditions of the plants themselves. In addition to the exogenous factors, the quality, origin and variety of the plant material effects concentration of polyphenolic compounds in olive leaves (Campeol et al., 2003). Of those metabolites, Salah et al. (2012) highlights the importance of oleuropein as a promising phenolic for the pharmaceutical industry in the future. Drought is the main danger to biological life all over the world through affecting physiology and biochemistry of plant. The effect of abiotic stress factors on physiological and biochemical indices of the plants vary depending on genotype, the amount of applied water and exposure time (Petridis et al., 2012).
Olea europaea L. belonging to the *Oleaceae* family is of the most important crops in Mediterranean countries on which they cover around 8 million ha (Guinda et al., 2004). Concerned with the various levels of water requirement, olive varieties may differ in their acclimatization mechanisms against water deficiency. Herein, it is aimed to monitor the

monthly and seasonal changes of total phenolic and total flavonoid composition of olives cultivars leaves induced by water scarcity.

MATERIALS AND METHODS

Plant material

An experiment was conducted on two different olive cultivars (cv. Kilis Yaglık and Gemlik) under Kilis ecological conditions between 2011-2012 years. The study was performed with three replicates and each replicate includes two trees. The trees were subjected to their agro-ecological conditions and climates in order to monitor the monthly and seasonal variations between the cultivars aged differently as a response to the irrigated and non-irrigated conditions. The leaf samples of the olive trees were harvested and dried under shadow.

Methanol extraction of leaf samples - 5 grams of dried and powdered leaf samples were stirred with 100 ml of methanol for 30 min. Then, the extracts were filtrated through Whatman No. 4 filter paper. Filtrated extracts were concentrated using a Rotary Evaporator.

Determination of total phenolic content - was realised according to the Folin-Ciocalteu reagent method (Singleton et al., 1999). The amount of total phenol was calculated as mg/g (Gallic Acid Equivalents) from calibration curve of Gallic acid standard solution.

Determination of total flavonoid content – was assessed using by aluminium chloride method using quercetin as a reference compound (Kumaran and Karunakaran, 2006). This method based on the formation of a complex flavonoid-aluminium. Total flavonoid content of plant leaves was expressed as mg quercetin equivalents (CE)/g of dried olive leave material.

Relative Water Content (RWC) was determined according to the methods proposed by Sanchez et al. (2004) and Turkan et al. (2005). The percentage of relative water content was calculated according to the following formula:

RWC = (Fw-Dw) / (Tw-Dw) x100 were,

(Fw - Fresh weight; Dw - Dry weight; Tw: Turgor weight).

Statistical analysis - all measurements were replicates. The data were subjected to the two-ANOVA and means comparison was analysed using Duncan's multiple range tests. Statistical analysis was performed using MSTATC (Michigan State University, East Lansing, MI). Differences were considered to be statistically significant at a level of $P < 0.05$.

RESULTS AND DISCUSSIONS

The current study was designed to examine the effects of irrigated and non-irrigated conditions on the relative water content, total phenol and flavonoid content of olive cultivars (cv. Kilis Yaglık and Gemlik). The same parameters in young and old trees of cv. Kilis Yaglık were also determined. It was of the main targets to determine the reactions against stress factors. The changes in contents of olive leaves according to cultivars and ages are shown in Table 1 and Table 2. Besides these results monthly changes of total phenolic contents, total flavonoid contents and relative water content in leaves of olive are given in Table 3.

Table 1.Total Phenolic contents, Total Flavonoid contents and Relative Water Content in leaves of olive cultivars under irrigated and non-irrigated conditions

	Cultivars			Irrigation		
	Kilis Yaglık	**Gemlik**	**LSD**	**Irrigated**	**Non-irrigated**	**LSD**
Total Phenolic (mg/g GAE)	103.35 **a**	97.14 **b**	0.0139	101.71 **a**	98.78 **b**	0.014
Total Flavonoid (mg/g QE)	73.23 **a**	70.81 **b**	0.399	72.79 a	71.26 **b**	0.399
Relative Water Content (%)	80.40 **b**	82.60 **a**	0.3335	79.90 **b**	83.10 **a**	0.334

Means in the same column by the same letter are not significantly different to the test of Duncan (α=0.05)

Table 2. Total Phenolic contents, Total Flavonoid contents and Relative Water content in leaves of different aged Kilis Yaglık cultivars under irrigated and non-irrigated conditions

	Kilis Yaglık			Irrigation		
	Young	Old	LSD	Irrigated	Non-irrigated	LSD
Total Phenolic (mg/g GAE)	103.3 a	101.26 **b**	0.0139	102.06 **b**	102.56 **a**	0.0139
Total Flavonoid (mg/g QE)	73.23 b	77.87 a	0.598	75.88 a	75.23 b	0.598
Relative Water Content (%)	80.61**a**	79.18 **b**	0.2209	79.16 **b**	80.636 **a**	0.2209

Means in the same column by the same letter are not significantly different according to Duncan test (α=0.05)

Table 3. Monthly changes Total Phenolic contents, Total Flavonoid contents and Relative Water Content in leaves of olive

Months	Total Phenolic (mg/g GAE)	Total Flavonoid (mg/g QE)	Relative Water Content (%)
January	120.95 **b**	74.57 **bc**	70.7 **f**
February	133.75 **a**	71.37 **de**	86.2 **a**
March	111.07 **c**	65.40 **f**	84.3 **ab**
April	119.47 **b**	66.85 **f**	75.8 **de**
May	100.07 **d**	73.27 **cd**	85.7 **a**
June	93.36 **e**	73.13 **cd**	88.0 **a**
July	80.69 **g**	78.91 **a**	87.9 **a**
August	78.16 **g**	77.54 **ab**	74.1 **ef**
September	95.92 **e**	68.71 **ef**	86.1 **a**
October	85.49 **f**	66.61 **f**	80.9 **bc**
November	82.13 **fg**	77.36 **ab**	80.4 **c**
December	101.90 **d**	70.56 **de**	78.2 **cd**
LSD:	4.145	3.491	3.837

Means in the same column by the same letter are not significantly different according to Duncan test (α=0.05)

Irrigation systems elicited statistically significant differences with respect to the total phenolic content between the cultivars and months. The highest total phenolic content was achieved in February while phenolic content decreased in October and November.

Kilis Yaglık accumulated more total phenolic content than cv. Gemlik and irrigated regime induced more biosynthesis of total polyphenols. In the current study, the age factors (old and young) significantly influenced the total phenolic content. The young aged trees accumulated more phenolic contents (Figure 1, 2).

In this context, the variations in total flavonoid content in leaves of different olive trees and different-aged tree groups under irrigated and non-irrigated conditions were determined.

According to the current results, statistically significant differences were determined with respect to the total flavonoid contents among the groups.

The highest total flavonoid contents were seasonally determined in July and then decreased in the months of March and April. Kilis Yaglık accumulated more flavonoid content than Gemlik and more flavonoid contents were obtained under irrigated conditions (Figure 3). Seasonal flavonoid contents in different age groups of Kilis Yaglık varied depending on the irrigation systems. In this context, more flavonoid contents were determined in old tree groups (Figure 4).

There were statistically significant differences between months and cultivars under non-irrigated conditions however the percentages of relative water content were closer to each other. Once compared to control group for each cultivar, variations in RWC between different periods have been determined in relation to the effects of stress conditions. RWC did not change until the mid of the year but then it started to decrease. The RWC in cv. Gemlik was higher than cv. Kilis Yaglık and the highest RWC was observed under non-irrigated conditions (Figure 5). Also, young tree groups of cv. Kilis Yaglık accumulated more RWC than the older ones (Figure 6).

Phenolic components are significant elements in struggle against abiotic and biotic stress factors (Ruiz and Romero, 2001; Dogan, 2005). Phenolic compound biosynthesis and accumulation was stimulated in response to the abiotic and biotic stress factors (Dixon and Paiva, 1995; Naczk and Shahidi, 2004). However, total phenolic content may demonstrate decline with increasing stress factors in different species and genotypes. Total phenolic and flavonoid contents of *Nigella sativa* L. are adversely influenced but salt at higher concentration stimulated biosynthesis of quercetin, apigenin and trans-cinnamic acid (Bourgou et al., 2010). In another study, the polyphenol contents of *Cynara scolymus* L. were significant increased with salt applications and the accumulation of cafeic acid and chlorogenic acid composition were positively affected (Rezazadeh et al., 2012). In addition, phenolic contents are significantly influenced by different age groups (Seemannová et al., 2006; Achakzai et al., 2009). Young, old and age factors influence the total phenolic and the contents decrease with the increasing periods (Padda and Picha, 2007). The relations between flavonoid biosynthesis and stress conditions have been investigated (Rezazadeh et al., 2012). The flavonoid contents were reported to increase and lipid peroxidation declined under salt stress conditions. Moreover, the alleviation roles of total flavonoid against salts stress tolerance have been reported (Chutipaijit et al., 2009).

RWC in tolerant genotypes or cultivars were less-influenced in comparison with less-tolerant ones and RWC variations in tolerant plants are generally non-significant (Turkan et al., 2005). *Acorus americanus* exposed to drought conditions included 35% less RWC than the control group (Romanello et al., 2008).

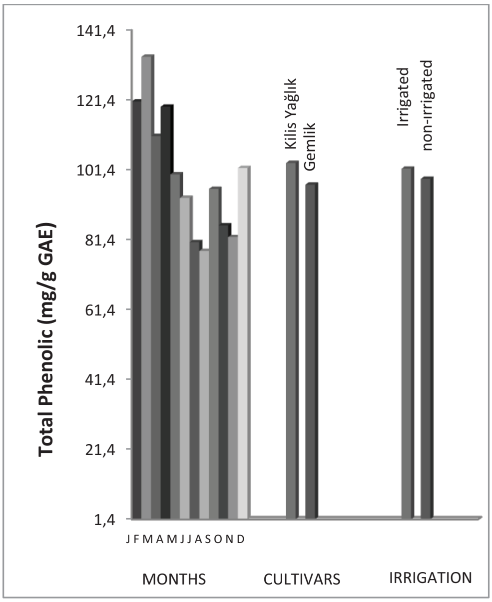

Figure 1. Monthly Total Phenolic content of olive leaves according to cultivars and irrigation

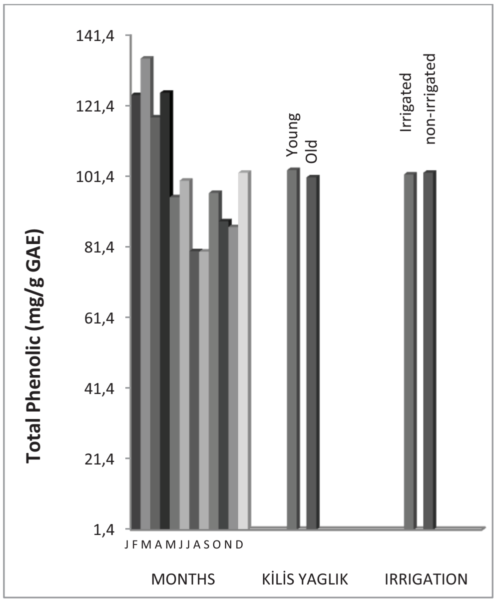

Figure 2. Monthly Total Phenolic content of olive leaves according to tree age and irrigation

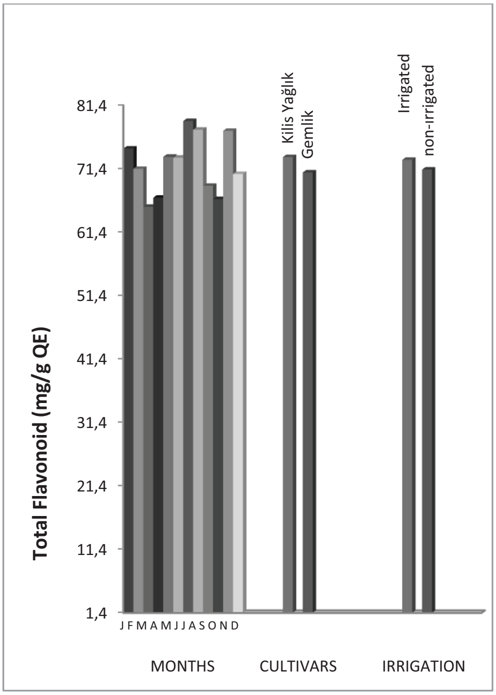

Figure 3. Monthly Total Flavonoid content of olive leaves according to cultivars and irrigation

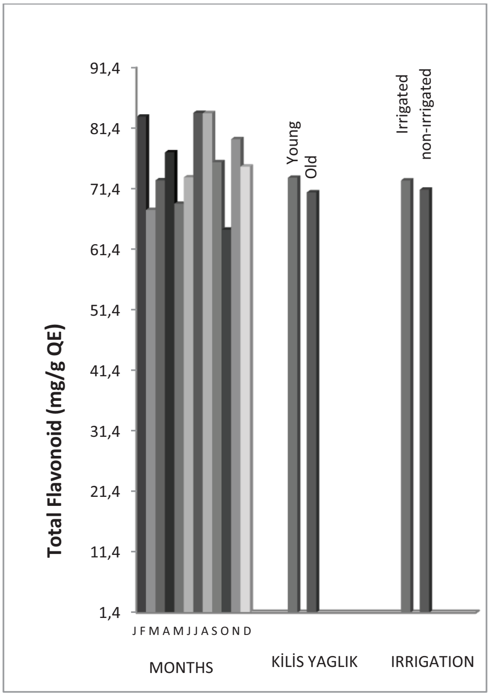

Figure 4. Monthly Total Flavonoid content of olive leaves according to tree age and irrigation

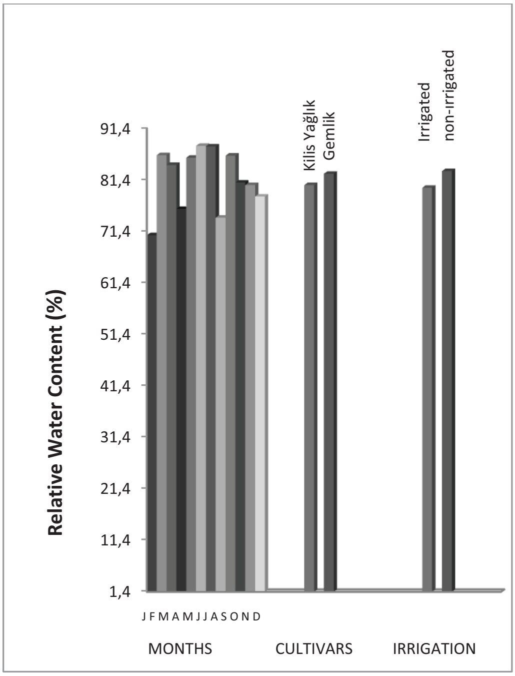

Figure 5. Monthly Relative Water content of olive leaves according to cultivars and irrigation

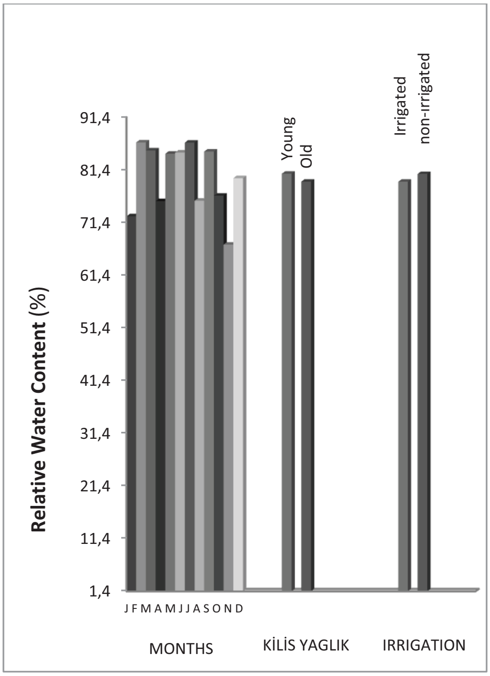

Figure 6. Monthly Relative Water content of olive leaves according to tree age and irrigation

CONCLUSIONS

To sustain their life span, plants have to evolve defense mechanisms against stress factors. In this context, these defense mechanisms may include the production, accumulation and secretion of secondary metabolites in addition to the primary metabolites. The variation concerned with metabolites may influence the agricultural properties such as growth and yield parameters and also qualitative parameters such as nutritional value and biological activities of the plant. Phenolic compounds are of the essential chemical parameters in identification and characterization of olive cultivars and they are significantly affected by harvest time and location, harvest techniques and method and olive genotypes or cultivars. Phenolic compounds influence the biological potential, tolerance and flavor of oil. In the current study, bioactive component content varied depending on the cultivars and maturity. Hence, factors such as maturity or cultivars should be taken into account in medicinal, pharmaceutical and food sectors.

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