ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 20(5) 2021, 43–61
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2021.5.5
ORIGINAL PAPER
Accepted: 14.10.2020

OLIVE ANTIOXIDANTS UNDER CLIMATIC CONDITIONS

Dhouha Saidana Naija✉[1], Samia Ben Mansour Gueddes[1], Imed Cheraief [2], Fathi Ben Mariem[1], Wafa Ghariani[1], Mohamed Braham[1]

[1] Laboratory of the Improvement of Olive Tree Productivity and Product Quality, Olive Tree Institute, Sousse Station, 4061, Tunisia
[2] Laboratory of Biochemistry, UR "Human Nutrition and Metabolic Disorder", Faculty of Medicine, Monastir 5019, Tunisia

ABSTRACT

Climate change has become a widespread serious phenomenon. Its effects are related to variability in local climates rather than in global climatic patterns. Mediterranean countries are the most concerned where olive tree constitutes one of the most dynamic cultivations. This work focuses on the research for new indicators of the adaptation of the olive tree to several climatic conditions. 'Chemlali' and 'Chetoui' represent the primary Tunisian olive tree cultivars. To adapt to different climatic conditions characterizing the north, the center and the south of the country (superior semiarid, inferior semiarid and inferior arid respectively), these varieties synthesize many interesting compounds that have been screened and compared. Indeed, the methanolic extracts from 'Chemlali' and 'Chetoui' leaves have been tested for their antioxidant activities. The chemical compositions of the extracts have been quantified in antioxidants. Both 'Chemlali' and 'Chetoui' exhibited a significant antioxidant activity, reaching 90%. However, Chemlali activity was more important in the inferior semiarid (80%) and in the inferior arid (70%), while 'Chetoui' activity was more significant in the superior semiarid and in the inferior arid. Total phenols of 'Chemlali' showed a triple content in the inferior arid comparatively to the superior semiarid. Additionally, flavonoids, o-diphenols, saponin and carotenoids of 'Chemlali' increased significantly in this area as opposed to those of 'Chetoui'. 2(3H) 5-methyl furanone, 4-vinyl methoxyphenol, and hexadecanoic acid known for their antioxidant activity and many others have been identified in these varieties.

Key words: olive tree, superior semiarid, inferior semiarid, inferior arid, antioxidants

INTRODUCTION

Nowadays, climate change has grown into a serious problem profoundly impacting our world. Climate change has actually affected precipitation intensity, water availability, landless vulnerability, agricultural productivity, food safety and quality, life quality and welfare in general. Mediterranean countries, mainly southern ones, seem to be the most endangered regions due to climate change harmful effects, given that drought in those areas has been more frequent, more intense and more lasting when compared to other parts.

The olive culture has been a characteristic feature of the Mediterranean region, for it has been part and parcel of people's life there, shaping the economy, society and even the environment at large. The olive oil production of the Mediterranean region has reached approximately 98% of the world production, and the consumption of this oil has rose to 82% of the world consumption [Aguilera et al. 2014].

Tunisia has ranked as the 4th world olive producer and 2nd in terms of plantation and exportation [Al-Dobai and Nasr 2016]. However, the country has been characterized by marked climate variability in time and space and by extreme aridity. Olive trees that have been cultivated especially in rain-fed conditions

✉ dhouhasaidana@yahoo.com

under the minimum rainfall have been often subjected during the summer to long periods of severe drought. According to Smirnoff [1993], the water stress has been often associated with an increase in the level of reactive oxygen species (ROS) describing the oxidative stress that has been additionally imposed. In this fact, drought has created oxidative stress in the plant cell due to elevated leakage of electrons to O_2 in the photosynthetic and respiratory systems, leading to an improvement in the production of ROS.

To adapt to this situation, olives have adopted, for sustainability, various oxidative stress defense mechanisms including the accumulation of phenolic chemicals, known for their interesting antioxidant properties [Kiritsakis and Shahidi 2017]. According to Popović et al. [2016], several phenolic compounds could be used as indicators of the adaptability of plant species to water deficit. Besides phenols, many other compounds have possessed antioxidant power, such saponins [Watson 2014], phytosterols [Hsu et al. 2017] and chlorophyll and derivates [Lanfer-Marquez et al. 2005] elements that have played an important role in drought stress tolerance.

The relationship between all these parameter and olive behavior under hard climatic conditions has not been well established, and the antioxidant molecules synthesized by the olive tree to overcome drought stress have not been well known, either.

Adaptation of plant species to drought can include phenological, morphological, physiological and metabolic changes [Bhattacharjee and Saha 2014]. The metabolic response has not been well comprehended. Physiological and biochemical comportments, including quantitative and qualitative changes of carbohydrates, proteins and lipids, have been detected in several plants submitted under diverse levels of water deficit. The aim of this study, therefore, was to identify new metabolites, indicators of the olive tree adaptability to hard climatic conditions, which may help in better understanding survival mechanisms in drought. The enhancement of antioxidant power in olives may augment their adaptive capacity, improve their performance and maintain their important position in the world as healthy food. In this fact, the primary Tunisian olives, 'Chemlali' and 'Chetoui', cultivated in different climatic conditions located in several areas, were studied for their antioxidant activities and the related phytochemicals that are indispensable to adapt to these conditions.

MATERIALS AND METHODS

Plant material

The olives *Olea europaea* (L.), 'Chemlali' and 'Chetoui', growing in rain-fed conditions, in flowering state (May 2012), were harvested from coastal areas situated in the north, the center and the south of Tunisia (Mornag, Chott Mariem and Zarzis respectively). The experimental sites belong to diverse bioclimatic zones (Tab. 1). Olive trees used in this study were equivalents in age, size and other culture conditions. Five olives were sampled of each variety in each region.

Climatic parameters, relatively to these sites, were detailed in Figure 1. Data were determined from a standard meteorological station neighboring to the experimental field. Accordingly, the climatic parameters that most discriminated the studied areas were maximal relative humidity and precipitation. The maximal relative humidity progressively decreased

Table 1. Climatology of different sampling sites

Region	Coordinates	Bioclimatic stage	Localisation
Mornag	36°40'51"N 10°17'25"E	superior semiarid	coastal north zone
Chott Meriem	35°56'08"N 10°33'26"E	inferior semiarid	coastal center zone
Zarzis	33°30'N 11°07'E	inferior arid	coastal south zone

from superior semiarid to inferior semiarid and inferior arid, reaching respectively 81.19, 75.70 and 30.83%. While precipitation, although nil in all areas during this month, was very high in superior semiarid (84.4 mm) and inferior semiarid (129.5 mm) compared to inferior arid (7.72 mm), in previous months. Accumulative amounts of precipitation of 190.3, 212.3 and 14.27 mm were respectively calculated.

Extraction of phytochemicals

Preparation of olive leaf samples. Fresh olive leaves were selected randomly from around the tree. Leaves were washed with water then dried in the stove at 40°C for 3 days. Next, dried leaves were pulverized in a blender in order to obtain a fine powder and stored in a dry place in the dark for extraction.

Extraction procedure. 5 g of dried and powdered leaves was refluxed with 50 ml of methanol (100%). The extracts were stirred during 24 h at room temperature at 120 rpm, then filtered through Whatman filter paper (No. 1) and concentrated with a vacuum evaporator. The extraction was repeated twice. The extract dried was re-suspended in methanol in a final concentration of 1 mg/mL.

Antioxidant capacity determination

DPPH radical-scavenging essay. To estimate free radical scavenging activities of the methanolic extracts from leaves of 'Chemlali' and 'Chetoui' cultivars, the method of Choi et al. [2002] was adopted.

ABTS$^{\bullet+}$ scavenging assay. The ABTS$^{\bullet+}$ test was performed according to the method of Yu et al. [2002].

Determination of total phenol content. Total phenols were quantified in each extract using the Folin-Ciocalteu method described by McDonald et al. [2001]. The total phenol contents were expressed as milligram of catechin equivalents per gram of dry weight (mg CE/g DW).

Determination of total flavonoid content. Total flavonoids contents were determined according to Chang et al. [2002] method. Total flavonoid levels were expressed as milligram of quercetine equivalents per gram of dry weight (mg QE/g DW).

Determination of total condensed tannin content. The condensed tannins were measured by adopting the method of vanillin in acidic medium, as described by Ba et al. [2010]. The results were expressed in milligram of catechol equivalents per gram of dry weight (mg EC/g DW).

Determination of ortho-diphenol content. According to Blekas et al. [2002] method, ortho-diphenol contents were determined. The results were expressed in milligrams of caffeic acid equivalent per gram of dry weight (mg CAE/g DW).

Saponin content. The contents of total saponins in foliar methanolic extract were determined as described by Baccou et al. [1977]. The content of total saponins was expressed in milligram of Dioscin equivalents per gram of dry weight (mg DE/g DW).

Chlorophyll a, chlorophyll b and carotenoid content. The absorbance of the methanol extracts (200 µL) was read at 400–700 nm. Chlorophyll a, b and total caroten have shown maxima absorbencies at 666, 653 and 470 nm respectively. The quantities of these pigments were determined according to Lichtenthaler and Wellburn [1983].

Phytosterol content. The dosage of phytosterol was based solely on the direct contact of the plant extract with Liebermann-Burchard reagent (LB), according to Araújo et al. [2013] method.

The phytosterol content was expressed in milligram of β-sitosterol equivalents per gram of dry weight (mg SE/g DW).

GC-MS. Analysis of the constituents of the extracts was conducted on a Hewlett-Packard GC-MS system described by Saidana et al. [2008].

Statistical analysis

All data presented the averages of triplicate analyses. The data were recorded as means ± standard deviations. Analysis of variance was performed by ANOVA procedures using SPSS 20. Significant differences between means were determined by the Tukey test, with $p \leq 0.05$ being regarded as significant. Principal Component Analysis (PCA) was carried out to evaluate the associations among phenolic parameters, chlorophyll, carotenoids, saponins, phytosterols, the antioxidant activity and the sampling sites.

RESULTS

Antioxidant capacity determination

DPPH radical-scavenging activity. T he antioxidant activity of the methanolic extracts from the ol-

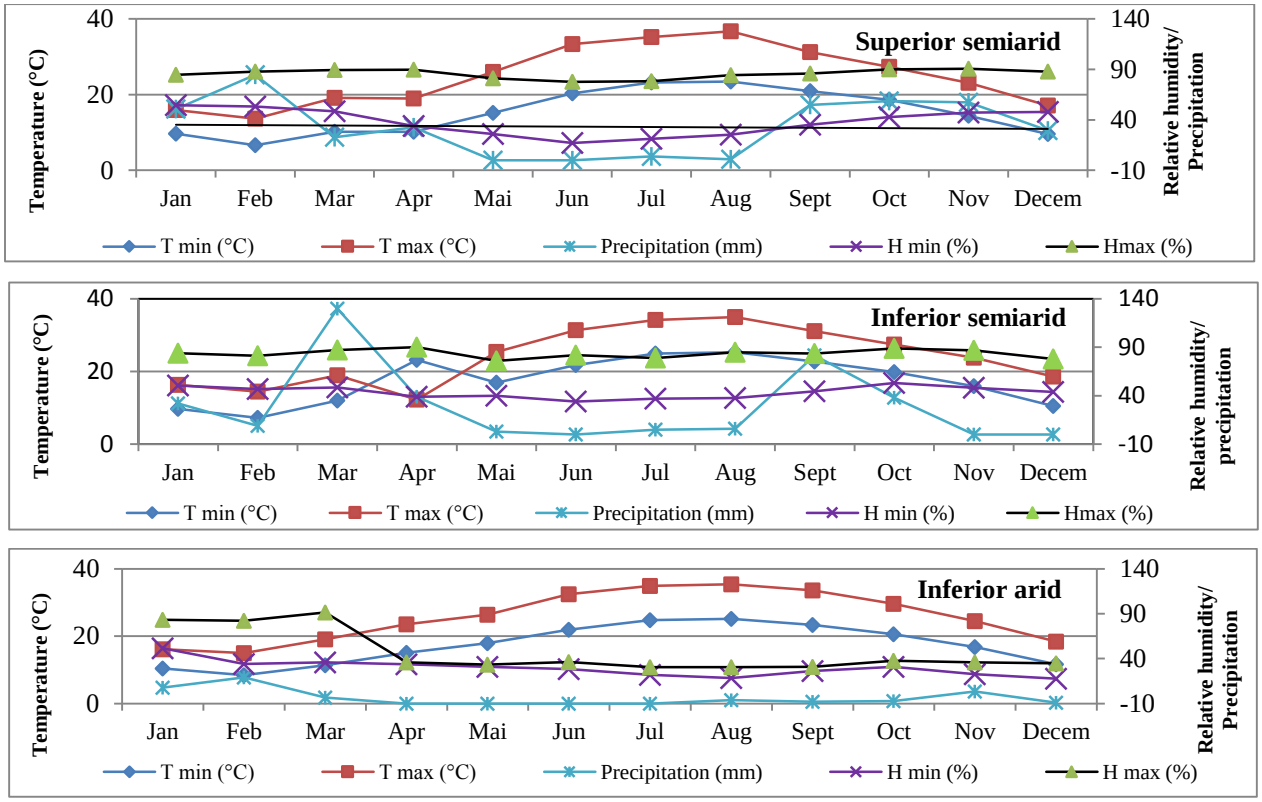

Fig. 1. Monthly climatic variations of coastal areas, situated in superior semiarid, inferior semiarid and inferior arid, during the trial period (the year 2012)

(T max: maximal temperature; T min: minimal temperature; H max: maximal relative humidity; H min: minimal relative humidity).

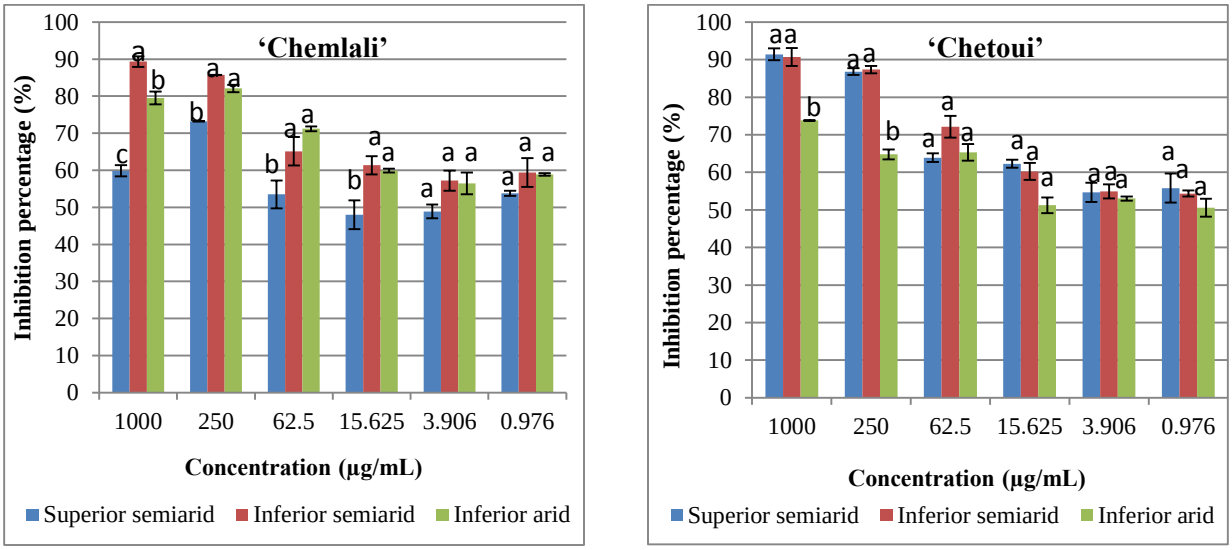

Fig. 2. DPPH radical scavenging activity (%) of methanolic extracts from 'Chemlali' and 'Chetoui', growing in superior semiarid, inferior semiarid and inferior arid

For each concentration, means with different letters were significantly different at 5%

ive leaves was very powerful against DPPH (Fig. 2). It reached its maximum in inferior semiarid for 'Chemlali' and in superior semiarid for 'Chetoui', reaching respectively 89.33 and 91.40 % at 1 mg/mL. The antioxidant activity of 'Chemlali' was increasing in inferior semiarid and inferior arid compared to superior semiarid.

Indeed, the unregistered activity increased from 59.83% in superior semiarid to 89.33% in inferior semiarid (increase of 33.01%), and to 79.49% in inferior arid (increase of 24.72%). This increase was noted for most tested concentrations.

For 'Chetoui', the most important antioxidant activities were those of superior semiarid and inferior semiarid, reaching 91.40 and 90.70% respectively. However, the antioxidant activity was lower in inferior arid for all tested concentrations. The difference was significant only for the two higher concentrations (73.78 and 64.75% at 1000 and 250 µg/mL).

ABTS$^{\bullet+}$ scavenging activity. For ABTS$^{\bullet+}$ radicals, the antioxidant activity was less effective than the one taking place against DPPH. It reached only a maximum of 36.01 and 42.09%, for 'Chemlali' and 'Chetoui' of inferior arid (Fig. 3).

For 'Chemlali', after 5 min of contact, the most important antioxidant activity was recorded in inferior arid (36.01%). However, it was only 24.02% in superior semiarid and 20.02% in inferior semiarid one, at 1000 µg/ml. After 30 min of contact, the antioxidant activity increased to 31.15% in superior semiarid while it decreases in inferior semiarid and inferior arid areas to 17.62% and 9.94%, respectively.

For 'Chetoui', the antioxidant activity reached its maximum in inferior arid (42.09%) after 30 min of contact, at a concentration of 1000 µg/mL, but this activity was only 24.20% after 5 min of contact.

Polyphenol content. 'Chemlali', growing in inferior arid, had the highest content in polyphenols, where it was tripled in comparison to the other (more than 45.56 mg CE/g DW) – Figure 4a.

However for 'Chetoui', inferior semiarid was distinguished by the highest cultivar in polyphenol content (23.56), followed by superior semiarid (15.15) then inferior arid (12.01 mg CE/g DW).

Flavonoid content. In all tested areas, 'Chemlali' appeared richer in flavonoids than 'Chetoui'. Flavonoid contents of 0.40; 1.71 and 6.72 mg CE/g DW were noted, in 'Chemlali' leaves, respectively in superior semiarid, in inferior semiarid and in inferior arid, while only 0.64, 0.52 and 0.90 mg CE/g DW of flavonoids were respectively noted for 'Chetoui' (Fig. 4 b).

Both tested varieties concentrated their flavonoid content in inferior arid, especially 'Chemlali' that had flavonoid content being seven times higher than that of 'Chetoui'. Thus, the difference in flavonoid content of both cultivars was highly significant at 5%.

Condensed tannin content. 'Chemlali' presented a high content in tannin especially in superior semiarid (12.54 mg EC/g DW) and inferior arid (11.50 mg EC/g DW). Nevertheless, 'Chetoui' species, found in inferior semiarid and inferior arid were characterized by a high amount of tannins (6.36 and 5.06 mg EC/g DW respectively).

In inferior arid, 'Chemlali' was the richest in tannins comparatively to 'Chetoui'; in superior semiarid, 'Chemlali' contained more than the double amount of tannins (Fig. 4c). The difference in condensed tannin content of both cultivars was highly significant.

O-diphenol content. The o-diphenol content in 'Chemlali' has progressively increased from superior semiarid to inferior arid, reaching 31.62 mg CAE/g DW (Fig. 4d). In contrast, 'Chetoui' presented the same amount of o-diphenol either in superior semiarid or in inferior semiarid; in inferior arid, it had smaller content (9.08 mg CAE/g DW). The o-diphenol content in 'Chetoui' was higher than that of 'Chemlali' both in superior semiarid and inferior semiarid. However, in inferior arid, 'Chemlali' presented much more o-diphenols than 'Chetoui', reaching more than the triple content (31.62 mg CAE/g DW).

Hence, the difference in o-diphenol content of both cultivars was highly significant.

Saponin content. Saponins were concentrated increasingly from superior semiarid to inferior arid for the 'Chemlali' species, where it reached 3.36; 5.62 and 6.29 mg DE/g DW respectively (Fig. 4e). For 'Chetoui', the saponin content was the highest in inferior arid (5.59 mg DE/g DW) comparatively to the other areas (4.78 mg DE/g DW). Contrarily to superior semiarid, saponin content for 'Chemlali' was more significant than of 'Chetoui' in inferior semiarid and inferior arid.

Phytosterol content. 'Chemlali' seemed to be richer in phytosterol in inferior semiarid (25.90 mg SE/g DW

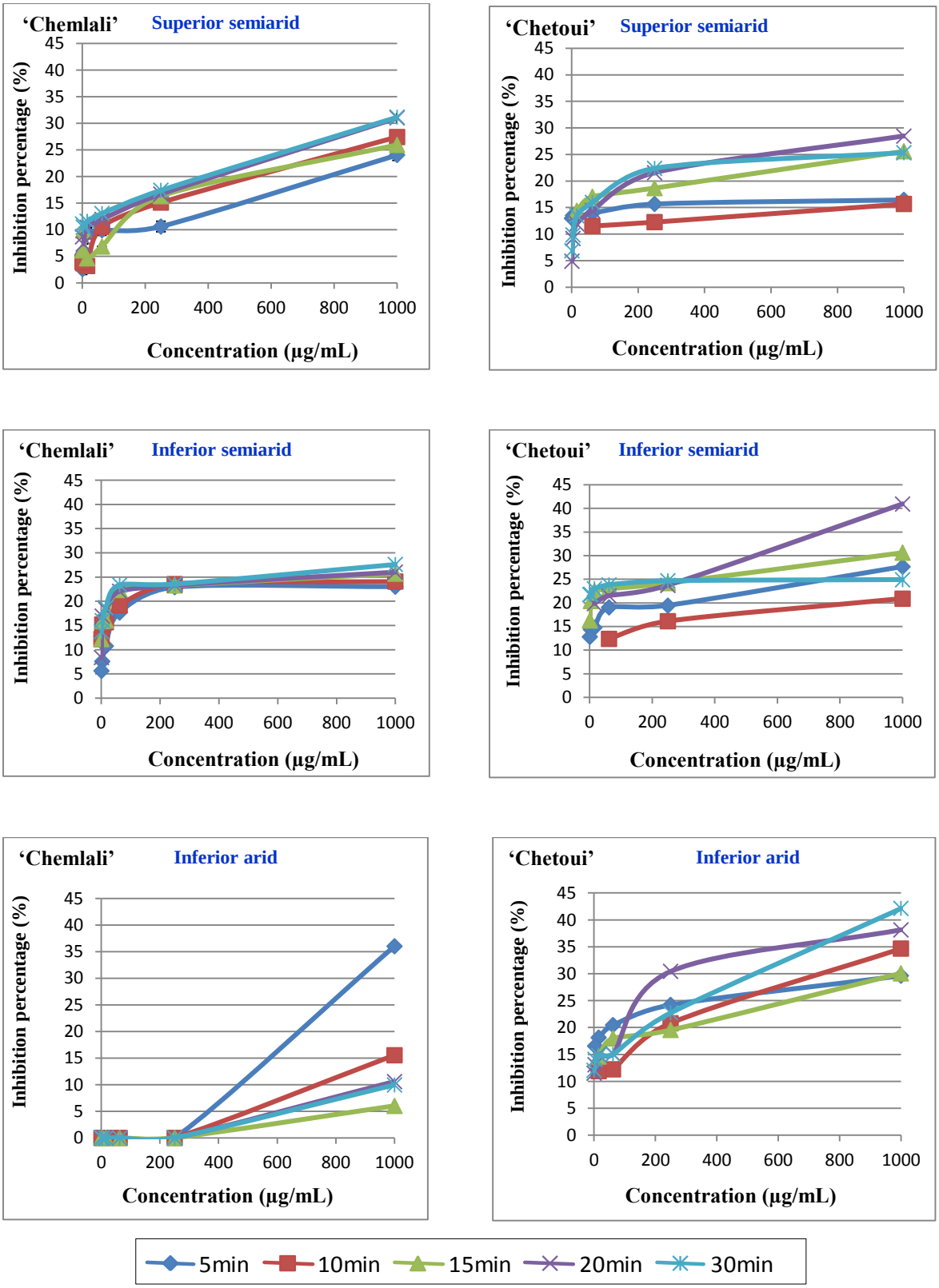

Fig. 3. ABTS$^{\bullet+}$ cation radical scavenging capacity (%) of methanolic extracts from 'Chemlali' and 'Chetoui', growing in superior semiarid, inferior semiarid and inferior arid, in function of time and concentrations

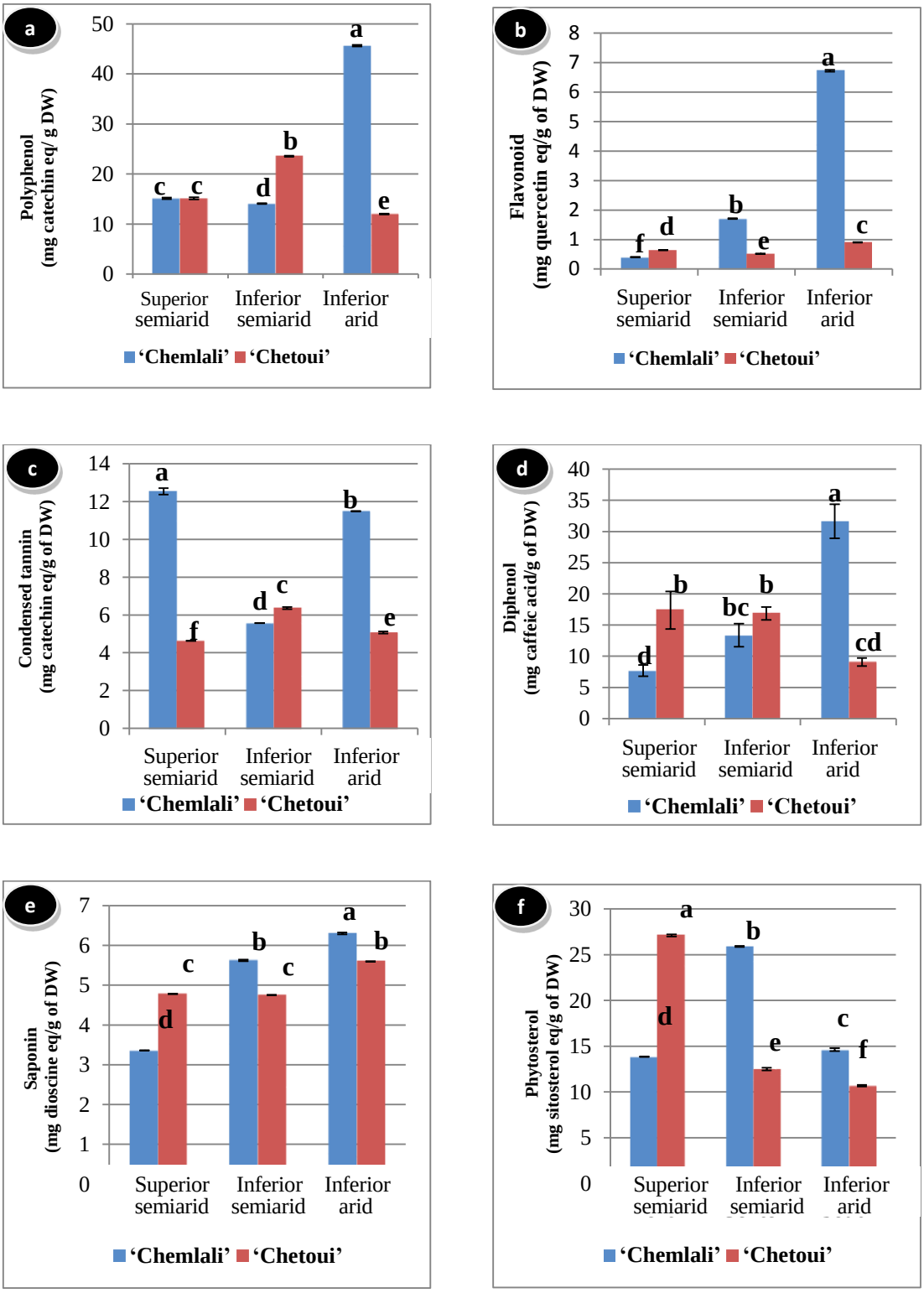

Fig. 4. Antioxidants content in both 'Chemlali' and 'Chetoui' cultivars, when grown in superior semiarid, inferior semiarid and inferior arid, expressed as mg of control equivalent/g of dry weight. For each cultivar, means with different letters were significantly different at 5%; a – polyphenol; b – flavonoid; c – condensed tannin; d – diphenol; e – saponin; f – phytosterol

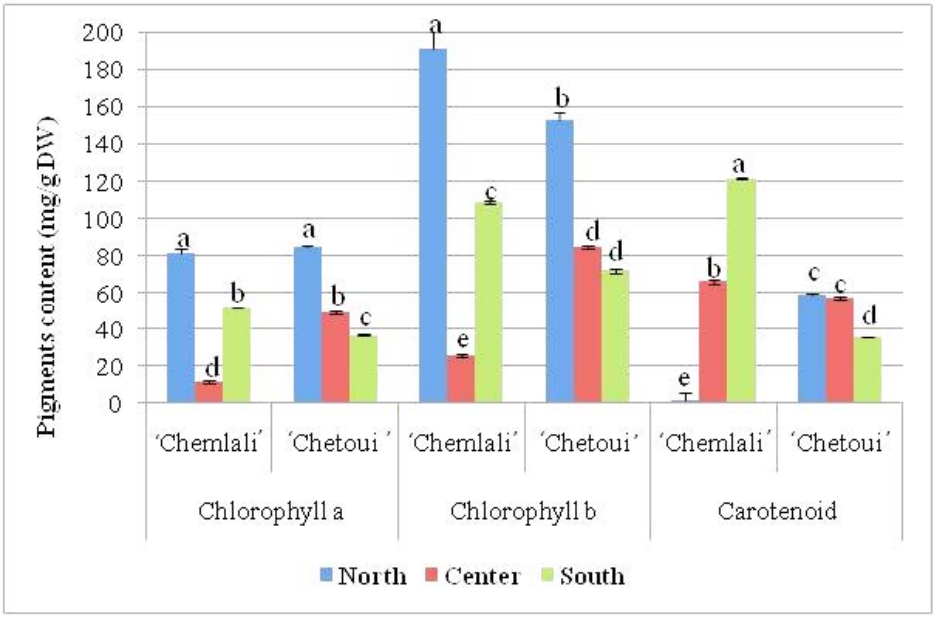

Fig. 5. Chlorophyll a, b and carotenoid content in both 'Chemlali' and 'Chetoui' cultivars, when grown in superior semiarid, inferior semiarid and inferior arid. Means with different letters indicated significantly differences at 5% for each photosynthetic pigment

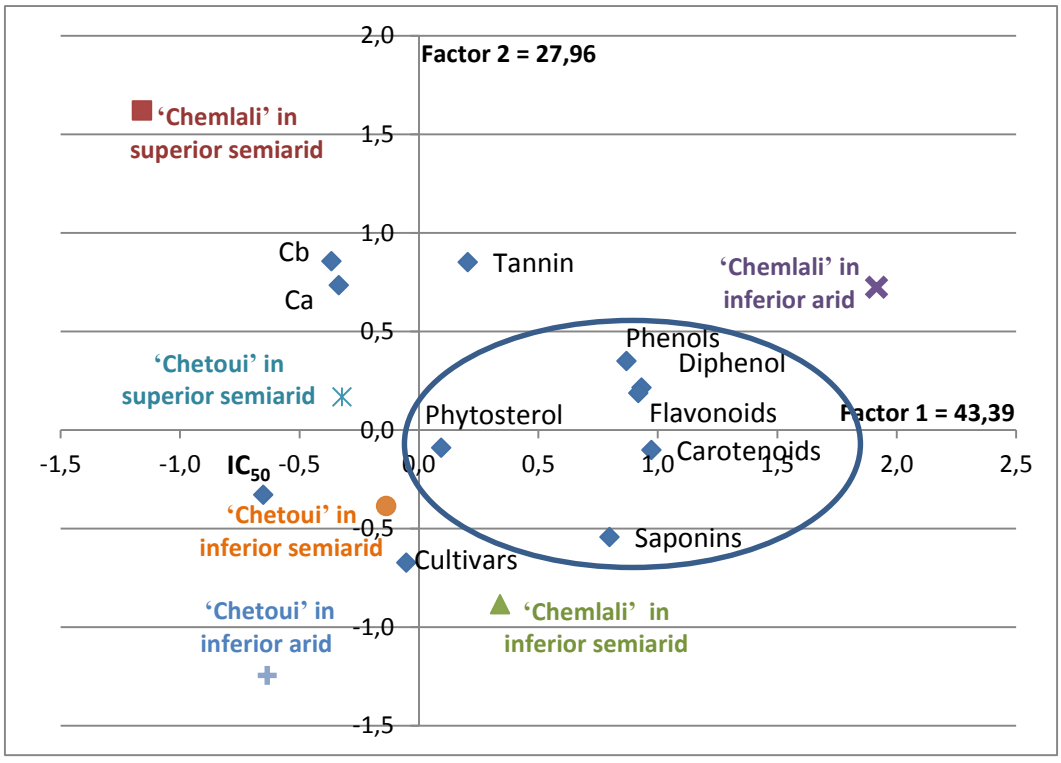

Fig. 6. PCA analysis of antioxidants in 'Chemlali' and 'Chetoui' leaves harvested in superior semiarid, inferior semiarid and inferior arid

and in inferior arid (14.62 mg SE/g DW). However, the level of phytosterol for 'Chetoui' was more important in superior semiarid and became smaller going to inferior arid (27.09; 12.50 and 10.68 mg SE/g DW respectively) – Figure 4f. A significant difference was recorded between both varieties in all these areas.

Chlorophyll a, b and carotenoid content. 'Chemlali' displayed the maximum of chlorophyll content in superior semiarid, either chlorophyll a (0.81 mg/g DW) or chlorophyll b (1.91 mg/g DW) – Figure 5. The chlorophylls a and b were concentrated then in cultivars growing in inferior arid, as 0.51 and 1.09 mg/g DW respectively.

In inferior semiarid, chlorophylls seemed to be very low reaching 0.11 and 0.37 mg/g DW respectively for chlorophylls a and b.

However, 'Chetoui' showed a decreasing gradient of concentration from superior semiarid to inferior arid for both types of chlorophylls (a and b). Indeed, chlorophyll a content was estimated to 0.85; 0.49 and 0.26 mg/g DW in superior semiarid, inferior semiarid and inferior arid (a reduction of 70 % from superior semiarid to inferior arid).

While chlorophyll b content was estimated to 1.53; 0.85 and 0.72 mg/g DW respectively in these areas (a reduction of 53% from superior semiarid to inferior arid).

On the other hand, 'Chemlali' has significantly increased its content in carotenoids in inferior arid, reaching 1.21 mg/g DW, which represented the double of its content when grown in inferior semiarid. In superior semiarid, carotenoids were found in a small quantity (0.01 mg/g DW).

Contrarily, 'Chetoui' displayed a decreasing level in its carotenoid content from superior semiarid (0.59 mg/g DW) to inferior arid (0.35 mg/g DW). 'Chetoui' thus contained more carotenoids in superior semiarid than 'Chemlali', which seemed to be richer in the other areas.

Chemometric evaluation. The Principal Component Analysis (PCA) was applied to the phenolics content and the antioxidant activities against DPPH radicals, summarized by the IC_{50} of the tested samples. The analysis was based on the correlation between the variables, from which virtual axes linearly correlated to existing variables were generated.

The variance levels explained by the PCA were 43.39 and 27.96% for Factor 1 (F1) and Factor 2 (F2), respectively (Fig. 6). As reported in the chemometric evaluation, phenols parameters, such as phenols, flavonoids, o-diphenols and tannins, were positioned closely due to the significant positive correlations among them according to both axis F1 and F2. These compounds were near 'Chemlali' of inferior arid.

The major indicators of antioxidant activity, such as phenols parameters, saponins, carotenoids, phytosterols were near the F1 axis, and belong to the same group. While, the IC_{50} were conversely correlated to this group according to both axis F1 and F2.

Chlorophyll a and b were positively correlated with 'Chemlali' and 'Chetoui', growing in superior semiarid. While, chlorophyll a, b and tannin appeared nearby, that supposes the existence of a close relationship between them.

Chemical composition of leaf olive extracts using GC-MS. The chemical composition of methanolic leaf extract from 'Chemlali' and 'Chetoui' cultivars, harvested in three different areas, located in superior semiarid, inferior semiarid and inferior arid, was analyzed by GC-MS (Tab. 2).

Identified compounds varied between 56 to 80% according to the cultivar and the studied area. 149 compounds were identified, of which 33 were common to both cultivars; while 69 compounds were identified in 'Chetoui', only 47 were recognized in 'Chemlali'.

According to Table 2, 2-furancarboxaldehyde (n° 78); 2-furanmethanol (n° 80); benzaldehyde (n° 93) and benzeneethanol (n° 100) were found in all the analyzed samples about the 'Chemlali' and 'Chetoui' species that were harvested in superior semiarid, inferior semiarid and inferior arid.

Additionally, many other compounds were identified in the olive leaves of both cultivars harvested in inferior arid. The compounds are found in important proportions and have according to many other studies, an antioxidant capacity such 2(3H)-5-methyl-furanone, (n° 77: 4.23 and 3.63% for 'Chemlali' and 'Chetoui' respectively); 4-vinyl-2-methoxy-phenol (n° 102: 3.04 and 11.35% respectively) and hexadecanoic acid (n°142: 3.63 and 3.85% respectively).

Other interesting compounds were specific of 'Chetoui' and are present in important proportions in

Table 2. Chemical composition of the methanolic extracts of 'Chemlali' and 'Chetoui' growing in superior semiarid (SS), inferior semiarid (IS) and inferior arid (IA)

N°	RT	Compounds (%)	'Chemlali'			'Chetoui'		
			SS	IS	IA	SS	IS	IA
Aliphatic hydrocarbons								
1	3.12	dichloromethane	1.57				0.71	
2	9.60	2-hexyne				0.17	0.26	
3	12.03	2,5-dimethyl-1,4-hexadiene					0.33	
4	15.93	5-methyl-1,2-hexadiene		0.29				
5	17.97	cyclopropane, 1,1-dimethyl-2-(2-methyl-1-propenyl)					0.33	
6	18.12	1-nonadecene						0.27
7	20.77	n-nonane			0.48			
8	25.88	1-octene, 2-methyl-		0.30				
9	29.77	borane, diethyl(1-ethyl-2-methyl-1-butenyl)-, (E)		0.34				
10	32.23	4-methyl-1,4-heptadiene		0.37				
11	33.50	(1,3Z,5E)-undeca-1,3,5-triene		0.20				
12	42.6	3-heptyne, 7-chloro			0.57			
Oxygenated hydrocarbons								
13	12.59	vitispirane		0.41	0.76		0.79	
14	19.07	(1'-propenyl)thiophene				0.19		
15	19.69	cyclohexene, 1-methoxy-						0.39
16	20.76	2-heptene, 2-methoxy-		0.49				
17	20.78	1,7,7-trimethyl-2-exo-(1-oxacyclohex-2-yloxy)bicyclo[2,2,1]heptane				0.84		
18	21.72	E-1,4-dimethyl-1,4-dihydroxy-cyclo hexan-2,5-diene						0.30
19	22.73	1,2-epoxy-1-vinylcyclododecene				0.22		
20	22.73	4-methyl-2-(3-methyl-2-butenyl)-furan						0.21
21	23.05	3,7,9-trioxabicyclo[4,2,1]nonane, 4,6-dimethyl-, exo					1.51	
22	25.89	2-(2-isobutenyl)-3-methylfuran						0.24
23	26.02	2H-pyran, 2-(bromomethyl)tetrahydro-		0.84				
24	27.43	2-(1,2-epoxycyclohexyl)-1-pentene						0.17
25	27.99	tert-butyl p-tolyl ether						0.44
26	31.84	9-octadecene, 1-methoxy-,				1.27		
Carbonyl compounds								
Ketones								
27	4.57	2,3-pentanedione			0.28			
28	8	2-pentanone			0.99			
29	12.09	2-acetyl furan						0.20
30	12.94	methanone, dicyclopropyl-		0.20		0.25		
31	13.33	4-cyclopentene-1,3-dione		1.38				
32	16.16	2(5H)-furanone	2.55	0.85		0.82	0.41	
33	17.25	cyclotene				1.64		0.31
34	17.26	3-methyl-1,2-cyclopentanedione					0.74	
35	17.54	3-(1-methylpropyl)-2-hydroxy-2-cyc lopenten-1-one		0.54				
36	17.57	manicone			0.56			0.38
37	17.58	3-(2-butyl)-1,2-cyclopentadione					0.56	

Table 2 cont.

N°	RT	Compounds (%)	'Chemlali'			'Chetoui'		
			SS	IS	IA	SS	IS	IA
Ketones								
38	18.23	3-ethyl-2-hydroxy-2-cyclopenten-1-one		0.27	0.46	0.24		
39	19.69	3,6-dihydro-5-methyl-2h-pyran-2-one	0.56		0.51			
40	20.17	hydroxy dimethyl furanone				2.40	2.73	0.61
41	20.95	(+)-(1r,4s,5s)-1-isopropyl-endo-4-methylbicy-clo[3,1,0]hexan-3-0ne]		0.15		0.15		
42	22.81	2H-inden-2-one, 1,4,5,6,7,7a-hexahydro-7a-methyl-						0.13
43	23.31	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl				5.93	0.9	1.51
44	25.76	4-[5-methyl-2-(1-methylethylidene) cyclohexyl]-3-cyclo-hexen-1-one		0.15				
45	26.04	2H-pyran-2-one, tetrahydro-6-propyl				1.11		
46	26.73	3-hydroxy-,beta,-damascone				1.05		1.49
47	26.93	6,6-dimethylcycloocta-2,4-dien-1-one		1.12				
48	26.94	1-propyl-cis-bicyclo(3,2,0)hept-2-en-3-one			0.83			
49	27.08	endo-7-hydroxy-8,8-dimethylbicyclo[4,3,0]non-1(9)-en-2-one						0.86
50	28.23	dihydro-,beta,-ionone		1.98				
51	29.2	dihydrojasmone		0.38	0.27	1.42		
52	33.52	(1S,15S)-bicyclo[13,1,0]hexadecan2-one						0.37
53	34.26	9-undecen-2-one, 6,10-dimethyl				0.17		
54	34.77	(1,5-dimethyl-6-bicyclo[3,2,0]heptyl) methyl acétone		0.11				
55	13.15	5-methyl-2-furfural		1.83		1.49	1.08	0.63
56	14.16	1,4-butanediol hexadecanal acetal			2			
57	19.87	1H-pyrrole-2-carboxaldehyde		0.98				
58	20.76	2-butene, 1,1-dimethoxy					0.33	
59	21.80	2,4 hexadienal			0.47			
60	26.20	5-(hydroxymethyl)furfural		3.36		2.88		3.01
61	29.38	1-cyclohexen-1-al		1.86			1.58	
62	31.14	2-methoxy-4-(methoxymethyl)-phenol						1.85
63	34.82	3,7-epoxy-2-methylene-6-methyloct-6-en-1-al						0.16
64	7.52	triethylamine		1.13	0.2	0.98	1.43	
65	11.91	4-acetylpyrazole		0.68	0.46	0.59		
66	17.69	dihydrouracil, 1-n-methyl				0.56		0.21
67	17.97	2(1H)-pyridinone, hydrazone				0.42		
68	19.36	methylthiourea						0.15
69	19.66	3-ethoxypyrazole		0.41				
70	20.57	N-methyl-4-ethoxypyrazole				1.06		0.55
71	23.05	ethyl methyl amphetamine		2.63				
72	23.05	2-diethylaminoethanol			0.79			
73	24.29	diethylnitrosamine		0.65				
74	24.33	isopropylmethylnitrosamine				0.63		
75	25.88	1-nitro-1-cyclohexene				0.22		
76	**29.48**	**2-methyl-3-oxime-1-cyclohexen-3-on**						**10.12**

Table 2 cont.

N°	RT	Compounds (%)	'Chemlali'			'Chetoui'		
			SS	IS	IA	SS	IS	IA
Terpenes								
Hemiterpenes								
77	**10.33**	**2(3H)-furanone, 5-methyl**		**2.71**	**4.23**	**1.64**		**3.63**
78	**11.29**	**2-furancarboxaldehyde**	**2.29**	**3.67**	**5.76**	**2.66**	**4.19**	**3.92**
79	14.26	oxirane, 2-ethyl-2-methyl				0.36		
80	**14.45**	**2-furanmethanol**	**2.46**	**7.56**	**3.19**	**6.27**	**3.56**	**2.71**
81	15.36	2-deuteroxymethyl furan				0.13		
82	16.22	3,4-dihydropyran	0.44		0.35			0.26
Monoterpenes								
83	**16.68**	**4-carene**			**3.06**			
84	21.50	3-menthene				0.69		
85	21.99	gamma,-campholenol		0.11				
86	24.44	isoeugenol				0.20		0.18
87	24.65	1,2,5,6,9,10-hexahydrocoronene						0.72
88	25.44	cyclooctene, 4-ethenyl		0.11				
89	33.83	isopinocamphone					0.15	
Sesquiterpenes								
90	**25.68**	**alpha,-gurjunene**		**0.97**				
91	38.18	farnesol						1.14
92	11.84	ionene			1.73			
93	**12.46**	**benzaldehyde**	**1.74**	**5.25**	**1.92**	**2.74**	**2.61**	**1.65**
94	15.01	1H-indene, 2,3-dihydro-1,1,4,6-tetramethyl						0.71
95	16.61	methyl salicylate		0.99				
96	17.68	1,2-benzenediol, 3-fluoro					0.2	
97	17.69	phenol, 2-chloro			0.45			
98	17.77	phenol, 2-methoxy	1.72	1.65	1.29	1.64	3.19	1.40
99	18.01	benzenemethanol			0.5			0.16
100	**18.53**	**benzeneethanol**	**0.56**	**1.21**	**1.57**	**0.50**	**0.63**	**0.32**
101	19.90	phenoL-D			0.52			0.28
102	**22.49**	**4-vinyl-2-methoxy-phenol**	**7.30**		**3.04**	**5.87**	**7.40**	**11.35**
103	23.06	trimecaine						1.48
104	23.07	N-phenethyl-N- methylacetamide				1.75		
105	23.36	2,6-dimethoxyphenol					0.81	
106	24.63	5-methylthio-3-(2-(3,4,5-trimethoxyphenyl) ethenyl) pyrazole	0.46		1.49			
Phenolic compounds								
107	24.99	4-vinylphenol		1.23				
108	24.99	2,3-dihydro-benzofuran				0.95		
109	25.34	benzoic acid		0.14				0.66
110	25.68	9,10-dimethyl-9,10-dihydro-9,10-diboraanthracene						
111	26.87	1,2-benzenedicarboxylic acid, butyl 2-ethylhexyl ester			2	1.36		3.23
112	27.08	1H-cyclopenta[1,3]cyclopropa[1,2]benzen-6(7H)-one, hexa-hydro-3a-hydroxy-3b-methyl-		0.66		0.78		
113	**28.82**	**catechol**			**3.39**			

Table 2 cont.

N°	RT	Compounds (%)	'Chemlali'			'Chetoui'		
			SS	IS	IA	SS	IS	IA
114	29.18	benzenethiol						1.26
115	31.12	benzeneacetic acid, 4-hydroxy-3-methoxy				1.36		
116	33.33	2-hydroxyfluorene						0.12
117	35.52	1,4-benzenediol		0.17		0.33		
118	37.22	1,3-nenzenediol, 5-methyl		0.22		0.39		
119	**24.99**	***trans*-o-coumaric acid**						**2.07**
Naphthalens								
120	15.05	naphthalene, 1,2,3,4-tetrahydro-1, 6,8-trimethyl			1.22			0.30
121	22.80	1-6-dimethylamino-2-phenyl-3-methyl-7-methoxynaphtha-lene			2.59		2.67	
122	23.88	3,5'-biacenaphthene	11.27		2.69			
123	24.07	1-phenyl-4-(1-phenylethenyl) naphthalene	37.77		10.12		7.52	
Alcohols								
124	9.79	3-furanol, tetrahydro				0.76		
125	16.89	3-cyclohexen-1-ol		0.24				
126	19.27	maltol	0.28		0.26	0.24	0.40	0.23
127	22.71	epi-photonerol A		0.48				
128	27.46	1-octadecanol				0.50		
129	27.54	1-(1,3-butadiene-2-yl)-cyclopentanol						
130	38.14	solanesol				0.54		
131	**39.65**	**vomifoliol**						**5.20**
Esters								
132	16.67	methyl salicylate					1.13	
133	22.34	5-formyl-2-furfurylmethanoate		0.21				
134	25.34	ammonium benzoate						0.27
135	31.13	acetic acid, (4-methyl-5-oxo-2(5H)-furany lidene), methyl ester, (E)		1.20				
136	41.59	*bis*(2-ethylhexyl) phthalate		0.98				
Fatty acids								
137	14.14	2-propenoic acid				0.16		
138	16.00	(R)-(+)-cyclohex-3-ene-1-carboxylic acid						0.15
139	19.89	4-hydroxybicyclo[3,1,0]hex-2-en-endo-6-carboxylic acid				0.68		
140	25.34	benzoic acid		0.14		0.18		
141	30.50	pentadecanoic acid				0.43		0.30
142	**32.72**	**hexadecanoic acid**		**2.11**	**3.39**	**3.63**		**3.85**
143	37.72	2-(1'-methylethenyl)pent-2-enoic acid				0.79		
144	39.19	octadecanoic acid		0.30		1.25		0.59
145	40.31	heptadecene-(8)-carbonic acid-(1)						0.66
Others								
146	13.33	dimethyl sulfoxide DMSO	3.77		0.33		1.83	1.53
147	28.00	tetravinylsilane				0.27		
148	**28.25**	**stibine, trimethyl**			**2.03**			
	Total (%)		79.93	55.87	66.75	65.9	58.25	74.17

The compounds the most abundant and interesting are in bold type; RT – retention time

inferior arid. Such compounds included 2-methyl-3--oxime-1-cyclohexen-3-on (n° 76: 10.12%); trans-o--coumaric acid (n° 119: 2.07%); vomifoliol (n° 131: 5.20%).

For 'Chemlali', a number of compounds were identified in inferior arid, they were 4-carene (n° 83: 3.06%); catechol (n° 113: 3.39%) and trimethyl stibine (n° 148: 2.03%).

DISCUSSION

The methanolic extracts of the olive leaves exhibited a very powerful antioxidant activity against DPPH, at 1 mg/mL (91.40%). Similarly, Ferreira et al. [2007] have found that the methanolic extract of olive tree leaves was very active against DPPH radicals where inhibitions varied from 87.1 to 100% at 5 mg/ml. Differently to 'Chetoui', the antioxidant activity of 'Chemlali' was increasing in inferior semiarid and inferior arid compared with superior semiarid. This suggests that 'Chemlali' has developed a sophisticated mechanism to overcome the inferior arid conditions. A reinforcement of antioxidants level is supposed. Both 'Chemlali' and 'Chetoui' exhibited a maximum antioxidant activity against $ABTS^{\bullet+}$ in inferior arid (36.01 and 42.09% respectively). The antioxidant activity against $ABTS^{\bullet+}$ was very weak if compared to DPPH, reaching a maximum of 42.09% only. The antioxidant activity varied according to the extracting solvent type and polarity, the isolation process, the purity of the active ingredients, in addition to the testing system [Meyer 1998].

To adapt to the arid climatic conditions, 'Chemlali' secreted much more polyphenols than those of the other areas did. However, being native to the north of Tunisia (Subhumid to superior semiarid), 'Chetoui' secreted a maximum of polyphenols reaching 23.56 mg CE/g DW in order to adapt to the climatic conditions characterizing the center zone of the country (inferior semiarid). This content decreased significantly in the south (inferior arid). 'Chemlali' seemed to have the most capacity to adapt to the arid climatic conditions by presenting the high amount of polyphenols, in parallel to a highest antioxidant activity. 'Chemlali' presented a high total phenol content, reaching 45.56 mg CE/g DW. According to Petridis et al. [2012], olive trees presented an increase in total phenol content in water stress condition from 25 to 40 mg CE/g DW. In June, the level of phenols increased specially for the olive tree receiving an amount of water equivalent to only 33% of field capacity, and reached 44 CE/g DW. However, Popović et al. [2016] have affirmed that the total phenol content of olive was affected by water stress provoked by polyethylene glycol (PEG 6000: 100 and 200 mOsm) for almost all tested genotypes, either in leaves or in roots.

Both tested varieties concentrated their flavonoid content in inferior arid, especially 'Chemlali', which testifies to the greater ability of 'Chemlali' to overcome the severe weather conditions that characterizes the inferior arid conditions. According to Brahmi et al. [2013], the amount of flavonoid depended greatly with olive cultivar. For 'Chemlali', the total flavonoid content was 3.77 mg CE/g DW, while for 'Neb Jmel' cultivar, it was 1.47 mg CE/g DW.

Olive leaves have proved to be rich in flavonoids, independently of the sampling parameters such as olive cultivar, leaf age or sampling date [Therios 2009]. The adaptive mechanism of flavonoids has been explained as the interception of UV accumulated during stress by flavonoids and the prevention from UV-B to reach the mesophyll and to affect photosynthesis. Lois and Buchanan [1994] have proposed that screening radiation accumulated in response to UV radiation by flavonoids, which show high absorbance in a region of 290–320 nm, constitutes an important protective effect against UV-B radiation.

'Chemlali' was the richest in tannins, comparatively to 'Chetoui', either in superior semiarid (12.54 mg EC/g DW) or in inferior arid (11.50 mg EC/g DW). In accordance with Brahmi et al. [2013], the amount of total condensed tannin depended with olive cultivar. Tannin was concentrated respectively as 0.44 and 0.32 mg EC/g DW in 'Chemlali' and 'Neb Jmel' leaves cultivated in the same conditions. Under drought stress, there is an increase in tannin content. Pizzi and Cameron [1986] explained that many trees originating from South Africa, characterized by drought areas, are considered as high producers of tannins. Tannins are considered as structural wood components in the same order to cellulose, hemicelluloses and lignin.

Plants have performed a complex antioxidant mechanism to prevent oxidative damages. This system includes both enzymatic and non-enzymatic

components. By catalyzing the reduction of H_2O_2 to H_2O and O_2, polyphenoloxidase helps plants to opposite to oxidative damages. This enzyme catalyzes, in the presence of oxygen, the o-hydroxylation of monophenols to o-diphenols, and the oxidation of o-diphenols to quinones [Rivero et al. 2001]. Accordingly, polyphenoloxidase plays a potential protective role with o-diphenol in the protective mechanism and modifications in their activities could be an indicator of important endogenous modifications. Similar results have demonstrated an increase in polyphenoloxidase activities in olive trees under drought stress [Boughalleb and Mhamdi 2011]. The increase of the o-diphenol content in 'Chemlali' leaves from superior semiarid to inferior arid, and the presence of a much more content of o-diphenols than in 'Chetoui', demonstrated the great adaptive capacity of 'Chemlali' to the hard climatic conditions that characterize these areas.

Saponins were concentrated both in 'Chemlali' and 'Chetoui' cultivars in inferior arid. According to Peiran et al. [2017], total saponin content of areal part organs of *Panax notoginseng* has significantly increased in water deficit (0.55 and 0.85 field capacity).

Moreover, *Agave salmiana* amplified its saponin content and consequently its antioxidant activity in response to water deficit induced via PEG [Puente-Garza et al. 2017]. Martinelli et al. [2013] proved interestingly a significant increase in erythrodiol and loganin, belonging to saponins family, in water stressed olives. In the same way, Berenguer et al. [2006] studied the effects of different water levels on olive oil quality and demonstrated the fastidious interest of these compounds due to their strong health benefits. Moreover, saponins are an important group of plant secondary metabolites known as phytoprotectants that defend plants against attack by microbes and herbivores. Several abiotic stress conditions revealed the decline of plants defense mechanisms and the enhancement of their susceptibility to pathogen infection [Suzuki et al. 2014]. Accumulation of saponins in arid conditions, in both olive cultivars, 'Chemlali' and 'Chetoui', may prevent the combination of biotic and abiotic stress.

'Chemlali' increased its phytosterol content in inferior semiarid and in inferior arid. Contrarily, 'Chetoui' showed a decrease in the level of phytosterol in these areas. Phytosterols are integral components of the lipid bilayer membrane in plants. They regulate membrane fluidity to influence its properties, functions and structure. According to Martinelli et al. [2013], besides phenol compounds, water scarcity affected the content of some of the major metabolites in olive fruits such as sterols, free fatty acids and terpens [Berenguer et al. 2006]. It has been found that the phytosterols content, namely b-sitosterol, the major one in rice seedlings, increased accordingly to the severity of water stress [Kumar et al. 2015].

Both 'Chemlali' and 'Chetoui' cultivars concentrated their chlorophyll a and b in superior semiarid, where the climatic conditions are the most favorable (0.81 and 0.85 mg/g DW respectively for chl a and 1.91 and 1.53 mg/g DW respectively for Chl b). Shivakrishna et al. [2018] demonstrated in *Arachis hypogaea* leaves, that the decrease in chlorophyll content was depended on an increasing concentration of polyethylene glycol-6000. Reduction in chlorophyll a level was much more important than the chlorophyll b [Shivakrishna et al. 2018]. The exposure of both olive cultivars 'Chemlali' and 'Chetoui' to the arid conditions have led to smaller chlorophyll a and chlorophyll b contents in comparison to superior semiarid. Pierantozzi et al. [2013] explained the reason for the decrease in chlorophyll content under water deficit conditions; they state that drought stress produce reactive oxygen species (ROS) such as O_2 and H_2O_2, which can cause lipid peroxidation and therefore chlorophyll deterioration.

The decrease in chlorophyll content was due to the changing color of the leaf from green to yellow. However, the loss of chlorophyll reduces the quantity of photons absorbed by the leaves, which improves the photoprotective and antioxidant capacity of the leaves.

In other way, the carotenoid content significantly increased in 'Chemlali' (1.21 mg/g DW), and decreased in 'Chetoui' (0.35 mg/g DW), in inferior arid comparatively to superior semiarid. According to Arji and Arzani [2008], the chlorophyll a, b and carotenoid contents in the olive tree decreased significantly under water stress. The reduction depended to water availability and tested cultivar. Carotenoids perform essential functions in photosynthesis and photoprotection mechanisms.

As well as their structural roles, carotenoids present an important antioxidant capacity by quenching firstly ^{3}Chl and $^1O_2$, inhibiting then lipid peroxidation, and subsequently stabilizing the membranes. Moreover,

carotenoids play a vital role in assembling the light recovery complex and in restricting the radiation dissipation of surplus energy [Hanci and Cebeci 2014].

The PCA analyze demonstrated strong positive correlations between the phenolic group (polyphenols, flavonoids, o-diphenols and tannin) and the other antioxidant compounds such carotenoids, saponins and phytosterols. These compounds were near 'Chemlali' of inferior arid indicating its richness in these compounds. Such compounds could contribute to the adaptation of 'Chemlali' to the arid conditions characterizing the south. Moreover, the antioxidant activity represented by IC_{50} was highly inversely correlated to phenols parameters, saponins, carotenoids and phytosterols. This pointed consequently that this group included powerful antioxidants. Varela et al. [2016] confirmed that in drought conditions, plants may accumulate a great content of secondary metabolites, such as polyphenols, which could be a strategy to tolerate drought stress given their great antioxidant power capable to control unnecessary ROS in tissues. Chlorophyll a and b were positively correlated with 'Chemlali' and 'Chetoui'of superior semiarid, indicating that in these areas, both cultivars were rich in chlorophyll, a fact that reflected a good photosynthesis capacity. Chlorophyll a, b and tannin appeared in close relationship. In accordance with Lewis and Yamamoto [1989], in several tannin-rich plants, the synthesis of tannin contributes greatly to the use of captured photosynthetic energy.

The chemical composition of the methanolic leaf extracts from 'Chemlali' and 'Chetoui' cultivars, located in superior semiarid, inferior semiarid and inferior arid, analyzed by GC-MS, demonstrated the presence of many active compounds. 2(3H)-5-methyl-furanone was identified in both cultivars growing in inferior arid. According to Kakinuma et al. [1986], 3(2H)-furanones have resulted in the development of an extremely potent antimutagen, due to the alteration of the proteins implicated in the mutated DNA repair system, through catching thiol groups; this aptness is associated with the strong antioxidant potency. 2-methoxy-4-vinylphenol, identified in 'Chemlali' and 'Chetoui' grown in inferior arid, is an aromatic compound used as a flavoring additive; it is found in *Coffea* ssp. and *Citus sinensis* [Polya 2003], known for their antioxidant power. While hexadecanoic acid, also identified in both olive cultivars of the inferior arid, was known as antioxidant, nematicide, pesticide, anti-androgenic flavor, hemolytic and 5-alpha-reductase inhibitor [Praveen Kumar et al. 2010]. Additionally, Oxime derivates, identified in 'Chetoui' growing in inferior arid, have largely been considered as antioxidants [Özyürek et al. 2014]. Oximes counterbalanced the oxidative stress status induced by the organophosphorus compounds and reverse the acetylcholinesterase inhibition that they caused [Worek et al. 2005]. Additionally, coumaric acid, identified in this cultivar when grown in Inferior arid, has exhibited an antioxidant activity which is nevertheless lesser than that of gallic and gentisic acids [Karamać et al. 2005]. Furthermore, it has been found that the vomifoliol, identified in 'Chetoui' of the inferior arid, was a compound related to abscisic acid and it has been considered as an endogenous regulator of stomatal aperture [Stuart and Coke 1975]. However, for 'Chemlali' of the inferior arid, 4-carene and catechol were identified. According to Aazza et al. [2011], δ-3-carene has been a good antioxidant standard (IC_{50} = 0.603 mg/mL), inhibiting strongly the acetylcholinesterase activity. The double bond position played an important role in the unregistered activity. Additionally catechol, which has been identified in olive leaves by Benavente-García et al. [2000], has been considered as a functional group of scavenging radicals. Thus, the antioxidants containing catechol are able to be protected from lipid peroxidation via their catechol part.

Being native to the center and the south of Tunisia, characterized by arid climatic conditions, 'Chemlali' has shown a strong antioxidant power (inhibition of 90% of radicals), following high content in polyphenols, flavonoids, condensed tannins, o-diphenols, saponins and carotenoids especially in Inferior arid site. This cultivar has triplicate its polyphenol content, especially o-diphenols to enhance its capacity to scavenge radicals and inhibit oxidative stress; has synthesized more tannin to consolidate its wood structure and has developed its flavonoid level to protect from excessive UV radiation in the Inferior arid.

Moreover, 'Chemlali' has strengthened its lipid bilayer membrane by developing its phytosterol content; has decreased its chlorophyll to minimize radiation perception and has synthesized more carotenoids to enhance its photosynthesis and photoprotection

capacities. Furthermore, 'Chemlali' has strengthened its protection to biotic stress which could be additionally imposed following an oxidative stress by increasing its foliar saponin content.

Chetoui, originating in subhumid and superior semiarid areas, has concentrated these compounds, which have contributed to an important antioxidant activity in this site. Nevertheless, this cultivar has displayed a significant decrease in the content of almost all the above cited compounds in Inferior arid, showing a lesser adaptive capacity.

CONCLUSIONS

To mitigate climatic change effects and adapt to global warming, the olive tree is significantly increasing its phenolic contents, flavonoids, saponins and especially o-diphenols. It synthesizes many interesting antioxidants which act synergically to scavenge radicals and avoid stresses. Nevertheless, olive behavior is greatly related to the cultivar. Moreover, the primary Tunisian olives, 'Chemlali' and 'Chetoui', growing in inferior arid, have condensed in their leaves many interesting antioxidants which could be considered as new indicators of adaptation to water scarcity. Such antioxidants are a real richness for eventual valorization in food, pharmacology and medicine following their great benefits to our health, or as bio-fertilizer enhancing plant adaptation to arid climatic conditions.

SOURCE OF FUNDING

The Laboratory of the Improvement of Olive Tree Productivity and Product Quality, Olive Tree Institute, Tunisia.

REFERENCES

Aazza, S., Lyoussi, B., Miguel, M.G. (2011). Antioxidant and antiacetylcholinesterase activities of some commercial essential oils and their major compounds. Molecules, 16, 7672–7690. https://doi.org/10.3390/molecules16097672

Aguilera, F., Ruiz, L., Fornaciari, M., Romano, B., Galán, C., Oteros, J., Ben Dhiab, A., Msallem, M., Orlandi, F. (2014). Heat accumulation period in the Mediterranean region: phenological response of the olive in different climate areas (Spain, Italy and Tunisia). Int. J. Biometeorol., 58, 867–876. http://dx.doi.org/10.1007/s00484-013-0666-7

Al-Dobai, S., Nasr, N. (2016). FAO preventive actions to the introduction and spread of OQDS – *Xylella fastidiosa* in NENA Region Crop Protection Officer. International Workshop on "*Xylella fastidiosa* and OQDS", Bari, 19–22.

Araújo, L.B.D.C., Silva, S.L., Galvão, M.A.M., Ferreira, M.R.A., Araújo, E.L., Randau, K.P., Soares, L.A.L. (2013). Total phytosterol content in drug materials and extracts from roots of *Acanthospermum hispidum* by UV-VIS spectrophotometry. Rev. Bras. Farmacogn., 23(5), 736–742. https://doi.org/10.1590/S0102-695X2013000500004

Arji, I., Arzani, K. (2008). Effect of water stress on some biochemical changes in leaf of five olive (*Olea europaea* L.) cultivars. Acta Hortic., 791, 523–526. http://dx.doi.org/10.17660/ActaHortic.2008.791.80

Ba, K., Tine, E., Destain, J., Cisse, N., Thonart, P. (2010). Étude comparative des composés phénoliques, du pouvoir antioxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt. Biotechnol. Agro. Soc. Environ., 14(1), 131–139 [in French].

Baccou, J.C., Lambert, F., Sauvaire, Y. (1977). Spectrophotometric method for the determination of total steroidal sapogenin. Analyst, 102(1215), 458–65. https://doi.org/10.1039/an9770200458

Bhattacharjee, S., Saha, A.K. (2014). Plant water-stress response mechanisms. In: Approaches to plant stress and their management, Gaur, R.K., Sharma, P. (eds.), Springer, India, 149–172. http://dx.doi.org/10.1007/978-81-322-1620-9_8

Benavente-García, O., Castillo, J., Lorente, J., Ortuño, A., Del Rio, J.A. (2000). Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chem., 68, 457–462. https://doi.org/10.1016/S0308-8146(99)00221-6

Berenguer, M.J., Vossen, P.M., Grattan, S.R., Connell, J.H., Polito, V.S. (2006). Tree irrigation levels for optimum chemical and sensory properties of olive oil. HortScience, 41(2), 42–432. https://doi.org/10.21273/HORTSCI.41.2.427

Blekas G., Psomiadou E., Tsimidou M., Boskou D. (2002). On the importance of total polar phenols to monitor the stability of Greek virgin olive oil. Eur. J. Lipid Sci. Technol., 104(6), 340–346. https://doi.org/10.1002/1438-9312(200206)104:6%3C340::AID-EJLT340%3E3.0.CO;2-L

Boughalleb, F., Mhamdi, M. (2011). Possible involvement of proline and the antioxidant defense systems in the drought tolerance of three olive cultivars grown under

increasing water deficit regimes. Agric. J., 6(6), 378–391. http://dx.doi.org/10.3923/aj.2011.378.391

Brahmi, F., Mechri, B., Dhibi, M., Hammami, M. (2013). Variations in phenolic compounds and antiradical scavenging activity of *Olea europaea* leaves and fruits extracts collected in two different seasons. Ind. Crops Prod., 49, 256–264. https://doi.org/10.1016/j.indcrop.2013.04.042

Chang, C.C., Yang, M.H, Wen, H.M., Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10(3), 178–182. https://doi.org/10.38212/2224-6614.2748

Choi, C.W., Kim, S.C., Hwang, S.S., Choi, B.K., Ahn, H.J., Lee, M.Y., Park, S.H., Kim, S.K. (2002). Antioxydant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay guided comparison. Plant Sci., 163(6), 1161–1168. https://doi.org/10.1016/S0168-9452(02)00332-1

Ferreira, I.C.F.R., Queiroz, M.J.R.P., Vilas-Boas, M., Estevinho, L.M., Begouin, A., Kirsch, G. (2007). Evaluation of the antioxidant properties of diarylamines in the benzo[b]thiophene series by free radical scavenging activity and reducing power. Bioorg. Med. Chem. Letters, 16(5), 1384–1387. https://doi.org/10.1016/j.bmcl.2005.11.035

Hanci, F., Cebeci, E. (2014). Investigation of proline, chlorophyll and carotenoids changes under drought stress in some onion (*Allium Cepa* L.) cultivars. Turk. J. Agric. Nat. Sci., S2, 1499–1504.

Hsu, C.C., Kuo, H.C., Huang, K.E. (2017). The effects of phytosterols extracted from *Diascorea alata* on the antioxidant activity, plasma lipids, and hematological profiles in Taiwanese menopausal women. Nutrients, 9(12), 1320. https://doi.org/10.3390/nu9121320

Kakinuma, K., Koike, J., Ishibashi, K., Takahashi, W., Takei, H. (1986). Structure-activity relationship and design of an antimutagen against the UV-induced mutation of *Escherichia coli.* Agric. Biol. Chem., 50(3), 625–631. https://doi.org/10.1080/00021369.1986.10867429

Karamać, M., Kosińska, A., Pegg, R.B. (2005). Comparison of radical-scavenging activities for selected phenolic acids. Pol. J. Food Nutr. Sci., 55(2), 165–170.

Kiritsakis, A., Shahidi, F. (2017). Olives and olive oil as functional foods: bioactivity, chemistry and processing. John Wiley, Sons, pp. 688.

Kumar, M.S., Ali, K., Dahuja, A., Tyagi, A. (2015). Role of phytosterols in drought stress tolerance in rice. Plant Physiol. Biochem., 96, 83–89. https://doi.org/10.1016/j.plaphy.2015.07.014

Lanfer-Marquez, U.M., Barros, R.M.C., Sinnecker, P. (2005). Antioxidant activity of chlorophylls and their derivatives. Food Res. Inter., 8–9(28), 885–891. http://doi.org/10.1016/j.foodres.2005.02.012

Lewis, N.G., Yamamoto, E. (1989). Tannins – their place in plant metabolism. In: Chemistry and significance of condensed tannins, Hemingway, R.W., Karchesy, J.J., Branham, S.J. (eds.). Springer, US, 23–46.

Lichtenthaler, H.K., Wellburn, A.R. (1983). Determination of total carotenoids and chlorophylls A and B of leaf in different solvents. Biochem. Soc. Trans., 11(5), 591–592. https://doi.org/10.1042/bst0110591

Lois, R., Buchanan, B.B. (1994). Severe sensitivity to ultraviolet radiation in an *Arabidopsis* mutant deficient in flavonoid accumulation. II. Mechanisms of UV resistance in *Arabidopsis*. Ptanta, 194(4), 504–509. https://doi.org/10.1007/BF00714463

Martinelli, F., Remorini, D., Saia, S., Massai, R., Tonutti, P. (2013). Metabolic profiling of ripe olive fruit in response to moderate water stress. Sci. Hortic., 159, 52–58. https://doi.org/10.1016/j.scienta.2013.04.039

McDonald, S., Prenzler, P.D., Antolovich, M., Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. Food Chem., 73(1), 73–84. https://doi.org/10.1016/S0308-8146(00)00288-0

Meyer, A.S., Heinonen, M., Frankel, E.N. (1998). Antioxidant interactions of catechin cyaniding, caffeic acid, quercetin and ellagic acid on human LDL oxidation. Food Chem., 61, 71–75.

Özyürek, M., Akpınar, D., Bener, M., Türkkan, B., Güçlü, K., Apak, R. (2014). Novel oxime based flavanone, naringin-oxime: Synthesis, characterization and screening for antioxidant activity. Chem-Biol. Inter., 212, 40–46. https://doi.org/10.1016/j.cbi.2014.01.017

Peiran, L., Diqiu, L., Tian-Rui, X., Ye, Y., Xiuming, C. (2017). Soil water stress attenuates the growth and development but enhance the saponin synthesis of *Panax notogesing* during flowering stage. Ind. Crops Prod., 108, 95–105. https://doi.org/10.1016/j.indcrop.2017.05.052

Petridis, A., Therios, I., Samouris, G., Koundouras, S., Giannakoula, A. (2012). Effect of water deficit on leaf phenolic composition, gas exchange, oxidative damage and antioxidant activity of four Greek olive (*Olea europaea* L.) cultivars. Plant Physiol. Biochem., 60, 1–11. https://doi.org/10.1016/j.plaphy.2012.07.014

Pierantozzi, P., Torres, M., Bodoira, R., Maestri, D. (2013). Water relations, biochemical – physiological and yield responses of olive trees (*Olea europaea* L. cvs. Arbequina and Manzanilla) under drought stress during the pre-flowering and flowering period. Agric. Water Manag., 125, 13–25. https://doi.org/10.1016/J.AGWAT.2013.04.003

Pizzi, A., Cameron, F.A. (1986). Flavonoid tannins – structural wood components for drought-resistance mechanisms of plants. Wood Sci. Technol., 20(2), 119–124.

Polya, G. (2003). Biochemical targets of plant bioactive compounds: A pharmacological reference, guide to sites of action and biological effects, CRC Press, pp. 864.

Popović, B.M., Štajner, D., Ždero-Pavlović, R., Tumbas Šaponjac, V., Ćanadanović-Brunet, J., Orlović, S. (2016). Water stress induces changes in polyphenol profile and antioxidant capacity in poplar plants (*Populus* spp.). Plant Phys. Biochem., 105, 242–250. https://doi.org/10.1016/j.plaphy.2016.04.036

Praveen Kumar, P., Kumaravel S., Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo.* Afr. J. Biochem. Res., 4(7), 191–195.

Puente-Garza, C.A, Meza-Miranda, C., Ochoa-Martínez, D., García-Lara, S. (2017). Effect of *in vitro* drought stress on phenolic acids, flavonols, saponins, and antioxidant activity in *Agave salmiana*. Plant Phys. Biochem., 115, 400-407.

Rivero, R.M., Ruiz J.M., García P.C., López-Lefebre L.R., Sánchez E., Romero L. (2001). Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. Plant Sci., 160, 315–321.

Saidana, D., Mahjoub, S., Boussaada, O., Chriaa, J., Mahjoub, M.A., Chéraif, I., Daami, M., Mighri, Z., Helal, A.N. (2008). Antibacterial and antifungal activities of the essential oils of two saltcedar species from Tunisia. J. Am. Oil Chem. Soc., 85, 817–826.

Shivakrishna, M.P., Reddy, K.A., Rao, D.M. (2018). Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. Saudi J. Biol. Sci., 25(2), 285–289. https://doi.org/10.1016/j.sjbs.2017.04.008

Smirnoff, N. (1993). The role of active oxygen in the response to water deficit and desiccation. New Phytol., 125, 27–58. https://doi.org/10.1111/j.1469-8137.1993.tb03863.x

Stuart, K.L., Coke, L.B. (1975). The effect of vomifoliol on stomatal aperture. Planta, 122(3), 307–310. https://doi.org/10.1007/BF00385281

Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., Mittler, R., 2014. Abiotic and biotic stress combinations. New Phytol., 203(1), 32–43. https://doi.org/10.1111/nph.12797

Therios, I.N. (2009). Olives. CABI, Wallingford, UK.

Varela, M.C., Arslan, I., Reginato, M.A., Cenzano, A.M., Luna, M.V. (2016). Phenolic compounds as indicators of drought resistance in shrubs from *Patagonian shrublands* (Argentina). Plant Physiol. Biochem., 104, 81–91. https://doi.org/10.1016/j.plaphy.2016.03.014

Watson, R.R. (2014). Polyphenols in plants: isolation, purification and extract preparation. Academic Press, pp. 360.

Worek, F., Thiermann, H., Szinicz, L., Eyer, P. (2005). Kinetic analysis of interactions between human acetylcholinesterase, structurally different organophosphorus compounds and oximes. Biochem. Pharmacol., 68(11), 2237–2248. https://doi.org/10.1016/j.bcp.2004.07.038

Yu, L., Scanlin, L., Wilson, J., Schmidt, G. (2002). Rosemary extracts as inhibitors of lipid oxidation and color change in cooked turkey products during refrigerated storage. J. Food Sci., 67, 582–585. https://doi.org/10.1111/j.1365-2621.2002.tb10642.x