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## Characterization and chemosystematics of Algerian thuriferous juniper (*Juniperus thurifera* L.)

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### Summary

Leaf essential oils (EO) of *Juniperus thurifera* L. collected at six locates from Aures Mountains in Algeria, were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The main components identified were: sabinene (5.2-19.78 %), terpinene-4-ol (5.43-9.37 %), elemol (0.69-7.61 %),  $\Delta$ -cadinene (3.26-6.11 %). Terpenoids data of our samples and those reported in other works realized by various authors were subjected to Principal Component Analysis (PCA), and Unweighted Pair Group Method with Arithmetic means (UPGMA) cluster was carried. This analysis revealed significant differences between *Juniperus thurifera* populations, and confirmed the clear separation of Algerian populations to the European and Moroccan populations. Algerian thuriferous juniper is more similar to *J. thurifera* from Moroccan populations, and different from that of essential oils obtained from European populations.

### Introduction

Thuriferous juniper (*Juniperus thurifera* L.), is an evergreen and a dioecious shrub or tree with scale leaves and bluish black berries at maturity, occurring from Algeria and Morocco over the Iberian Peninsula and the Pyrenees to the French and Italian Alps and to Corsica (GAMISANS et al., 1994; GAUQUELIN et al., 1988; GAUQUELIN et al., 2003). In Algeria, the thuriferous juniper is extremely rare and only localized in the Aures mountains with a number of scattered and often very large trees that are probably the remains of formerly more extensive stands (VELA and SCHÄFER, 2013).

*Juniperus thurifera* is a morphologically variable species, perhaps as a result of long-term isolation of disjunct populations. MAIRE (1926) was the first to distinguish the North African and European populations of *J. thurifera*. Later, based on morphometric characters such as the size of the cones and the number of seeds per cone. GAUQUELIN et al. (1988) recognized both entities as subspecies; *J. thurifera* subsp. *africana* (Maire) Gauquelin in North Africa, and *J. thurifera* subsp. *thurifera* in the European range of the species. Of the latter, there are 3 varieties: var. *thurifera* on the Iberian Peninsula, var. *gallica* De Coincy in the Alps and var. *corsicana* Gauquelin in Corsica (GAUQUELIN et al., 2003). The distinction of North African and European populations of *J. thurifera* is validated by ROMO and BORATYNSKY (2007).

Studies based on essential oils composition, random amplified polymorphic DNA (RAPD) and morphometric data (ADAMS, 1999; ADAMS et al., 2003; BARRERO et al., 2004; ACHAK et al., 2008; 2009; BAHRI et al., 2013; BORATYNSKY et al., 2013), supported the clear differentiation of *J. thurifera* subsp. *africana* from Morocco against the European populations, but without studying the Algerian ones, which are generally empirically assimilated to the Moroccan taxon. The study achieved by TERRAB et al. (2008), based on genetic polymorphism (AFLP), has shown that The Algerian population was genetically more closely related to the European than to the Moroccan

ones, probably due to dispersal events from Europe to Algeria, and the Moroccan populations should be recognized as a distinct subspecies (*J. thurifera* L. subsp. *africana* (Maire) Romo and Boratynsky). A taxonomic synthesis, completed by a brief morphological study of fruits based on herbarium samples was performed by VELA and SCHÄFER (2013), concludes with the desirable distinction of a Moroccan taxon (subsp. *africana*) and an Algerian one (*Juniperus thurifera* var. *aurasiaca* Vela & P. Schäf.). It is distinguishable as intermediate between subsp. *thurifera* and subsp. *africana*, more similar to the first one following molecular properties but sharing partial polymorphism with the second and more similar to the latter one following some morphological elements as fruit size average but with a variation amplitude partially sharing numerical values with the first. But above all, the polymorphism of Algerian populations is very poorly known and would be deeply studied in the future (VELA and SCHÄFER, 2013).

The aim of the present study was to characterize the Algerian Thuriferous juniper and sought its taxonomic status. We are based on the chemical composition of the essential oils isolated from the leaves of *Juniperus thurifera* L. collected at different locations in Aures mountains (Algeria), and compare these data with other studies on the chemical variability of essential oils from European and Moroccan populations.

### Material and methods

#### Plant material

Leaves of *juniperus thurifera* L. were collected in March, 2010, from different localities around the Aures Mountains, in Algeria (Tkout1 at 1450 m of altitude, Tkout2 at 1700 m, Baâli at 1750 m, Tizi nerrsas at 1500 m, Tibhirin at 1500 m, and Chelia at 1700 m of altitude). A voucher specimen is deposited in the herbarium of the Laboratory of Natural Resource Valorization, Faculty of Biology, Farhat Abbes University, Setif, Algeria.

#### Isolation and analysis of the essential oils

The plant material was submitted to hydro distillation for 3 h, according to TUMEN et al. (2010). The prepared volatile oils were dehydrated over anhydrous sodium sulphate and stored in sealed glass vials at 4-5 °C prior to analysis. Yield based on dry weight of the sample was calculated.

The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard model 5971, equipped with a DB5 MS column (30 m X 0.25 mm; 0.25  $\mu$ m), programming from 50 °C (5 min) to 300 °C at 5 °C/min, with a 5 min hold. Helium was used as the carrier gas (1.0 mL/min); injection in split mode (1:30); injector and detector temperatures, 250 and 280 °C, respectively. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180 °C; MS data were acquired in the scan mode in the *m/z* range 33-450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library (NIST, 2002) and those described by ADAMS (2001) as well as on comparison of

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their retention indices either with those of authentic compounds or with literature values (ADAMS, 2001).

### Statistical analysis

To examine the phytochemical diversity based on the content (%) of chemical constituents in essential oil among the studied six populations; these were first subjected to Principal Components Analysis (PCA) to examine the relationships among the compounds and identify the possible structure of the population. Cluster analysis (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to seek for hierarchical associations among the populations. Statistical analyses were carried out using STATISTICA 8 software.

## Results and discussion

### Variability of Algerian *J. thurifera* essential oils

Volatile oil yield of the leaves of the investigated *J. thurifera* populations is summarized in (Tab. 1). The yield varied from 0.40 to 0.53 % in different populations of *J. thurifera*. Maximum essential oil yield was noticed in Tkout1 population (0.53 %), followed by Tizi nersas (0.48 %), the minimum essential oils yield was noticed in Chelia population (0.40 %), which is very low compared to the yield obtained by ACHAK et al. (2008; 2009) and BAHRI et al. (2013) from the same species.

Inter-population variation of essential oil yield is quite common phenomenon and encountered earlier in several other plant species. These variations might be due to climatic conditions of the growing site, pedoclimatic variation or due to difference in the genetic makeup of the *J. thurifera* populations.

The volatile oils of all six *Juniperus thurifera* populations were characterized and identified by gas chromatography and gas chromatography-mass spectrometry. The relative percentages of the constituents are listed in the Tab. 1. Seventy nine constituents, representing 90.2-98.7 % of the total oil composition, were identified. Seventeen constituents were monoterpene hydrocarbons accounting (18.6-40.1 %) of the EO, 17 constituents were oxygenated monoterpenes (10.3-24 %), 22 constituents were sesquiterpene hydrocarbons (15.1-23.8 %), 18 constituents were oxygenated sesquiterpenes (18-36.8 %), two constituents were diterpene hydrocarbons (0.2-1.7 %), and three constituents were oxygenated diterpenes (0.5-1.8 %).

The main components identified were: Sabinene (5.2-20 %), terpinene-4-ol (5.4-9.4 %), Elemol (0.7-7.6 %),  $\Delta$ -cadinene (3.3-6.1 %), linalyl acetate (0.9-6.2 %),  $\gamma$ -terpinene (2.6-3.9 %),  $\alpha$ -pinene (2.6-

3.9 %) and myrcene (1.2-3.3 %). Leaf EO of *Juniperus thurifera* in deferent regions of Morocco are rich in sabinene (12.2 % to 45.8 %),  $\alpha$ -pinene (4 % to 17.1 %) and terpinene-4-ol (2.6 % to 16.9 %) (ADAMS et al., 2003; ROMAN et al., 2008; 2009; BAHRI et al., 2013). The major constituent of the volatile oils obtained from branches of Moroccan *Juniperus thurifera* is  $\beta$ -pinene (36.26 %) (MANSOURI et al., 2010).

Twenty-two compounds showed statistically significant variations among the six locations, the components identified (sabinene,  $\alpha$ -cadinol, valencene, elemol, linalyl acetate, and linalool) show a significant variability of terpenoid (Fig. 1).

The principal component analysis (PCA) performed on the correlation matrix of the 79 variables showed that the first three axes explained 74.3 % of the observed variation. This analysis allowed recognizing two distinct EO types based on the content of sabinene, linalyl acetate, linalool,  $\gamma$ -terpinene, myrcene, bulnesol, valencene,  $\gamma$ -eudesmol, epi- $\alpha$ -cadinol, epi- $\alpha$ -muurolol, and 4-epi-abietal.

The first group was represented by four populations (Tkout1, Baâli, Tizi nersas, Tibhirine), located on the positive part of axis one, characterized by high concentrations of sabinene, linalyl acetate, linalool,  $\gamma$ -terpinene, myrcene, and bulnesol. This group is opposed to the second group, formed by two populations (Chelia and Tkout2), which is characterized by high concentrations of valencene,  $\gamma$ -eudesmol, epi- $\alpha$ -cadinol, epi- $\alpha$ -muurolol, and 4-epi-abietal (Fig. 2).

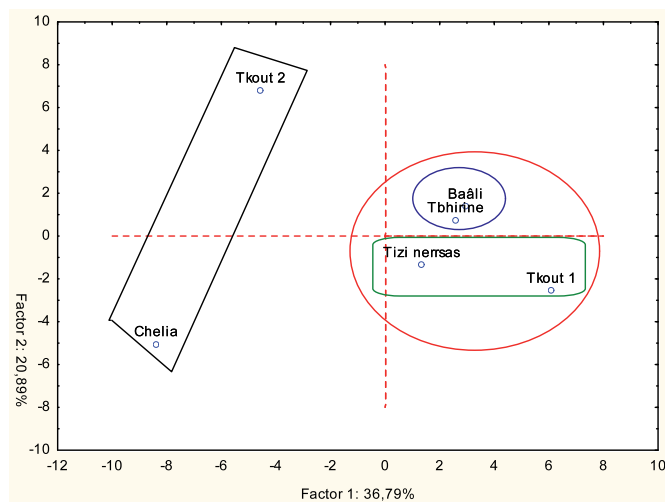


Fig. 2: Projection of Algerian *Juniperus thurifera* populations on the factor plane (1x 2).

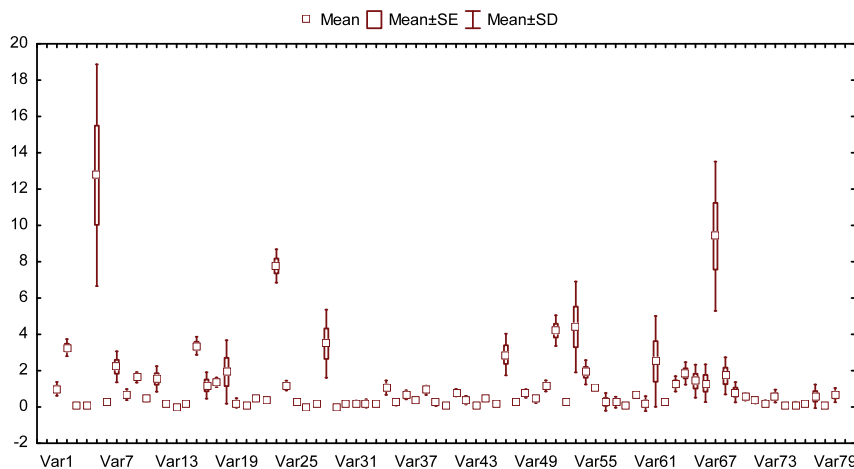


Fig. 1: Chemical Variability of main compounds of *Juniperus thurifera* (Var 1 to Var 79 are mentioned in the Tab. 1).

**Tab. 1:** Composition of the leaf essential oils of *J. thurifera* from Aures mountains in Algeria.

	Compound	Ki	Tk1	Tk2	Tz	Ba	Tb	Ch
Var1	$\alpha$ -thujene	924	1.2	0.8	1.1	1.5	0.8	0.5
<b>Var2</b>	<b><math>\alpha</math>-pinene</b>	<b>932</b>	<b>3.3</b>	<b>2.6</b>	<b>2.62</b>	<b>3.47</b>	<b>3.1</b>	<b>3.9</b>
Var3	fenchene	946	0.1	tr	0.1	0.1	0.1	-
Var4	camphene	947	0.1	0.1	0.1	0.1	0.1	tr
<b>Var5</b>	<b>sabinene</b>	<b>975</b>	<b>17.9</b>	<b>8.3</b>	<b>11.3</b>	<b>20.0</b>	<b>9.0</b>	<b>5.2</b>
Var6	$\beta$ -pinene	979	0.3	0.3	0.3	0.3	0.3	0.4
Var7	myrcene	991	2.8	1.7	2.0	3.3	1.7	1.2
Var8	$\Delta$ -3-carene	1011	0.2	1.5	0.9	0.5	1.0	0.8
Var9	$\alpha$ -terpinene	1018	1.6	1.3	1.7	1.8	1.9	1.2
Var10	p-cymene	1026	0.5	0.2	0.3	0.7	0.4	0.5
<b>Var11</b>	<b>limonene</b>	<b>1031</b>	<b>1.4</b>	<b>3.2</b>	<b>2.5</b>	<b>2.0</b>	<b>1.2</b>	<b>0.7</b>
Var12	$\beta$ -phellandrene	1031	0.2	-	0.2	0.2	0.2	0.2
Var13	(Z)- $\beta$ -ocimene	1040	0.10	-	-	-	-	-
Var14	(E)- $\beta$ -ocimene	1050	0.3	-	0.2	0.3	0.2	-
<b>Var15</b>	<b><math>\gamma</math>-terpinene</b>	<b>1062</b>	<b>3.2</b>	<b>2.6</b>	<b>3.5</b>	<b>3.6</b>	<b>3.9</b>	<b>2.6</b>
Var16	cis-sabinene hydrate	1068	0.8	0.8	1.6	0.6	2.3	0.6
Var17	terpinolene	1088	1.3	1.2	1.5	1.5	1.5	0.9
<b>Var18</b>	<b>linalool</b>	<b>1096</b>	<b>4.6</b>	<b>0.3</b>	<b>0.4</b>	<b>2.5</b>	<b>1.6</b>	<b>0.4</b>
Var19	cis-thujone	1100	0.1	0.4	-	0.3	0.6	-
Var20	trans-thujone	1111	-	0.27	-	-	-	0.3
Var21	cis-p-menth-2-en-1-ol	1122	0.4	0.3	0.5	0.4	0.6	0.3
Var22	trans-menth-2-en-1-ol	1141	0.3	0.2	0.3	0.3	0.4	0.3
Var23	<b>terpinene-4-ol</b>	<b>1177</b>	<b>7.2</b>	<b>5.4</b>	<b>7.6</b>	<b>7.5</b>	<b>9.4</b>	<b>7.1</b>
Var24	$\alpha$ -terpineol	1195	1.6	0.6	1.1	1.1	1.1	0.9
Var25	verbanone	1205	0.2	0.2	0.3	0.3	0.4	0.3
Var26	trans piperitol	1209	0.1	-	-	-	-	-
Var27	nerol 1 (80)	1224	0.3	-	0.2	0.2	0.2	-
Var28	linalyl acetate	1249	6.2	1.2	3.4	3.1	3.7	0.9
Var29	pregeijerene	1280	0.12	-	-	-	-	-
Var30	bornyl acetate	1284	0.1	-	-	0.1	0.1	0.4
Var31	isobutyl benzene	1287	0.3	-	0.3	-	0.3	-
Var32	decan-2,4-dien-1-ol	1312	0.3	0.2	-	0.5	0.1	-
Var33	$\delta$ -elemene	1338	0.2	0.2	0.2	0.1	0.2	-
Var34	$\alpha$ -terpinene acetate	1343	0.7	0.8	1.7	0.8	1.1	0.1
Var35	neryl acetate	1362	0.4	-	0.3	0.2	0.2	-
Var36	geranyl acetate	1381	1.0	0.3	0.6	0.8	0.6	0.3
Var37	$\beta$ -elemene	1391	0.3	0.4	0.3	0.4	0.4	0.2
Var38	$\beta$ -caryophyllene	1419	0.9	1.1	0.9	1.0	1.2	0.5
Var39	$\gamma$ -elemene	1429	0.4	0.4	0.2	0.1	0.4	-
Var40	cadina-3,5-diene	1448	0.1	0.1	0.1	0.2	0.1	-
Var41	$\alpha$ -humulene	1454	0.5	1.0	0.8	0.9	1.1	0.7
Var42	cis muurola-4(14),5-diene	1460	0.1	0.5	0.2	0.4	0.7	0.3
Var43	cadina-1(6),4-diène	1460	0.1	0.2	-	0.2	0.1	-
Var44	$\gamma$ -muurolene	1477	0.3	0.6	0.5	0.5	0.5	0.5
Var45	curcumene	1478	0.2	-	0.3	-	0.2	0.2
Var46	<b>germacrene-D</b>	<b>1480</b>	<b>1.9</b>	<b>2.7</b>	<b>4.4</b>	<b>3.1</b>	<b>3.3</b>	<b>1.6</b>

Var47	trans muurola-4(14),5-diene	1494	0.2	0.4	0.3	0.5	0.2	0.4
Var48	$\alpha$ -muurolene	1500	0.8	1.1	1.1	0.8	0.6	0.4
Var49	$\beta$ -curcumene	1516	0.5	0.2	0.7	0.2	0.4	0.2
Var50	$\gamma$ -cadinene	1514	0.7	1.8	1.0	1.5	1.2	1.4
Var51	<b><math>\Delta</math>-cadinene</b>	<b>1523</b>	<b>3.3</b>	<b>6.1</b>	<b>4.1</b>	<b>5.6</b>	<b>3.9</b>	<b>4.1</b>
Var52	$\alpha$ -cadinene	1539	0.2	0.4	0.2	0.3	0.3	0.3
Var53	<b>elemool</b>	<b>1550</b>	<b>4.7</b>	<b>5.8</b>	<b>0.7</b>	<b>3.9</b>	<b>7.6</b>	<b>5.2</b>
Var54	germacrene-B	1561	2.5	2.3	2.0	1.2	2.6	1.2
Var55	germacrene-D-4ol	1576	0.8	1.9	1.1	0.9	1.1	1.1
Var56	Caryophyllene Oxide	1585	0.1	-	1.1	0.2	-	-
Var57	cedrol	1596	0.1	0.1	0.1	0.1	0.2	0.8
Var58	humulene epoxyde II	1607	0.2	0.2	0.1	-	-	0.1
Var59	$\beta$ -oplopenone	1608	0.5	1.2	0.7	0.6	0.5	0.9
Var60	epi-cedrol	1613	0.92	-	-	-	-	-
Var61	valencen	1619	0.5	2.5	3.1	1.4	0.9	6.6
Var62	1,10-diepi-cubenol	1627	0.3	0.3	0.2	0.2	0.4	0.3
Var63	1-epi-cubenol	1629	1.1	1.3	1.0	1.8	0.8	1.6
Var64	$\gamma$ -eudesmol	1632	1.3	2.1	2.4	1.1	2.	2.4
Var65	epi- $\alpha$ -cadinol	1640	0.9	2.9	1.1	0.8	1.2	3.0
Var66	epi- $\alpha$ -muurolol	1642	0.9	3.7	0.7	0.8	1.0	3.1
Var67	<b><math>\alpha</math>-cadinol</b>	<b>1663</b>	<b>6.1</b>	<b>13.5</b>	<b>10.6</b>	<b>5.5</b>	<b>9.2</b>	<b>15.7</b>
Var68	bulnesol	1666	3.2	1.8	0.9	1.9	2.0	0.6
Var69	gurjunene	1670	0.8	1.1	0.9	0.8	1.6	-
Var70	$\gamma$ -gurjunene	1678	0.3	0.6	0.6	0.8	0.4	0.7
Var71	epi- $\alpha$ -bisabolol	1686	0.3	0.4	0.4	0.1	0.4	0.6
Var72	2-pentadecanone	1698	0.3	0.3	-	-	0.2	0.4
Var73	(Z,Z)-farnesol	1714	0.5	0.3	0.4	0.2	1.0	0.9
Var74	8- $\alpha$ -acetoxyl elemol	1789	0.1	0.2	-	-	0.1	-
Var75	manoyl oxide	1989	0.4	0.2	-	-	0.2	-
Var76	13-epi-manoyl oxide	1992	0.3	0.6	0.1	0.3	0.2	-
Var77	Abietatriene	2090	0.3	0.5	0.4	0.2	0.3	1.7
Var78	phytol	2109	0.2	0.2	-	-	0.1	-
Var79	4-epi-abietal	2299	0.7	1.1	0.3	0.4	0.6	1.3
Monoterpene hydrocarbons			35.3	24.5	29.8	40.1	27.9	18.6
Oxygenated monoterpenes			24.0	10.3	16.7	18.3	20.6	12.3
Sesquiterpene hydrocarbons			15.1	23.8	21.9	20.2	20.4	19.5
Oxygenated sesquiterpenes			22.4	36.2	21.7	18.0	27.7	36.8
Diterpene hydrocarbons			0.5	0.7	0.4	0.2	0.4	1.7
Oxygenated diterpenes			1.4	1.8	0.5	0.6	1.0	1.3
Yield (%)			0.53	0.46	0.48	0.45	0.46	0.40
Total %			98.7	97.3	91.0	97.4	98.0	90.2

(Tk1: Tkout 1, Tk2: Tkout 2, Tz: Tizi nerrsas, Tb: Tibhirin, Ch: Chelia, Ba: Baâli).

Mono and sesquiterpenoids variability reflects the heterogeneity of the genetic structure of population (DODD and POVEDA, 2003; LIMA et al., 2010; SHANJANI et al., 2010). HANNOVER (1992) provides evidence that terpene chemotypes are strongly controlled by genetic factors; he also reported instances of environmental variation in terpene expression under extreme habitat conditions. A prevalence of monoterpene hydrocarbons compared to other

components was noted in Tkout1, Baâli, Tibhirin, and Tizi nerrsas populations, while its reverse trend could be seen in Tkout2 and Chelia populations, which were dominated by sesquiterpene hydrocarbons (Tab. 1). Monoterpenes and sesquiterpene hydrocarbons were strongly related to chemical balance in soils (organic matter, phosphor and base saturation). The chemovariation observed appears to be environmentally determined (LESJAK et al., 2013).

Influence of environmental factors in the chemical composition of essential oils have also been reported in the genus *Juniperus* (DODD and POVEDA, 2003; LIMA et al., 2010; SHANJANI et al., 2010; LOŽIENE and LABOKAS, 2012; LESJAK et al., 2013), *Cupressaceae* family (OTTAVIOLI, 2009), and are well known for other family (HAIDER et al., 2004; KAROUSOU et al., 2005; CUARDO et al., 2006; LEI et al., 2010; DJABOU et al., 2012).

The dendrogram based on UPGMA clustering (Manhattan distance), shows the presence of two groups (Fig. 3) that confirms result obtained from PCA analyses. The first cluster is divided into two sub-groups based on the content of terpinene-4-ol, germacrene-D,  $\alpha$ -terpinene acetate, cis-sabinene hydrate. The first sub-group formed by Tkout1 and Baali, characterized by low concentration of these constituents unlike for Tizi nerssas and Tibhirin populations.

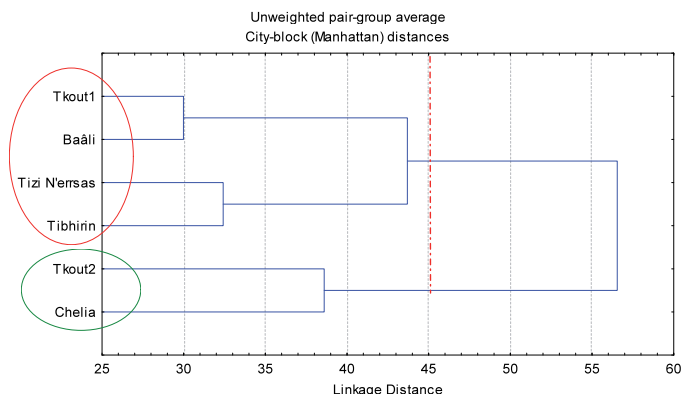


Fig. 3: Dendrogram of Algerian *Juniperus thurifera* populations, based on Manhattan Similarity distance.

**Characterization and chemosystematics**

Leaf essential oils are extremely useful for the analyses of populational differentiation, hybridization and introgression and in assigning individual plants to a species (ADAMS, 2010).

To compare the Algerian thuriferous juniper with other populations, terpenoids data of our samples and those reported in the work of ADAMS (1999), ADAMS et al. (2003), ACHAK et al. (2009), OTTAVIOLI (2009) and BAHRI et al. (2013) were subjected to PCA, and UPGMA cluster was carried. This analysis revealed significant differences between *Juniperus thurifera* populations.

The PCA performed on the correlation matrix of the 96 variables based on the oil composition resulted in 38 factors of which the first three accounted for 47.4 % of the variance among the 39 populations, shows that *J. thurifera* populations are divided into three distinct groups (Fig. 4). The Algerian populations separated by 27.30 % of the variance in the chemical composition of essential oils. However, the Moroccan populations accounted for about 12.66 % of the variance (Fig. 4).

The UPGMA based on the Unweighted pair-group average distance and the City-block (Manhattan) (Fig. 5), has divided 39 populations of *J. thurifera* into two clads, confirmed the separation of the North African populations from the European populations.

Then, some qualitative differences in chemical composition can be deduced. The leaf essential oil composition of European populations was higher in limonene (30-75 %) and lower in sabinene (0-9,7 %) (ADAMS et al., 2003; ACHAK et al., 2008; OTTAVIOLI, 2009). However, the composition of the oils from Algerian populations was similar to those reported for oils of *J. thurifera* from Moroccan populations (ADAMS et al., 2003; ACHAK et al., 2008; BAHRI et al., 2013). The concentration of  $\alpha$ -pinene and sabinene were greater in the

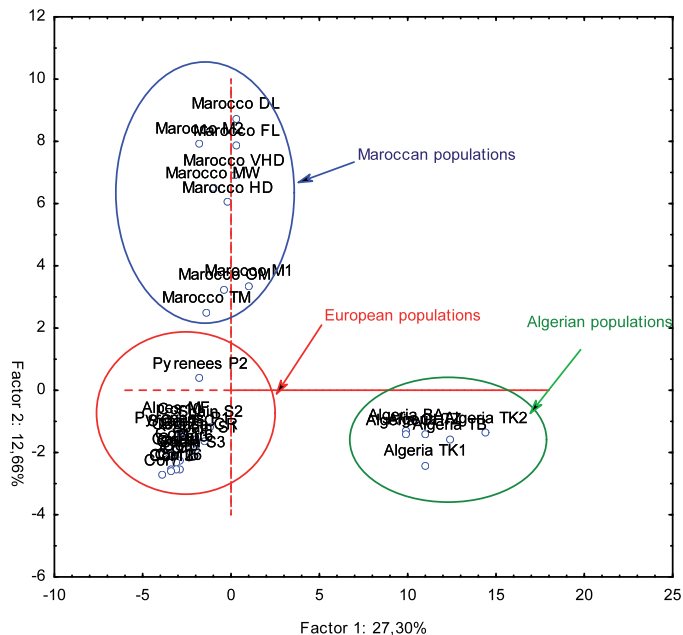


Fig. 4: Projection of *Juniperus thurifera* populations on the factor plane (1x 2).

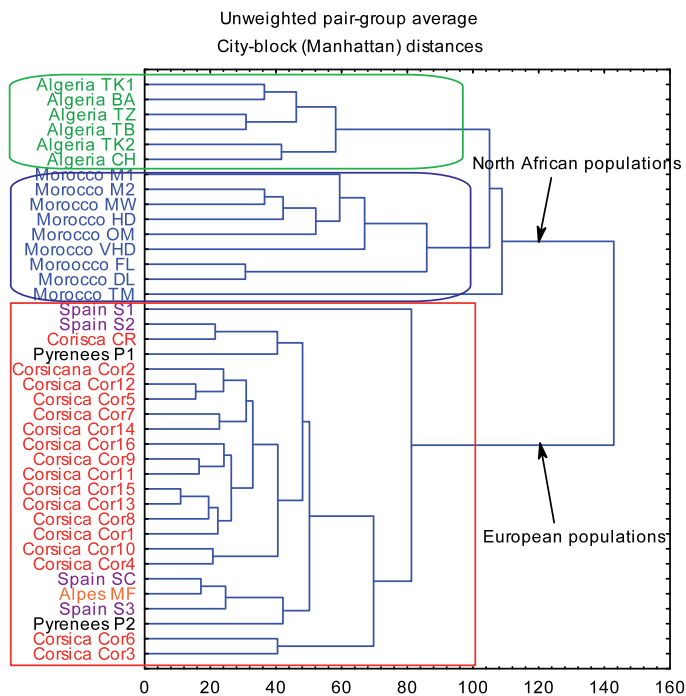


Fig. 5: Dendrogram of *Juniperus thurifera* populations, based on Manhattan Similarity distance (ADAMS, 1999: Spain S3 = 2 Km e Ruidera); (ADAMS et al., 2003: Spain SC = Consuegra, Spain S1 and S2 = Ruidera); Morocco M1 and M2 = Atlas Mts, Morocco TM = Tizi-n-Tichka, Morocco OM = Oukaimeden; Pyrenees P1 and P2 = Pyrenees, France; Corsica CR = Corse, Island); (ACHAK et al., 2009: Morocco FL = fresh leaves; Morocco DL = dried leaves, Ait Lkak Oukaimeden, Atlas Mts), (OTTAVIOLI, 2009: Corsica Cor 1-16 = Corse) and (BAHRI et al., 2013: Morocco VHD and MW= Morocco)

oil of the Moroccan populations, conversely, the concentrations of  $\Delta$ -cadinene and  $\alpha$ -cadinol were greater in the oil of Algerian population.

Should be recognized that Algerian populations as sub-group distinctive from the European and Moroccan populations or do they merely represent geographical interspecific variation?

Algerian *Juniperus thurifera* is distinguishable as intermediate between subsp. *africana* and subsp. *thurifera*. More similar to the first one following some morphological elements as fruit size average but with a variation amplitude partially sharing numerical values with the latter (VELA and SCHÄFER, 2013). The results of the present study confirmed this similarity between Moroccan populations subsp. *africana* and Algerian populations called *Juniperus thurifera* var. *aurasiaca* VÉLA & P. Schäf.

In view of the data presented in this study, it is apparent that Algerian populations is not related to subsp. *thurifera* (*sensu stricto*), but to subsp. *africana*.

However, Terpenes are generally not as useful in making phylogenetic decisions because several terpenes may be controlled by a single enzyme (ADAMS, 2010). FARJON (2005) considered species based on the chemistry of terpenes analysis as based on inconclusive evidence, but DNA sequence data certainly can (which is the main reason for their superiority).

The study achieved by TERRAB et al. (2008), based on genetic polymorphism, has shown that Algerian populations are distinct from the Moroccan ones and more related to European populations.

Intra- and inter-population morphological variability throughout this vast territory fragmented (Spain, Pyrenees, Alps, Corsica and Morocco) has recently been investigated by BORATYŃSKI et al. (2013) and gives results congruous with genetic pattern obtained on the same whole Europe/Morocco (TERRAB et al., 2008), the Algerian population excluded.

Finally, it is true that adding up the number of terpene differences may or may not give a good estimation of divergence. But, the number and scope of terpene differences between the Algerian and European populations indicate considerable differentiation. Additional research, using morphological variability, should help in elucidating these relationships.

## Conclusions

In brief, essential oils analysis carried out on six populations of *J. thurifera* showed both inter-population variability in their terpenoid content, with abundance of sabinene, terpinene-4-ol, elemol... The PCA and UPGMA analysis allowed recognizing two distinct EO types based on the content of monoterpene and sesquiterpene hydrocarbons, the chemovariation observed appears to be environmentally determined. VÉLA and SCHÄFER (2013), proposed to call the Algerian population by *Juniperus thurifera* var. *aurasiaca* VÉLA & P. Schäf, in the varietal rank, which will allow us to consider now as belonging European subset (subsp. *thurifera*) or Moroccan subset (subsp. *africana*) or equal with both. In view of the results of this study, it seems that the Algerian populations are much more similar to the Moroccan populations, but it is still early to specify its taxonomic status. Therefore, we support the proposition of VÉLA and SCHÄFER (2013), and the conflict between chemical and genetical data should be resolved on the morphological level.

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## References

- ACHAK, N., ROMAN, A., ALIFRIQI, M., ADAMS, P.R., 2008: Effect of the leaf drying and sources on the essential oil composition of *Juniperus thurifera* L. Var. *africana* (Maire.) from Tensift Alhouz Marrekch (Morocco). *J. Ess. Oil Res.* 20, 200-204.
- ACHAK, N., ROMAN, A., ALIFRIQI, M., ADAMS, P.R., 2009: chemical studies of leaf essential oil of three species of *Juniperus* from Tensift Alhouz Marrekch (Morocco). *J. Ess. Oil Res.* 21, 337-341.
- ADAMS, P., 1999: Systematics of multi-seeded eastern hemisphere *Juniperus* based on leaf essentials and RAPD DNA fingerprinting. *Biochem. Syst. Ecol.* 27, 709-725.
- ADAMS, R.P., MUMBA, L.E., JAMES, S.A., PANDEY, R.N., GAUQUELIN, T., BADRI, W., 2003: Geographic variation in the leaf oils and DNA Fingerprints (RAPDs) of *Juniperus thurifera* L. from Morocco and Europe. *J. Ess. Oil Res.* 15, 148-154.
- ADAMS, R.P., 2001: Identification of Essential oil components by Gas chromatography/Mass spectroscopy. Allured Publishing Corporation, USA.
- ADAMS, R.P., KAUFFMAN, M.E., 2010: Geographic variation in the leaf essential oils of *Juniperus grandis* (Cupressaceae) and comparison with *J. occidentalis* and *J. osteosperma*. *Phytologia.* 92(2), 167-185.
- BAHRI, F., HARRAK, R., ACHAK, N., ROMANE, A., 2013: Chemical composition and antibacterial activities of the essential oils isolated from *Juniperus thurifera* L. var. *Africana*. *Nat. Prod. Res.* DOI: 10.1080/14786419.2012.755678.
- BARRERO, A.F., QUILEZ DEL MORAL, J.F., HERRDOR, M.M., AKSSIRA, M., BENNAMARA, A., AKKAD, S., AITIGRI, M., 2004: Oxygenated diterpenes and other constituents from Moroccan *Juniperus phoenicea* and *Juniperus thurifera* var. *Africana*. *Phytochemistry* 65, 2507-2515.
- BORATYŃSKI, A., JASINSKA, A.K., MARCYSIAK, K., MAZUR, M., ROMO, A.M., BORATYŃSKA, K., SOBIERAJSKA, K., ISZKUŁO, G., 2013: Morphological differentiation supports the genetic pattern of the geographic structure of *Juniperus thurifera* (Cupressaceae). *Plant Syst. Evol.* 299, 773-784.
- CUARDO, M.A., OLIVEIRA, C.B.A., JESUS, J.G., SANTOS, S.C., SERAPHIN, J.C., FERRI, P.H., 2006: Environmental factors influence on chemical polymorphism of the essential oils of *Lychnophora ericoides*. *Phytochemistry* 67, 2363-2369.
- DJABOU, N., MUSELLI, A., ALLALI, H., DIB, M.A., TABTI, B., VARESI, L., COSTA, J., 2012: Chemical and genetic diversity of two Mediterranean subspecies of *Teucrium polium* L. *Phytochemistry* 83, 51-62.
- DODD, R.S., POVEDA, M.M., 2003: Environmental gradients and population divergence contribute to variation in cuticular wax composition in *Juniperus communis*. *Biochem. Syst. Ecol.* 31(11), 1257-1270.
- FARJON, A., 2005: A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew. Richmond, Surrey, UK.
- GAMINSAS, J., JEANMONOD, D., REGATO, P., GRUBER, M., 1994: Le Génévrier thurifère (*Juniperus thurifera* L.) en Corse. *Candollea* 49, 600-607.
- GAUQUELIN, T., BERTAUDIÈRE, V., CAMBECEDES, C., LARGIER, G., 2003: Le Jeneverier thurifère (*Juniperus thurifera* L.) dans les pyrenees: état de conservation et perspectives. *Acta Bot. Barc.* 49, 83-94.
- GAUQUELIN, T., IDRISSE HASSANI, M., LEBRETON, P., 1988: Le génévrier thurifère (*Juniperus thurifera* L.) (Cupressacées), analyse biométrique et biochimique, propositions systématiques. *Oecologia Mediterranea* 14, 31-42.
- HAIDER, F., DWIVEDI, P., SINGH, S., NAQVI, A.A., BAGACHI, G., 2004: Influence of transplanting time on essential oil yield and composition in *Artemisia annua* plants grown under the climatic conditions of subtropical north India. *Flavour Frag. J.* 19, 51-53.
- HANNOVER, J.W., 1992: Applications of terpene analysis in forest genetics. *New Forests* 6, 159-178.

- KAROUSOU, R., KOUREAS, D.N., KOKKINI, S., 2005: Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja thymbra* in NATURA 2000 sites of Crete. *Phytochemistry* 66, 2668-2673.
- LEI, H., WANG, Y., LIANG, F., SU, W., FENG, Y., GUO, X., WANG, N., 2010: Composition and variability of essential oils of *Platycladus orientalis* growing in China. *Biochem. Syst. Ecol.* 38, 1000-1006.
- LESJAK, M.M., BEARA, I.N., ORČIĆ, D.Z., RISTIĆ, J.D., ANAČKOV, G.T., BOŽIN, B.N., MIMICA-DUKIĆ, N.M., 2013: Chemical characterisation and biological effects of *Juniperus foetidissima* Willd. *Food Sci. Tech.* 53(2), 530-539.
- LIMA, A.S., TRINDADE, H., FIGUEIREDO, A.C., BARROSO, J.G., PEDRO, L.G., 2010: Volatile and molecular analysis of *Juniperus brevifolia* (Seub.) Antoine, an Azorean endemic species. *Biochem. Syst. Ecol.* 38, 621-629.
- LOŽIENE, K., LABOKAS, J., 2012: Effects of abiotic environmental conditions on amount and enantiomeric composition of  $\alpha$ -pinene in *Juniperus communis* L. *Biochem. Syst. Ecol.* 44, 36-43.
- MAIRE, R., 1926: Contribution à l'étude de la flore de l'Afrique du Nord, Fascicule 10. *Bull. Soc. Hist. Nat. Afr. Nord.* 17, 125-126.
- MANSOURI, N., SATRANI, B., GHANMI, M., EL-GHADRAOUI, L., AAFI, A., FRAH, A., 2010: Valorisation des huiles essentielles de *Juniperus thurifera* et de *Juniperus oxycedrus* du Maroc. *Phytothérapie* 8, 166-170.
- NIST., 2002: Mass spectral search program for the NIST/EPA/NIH Mass spectral Library. Vers.2.0. Fireash data, USA.
- OTTVIOLO, J., 2009: Contribution de la RMN 13C à l'analyse d'huiles essentielles et d'oléorésines: caractérisation de genévriers et du pin maritime de Corse. Thèse de Doctorat, Université de Corse Pascal Paoli, Corse.
- ROMO, A., BORATYNSKI, A., 2007: Nomenclatural note on *Juniperus thurifera* subsp *africana* (Cupressaceae). *Ann. Bot. Fennici* 44, 72-75.
- SHANJANI, P.S., MIRZA, M., CALAGARI, M., ADAMS, R.P., 2010: Effects drying and harvest season on the essential oil composition from foliage and berries of *Juniperus excelsa*. *Industrial Crops and Products* 32(2), 83-87.
- TERRAB, A., SCHÖNSWETTER, P., TALAVERA, S., VELA, E., STUESSY, T.F., 2008: Range-wide phylogeography of *Juniperus thurifera* L., a presumptive keystone species of western Mediterranean vegetation during cold stages of the Pleistocene. *Molecular Phylogenetics and Evolution* 48, 94-102.
- TUMEN, I., HAFIZOGLU, H., PRANOVICH, A., REUNANEN, M., 2010: Chemical constituents of cones and leaves of cypress (*Cupressus sempervirens* L.) grown in Turkey. *Fresenius Environmental Bulletin* 19 (10), 2268-2276.
- VELA, E., SCHÄFER, P.A., 2013: Typification de *Juniperus thurifera* var. *africana* Maire, délimitation taxonomique et conséquences nomenclaturales sur le Genévrier thurifère d'Algérie. *Ecologia mediterranea* 39(1), 69-80.

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