

Terpene markers reveal inter- and intra-population variation in southern Atlas cedar (*Cedrus atlantica*) in northeastern Algeria

Original Article

Abstract:

The needle essential oils of *Cedrus atlantica* (Pinaceae) from two cedar forests (Chélia and Tuggurt) located in northeastern Algeria were analyzed using terpene markers. Essential oils were extracted by steam distillation and analyzed by GC–MS. A total of 43 volatile compounds were identified, with 36 in oils from Chélia (Aurès forest) and 14 in oils from Tuggurt (Belezma National Park), accounting for 92.04% and 92.91% of the total oil composition, respectively. Marked variation in terpene composition was observed both between populations and among individuals within populations. Oils from Chélia were dominated by sesquiterpenes, particularly hydrocarbon sesquiterpenes (28.22%), and showed high intraspecific variability, defining three main chemotypes: linalool, aromadendrene-2, and *trans*-caryophyllene. In contrast, Tuggurt oils were dominated by monoterpenes (21.43%) and displayed a more homogeneous profile, largely characterized by β -myrcene. PCA and K-means clustering revealed clear chemical differentiation among populations, suggesting distinct chemotypes associated with environmental differences.

Key words:

Algeria, *Cedrus atlantica*, chemotypes, ecotypes, GC–MS, terpene markers

Apstrakt:

Terpenski markeri otkrivaju međupopulacionu i unutarpopulacionu varijabilnost atlaskog kedra (*Cedrus atlantica*) u severoistočnom Alžiru

Etarska ulja iglica vrste *Cedrus atlantica* (Pinaceae) iz dve kedrove šume (Chélia i Tuggurt) u severoistočnom Alžiru analizirana su primenom terpenskih markera. Etarska ulja su izolovana destilacijom vodenom parom i analizirana GC–MS metodom. Ukupno su identifikovana 43 isparljiva jedinjenja, od kojih 36 u uljima iz Chélia (šuma Aurès) i 14 u uljima iz Tuggurt (Nacionalni park Belezma), što čini 92,04% i 92,91% ukupnog sastava ulja, tim redom. Uočena je izražena varijabilnost u terpenskom sastavu kako između populacija, tako i među jedinkama unutar populacija. Uljima iz Chélia dominirali su seskviterpeni, naročito ugljovodonični seskviterpeni (28,22%), uz visoku intraspecijsku varijabilnost, što je dovelo do izdvajanja tri glavna hemotipa: linalol, aromadendren-2 i *trans*-kariofilen. Nasuprot tome, uljima iz Tuggurt dominirali su monoterpeni (21,43%) i pokazala su homogeniji profil, uglavnom karakterisan β -mircenom. PCA i K-means klaster analiza ukazale su na jasnu hemijsku diferencijaciju između populacija, što sugerise postojanje različitih hemotipova povezanih sa razlikama u uslovima sredine.

Ključne reči:

Alžir, *Cedrus atlantica*, hemotipovi, ekotipovi, GC–MS, terpenski markeri

Introduction

Atlas cedar (*Cedrus atlantica* Manetti) is endemic to North Africa, classified as a rare species and listed since 2013 as endangered on the IUCN Red List of Threatened Species (Thomas, 2013). It occurs spontaneously and represents a valuable forest species within the mountain ranges of Algeria

and Morocco (Azami, 2021). Atlas cedar exhibits a broad ecological amplitude, occurring between 1,200 and 2,400 meters in elevation and thriving under sub-humid to per-humid climatic conditions (Demarteau et al., 2007). Its ecological and silvicultural importance lies in its wide ecological plasticity, low susceptibility to fire, superior wood quality, and notable resilience to environmental and

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climatic constraints (Rabhi et al., 2018). *Cedrus atlantica* essential oil has demonstrated antibacterial and antifungal activities *in vitro* (Bennouna et al., 2020) and is reported to be traditionally applied for dermatological and hair-related indications. It is administered as an anthelmintic and used in veterinary practice to manage cutaneous disorders among local populations (Bellakhdar, 1997).

In Algeria, *C. atlantica* covers an estimated area of approximately 2,089 km², primarily distributed across the Tellian and Saharan Atlas ranges, starting from 1,300 m on the northern slopes and 1,400 m on the southern slopes (Benhizia et al., 2023). The occurrence of distinct ecotypes, shaped by climatic and edaphic factors, along with the presence of distinct morphotypes (e.g., *glauca* and *viridis*), suggests geographical and individual differentiation, likely of genetic origin (Harfouche & Nedjahi, 2003).

The Aurès region contains the most significant stand in Algeria, covering 17,000 hectares and mainly confined to the Chélia (8,000 ha) and Belezma (5,000 ha) massifs, under relatively xeric conditions. These forests, located in the southernmost part of the *C. atlantica* range, have undergone an alarming regression over the past century due to the combined effects of climate change, overexploitation, and overgrazing (Bentouati, 2008). Since the 1980s, a large-scale dieback phenomenon has been observed throughout its range, characterized by chronic water stress, massive defoliation, and high tree mortality. It reaches a particularly advanced stage in Belezma, affecting up to 90% of the stands (Sarmoum et al., 2019).

Moreover, secondary metabolites play crucial roles in forest trees' physiology and ecology, and can serve as biochemical or genetic markers once their biosynthetic pathways are well characterized (Hanover, 1992). Among these, terpenoid, polyphenolic, and resinous compounds are widely employed in chemotaxonomic analyses and studies of genetic variability (Prat et al., 2006). Terpenes, being genetically determined metabolites, are particularly valuable for detecting intraspecific variation (Fady, 1990). They represent the most abundant and structurally diverse class of plant secondary compounds, with more than 80,000 molecules identified (Christianson, 2017). Beyond their taxonomic significance, terpenes play essential roles in plant growth and development, chemical communication, and defense mechanisms, mediating both plant-plant and plant-environment interactions. They also contribute to resistance against pathogens and act as signaling compounds in response to biotic and abiotic stress (Raber et al., 2021).

The terpenoid composition of conifer species can vary both among species and within the

same species, depending on intrinsic (genetic and physiological), extrinsic (environmental and ecological), and technological (extraction or analytical methods) factors (Özcan & Chalchat, 2008). Pronounced variability is frequently observed between different species or ecotypes, reflecting their adaptive responses to distinct environmental conditions (Tholl et al., 2005).

Monoterpenes and sesquiterpenes are recognized as reliable chemotaxonomic markers for differentiating conifer species and even populations within the same species. These compounds are often species-specific or confined to closely related taxonomic groups (Lerdau & Gray, 2003), particularly within the genera *Pinus* (Zavarin et al., 1991), *Abies* (Fady et al., 1992), and *Cedrus* (Canard et al., 1997).

Several studies conducted in Algeria and Morocco have documented multiple chemical profiles of *C. atlantica* essential oils using diverse analytical techniques, highlighting a substantial chemical variability (Aberchane et al., 2003; Lahlou, 2003; Boudarene et al., 2004; Derwich et al., 2010; Rhafouri et al., 2014; Benouaklil et al., 2017 & Bensaid et al., 2021). Nevertheless, a comparative investigation of the essential oil composition within and among natural populations of this species has not yet been undertaken.

The present study investigates the chemical composition of *C. atlantica* needle essential oils from the southernmost part of the *C. atlantica* range (Aurès region, Northeast Algeria), using terpene markers and gas chromatography-mass spectrometry (GC-MS) analyses. The main objective is to assess the intra- and inter-population variability in oil composition, considering differences related to geographical location and forest ecological conditions.

Materials and Methods

Study sites

The study was conducted in northeastern Algeria, within two *C. atlantica* forests located approximately 60 km apart in a straight line: the Tuggurt Cedar Forest (Belezma National Park, biosphere reserve) and the Chélia Forest (Aurès region) (**Fig. 1**). The Tuggurt Cedar Forest covers an area of approximately 680 ha, situated on the northern slope of the Belezma Forest at elevations ranging from 1,300 m to 1,805 m. The climate is semi-arid, with a mean annual precipitation of 346 mm and an average annual temperature of 15.1 °C (Benzina et al., 2021). The Chélia Cedar Forest extends over 2,632 ha on the southern slope of the Aurès Mountains, between 1,400 m and 2,300 m in altitude (**Tab. 1**). The climate there is sub-humid, characterized by

a mean annual rainfall of 533 mm and an average temperature of 16.6 °C (Tafer et al., 2022). Both study areas are underlain by decalcified rendzina soils, a typical feature of cedar ecosystems in this region (Abdessemed, 1981).

Plant material and essential oil extraction

Samples were collected from twelve *C. atlantica* trees of varying ages, distributed across the two study sites, with six trees per site. Needle samples were randomly harvested from the lower branches on all sides of each tree and immediately placed in sterile polyethylene bags. Samples were then transported to the laboratory for distillation on the same day. The collection was carried out early in the morning, when the concentration of plant secondary metabolites is generally at its peak (Sadou et al., 2015).

Essential oils (EO) were extracted by hydrodistillation using a steam distillation apparatus. For each sample, approximately total mass of 10 kg of fresh needles were distilled for 4 hours. EO is subsequently separated from the aqueous phase by adding anhydrous sodium sulfate (Na_2SO_4). The EO was kept in sealed dark vials refrigerated at 4 °C until further analysis. EO yields were estimated on a dry-weight (w/w) basis. The voucher specimen has been deposited at the laboratory "Biodiversity, Biotechnology and Sustainable Development" at the University of Batna 2, Algeria.

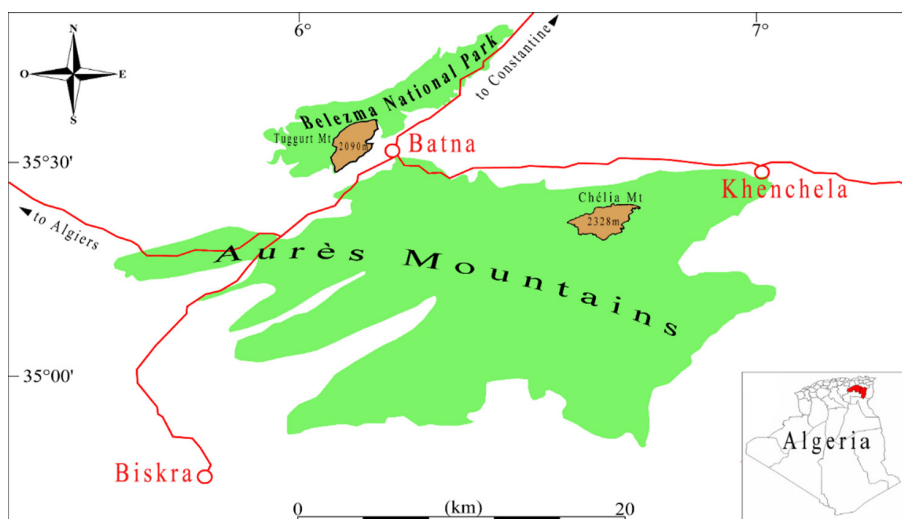


Fig. 1. Location of the two Atlas cedar (*Cedrus atlantica* Manetti) forests studied in the Aurès region (Tuggurt and Chélia mountains, northeast Algeria).

Gas chromatography – mass spectrometry (GC-MS)

The gas chromatography-mass spectrometry analysis (GC-MS) was performed using a Perkin Elmer Clarus 500 (U.S.A.), equipped with an AOC-20i auto sampler and a gas chromatograph coupled to a mass spectrometer, fitted with an Elite-5MS capillary column (5% diphenyl 95% dimethylpolysiloxane), 30 m in length, 0.25 mm in diameter, and 0.25 µm film thickness (Sabithira & Udayakumar, 2017). Helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL min⁻¹. The oven temperature was programmed as follows: an initial temperature of 40 °C held for 2.5 min, then increased at 5 °C min⁻¹ to 90 °C and at 10 °C min⁻¹ to 250 °C for 2 min. 1 µL of essential oil diluted in hexane (1:3, v/v) was injected in splitless mode. Mass spectra were obtained by electron ionization (EI+) at 70 eV using SCAN mode acquisition. Interpretation of GC-MS results was conducted using the National Institute of Standards and Technology (NIST) database, which

Table 1. General characteristics of the two studied populations of *Cedrus atlantica* Manetti in the Aurès region (northeast Algeria).

Population	Chélia forest	Tuggurt forest
Latitude (N)	35°18'31"	35°34'72"
Longitude (E)	06°37'37"	06°03'29"
Exposure	SE/SW	N/NW
Altitude (m)	1700 – 1900	1400 – 1650
Dieback (%)	20	65
Soil type	Decalcarized rendzinas	Decalcarized rendzinas
Bioclimate	Cold subhumid	Cold semi-arid

contains over 62,000 mass spectral libraries (Abirami & Rajendran, 2012). The relative percentage of each component was calculated by comparing the relative peak area of its chromatographic peak to the total area of detected peaks.

Statistical analysis

Statistical analyses were performed in Python 3.13.3 to evaluate variability in the terpene composition of *C. atlantica* essential oils. A Principal Component Analysis (PCA) was first performed to reduce data dimensionality and identify the main components explaining the observed variance among samples. Terpene concentration data were standardized (centered and scaled to unit variance) to account for differences in magnitude among variables and to ensure comparability. All detected terpene compounds exceeding the analytical threshold were included in the analysis, and no a priori variable exclusion was applied, as the objective was to capture the overall chemical structure. Subsequently, Hierarchical Cluster Analysis (HCA) using Ward’s linkage method and K-Means clustering based on the Elbow criterion were applied to classify samples according to the similarity of their terpene profiles.

Results

Terpene composition in cedar atlas essential oils

The GC-MS analysis of Atlas cedar needles' essential oils allowed the identification of 43 volatile compounds, including 36 in oils from Chélia and 14 in oils from Tuggurt, representing 92.04% and 92.91%, respectively, of the total oils' composition. According to their structure, terpene compounds can be classified into five categories: monoterpenes (8 compounds), oxygenated monoterpenes (4), sesquiterpenes (15), oxygenated sesquiterpenes (8), and other compounds (8). In general, Chélia essential oils showed intraspecific diversity (component types) compared with Tuggurt essential oils (Tab. 2).

Table 2. Chemical composition of needle essential oils of Atlas cedar (*Cedrus atlantica* Manetti) from two cedar forests in northeast Algeria (C1–T6: sampled trees).

Peak	Retention time (mn)	Components	Chélia forest						Tuggurt forest					
			C1	C2	C3	C4	C5	C6	T1	T2	T3	T4	T5	T6
Monoterpenes														
1	8.07	α-pinene	-	-	-	-	16.59	-	-	-	-	32.45	-	-
2	8.08	1R-α-pinene	-	-	-	-	-	-	6.56	-	-	-	-	-
3	8.09	α-Thujene	0.88	-	-	-	-	-	-	-	-	-	-	-
4	8.42	Camphene	-	-	-	-	-	-	-	-	-	1.84	-	-
5	9.05	β-pinene	-	-	-	-	-	-	0.56	-	-	2.14	-	-
6	9.32	β-Myrcene	5.70	-	-	-	7.25	1.00	21.48	-	-	44.02	-	-
7	10.15	d-Limonene	1.96	-	-	-	6.81	-	3.47	-	-	15.10	-	-
8	11.40	p-menth-1-ene	-	-	-	-	-	-	-	-	-	0.93	-	-
Oxygenated monoterpenes														
9	11.58	Linalool	36.08	-	-	-	-	-	-	-	-	-	-	-
10	13.39	α-terpineol	0.86	-	-	1.70	-	-	-	-	-	-	-	-
11	15.15	Endo Bornyl acetate	-	-	-	6.12	-	4.16	-	-	-	-	-	-
12	16.71	Geranyl acetate	-	-	-	-	-	1.41	-	-	-	-	-	-
Sesquiterpenes														
13	17.51	trans-Caryophyllene	2.18	-	-	1.41	29.81	20.22	-	-	-	1.50	-	-

14	17.65	β-Copaene	-	-	-	-	0.90	-	-	-	-	-	-	-
15	17.65	Germacrene D	0.18	-	-	1.60	3.15	13.94	-	-	-	-	-	-
16	17.93	trans-β-Farnesene	1.25	-	-	-	-	8.46	-	-	-	-	-	-
17	17.94	(E)-β-Farnesene	-	-	-	-	9.11	-	-	-	-	-	-	-
18	18.06	α-Humulene	-	-	-	-	7.45	5.37	-	-	-	-	-	-
19	18.38	γ-Muurolene	-	-	-	-	1.02	1.70	-	-	-	-	-	-
20	18.58	α-Helminthene	-	-	-	-	0.62	-	-	-	-	-	-	-
21	18.75	γ-Cadinene	-	-	-	-	0.65	4.46	-	-	-	-	-	-
22	18.81	β-Bisabolene	-	-	-	1.12	1.65	-	-	-	-	-	-	-
23	18.81	Bergamotene	-	-	-	1.20	-	1.31	-	-	-	-	-	-
24	19.09	α-Copaene	-	-	-	-	2.89	0.00	-	-	-	-	-	-
25	19.09	σ-Cadinene	-	-	-	-	-	5.54	-	-	-	-	-	-
26	20.22	Aromadendrene 2	-	-	-	39.81	-	-	-	-	-	-	-	-
27	21.12	β-Selinene	-	-	-	2.31	-	-	-	-	-	-	-	-
Oxygenated sesquiterpenes														
28	18.74	Geranyl propionate	0.16	-	-	-	-	-	-	-	-	-	-	-
29	18.87	Phenol. 2.6-bis (1.1-dimethylethyl)-	-	-	-	-	1.81	-	-	-	-	-	-	-
30	19.62	Nerolidol	-	-	-	17.66	-	-	-	-	-	-	-	-
31	20.09	(-)-Caryophyllene oxide	-	-	-	-	0.64	0.52	-	-	-	-	-	-
32	20.11	α-Sinensal	-	-	-	22.00	-	-	-	-	-	-	-	-
33	21.07	τ-Cadinol	-	-	-	-	-	1.02	-	-	-	-	-	-
34	21.28	Asarone	-	-	-	1.90	-	-	-	-	-	-	-	-
35	14.50	trans-Sesquilandulyl acetate	0.09	-	-	-	-	-	-	-	-	-	-	-
Other compound														
36	4.43	Tropilidene	-	18.33	13.00	-	-	-	-	-	-	-	-	-
37	4.45	Toluene	-	-	-	-	-	5.74	9.61	15.02	12.55	-	5.58	-
38	4.85	Hexanal	20.30	72.45	39.88	-	-	20.15	35.39	71.93	75.00	-	18.45	-
39	5.02	Oxirane, (propoxymethyl)	-	-	-	-	-	-	-	-	-	-	-	93.99
40	21.07	Orthosilicate	-	-	-	1.60	-	-	-	-	-	-	-	-
41	27.06	Phthalic acid	12.83	-	38.51	-	3.84	-	12.06	5.76	-	-	70.97	-
42	27.17	Phthalic acid diethyl ester	-	-	-	-	-	-	-	-	-	-	-	0.82
43	27.28	Tuaminoheptane	-	-	-	-	-	-	-	-	-	-	-	0.25
Percentage (%)			82.47	90.78	91.39	98.43	94.19	95.00	89.13	92.71	87.55	97.98	95.00	95.06

Chélia oils were characterized by their high sesquiterpene content, particularly hydrocarbon sesquiterpenes (28.22%), whereas Tuggurt oils were marked by monoterpenes (21.43%). The other compounds accounted for 41.11% and 71.23%, respectively (**Fig. 2**).

Three chemical profiles were identified in Chélia oils: linalool at 36.08%, aromadendrene 2 at 39.81%, and *trans*-caryophyllene at 29.81%. β -myrcene (5.75%) was also noted in sample C1, as well as significant proportions of α -sinensal (22.00%) and nerolidol (17.66%) in sample C4. Furthermore, the samples C5 and C6, dominated by *trans*-caryophyllene, produced more molecules than others did, such as α -pinene (16.59%) and germacrene D (13.94%), as well as lower or higher amounts of (E)- β -farnesene (9.11%) and *trans*- β -farnesene (8.46%), respectively (**Tab. 2**).

The Tuggurt oils were characterized by β -myrcene with a maximum of 44.02%, followed by α -pinene (32.45%), D-limonene (15.10%), and 1R- α -pinene (6.56%), respectively. The remaining samples, namely C2, C3, T2, T3, T5, and T6, showed zero detectable terpene yield under the same extraction conditions and were considered outliers (**Tab. 2**).

Variation of terpene profiles in essential oils of Atlas cedar

Principal component analysis (PCA) of the terpene profiles revealed that the first two components explained 44.70% of the total variance: 24.10% for the first axis and 20.60% for the second (**Fig. 3**).

The first principal component (PC1) was positively correlated with γ -muurolene (0.999),

α -humulene (0.995), *trans*-caryophyllene (0.987), germacrene D (0.884), γ -cadinene (0.827), geranyl acetate (0.730), δ -cadinene (0.730), τ -cadinol (0.730), and *trans*- β -farnesene (0.704), while it was negatively correlated with β -pinene (-0.190), α -thujene (-0.188), linalool (-0.188), geranyl propionate (-0.188), *trans*-sesquilandulyl acetate (-0.188), and 1R- α -pinene (-0.165). The second principal component (PC2) was positively correlated with aromadendrene 2 (0.965), β -selinene (0.965), nerolidol (0.965), α -sinensal (0.965), asarone (0.965), α -terpineol (0.898), endobornyl acetate (0.888), and bergamotene (0.754), while it was negatively associated with *d*-limonene (-0.460), α -pinene (-0.445), and β -myrcene (-0.378). PC1 discriminates mainly based on sesquiterpenes, whereas PC2 discriminates based on a mixture of monoterpenes and sesquiterpenes (**Fig. 3**).

On the second PC1-PC2 factorial plane, the dispersion of individuals is more pronounced in the Chélia cedar population, particularly in C3, C4, C5, and C6, which are characterized by three chemotypes: linalool, aromadendrene 2, and *trans*-caryophyllene, indicating significant chemical variability within the analyzed samples. The Tuggurt population appears more homogeneous, with individuals clustered more closely (T1 and T4) and characterized by β -myrcene. This reveals the presence of two distinct groups: one of sesquiterpenes and the other of monoterpenes and oxygenated compounds, except for outliers (**Fig. 4**).

Overall, hierarchical ascending classification (HAC) indicates significant intraspecific chemical variation. Hierarchical cluster analysis was performed using Ward's method and Euclidean distance.

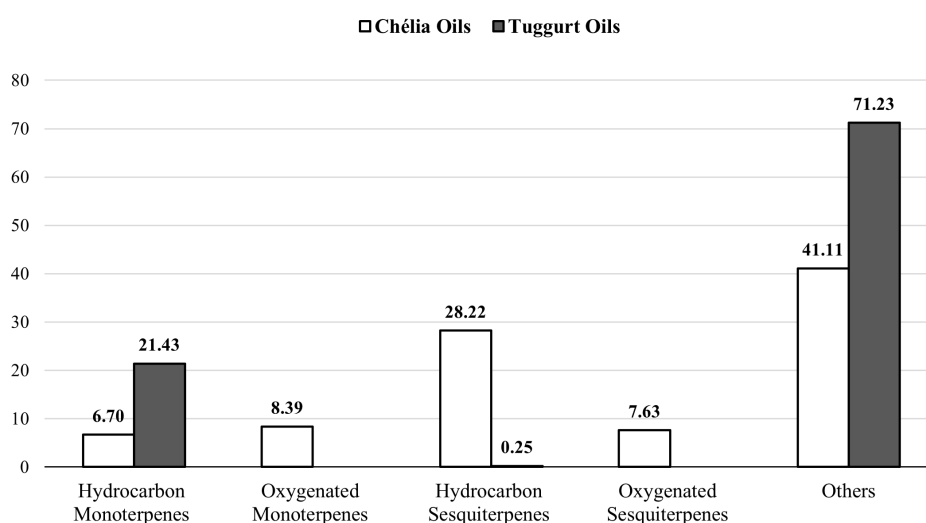


Fig. 2. Percentages of terpene classes identified in essential oils of Atlas cedar (*Cedrus atlantica* Manetti) from Tuggurt and Chélia forests (northeastern Algeria).

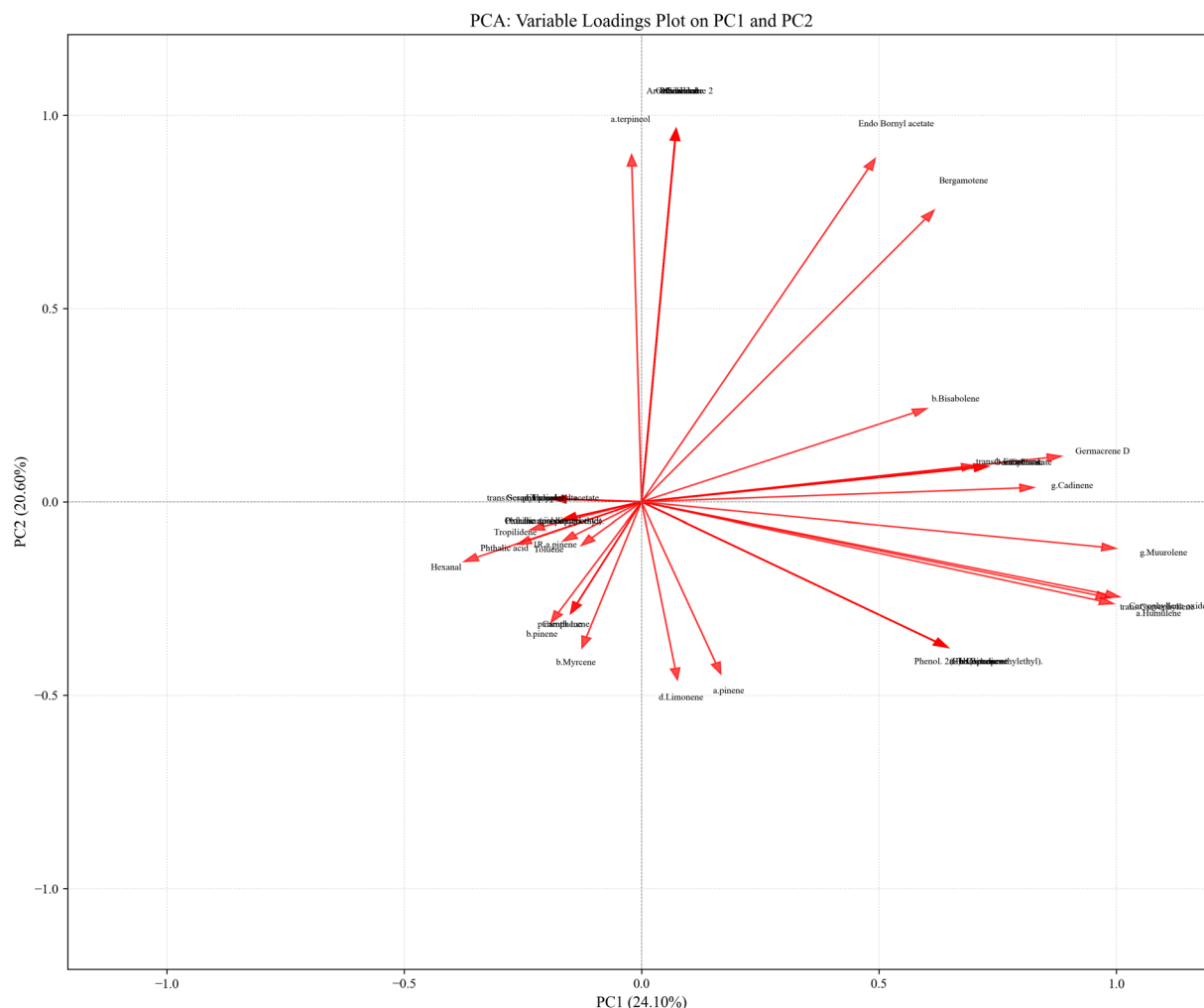


Fig. 3. PCA projection on the factorial plane of terpene components in essential oils of Atlas cedar (*Cedrus atlantica* Manetti) from northeast Algeria.

Based on visual inspection of the dendrogram, a distance threshold of 10.93 was selected, resulting in two main clusters (**Fig. 5**):

(i) the first cluster includes samples T1, T2, T3, T5, and T6 from Tuggurt, as well as C1 and C2, C3 (hidden points) from Chélia. Within this cluster, we note similar subgroups such as (T2, T3) and (C2, C3). These are considered outliers. Indeed, the closer we get to the threshold, the more the samples tend to diverge at different distances.

(ii) The second major group includes sample T4 from Tuggurt and C4, C5, and C6 from Chélia. All these samples crossed the threshold, highlighting their divergence, except for C5 and C6, which form a similar subgroup.

The application of K-means clustering ($k=3$), using the Elbow Method, forces the data into three distinct clusters (**Fig. 6**): the first one (0) includes the C5 and C6 samples from Chélia; the second cluster (1), includes a single sample C4, and the third (2),

which groups both the typical Tuggurt samples (T1 and T4), and the C1 sample from Chélia dominated by linalool (oxygenated monoterpene), disregarding outliers. The assignment of Chélia samples to various groups highlights a more pronounced intraspecific chemical diversity compared to the relative homogeneity observed in the Tuggurt forest (**Fig. 6**).

Discussion

GC/MS analysis of essential oils extracted from the leaves of *Cedrus atlantica* identified forty-three volatile constituents across the two studied populations, with 36 compounds in the Chélia samples and 14 in the Tuggurt samples, accounting for more than 92% of the total oil composition in both cases. These constituents were distributed among four chemical classes: hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, and oxygenated sesquiterpenes, indicating a terpenoid-dominated chemical profile.

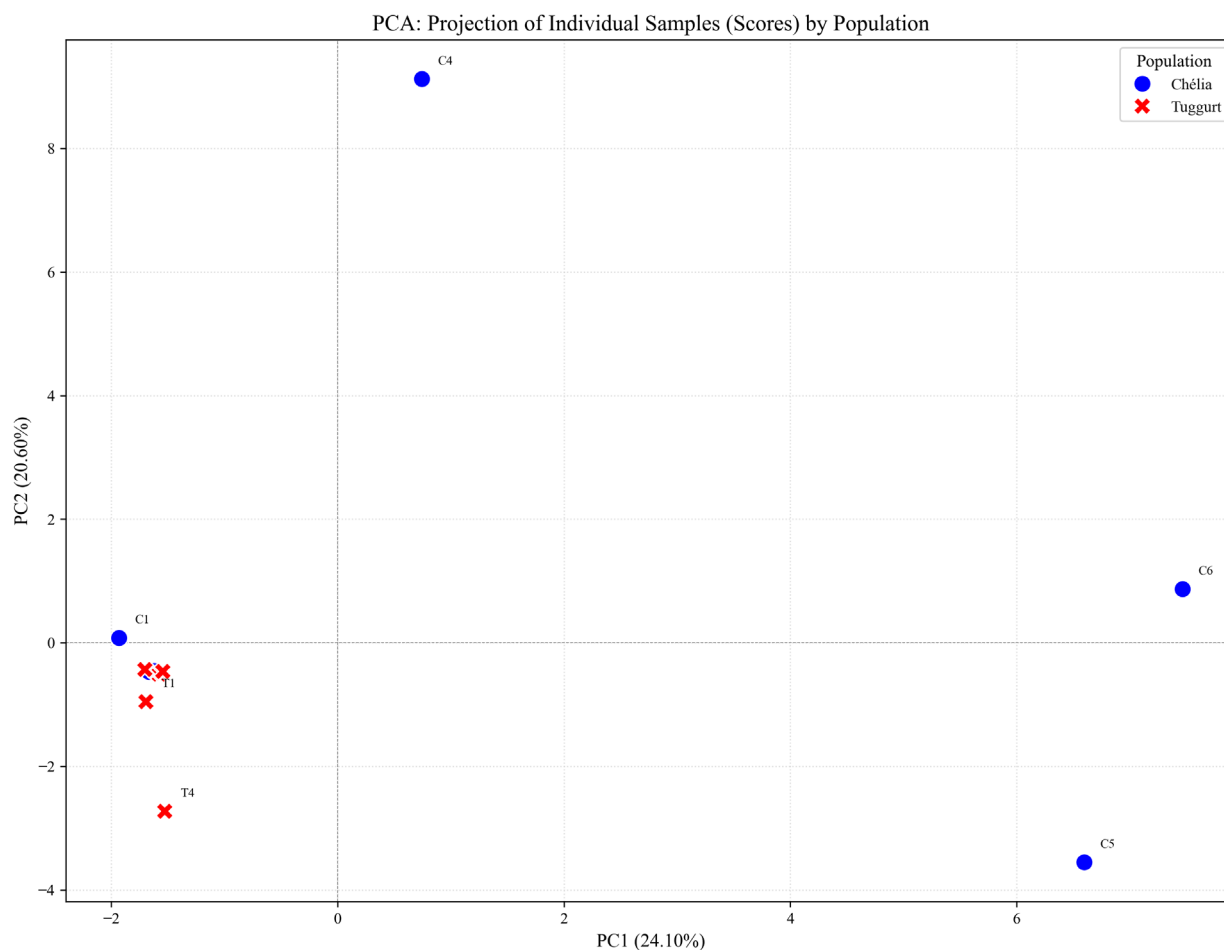


Fig. 4. PCA projection on the factorial plane of tree samples based on essential oil composition of Atlas cedar (*Cedrus atlantica* Manetti) from northeast Algeria.

Significant qualitative and quantitative differences were observed between the two populations. Oils from the Chélia forest were characterized by higher proportions of sesquiterpenes, including aromadendrene-2, linalool, *trans*-caryophyllene, α -sinensal, nerolidol, germacrene D, and farnesene isomers, whereas oils from Tuggurt were dominated by monoterpene hydrocarbons, mainly β -myrcene, α -pinene, D-limonene, and 1R- α -pinene. Sesquiterpene and diterpene fractions were absent in the Tuggurt samples, which were largely composed of non-terpenoid compounds. These results indicate marked intraspecific chemical variability between the two populations. This variability can be attributed to differences in environmental and ecological conditions between the two sites. Edaphoclimatic factors, including soil characteristics, precipitation, temperature, relative humidity, and wind exposure, are known to influence secondary metabolite biosynthesis and accumulation in plants (Pant et al., 2021).

The comparative profiling of the essential oils from the two cedar populations highlighted

marked intra- and inter-population heterogeneity in their chemical composition. This increases the complexity of identifying molecular markers that discriminate ecological gradients. According to the scientific literature, the higher sesquiterpene diversity observed in the Chélia population may be related to environmental differences between the two sites, although no direct measurements of stress factors were performed in this study. The predominance of monoterpenes in the Tuggurt population likely reflects adaptation to its semi-arid climate (~346 mm annual rainfall) rather than the protected status of the area, as climatic stressors may play a more decisive role than reduced anthropogenic disturbance. This pattern is consistent with the well-documented effects of environmental stress factors on the biosynthesis and composition of plant essential oils (Pant et al., 2021).

In addition to environmental effects, genetic background, ecotype differentiation, and long-term acclimation to local conditions contribute to the formation of distinct chemotypes. Despite the difference in the type of organ studied and differences

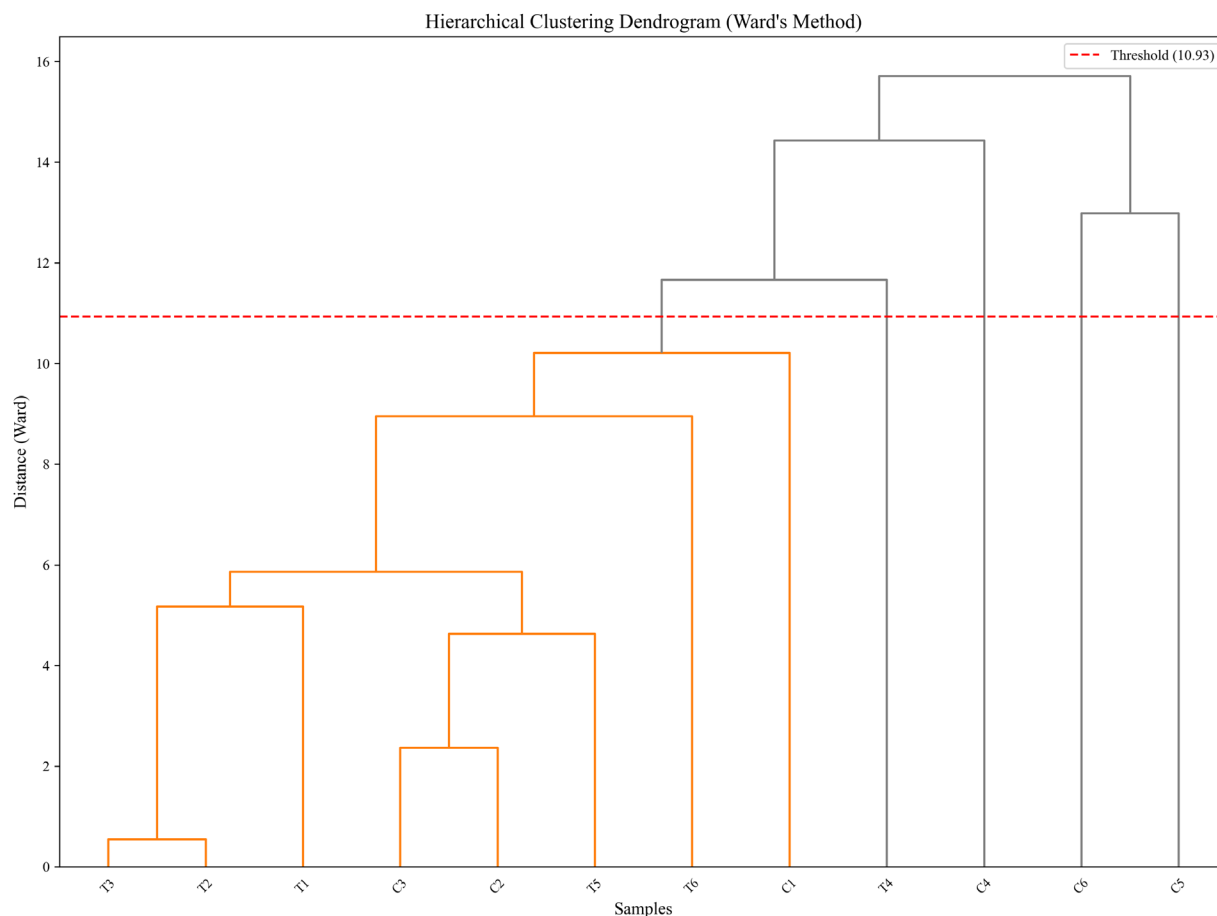


Fig. 5. Hierarchical cluster analysis dendrogram of Atlas cedar (*Cedrus atlantica* Manetti) tree samples based on essential oil composition from northeast Algeria.

in methods and analysis techniques, previous studies have reported multiple chemotypes in *C. atlantica* essential oils from Algerian and Moroccan populations (**Tab. 3**), including α -pinene-dominated and himachalene-rich profiles (Yassaa et al., 2000; Lahlou, 2003; Boudarene et al., 2004; Derwich et al., 2010; Ainane et al., 2019; Belkacem et al., 2021). The present results are generally consistent with this chemical polymorphism.

Comparison with other species of the genus further supports the chemotaxonomic relevance of these findings. *Cedrus libani* and *C. deodara* essential oils are generally characterized by germacrene D and β -caryophyllene as dominant constituents (Saab et al., 2009), whereas *C. brevifolia* oils are mainly composed of α -pinene and limonene (Boutos et al., 2020). In contrast, the Chélia population of *C. atlantica* exhibited a profile dominated by linalool, aromadendrene-2, and *trans*-caryophyllene, indicating the presence of a distinct chemotype in the Aurès region.

Overall, the observed chemical variability between the Chélia and Tuggurt populations reflects the combined effects of environmental conditions

and genetic factors. Essential oil composition, therefore, provides relevant chemotaxonomic and ecological information for the differentiation of *C. atlantica* populations and for the characterization of their potential as sources of bioactive compounds.

Terpenes appear to be useful chemotaxonomic markers for discriminating Atlas cedar ecotypes and highlight a previously undocumented chemical diversity within this endangered species. This variability has direct implications for conservation and sustainable management, as chemically distinct populations may respond differently to environmental stress and climate change. Integrating terpenes markers into monitoring and restoration programs would therefore enhance the preservation of the genetic and adaptive potential of *C. atlantica*. These findings should be confirmed using larger sample sizes and complementary genetic analyses. Future work combining chemical, genomic, and ecological data is needed to clarify the functional significance of these chemotypes and to support informed decision-making for the conservation of cedar forests in North Africa.

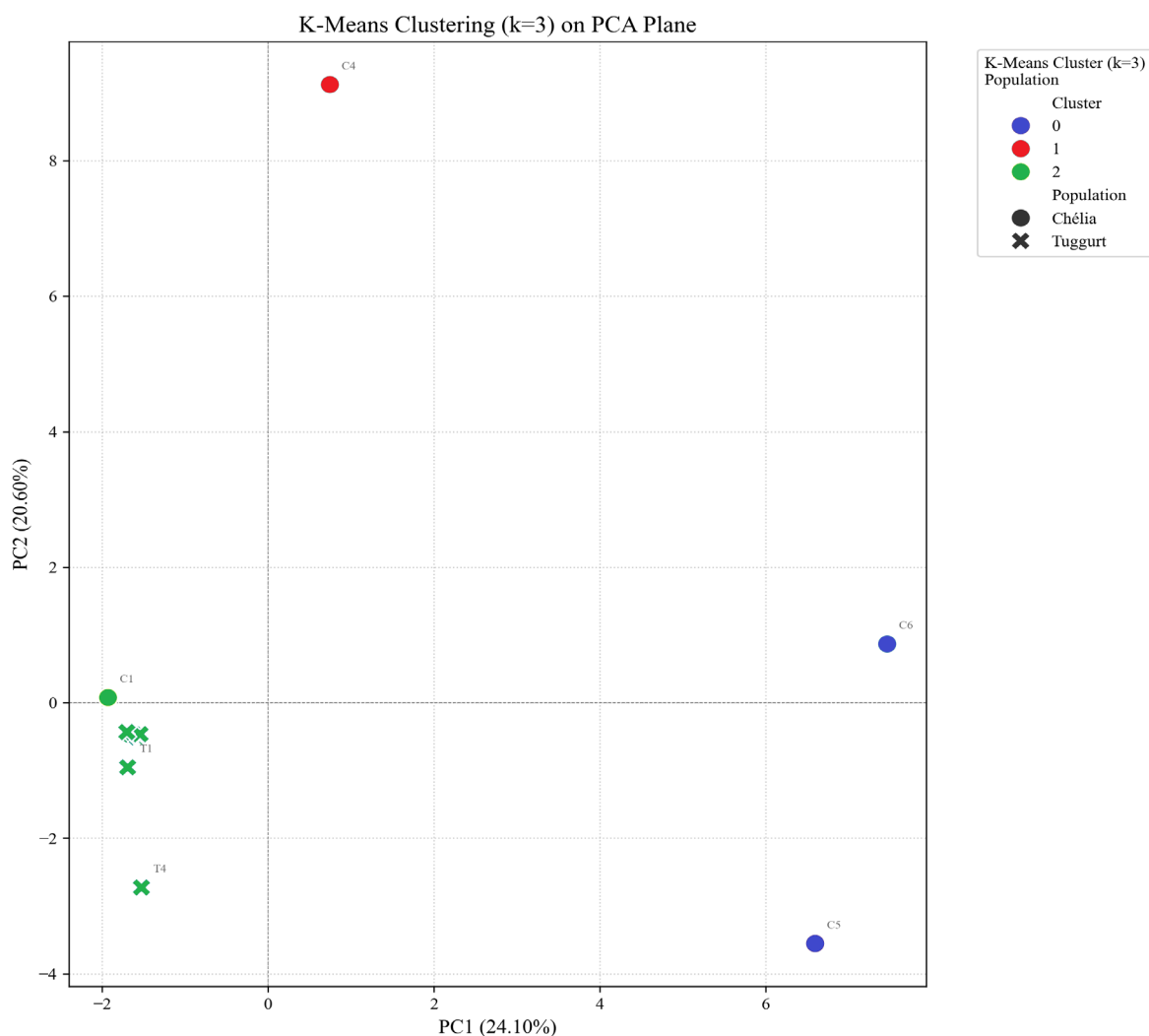


Fig. 6. K-means clustering of samples of needles of Atlas cedar

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Table 3. Comparison of the results of analysis of essential oils of the Atlas Cedar *Cedrus atlantica* in its distribution area (Algeria and Morocco).

Organ studied	Country	Forest (region)	Components	Authors
Needles	Algeria	El-Hamma botanical garden (Algiers)	α -Pinene (53.3%), camphene (12.6%), Myrcene (12.9%), R (+) Limonene (11.3%).	Yassaa et al. (2000)
Needles		Bainem Forest (Algiers)	α -pinene (17.7%), caryophyllene oxide (10.3%), β -caryophyllene (9.1%), α -terpineol (6.7%).	Boudarene et al. (2004)
Needles and branches		Chr��a Forest (Blida)	β -himachalene (30.1%), α -himachalene (16.4%), longifolene (14.5%).	Benouaklil et al. (2017)
Needles, branches and cones		Ch��lia Forest (Khenchela)	cis- α -bisabolene (19.6%), perhydrofarnesyl acetone (4.8%), spathulenol (4.5%), α -pinene (0.31%)	Bensaid et al. (2021)
Cones		Akfadou Forest (Tizi Ouzou)	α -pinene (81.5%), sabinene (3.2%), β -phellandrene (2.5%), β -pinene (1.95%)	Belkacem et al. (2021)
Needles	Morocco	Atlas median Forest	α -pinene (14.9%), himachalene (10.2%), β -himachalene (9.9%), σ -himachalene (7. 6%)	Derwich et al. (2010)
Wingless seeds		Various forests	<u>Mainly</u> : α -pinene, manool, bornyl acetate, β -pinene, α -terpinene	Rhafouri et al. (2014)
Winged seeds			<u>Mainly</u> : manool, α -pinene, 6-comphenol, and β -pinene	
Needles		Oued Cherrat Arboretum (Rabat)	α -pinene (34.1%), β -pinene (31.7%), myrcene (17.2%), limonene (5.2%)	Lahlou (2003)
Unspecified		Various forests	β -himachalene (31.2%), α -himachalene (15.6%), γ -himachalene (14.5%)	Ainane et al. (2019)
Unspecified		Unspecified	β -himachalene (50.0%), α -himachalene (19.4%),	Oualdi et al. (2025)
Needles		Algeria	Ch��lia Forest (Aur��s)	linalool (36.1%), <i>trans</i> -caryophyllene (29.8%), α -sinensal (22.0%), nerolidol (17.7%), α -pinene (16.6%), germacrene D (13.9%), (E)- β -farnesene (9.1%) and <i>trans</i> - β -farnesene (8.5%)
		Tuggurt Forest (Belezma)	β -myrcene (44.0%), α -pinene (32.5%), d-limonene (15.1%), 1R- α -pinene (6.6%)	

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