

# Volatiles Variation of Two Major Cultivars of *Olea europaea* L. Cultivated in Mediterranean and Arid Regions of Algeria

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**Abstract:** The chemical compositions of the volatile fractions, produced by the flowers and leaves of two *Olea europaea* L. cultivars (Chemlal and Sigoise) were investigated. The plant materials were harvested from two different zones in Algeria (Mediterranean and arid regions). The samples were extracted by hydrodistillation using a Clevenger apparatus. GC-MS analysis allowed the identification of 51 compounds. According to the geographic origin and the cultivar, qualitative and semi-quantitative variations were observed, especially with the abundance of the main compounds. Overall, the principal component analysis showed that the environmental condition was mainly affecting the production of the major compounds. Throughout the two regions, the main substances in the samples from the Mediterranean region were (*Z*)-jasmone (51.8%), nonanal (32.4%), theaspirane II (23.7%) and phenylethyl propionate (17.9%). While those of the arid region were bornyl acetate (26.8%), 3-ethenyl pyridine (17.2%), (*E,E*)-2,4-heptadienal (16.9%) and (*E*)-geranylacetone (14.4%), with a significant correlation between the flower volatiles, more than 86% in both regions. These results can add a valuable insight into the effect of arid climate on the production of volatiles by *Olea europaea*.

**Keywords:** *Olea europaea* L; cultivars; flower volatiles; (*Z*)-jasmone; nonanal; Arid region. ©2021 ACG Publications. All right reserved.

## 1. Introduction

*Olea europaea* L. is the most cultivated fruit tree in Northern Algeria (Mediterranean zone), an important olive oil producer in the world (9<sup>th</sup> position) [1-2]. At present, the Algerian Government is supporting programs on *Olea europaea* trees cultivation, in different regions of the country [3]. However, due to the large surface of Algeria (the largest country in Africa), a geographical diversity produces a range of different climates conditions. These conditions affected *O. europaea* trees

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growing, especially in the arid regions, which covers 80% of the country (Sahara of Algeria) [4]. Concerning genetic diversity or characteristic of *O. europaea* cultivars, a scarce exploitation in this vast area is reported by the literature [5].

Regardless of the wide use of olive oil in therapy all around the world, many researchers focused on the extremely high biological potential of *O. europaea* leaf extracts, i.e. hypotensive, antidiabetic, anticancer and antioxidant [6-7]. The main responsible for these activities was the presence of various hydroxytyrosol derivatives (e.g., oleuropein) and flavone glycosides as a bio-alternative to the synthetic antioxidants [6-7].

In parallel, several studies have focused on the volatile compounds of *O europaea* leaves (VOLs), which help in communication between the tree and the environment (plant resistance against both herbivores and pathogens) [8-9]. For example, Flamini *et al* [10] and Campeol *et al* [11] investigated the VOLs from different cultivars in various geographic sites in Italy. Further papers studied the VOLs at different harvest times to demonstrate the seasonal variation of these compounds, together with the influence of agronomical practices [12-13]. On the other hand, Brahimi *et al* [14] studied the biological activities of VOLs in certain Tunisian cultivars.

In Algeria, less attention has been paid to *O. europaea* leaf phytochemicals, especially volatile compounds [15]. Therefore, the VOLs and those of flowers (VOFs) need detailed chemical clarification and estimates of possible changes related to the diversity of the Algerian climates.

The genetic factors for the major cultivars that can lead to the formation of different chemotypes with an important biological potential, also need to be taken into account [14, 16]. According to Hauvill [17], there are 150 more or less abundant cultivars across the country, but they are not yet fully genetically characterized by the Algerian ministry of agriculture [2-3].

Consequently, this study is the first step to investigate the chemical variation of VOFs and VOLs in Algeria. Furthermore, it will provide information about the physiological characteristics of the major Algerian cultivars: Chemlal and Sigoise, in particular about the effect of geoclimatic conditions on the production of these compounds.

## 2. Materials and Methods

### 2.1. Plant Materials

Flowers and leaves were collected from five *Olea europaea* L. trees for each cultivar (10 years old); the selected trees were chosen to cover one plot of the orchard. These two local Algerian cultivars were leaders among the documented 36 throughout the country (Chemlal, comprising 40% and Sigoise 25% of the orchards) [2, 15]. The botanical identification of the collected material was performed at INRAA (National Institute of Agronomic Research of Algeria) under the owners of the two chosen farms. The first farm is located in the North of Algeria, at Cap Djinet, Boumerdes state; this state is well known for the olive cultivation since it belongs to the Mediterranean zone. The second one, named Algeria Horizon Agritech, is located in Southern Algeria, at Hassi Ben Abdallah, Ouargla state, which holds a project that counts 100.000 olive trees in the desert of Algeria. The two geographical sites are far away from industrial zones. The samples were gathered during the flowering stage (April 2019).

### 2.2. Climatic Characteristics of the Experimental Sites

*Ouargla region* (31° 56'57 " North, 5° 19'30 " East, 140 m) in the desert of Algeria, is one of the hottest and driest regions in the world. Its hyperarid climate is characterised by a very low precipitation rate (annual rainfall average, 38.7 mm), very high evaporation (2138 mm/year) and high thermal amplitudes (annual temperature average 23.03°C). It has very long, extremely hot and dry summers, with short winters [18].

*Boumerdes region* (3.46667 36° 46' 0" North, 3° 28' 0" East, 25 m) has a considerable agricultural potential, due to the typical Mediterranean climate, with dry summers and wet winters. The region is characterised by a mean annual temperature of 18°C. Unlike the first region, Boumerdes has a high yearly rainfall of 739 mm and a lesser evaporation rate (1214-1569 mm/year) [18].

Soil samples from the two experimental sites were air dried, ground and sieved (2 mm). The analyses, using a pH meter and a conductivity meter, were performed on aqueous soil extracts (soil to water ratio 1:5) [19]. The pedoclimatic characteristics of this period are shown in Table 1.

**Table 1.** Pedoclimatic characteristics of the experimental sites during the study period (April 2019)

	Average temperatures	Precipitation mm	Relative humidity %	pH	EC dS/m	ST
<b>Ouargla</b>	30.2°C	≤8	25.9	P1 7.53 to 7.94 P2 7.62 to 8.18	0.6 to 0.8 <0.6	Fine sand Medium sand
<b>Boumerdes</b>	15.7°C	≥60	60	P1 7.9 to 8.17 P2 7.78 to 8.07	<0.6 <0.6	Clayey silt Clayey silt

P1: plot 1= 20 to 40 cm depth, P2: plot 2= 40 to 60 cm depth, EC: electrical conductivity at 25°C, pH: potential of hydrogen, ST: Soil type [18].

### 2.3. Preparation of the Volatile Samples

Eight samples of 100 g each, were prepared from fresh *O. europaea* Flowers (F11, F12, F21 and F22) and dried Leaves (L11, L12, L21 and L22). In the codes the first number represents the region (1: the Mediterranean, 2: the arid ones), while the second one represents the cultivar (1: cv. Chemlal, 2: cv. Sigoise). The extraction of volatile compounds was carried out by hydrodistillation in a Clevenger apparatus for 3 h [20]. Volatile compounds were obtained by liquid-liquid extraction of the distillate with 2 ml of *n*-hexane. All the samples were stored in hermetically sealed glass vials at -18°C until analysis [10, 21].

### 2.4. Chromatographic Analyses

The volatile fractions were analyzed at the Department of Pharmacy in the University of Pisa. GC-EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. The injector and transfer line temperatures were set at 220 and 240°C respectively. The oven temperature was programmed from 60°C to 240°C at 3°C/min. Helium was used at 1 mL/min with a split ratio of 30:1. The MS acquisition parameters were: full scan mode, with a scan range of 35-350 amu, scan time 1.0 sec and fragmentation energy 70 eV. Identification of the constituents was based on the comparison of their retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 14, Adams 2007) [22-23] and home-made library mass spectra built up from pure substances and components of known oils and MS literature data (Adams 2007) [23]. The levels of the identified volatiles were expressed relative abundance [10].

### 2.5. Statistical Analysis

A comparative study using the multivariate statistical analysis technique Principal Component Analysis (PCA) was applied to highlight the relationships between the chemical constituents-cultivars and geographic site-cultivars based on the major compounds of VOFs (volatiles of *O. europaea* flowers) and VOLs (volatiles of *O. europaea* leaves) using the software XLSTAT 2016 from Addinsoft.

## 3. Results and Discussion

The chemical compositions of the studied samples, volatiles of *O. europaea* flowers (VOFs) and leaves (VOLs), are reported in Tables 2 and 3, respectively. Overall, fifty-one compounds were identified, accounting from 93.2 to 99.5% of VOLs and from 99.7 to 99.8% of VOFs. As showed, the

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identified constituents varied according to both type of cultivar (Chemlal or Sigoise), selected organ (leaves or flowers) and the geographic region (Mediterranean or arid).

**Table 2.** Compositions (%) of *Olea europaea* L. flower volatiles from Northern and Southern Algeria

No	Compound	LRI	F11	F12	F21	F22
1	hexanal	802	-	-	4.5	2.8
2	( <i>E</i> )-2-hexenal	856	-	-	4.4	-
3	( <i>Z</i> )-3-hexen-1-ol	857	-	-	2.7	-
5	<i>n</i> -nonane	900	-	-	0.8	0.8
6	heptanal	903	-	-	1.9	1.2
7	( <i>Z</i> )-2-heptenal	958	-	-	2.4	-
8	benzaldehyde	963	-	-	3.1	-
9	3-ethenyl pyridine	969	5.5	-	11.6	<b>17.2</b>
12	octanal	1002	-	-	1.9	-
13	( <i>E,E</i> )-2,4-heptadienal	1011	-	-	1.5	-
14	<i>p</i> -cymene	1028	3.7	8.1	2.7	2.8
15	limonene	1032	6.5	10.2	3.2	3.5
16	phenylacetaldehyde	1045	2.7	-	-	-
17	1-octanol	1071	-	-	1.6	-
18	<i>n</i> -undecane	1100	-	-	2.6	3.3
20	nonanal	1103	11.7	<b>32.4</b>	12.8	13.2
24	menthone	1154	-	-	4.5	4.5
27	methyl chavicol	1197	-	-	-	3.0
29	<i>n</i> -dodecane	1200	-	-	4.2	4.7
34	bornyl acetate	1287	-	-	<b>20.2</b>	<b>26.8</b>
37	<i>n</i> -tridecane	1300	-	-	-	3.7
41	phenylethyl propionate	1351	<b>17.9</b>	-	-	-
44	( <i>Z</i> )-jasmone	1395	<b>51.8</b>	<b>49</b>	13.2	9.4
45	<i>n</i> -tetradecane	1400	-	-	-	2.8
	<b>TOTAL</b>		<b>99.8</b>	<b>99.7</b>	<b>99.8</b>	<b>99.7</b>

LRI: Linear retention indices (DB-5 capillary column), \*F11: Flowers of Chemlal from the Mediterranean zone; F12: Flowers of Sigoise from the Mediterranean zone; F21: Flowers of Chemlal from the arid zone; F22: Flowers of Sigoise from the arid zone.

By comparing, the VOLs (L11, L12, L21 and L22) and VOFs (F11, F12, F21 and F22), the former were richer in constituents, 42 vs. 24 volatiles. Among them, 15 compounds were common to both VOLs and VOFs, 27 compounds were found in VOLs only and 9 compounds were exclusive of VOFs. Some of these compounds were unique in no more than one cultivar or one geographic region. Mostly, the VOFs and VOLs were more numerous in the plant material collected in the arid region (F2, L2) than from the Mediterranean region (F1, L1). Regardless of the harvesting area, Chemlal flowers (F11 and F21) and leaves (L11 and L21) were richer in chemicals than Sigoise (F12, F22, L12 and L22) in both regions. Comparing the two cultivars, in the case of VOFs, 19 compounds were present in F21 and only 7 in F12, while 15 constituents were identified in F22 and only 4 in F12. For VOLs, 30 compounds were detected in L21 and 22 in L11, while 21 chemicals were reported for L22 and 19 for L12.

VOLs and VOFs can be sorted into the 10 chemical classes listed in Table 4. Aldehydes and ketones constituted the major class of VOFs, from 26.6% up to 81.4%, except for F22, which exhibited oxygenated monoterpenes as the most abundant class (31.3%). In particular, monoterpenes, hydrocarbons, phenylpropanoids and alcohols were present only in F2 volatiles. Apocarotenoids (from 28.8 to 51.7%) followed by aldehydes/ketones (from 23.5 to 44.7%) were the most represented chemical classes of VOLs. On the other hand, apocarotenoids and oxygenated sesquiterpenes were not found in any of VOF samples. However, most of the detected compounds exhibited low relative abundances ( $\leq 5\%$ ) and just a few of them were present as the major ones, such as (*Z*)-jasmone (51.8%), nonanal (32.4%), bornyl acetate (26.8%), theaspirane II (23.7%) and (*E,E*)-2,4-heptadienal (16.9%).

**Table 3.** Compositions (%) of *Olea europaea* L. leaf volatiles from Northern and Southern Algeria

No	Compounds	LRI	L11	L12	L21	L22
1	hexanal	802	2.4	-	-	-
2	( <i>E</i> )-2-hexenal	856	4.8	2.7	2.4	6.5
3	( <i>Z</i> )-3-hexen-1-ol	857	1.1	1.3	-	-
4	1-hexanol	869	0.8	-	-	-
5	<i>n</i> -nonane	900	0.5	-	0.3	-
6	heptanal	903	0.8	-	0.4	-
8	benzaldehyde	963	2.7	3.0	1.9	3.5
9	3-ethenyl pyridine	969	4.4	-	1.9	1.9
10	6-methyl-5-hepten-2-one	987	-	-	1.8	4.1
11	( <i>E,Z</i> )-2,4-heptadienal	997	-	-	1.5	7.1
13	( <i>E,E</i> )-2,4-heptadienal	1011	3.2	3.0	4.6	<b>16.9</b>
14	<i>p</i> -cymene	1028	0.7	2.7	-	-
15	limonene	1032	1.1	4.2	-	-
16	phenylacetaldehyde	1045	2.6	3.2	-	-
17	1-octanol	1071	1.4	1.7	2.3	3.6
19	linalool	1101	-	-	0.7	-
20	nonanal	1103	<b>26.9</b>	11.6	3.1	4.7
21	phenylethyl alcohol	1111	8.8	3.4	-	-
22	1,4-dimethyl-3-tetrahydroacetophenone	1150	-	-	0.9	-
23	( <i>E,E</i> )-2,6-nonadienal	1153	-	-	0.8	-
24	menthone	1154	-	1.7	0	-
25	( <i>E</i> )-2-nonenal	1163	-	-	1.2	-
26	2-phenylethyl formate	1177	3.2	-	-	-
28	safranal	1198	-	-	1	1.4
30	$\beta$ -cyclocitral	1222	-	-	1	0.9
31	1,2-benzisothiazole	1223	2.4	1.6	-	-
32	carvone	1244	-	-	0.9	1.2
34	( <i>E</i> )-2-decenal	1262	1.3	-	1.2	0.6
35	( <i>E,Z</i> )-2,4-decadienal	1293	0	-	0.9	1.3
36	theaspirane I	1298	8.9	18.2	1.2	3.7
38	<i>p</i> -vinylguaiaicol	1314	-	2.7	2.8	1.3
39	theaspirane II	1315	<b>17.7</b>	<b>23.7</b>	0	13.7
40	( <i>E,E</i> )-2,4-decadienal	1317	-	-	5.4	-
41	phenylethyl propionate	1351	-	3.4	1.2	-
42	eugenol	1358	1.6	-	-	-
43	( <i>E</i> )- $\beta$ -damascenone	1382	-	4.0	7.4	3.9
46	dihydrodehydro- $\beta$ -ionone	1422	-	-	11.9	3.9
47	( <i>E</i> )-geranylacetone	1454	-	2.0	<b>14.4</b>	6.8
48	( <i>E</i> )- $\beta$ -ionone	1488	2.2	2.7	9.7	4.8
49	dihydroactinidiolide	1536	-	-	5.1	3.3
50	caryophyllene oxide	1581	-	-	2.6	-
51	benzophenone	1627	-	-	2.7	-
	<b>TOTAL</b>		<b>99.5</b>	<b>96.8</b>	<b>93.2</b>	<b>95.1</b>

LRI: Linear retention indices (DB-5 capillary column), \*L11: Leaves of Chemlal from the Mediterranean zone; L12: Leaves of Sigoise from the Mediterranean zone; L21: Leaves of Chemlal from the arid zone; L22: Leaves of Sigoise from the arid zone.

(*Z*)-jasmone was the main constituent in F1 fractions, with percentages ranging from 49.0 and 51.8% in F11 and F12 respectively. Conversely, lesser amounts of this substance were observed in F2 (13.2 and 9.4% in F21 and F22, respectively). It can be noted that the cv. Chemlal showed greater amounts of (*Z*)-jasmone than the cv. Sigoise in both regions. Zhang *et al* [24] have found (*Z*)-jasmone in the flowers of *Olea europaea* L. of two Chinese and Mediterranean cultivars, but at much lower relative contents (from 7 to 0.15%). Therefore, the present study renowned that (*Z*)-jasmone, with its

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high levels in both cultivars, could represent a chemical marker for their flowers in the Mediterranean zone of Algeria. Due to this high relative amounts, and because it was demonstrated to be electrophysiologically active and repellent to herbivores, (*Z*)-jasmone may represent a defense compound for *O. europaea* [25].

The F2 fractions were characterized by the presence of the oxygenated monoterpene bornyl acetate as the major constituent (26.6 and 20.2% in F22 and F21, respectively). Although, the significant appearance of this compound in F2, it was totally absent among F1 volatiles. As published by Cano-Lamadrid *et al* [26] and Veillet *et al* [27], bornyl acetate was detected in the volatile composition of olive oil (very low amounts). While, revealing this monoterpene in the present study, could be an indication of the biological potential of VOFs, since this volatile exhibits anti-inflammatory properties and it is used as an analgesic [9, 28-29].

In addition, VOFs contained considerable amounts of nonanal, ranging from 32.4 to 11.7% in F12 and F22, respectively. Indeed, nonanal was the second main compound of F12. This aldehyde was also an important constituent of VOLs (from 26.9 to 3.1%) for both regions. However, this compound was more abundant in samples from the Mediterranean area (26.9 and 11.6% in L11 and L12, respectively). Previous studies have reported, nonanal as the main compound in leaves samples of different *O. europaea* cultivars from different regions (Italy, Tunisia and Portugal) [10-13].

Similar amounts of nonanal in Sigoise leaves from the Mediterranean region (11.6%) were noticed compared to the Tunisian cultivar Chemlali (11.23%) in the same season (April 2013) and the Italian cultivars Leccino (11.5%) and Cipressino (11.8%), but in November only [11-12]. Other studies proved the antibacterial and antifungal potential of nonanal [30]. For example, Zhang *et al* [31] reported that nonanal could significantly inhibit the mycelial growth of *Penicillium cyclopium*. Zavala-Sánchez *et al* [32] demonstrated that nonanal isolated from *A. ludoviciana* was the compound responsible for the antidiarrhoeal activity.

**Table 4.** Chemical classes (%) of *Olea europaea* L. flower and leaf volatiles from Northern and Southern Algeria

Chemical classes	F11	F12	F21	F22	L11	L12	L21	L22
Monoterpene hydrocarbons	10.2	18.3	5.9	6.3	1.8	6.9	-	-
Oxygenated monoterpenes	-	-	24.7	31.3	-	1.7	1.6	1.2
Oxygenated sesquiterpenes	-	-	-	-	-	0	2.6	-
Nitrogen/sulfur derivatives	5.5	-	11.6	17.2	6.8	1.6	1.9	1.9
Phenylpropanoids	-	-	-	3.0	1.6	-	-	-
Apocarotenes	0	0	0	0	28.8	50.6	51.7	42.4
Non-terpene derivatives	84.1	81.4	57.6	41.9	60.5	36	35.4	49.6
	-	-	7.6	15.3	0.5	-	0.3	-
Non-terpene hydrocarbons	66.2	81.4	45.7	26.6	44.7	23.5	28.8	44.7
Non-terpene aldehydes/ketones	-	-	4.3	-	12.1	9.1	5.1	4.9
Non-terpene alcohols/phenols/ethers	17.9	-	-	-	3.2	3.4	1.2	-
Non-terpene esters								
<b>Total identified</b>	<b>99.8</b>	<b>99.7</b>	<b>99.8</b>	<b>99.7</b>	<b>99.5</b>	<b>96.8</b>	<b>93.2</b>	<b>95.1</b>

\*F11: Flowers of Chemlali from the Mediterranean zone; F12: Flowers of Sigoise from the Mediterranean zone; F21: Flowers of Chemlali from the arid zone; F22: Flowers of Sigoise from the arid zone. L11: Leaves of Chemlali from the Mediterranean zone; L12: Leaves of Sigoise from the Mediterranean zone; L21: Leaves of Chemlali from the arid zone; L22: Leaves of Sigoise from the arid zone.

Monoterpene hydrocarbons, detected as the third chemical class in VOFs, ranged from 18.3 to 5.9%, with a relevant presence of limonene (from 10.2 to 3.2%) and *p*-cymene (from 8.1 to 2.7%). Considerable higher percentages of these chemicals were found in F12 and F11 compared to F22 and F21. Besides, these two compounds were present at much lower levels in VOLs.

Comparing to the arid region, the Mediterranean climatic conditions stimulated much more the production of these natural products, particularly by cv. Sigoise for both VOFs and VOLs. Flamini *et al* [10] detected both *p*-cymene and limonene among the volatiles of the Italian olive paste obtained by

stone milling of the ripe fruits. Similarly, Malheiro *et al* [13] reported the detection of these two monoterpenes in the volatile composition of cv. Cobrançosa (Portuguese cultivar) olive leaf at different harvesting times. Limonene is a common constituent of various plant essential oils and it has been found to have numerous medicinal benefits (antioxidant properties, an excellent dietary source for cancer prevention and a slight cytotoxic activity toward normal cells) demonstrated both in human and animal studies [33-34].

On the contrary, Zhang *et al* [23] did not report the existence of nonanal neither limonene nor *p*-cymene in VOFs of both the Chinese and the Mediterranean cultivars. So, this study is the first report for these compounds in *O. europaea* flowers. Other compounds were missing in one fraction or found in only one fraction, i.e. 3-ethenyl pyridine, with the exception of F12 and L12, was present in all the other VOFs and VOLs samples from 17.2 to 5.5% and from 4.4 to 1.9%, respectively. In the case of F22, the same cultivar in the other region, it showed the highest amount (17.2%), which made this chemical the second main constituent of this sample. As nonanal, this aromatic amine was previously reported in VOLs of the Tunisian cultivars Chemlali as a major constituent through the vegetative cycle [12]. Two compounds were unique, being present only in F11, namely phenylethyl propionate (17.9%) and phenylacetaldehyde (2.7%). Phenylethyl propionate can be considered as the second main constituent of this sample. Although, it was not found in VOLs during previous studies [10-14].

Furthermore, it was noted the exclusive presence in the arid region (F2) of several compounds in VOFs, namely, *n*-dodecane, menthone, *n*-undecane, hexanal, heptanal and *n*-nonane. Similar relative amounts were noted for F21 and F22 but no significant differences for menthone and *n*-nonane. *n*-Dodecane reached here the highest amount (4.7%). This hydrocarbon was also found in the virgin olive oil of the Tunisian cultivar Chemlali [35]. Sample F21 showed the highest amount of heptanal and hexanal, together with other five exclusive aldehydes: (*E*)-2-hexenal (4.4%), benzaldehyde (3.1%), (*Z*)-2-heptenal (2.4%), octanal (1.9%), (*E,E*)-2,4-heptadienal (1.5%), and two alcohols: (*Z*)-3-hexen-1-ol (2.7%) and 1-octanol (1.6%). On the other hand, F22 showed higher amounts of hydrocarbons *n*-dodecane, *n*-undecane and *n*-nonane, together with other exclusive hydrocarbons such as *n*-tetradecane (2.8%) and *n*-tridecane (3.7%). Among all VOFs, F22 is the only sample that contains a phenylpropanoid derivative, methyl chavicol (3.0%).

In relation to leaves composition, hexanal was present in only L11 (2.4%). Malheiro *et al* [13] report low amounts of this compound ( $\leq 1\%$ ) in two Portuguese cultivars: Cobrançosa and Madural at different seasons. On the contrary, (*E*)-2-hexenal, which was detected in F21 only, was present in all the VOLs at appreciable percentages (From 6.5 to 2.4%); L11 showed a higher amount than L21, whereas L22 recorded the highest amount (6.5%), a value very close to that of the Italian cultivar Frantoio (6.4%) in July [11]. This C<sub>6</sub> aldehyde was reported as the most abundant volatile in many VOLs cultivars [11-12]. Concerning alcohols, 1-octanol (from 3.6 to 1.4%) was higher in L2 than L1, while (*Z*)-3-hexen-1-ol was detected only in region L1, in similar amounts in the two cultivars.

Some other compounds were detected only in VOLs of the Mediterranean region, particularly three of them, namely dihydroactinidiolide (5.1-3.3%), dihydrodehydro- $\beta$ -ionone (11.9-3.9%) and phenylethyl alcohol (8.8-3.4%). The rest, (*E,Z*)-2,4-decadienal, carvone,  $\beta$ -cyclocitral, 6-methyl-5-hepten-2-one, phenylethyl alcohol and (*E,Z*)-2,4-heptadienal were present at slightly lower levels compared to the previous three in both L21 and L22. In fact, dihydrodehydro- $\beta$ -ionone (11.9%) was the second main constituent in L21, while phenylacetaldehyde was detected in one flower fraction F11. 1-Hexanol, detected in fraction L11, in small amounts (0.8%) was reported among VOLs in previous studies [11-12]. Several compounds exclusive of VOLs, with relatively high percentages, were theaspirane I (from 18.2 to 1.2 %) and theaspirane II (from 23.7 to 13.7%). These two compounds were among the main constituents characterizing cv. Sigoise (L1), which showed its highest amounts (23.7 and 18.2%). Also, a higher percentage in L1 than in L2 was noted for theaspirane II, not detectable in L21. Moreover, theaspirane I and theaspirane II are well known as the main compounds of VOLs from Italian and Tunisian *O. europaea* cultivars [10-12, 14]. On the other hand, the (*E*)- $\beta$ -ionone in L2 showed higher amount than in L1, with an important percentage in L21 (9.7%). Minor compounds characterizing L21 were caryophyllene oxide (2.6%), benzophenone (2.7%), (*E,E*)-2,4-decadienal (5.4%), (*E*)-2-nonenal (1.2%), (*E,E*)-2,6-nonadienal (0.8%), 1,4-dimethyl-3 tetrahydroacetophenone (0.9%) and linalool (0.7 %).

*n*-Nonane and heptanal, present in the flowers of the arid region only, in the case of cv. Chemlal leaves, they were detected in both regions. L1 showed higher amounts (0.5 and 0.8%) than L2 (0.3 and

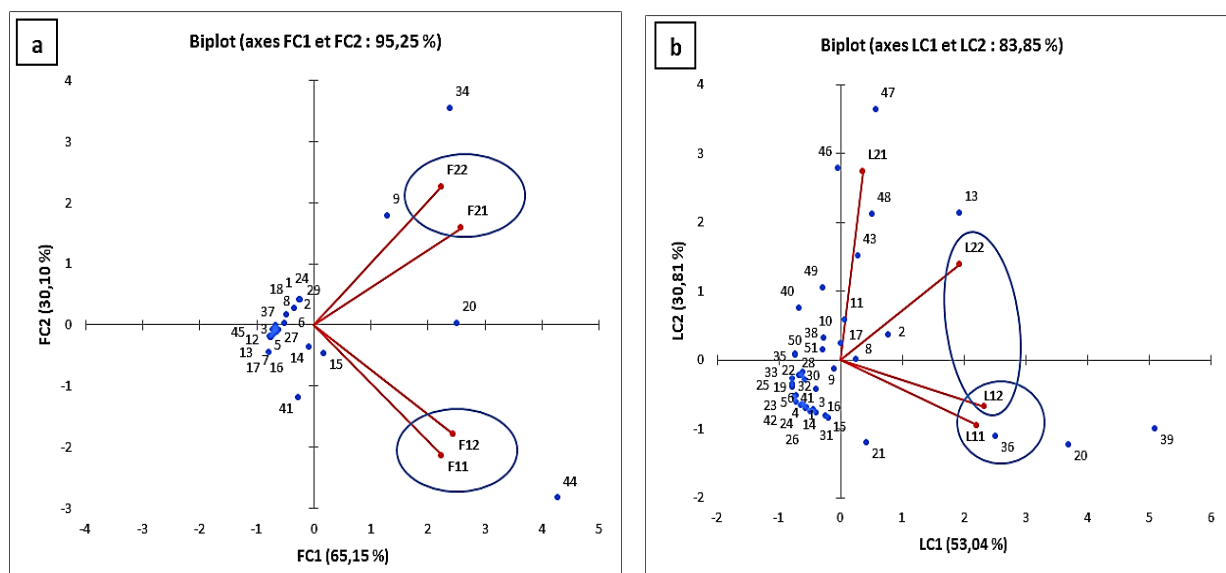
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0.4%), respectively. Other important volatiles, such as benzaldehyde (3.5-1.9%) and (*E,E*)-2,4-heptadienal (16.9-1.5%), were present in all VOLs. In detail, L22, showed the highest amounts of (*E,E*)-2,4-heptadienal and benzaldehyde. The arid region showed higher amounts, which made (*E,E*)-2,4-heptadienal the main compound of L22 fraction (16.9%). On the other hand, in the Mediterranean region, the same cultivar showed almost equal amounts (about 3%) of both volatiles. These two compounds were also present in the flower sample F21. Moreover, other compounds, i.e. (*E*)-geranylacetone, (*E*)- $\beta$ -damascenone and *p*-vinylguaiaicol were detected in all VOLs, except L11. Among these compounds, (*E*)-geranylacetone was the principal compound characterised the arid region in cv. Chemlal (14.4%).

Principal Component Analysis (PCA) was applied to the VOFs and VOLs data using the correlation matrix from Table 2 and 3 to obtain a more simplified view of the total compositions.

Figure 1a and Figure 1b showed the results obtained from PCA for the 42 compounds in VOLs and the 24 ones in VOFs. The cumulative percentage variance described by the first two principal components extracted from the VOFs (axes FC1 and FC2) and the VOLs (axes LC1 and LC2), explained 95.25 and 83.85% of the total variance of VOFs and VOLs, respectively. For VOFs, the first principal component (FC1) accounted for the 65.15% and the second one (FC2) accounted for an additional 30.10% of the total variance of flowers volatiles. The main compounds were unrelated: each one fell in the same region quadrant. The minor compounds were placed in the upper and lower left quadrants, close to the centre, and none of them characterised either the region nor cultivar.

The two regions were clearly separated from each other; the matrix of correlation shows that the correlation between F11 and F12 was 86% and between F21 and F22 was 92%. Also, the results indicate that the compositions of VOFs obtained from the same region are characterised by the same main compounds. In line with this results, several studies have demonstrated that the soil and the climate conditions have much more influence than the genetic factors among the cultivars [16, 36-38]. In parallel, for VOLs, the first and second principal components LCs explained 53.04% (LC1) and 30.81% (LC2) of the variance, across the samples. A correlation between L22 and L12 was noticed. Sigoise responded similarly in the two different geographic sites of Algeria, probably because the environmental factors have less impact on the volatiles production of this cultivar, while for Chemlal this not applies (chemical similarity). The PCA revealed a weaker inter-relationship between the compositions of VOFs and VOLs.



**Figure 1.** (a): Principal component analysis of volatiles of *Olea europaea* L. flowers (VOFs); (b): Leaves (VOLs)

Similarly to all secondary plant metabolites, volatiles are biosynthesized through enzymatic pathways. Their formation is therefore monitored by enzymes and influenced by several factors [8].



Previous researches on *O. europaea* leaves, including the type of cultivar [10], time of year [11-13], and geographic origin [14] have confirmed this.

Indeed, cv. Chemlal, the dominating cultivar in Algeria [15], showed a richer volatile compositions compared to cv. Sigoise for both flowers (VOFs) and leaves (VOLs). This can provide information about the plant physiology. Probably, the genetic characteristics of this cultivar has different enzymes levels and enzyme activity than cv. Sigoise, which are in turn responsible for the qualitative and quantitative composition of volatiles compound. Furthermore, interesting differences related to a strong environmental control were observed.

The flowers from the Mediterranean region were found to be rich in (*Z*)-jasmone, while the ones from the arid region were rich in bornyl acetate. Many studies report the high defence level of these two compounds and that their production is related to environmental stress, which induce the expression of various genes to activate defence-related pathways that result in the release of defence chemicals [8-9, 28-29]. Our study confirmed that the environmental factor is affecting the chemical composition of *O. europaea* flowers, which led to different chemotypes.

Some compounds shared by leaves and flowers, such as nonanal, limonene and *p*-cymene, are an evidence that the olive tree release substantial quantities of the same volatiles from leaf and floral organs [8, 16]. However, the study of the physiology and function of *O. europaea* floral organs is still in its infancy. More work is required for a detailed knowledge of the chemistry of floral fragrances of the olive tree [24].

In addition, the leaves from the Mediterranean region were richer in highly volatile compounds, such as the low-molecular weight aliphatic C6-C7 aldehydes and alcohols. Probably, their absence or a low percentages in the material from the arid zone is due to the extremely hard climate (high temperature, strong sand winds, important evaporation rate), where the low-molecular compounds easily evaporated into the atmosphere when there are no barriers to diffusion [16, 37]. Muzzalupo *et al* [39] study showed that the concentrations of hexanal, (*E*)-2-hexenal, and 1-hexanol were significantly affected by humidity. Brahmi *et al* [14] reported that the environmental factors appeared to influence the formation of C6-compounds in *O. europaea* leaves. Many of these compounds, are produced enzymatically by the lipoxygenase (LOX) pathway, the enzyme family involved in the formation of C6 volatiles in plant leaf, also called “green volatiles” [8, 37]. For example, hexanal is the oxidation product of linoleic acid and may derive from either LOX action or from chemical oxidation.

On other hand, the same samples were devoid of the higher molecular weight compounds of VOLs, such as caryophyllene oxide, benzophenone, dihydroactinidiolide and dihydrodehydro- $\beta$ -ionone, which were detected only in the leaves from the arid region. Probably due to changes in enzymes activity, which could be influenced by DNA adaptation in a new environment that affect physiological characteristics of the plants, especially in the arid region of Algeria.

The samples showed a different volatile composition that resulted strongly related to the different sites of cultivation and their characteristics. Actually, besides intrinsic factors, many extrinsic ones have influenced their production, such as light, precipitation, growing site (altitude, latitude) and nature of the soil (pH, constituents) [40]. The Mediterranean zone in Algeria from the Atlas Mountains has an environment similar to its Northern Africa neighbors (Tunisia and Morocco) and other regions in Southern Europe such as Italy, the Greek Islands, Portugal and Spain [14]. Certainly, these very close environments are reflected by the similarities in the volatile profile of *O. europaea*, especially for the main constituents, namely (*E*)-2-hexenal, nonanal, theaspirane I, 3-ethenyl pyridine and other minor ones, such as heptanal, octanal, linalool [13-14]. Some substances were unique in the Algerian cultivars, such as phenylethyl propionate. Others were produced in the arid region only, such as the aromatic amine 3-ethenyl pyridine, which was among the major VOFs.

#### 4. Conclusion

To the best of our knowledge, the present study has provided for the first time important information about the volatile profiles of *Olea europaea* L. flower and leaf of the most abundant cultivars in Algeria, cultivated in Mediterranean and Saharan regions. This can lead to an important source of variable natural products, potentially bioactive. Besides, it revealed the relationship between the volatiles and their geographic origin that caused the different volatile bouquets. This work could be

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a key to the large cultivation project of 100.000 olive trees in southern Algeria by providing information on how the plant physiology changes in the adaption to extremely hard conditions, such as those present in the arid state Ouargla in Algeria. Certainly, the present research will be very helpful for sustainable agriculture in the Algerian Sahara. Cosmetics, food and pharmaceutical industries will benefit from the presence of the main volatile compounds in *O. europaea*, in particular of jasmone in the production of perfumes and as a food flavoring, or bornyl acetate as an analgesic.

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