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Research Article

Chemotaxonomic Study of Four Subspecies of *Pinus nigra* Arn. Grown in Common Garden Based on Essential Oil Composition

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The aim of this study was to investigate the chemical diversity of *Pinus nigra* Arn. essential oils. The research was carried out on the needles collected from eighteen provenances of black pine grown in common garden located in West-Northern Tunisia and belonging to four different subspecies (*Pinus nigra* subsp. *nigra*, *Pinus nigra* subsp. *salzmannii*, *Pinus nigra* subsp. *pallasiana*, and *Pinus nigra* subsp. *laricio*). Essential oil yields ranged from 0.19% to 0.68%. The obtained essential oils have been analyzed by GC-FID and GC-MS apparatus. Twenty-three constituents accounting about 98% of total essential oil composition were identified. The essential oil compositions appeared to be very different according to their origin. Thus, five main essential oil chemotypes were identified in *Pinus nigra* plants: caryophyllene oxide, camphene, *ß*-caryophyllene, *α*-amorphene, and germacrene D. The chemotaxonomic value of the essential oil compositions was discussed in relation to the results of the multivariate statistical test, including a detailed survey of the available literature data.

1. Introduction

Pinus nigra Arnold (black pine) is a circum-Mediterranean *species* belonging to the *Pinaceae* family. The *species* is divided into six subspecies: *Pinus nigra* subsp. *nigra* (Arn.), *Pinus nigra* subsp. *salzmannii* (Dun.), *Pinus nigra* subsp. *dalmatica* (Vis.), *Pinus nigra* subsp. *pallasiana* (Lamb.), *Pinus nigra* subsp. *mauretanica* (Mair. & Pey.), and *Pinus nigra* subsp. *laricio* (Poir.) [1]. This *species* is discontinuously distributed from Southwest Europe to Asia Minor, extending to the Crimea, and is also found in North Africa (Morocco and Algeria). Pine essential oils were widely used as fragrances, flavoring, intermediates in the synthesis, and beverages. Traditional therapeutic and pharmaceutical uses of its oils have been recorded all around the world. Pine needle essential oils were mainly used in folk medicine for the treatment of cardiovascular and cholesterol-lowering benefits and respiratory infection, and they have powerful antioxidant and anti-inflammatory effects and the ability to enhance microcirculation by increasing capillary permeability [2]. Black pine was characterized by a genetic, morphological, phenotypic, and biochemical diversity. The chemical composition and the intraspecific variation of *Pinus nigra* essential oil have been the subject of numerous studies [3–11]. According to analysis of variance, these investigations indicated a significant variability in chemical composition of black pine essential oils between provenances and subspecies. In Tunisia, four subspecies from eighteen provenances of *Pinus nigra* have been introduced in a common garden in the northwest of the country since 1966.

Therefore, and based on the chemotaxonomic classification tool which was known as one of the most important guides for researchers in their studies for new industrial and medicinal plants, this work was conducted to study, for industrial and medicinal plants. This work was conducted to study, for the first time, the variability in essential oils obtained from the needles of 18 provenances of *Pinus nigra* growing under humid bioclimatic conditions in the northwest of Tunisia. This study could be helpful to highlight the variability of intraspecific terpene profile of *Pinus nigra* species growing in the southern limit of its range.

2. Materials and Methods

2.1. Plant Material. Eighteen samples of *Pinus nigra* needles were collected in April 2015 from the Souiniet common garden located in the Khroumirie region in West-Northern Tunisia (8°48′E, 35°54′N, 492 m). It is characterized by an annual temperature of 15.6°C and a mean rainfall of 1534 mm/year. The eighteen samples corresponding to eighteen provenances from different geographic origins have been planted since 1966 in provenance trials experimental sites (Table 1). The identification of the plant material was done by Professor Mohamed Larbi Khouja, and the voucher specimens ($PN2020_i$, where *i* represents the provenance number varied from 1 to 20) of the plants were deposited at the Herbarium of INRGREF (Tunisia).

2.2. Essential Oil Isolation and Analysis. Needles from the eighteen *Pinus nigra* provenances were subjected to hydrodistillation in a Clevenger-type apparatus for 3 h in order to isolate essential oils. The essential oils were measured directly in the extraction burette, and the amount of oil obtained (%) was calculated as volume (ml) of essential oils per 100 g of dry plant material. The essential oils were dehydrated over anhydrous Na_2SO_4 and kept in a cool and dark place prior to analysis. Analysis of the essential oils were carried out by combination of gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) according to a standard analytical procedure using a DB-5MS column (30 m, 0.25 mm, and 0.25 μm film thickness). The flow rate of the carrier gas (helium) was 1.0 ml/min. The GC oven temperature started at 100°C and then held for 1 min at 260°C and then for 10 min with a program rate of 4°C min^{-1}. Besides, the injector and detector temperatures were set at 250 and 230°C, respectively. The mass range was scanned from 50 to 550 amu. A sample of 1.0 μl was injected, using split mode (1 : 100). Compounds of the essential oils were identified by both their Kovats indices and mass spectra. Kovats indices were calculated by linear interpolation relative to retention times of *n*-alkanes (C_8–C_{24}). Mass spectra were matched with the reference spectra from the Wiley/NIST database, published data, and the spectra of authentic compounds. Relative amounts of individual components were calculated based on GC peak areas without FID response factor correction [2].

2.3. Chemometric Analysis. Principal component analysis (PCA) was performed on all the essential oil chemical compositions regrouped for the eighteen provenances ($n = 20$ replications). All the chemometric analyses were performed using XLSTAT software for Windows (v.2013.2.03; Addinsoft, New York, USA).

2.4. Statistical Analysis. Data were analyzed using the GLM procedure (general linear models) of the SAS (9.0) program. An analysis of variance test of the studied parameters was performed. All values are the mean of three replications. Principal component analysis was evaluated with the R (version 3.1.1) program.

3. Results and Discussion

Statistical analysis showed a high variability between the four studied subspecies ($p < 0.0001$). The highest yield was recorded by needles of *nigra* subspecies (Table 2), while the lowest values were reached by both *calabrica* and *salzmannii* subspecies. Furthermore, a significant variability was recorded between the eighteen provenances. The most important oil yield (0.68%) was reached by needles from *P*6 (*Pinus nigra austriaca*; Puget-Théniers, France) while *P*2 (*Pinus nigra calabrica*; Trenta, Italy) showed the lowest oil yield with 0.19%.

The results of the identified compounds by GC-MS are shown in Table 3. Twenty-three constituents accounting about 98% of total essential oil composition were identified. The essential oil compositions appeared to be very different among provenances. The major essential oil components were especially variable in occurrence and concentration among the different provenances, ranging from almost absent in some samples to more than 90% of the total essential oil composition in others.

There appear to be five basic essential oil chemotypes in *Pinus nigra* plants investigated (Figure 1): (a) caryophyllene oxide as the major component (provenances Trenta (22.86%), Les Barres (41.53%), Cosenza (80.54%), Kustendil (88.51%), Alaçam (75.11%), Crimée (41.85%), St Guilhem (38.71%), Cazorla (66.49%), Olette (87.74%), Tavola (90.05%), and Marghese (90.85%)); (b) camphene as the major compound (provenances Brouzet-lès-Alès (19.95%) and Catanzaro (38.07%)); (c) β-caryophyllene (provenances Bois Frerot (42.82%), Grancia (32.93%), and Les Barres (51.10%)); (d) α-amorphene (Les Barres (leint) (26.04%)); and (e) germacrene D (Puget-Théniers (27.13%)). Only the essential oils from Brouzet-lès-Alès (*P*1), Puget-Théniers (*P*6), and Catanzaro (*P*10) provenances were more rich in monoterpenes than sesquiterpenes, while the oils from the other provenances (*P*2, *P*3, *P*4, *P*5, *P*8, *P*9, *P*11, *P*12, *P*13, *P*14, *P*16, *P*17, *P*18, *P*19, and *P*20) had more sesquiterpenes than monoterpenes.

The results of the principal component analysis showed that β-caryophyllene, caryophyllene oxide, linalool, myrcenol, and γ-muurolene were the most significant variables for the classification of the *Pinus nigra* essential oils. These parameters were considerably loaded into the two major principal components (Dim 1 and Dim 2) explaining more than 50% of the variance. According to the analysis, five different groups were revealed (Figure 2). The first group contained *P*6, *P*10, and *P*19 samples, which had the main concentrations of β-caryophyllene (15.65, 23.28, and 51.10%, respectively) and the lowest rate of caryophyllene oxide (1.23, 8.33, and 0.99%, respectively). On the other hand, the second group enclosed only *P*12 and *P*13 samples, which were characterized by its high amount of both β-caryophyllene (30.29–32.16%) and caryophyllene oxide

Table 1: Geographic origin of the eighteen provenances of *Pinus nigra* Arn.

Subspecies	Code	Provenances	Country of origin	Altitude (m)	Latitude	Longitude
salzmannii	*P*1	Brouzet-lès-Alès	France	—	44°07 N	4°05 E
	*P*12	St Guilhem	France	350–400	43°41 N	3°35 E
	*P*16	Cazorla	Spain	1500	37°50 N	3°00 O
	*P*18	Olette (Pyr-Orient)	France	—	42°36 N	2°14 E
laricio	P2	Trenta	Italy	1050	39°25 N	16°35 E
	P3	Les Barres	France	150	47°50 N	2°45 E
	P4	Cosenza	Italy	1300	39°15 N	16°17 E
	P10	Catanzaro	Italy	—	38°54 N	16°34 E
	P14	Grancia	Italy	850	39°41 N	16°58 E
	P17	Tavola	Italy	950	39°25 N	16°35 E
	P19	Les Barres	France	150	47°50 N	2°45 E
	P5	Bois Frerot (Ardennes)	France	100	—	—
	P11	Les Barres (leint)	France	150	47°50 N	2°45 E
	P20	Marghese (Corse-du-Sud)	France	1100	41°39 N	9°12 E
nigra	P6	Puget-Théniers	France	1600	33°52 N	4°04 E
	P8	Kustendil	Bulgaria	—	43°57 N	6°53 E
pallasiana	P9	Alaçam	Turkey	800–1000	39°35 N	28°35 E
	P13	Crimée	Russia	500	44°33 N	34°17 E

Table 2: Essential oil yields of *Pinus nigra* Arn. needles.

Provenance	Oil yield (%)
*P*1	$0.24^{j} \pm 0.01$
*P*2	$0.19^{k} \pm 0.01$
*P*3	$0.30^{g} \pm 0.02$
*P*4	$0.37^{f} \pm 0.01$
*P*5	$0.41^{e} \pm 0.03$
*P*6	$0.68^{a} \pm 0.05$
*P*8	$0.38^{f} \pm 0.01$
*P*9	$0.41^{e} \pm 0.02$
*P*10	$0.51^{c} \pm 0.05$
*P*11	$0.26^{i} \pm 0.04$
*P*12	$0.47^{d} \pm 0.08$
*P*13	$0.41^{e} \pm 0.01$
*P*14	$0.46^{d} \pm 0.03$
*P*16	$0.30^{g} \pm 0.01$
*P*17	$0.66^{b} \pm 0.02$
*P*18	$0.40^{e} \pm 0.01$
*P*19	$0.27^{h} \pm 0.02$
*P*20	$0.37^{f} \pm 0.01$

Means with different letters in the same column were significantly different at $p < 0.05$. Results are expressed as mean ± standard deviation of 3 determinations.

(38.71–41.85%). The third group regrouped *P*1, *P*5, *P*11, and *P*14, which showed the most important amount of *γ*-muurolene (2.23–4.02%). The fourth group contained *P*2 and *P*3 oils characterized by the highest amount of linalool (5.83 and 2.97%, respectively). The fifth group regrouped all the other samples studied, which showed the highest rate of caryophyllene oxide.

When considering the variability between the four studied subspecies, statistical results showed that oils from *nigra* subsp. were the richest in *α*-pinene. This richness is related to the high amount found in *P*6 (Puget-Théniers, France) oil (19.34%).

The results of the principal component analysis revealed the presence of three groups (Figure 3)—the first group regrouped *laricio* and *salzmannii* subspecies, which showed the highest rate of camphene and limonene; the second group contained *pallasiana* subsp., which demonstrated the most important amount of caryophyllene oxide; and the third one enclosed only *nigra* subsp. representing the highest amount of *α*-pinene.

Various terpenoid compounds, which are characteristic constituents of conifers, have been reported in *Pinus nigra* needles. Several studies that noted the chemical composition of the essential oils extracted from needles of *Pinus* species growing in Tunisia (*Pinus halepensis*, *Pinus pinea*, and *Pinus pinaster*) found that *β*-caryophyllene, amorphene, limonene, and germacrene D were the major components in all essential oils of pine needles [2, 12–14]. These results were supported by our study.

In our study, caryophyllene oxide was the major compound in oils of *pallasiana* subsp. from Turkey. These results were not similar to those found by Dogan and Bagci [15] that demonstrated that the main compounds of this subspecies were *α*-pinene, limonene, and *β*-caryophyllene. In the same context, Sezik et al. [11] proved that *α*-pinene and *β*-pinene were the main constituents of *Pinus nigra* essential oils from Turkey.

Several studies investigated the chemical composition of Italian essential oils. Macchioni et al. [10] indicated that *α*-pinene was the principal constituent. In the same context, Bader et al. [16] claimed that the essential oils from *laricio* subsp. were rich in *α*-pinene, *β*-caryophyllene, and germacrene-D. According to our results, the main compounds in Italian oils were *β*-caryophyllene, camphene, and caryophyllene oxide.

Pinus nigra subsp. *laricio* essential oils from Corsica were investigated by Rezzi and co-workers [9] who demonstrated that *α*-pinene, manoyl oxide, and germacrene-D were found

Table 3: Chemical composition of essential oils from *Pinus nigra* Arn.

No.	Compounds (%)	KI	*P*1	*P*2	*P*3	*P*4	*P*5	*P*6	*P*8	*P*9	*P*10	*P*11	*P*12	*P*13	*P*14	*P*16	*P*17	*P*18	*P*19	*P*20
1	α-Pinene	939	—	—	—	—	—	19.34	—	—	—	—	2.16	2.12	—	—	—	—	—	—
2	Verbenol	952	0.40	2.17	0.81	—	—	—	0.66	—	—	0.62	—	—	0.80	—	0.15	—	0.03	—
3	Myrcenol	974	4.19	3.56	1.86	—	0.80	—	—	—	—	1.41	—	—	1.60	1.05	—	—	0.05	0.12
4	3-Udecyne	988	0.23	0.77	0.88	—	—	—	0.98	—	—	0.30	—	—	0.36	—	0.23	—	—	—
5	Linalool	1082	0.45	5.83	2.97	—	0.58	—	0.46	1.08	—	1.06	0.43	—	0.74	—	0.16	—	—	0.16
6	Limonene	1089	1.82	1.49	0.91	—	2.53	0.47	—	—	1.33	1.71	2.96	1.64	2.85	—	—	0.12	1.40	0.21
7	Geranyl acetate	1101	3.51	1.20	0.73	0.87	1.20	—	—	—	—	1.13	—	—	0.84	—	—	0.12	0.06	0.18
8	Camphenol acetate	1117	0.95	2.20	0.60	1.39	—	—	0.95	—	0.40	0.53	—	—	1.70	—	0.31	—	0.07	0.11
9	3-Decyne	1152	0.89	2.17	8.19	—	0.52	0.29	1.74	9.72	0.37	1.14	1.17	1.82	0.85	5.39	3.42	1.88	0.08	0.42
10	Limonene oxide	1168	14.21	8.14	6.88	—	4.18	0.19	0.84	1.87	0.40	7.09	0.75	0.49	4.15	5.38	0.87	0.24	0.17	0.23
11	β-Phenethyl butyrate	1295	2.81	2.33	0.53	—	0.89	23.17	—	0.48	8.32	5.39	2.61	0.83	4.82	—	0.35	0.34	15.88	—
12	5,9-Tetradecane	1328	0.53	—	0.85	—	—	—	—	—	—	—	0.59	0.50	—	—	—	—	0.05	0.09
13	β-Caryophyllene	1418	13.72	4.75	1.27	—	42.82	15.65	—	0.80	23.28	12.89	32.16	30.29	32.93	0.79	—	1.28	51.10	0.70
14	α-Amorphene	1422	6.69	7.38	0.59	—	11.22	—	—	—	2.60	26.04	0.52	2.46	8.30	—	—	3.98	—	—
15	Bicyclogermacrene	1438	0.82	3.14	5.24	—	1.44	0.21	1.05	0.65	1.21	2.20	3.33	3.80	0.72	1.25	0.57	—	0.12	0.09
16	γ-Muurolene	1441	2.92	1.36	2.07	0.98	3.82	1.92	—	0.90	2.16	4.02	1.49	1.44	2.23	—	—	1.53	2.08	0.43
17	α-Caryophyllene	1460	1.59	1.52	5.29	1.92	1.98	—	1.79	0.64	—	1.58	8.84	9.44	1.74	1.11	1.20	—	—	0.57
18	Caryophyllene oxide	1466	16.10	22.86	41.53	80.54	8.14	1.22	88.51	75.11	8.33	10.33	38.71	41.85	23.75	66.49	90.05	87.74	0.99	90.85
19	Germacrene D	1488	3.28	3.09	2.46	2.51	0.77	27.13	—	0.76	10.89	0.41	1.08	0.86	0.44	7.11	0.16	0.16	23.75	2.46
20	Farnesene epoxide	1509	0.59	8.13	1.34	—	—	—	—	1.17	—	0.45	—	—	—	—	—	—	—	—
21	γ-Cadinene	1526	0.29	2.28	3.85	—	0.64	—	—	0.94	—	1.22	—	0.46	1.01	—	0.20	0.25	0.05	0.12
22	Camphene	1578	19.95	8.40	6.70	9.17	3.25	—	—	0.73	38.07	16.77	0.42	—	5.08	5.61	0.15	0.17	2.06	0.43
23	α-Cadinol	1652	2.05	5.23	2.80	0.62	13.23	8.41	1.02	2.34	0.64	1.72	0.77	—	2.08	3.82	0.18	0.18	0.06	0.81
	Monoterpenes	—	47.81	33.88	23.32	12.55	13.31	47.13	1.96	4.44	50.69	30.2	7.8	5.11	16.5	19.15	1.49	0.81	27.52	3.79
	Sesquiterpenes	—	44.77	56.65	63.98	84.06	83.29	27.41	92.37	82.55	38.22	60.45	85.82	89.74	72.76	73.46	92.2	94.96	54.40	93.57

KI: Kovats indices calculated in regard to standards mixture of hydrocarbons (C_8–C_{24}) for 4.790–48.969 min. %: percentage calculated by GC-FID on DB-5MS column.

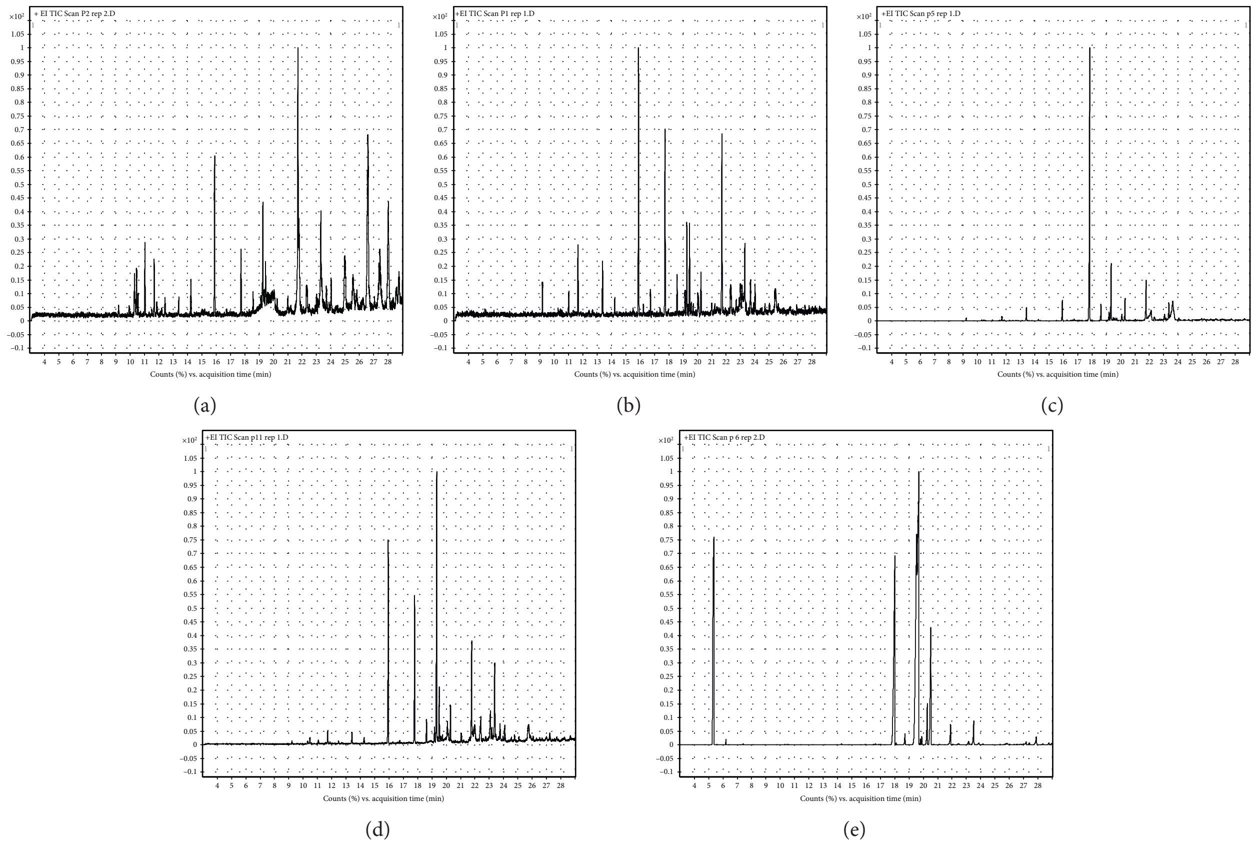

Figure 1: GC-FID chromatograms of the five chemotypes of *Pinus nigra* essential oils: (a) caryophyllene oxide, (b) camphene, (c) *ß*-caryophyllene, (d) *α*-amorphene, and (e) germacrene D.

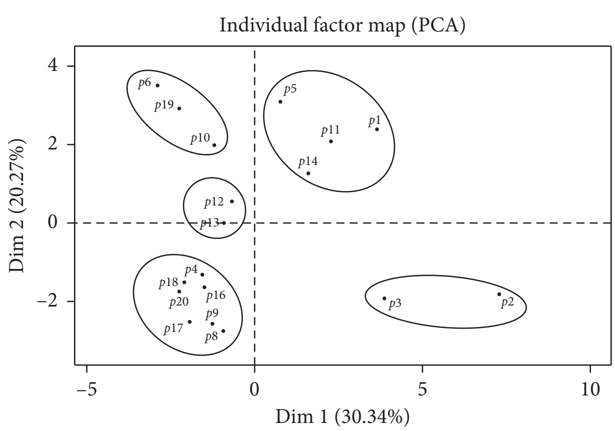

Figure 2: Individual factor map obtained from the PCA of data about the composition of *Pinus nigra* essential oils from 18 provenances.

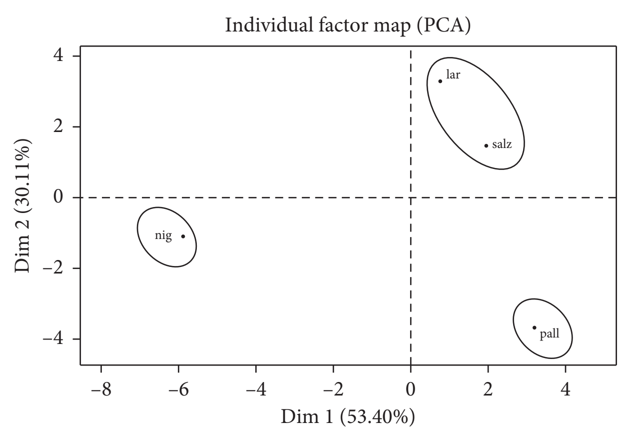

Figure 3: Individual factor map obtained from the PCA of data about the composition of *Pinus nigra* essential oils from four subspecies. lar: *laricio*, nig: *nigra,* salz: *salzmannii*, pall: *pallasiana.*

to be the main constituents. These findings were not in accordance to that found by our research team. In our case, *laricio* subsp. essential oils showed three main compounds, which were *β*-caryophyllene, *α*-amorphene, and caryophyllene oxide.

According to Jurc et al. [17], the terpene profile of *Pinus nigra* subsp. *salzmannii* from France was characterized by a high amount in *γ*-cadinene and *δ*-cadinene. Our results differ considerably from those reported previously. In this study, oils from *salzmannii* subsp., France, were characterized by a significant rate of camphene and caryophyllene oxide.

In most previous literature that focused on *Pinus nigra* essential oils composition, *α*-pinene was mentioned as the major compound, while in our study, this compound was absent in almost all the studied provenances excepting *nigra* subsp. *austriaca* var. that was mainly composed of germacrene and α-pinene. This finding was supported by Jurc et al. [17].

These comparisons with earlier studies indicated that, when planted in a common garden in Tunisia, where the pedoclimatic conditions were constant, *Pinus nigra* from four subspecies and deriving from eighteen provenances has undergone a significant change in its chemotypes. Plants of black pine seem to be adapted to local climate and soil conditions. This finding was supported by Amri et al. [12] who mentioned that there was a significant difference in the chemical composition of the essential oils from *Pinus nigra* subsp. *laricio* grown in Tunisia and those from other countries.

It has long been known that pedoclimatic conditions affect the volatile oil content as well as its chemical composition. Ormeño and Fernandez [18] mentioned that both biotic and abiotic conditions influence terpenoid production in plants, especially light and temperature. On the other hand, Staudt and Lhoutellier [19] determined the effect of these two environmental factors on monoterpene and sesquiterpene leaf emissions. In addition, water availability was known to be one of the most important environmental factors controlling volatile organic compounds from plants [20].

Under the same pedoclimatic conditions of the studied provenance trials, *Pinus nigra* plants showed a significant chemical variability. This could be explained by an eventual genetic variability among the eighteen provenances.

4. Conclusion

From the present study, we can conclude that the analyzed essential oils belonged to five different chemotypes. In addition, the obtained results showed differences in the quantitative and qualitative composition. Caryophyllene oxide was the main component of most of the studied oils. Therefore, this type of study will be useful for pharmacologists to promote pharmaceutical products and resolve several economic problems.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Sondes Fkiri and Faten Mezni performed some practical experiments, wrote and coordinated all the analysis, and calculated the results, statistics etc. Ghayth Rigane followed and checked the obtained results. Hanene Ghazghazi participated in some practical experiments. Ridha Ben Salem, M. Larbi Khouja, Zouheir Nasr, and Abdelhamid Khaldi supervised the scientific paper.

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References

[1] P. Quézel and F. Médail, "Ecologie et biogéographie des forêts du bassin méditerranéen," *Collection Environnement*, Elsevier, Amsterdam, Netherlands, 2003.

[2] S. Fkiri, H. Ghazghazi, H. Ghazghazi et al., "Chemical composition and biological activities essential oil from the needles African of *Pinus pinaster* Var," *Revue Roumaine de Chimie*, vol. 64, no. 6, pp. 511–518, 2019.

[3] M. Arbez, C. Bernard-dagan, and C. Fillon, "Variabilité intraspécifique des monoterpènes de *Pinus nigra* Arn.: bilan des premiers résultats," *Annales des Sciences Forestières*, vol. 31, no. 1, pp. 57–70, 1974.

[4] S. Gerber, *Chimiotaxinomie Et Hybridation Inter-Raciale Chez Les Pins Noirs*, Dissertation, National Institute of Agronomy, Paris, France, 1989.

[5] M. Paci, M. Michelozzi, and V. Vilrich, "Contentuto di monoterpeni di oteoresine corticali in provenienze diverse di *Pinus nigra* Arn," *Annali Accademia Italiana di Scienze Forestali*, vol. 38, pp. 223–231, 1990.

[6] V. Roussis, P. V. Petrakis, A. N. Ortiz, and B. E. Mazomenos, "Volatile constituents of needles of five *Pinus* species grown in Greece," *Phytochemistry*, vol. 39, pp. 57–361, 1995.

[7] A. R. Zara, R. S. Dodd, and E. Zavarin, "Genetic diversity in foliar terpenoids among natural populations of European black pine," *Biochemical Systematics and Ecology*, vol. 24, pp. 325–339, 1996.

[8] O. Politeo, M. Skocibusic, A. Maravic, M. Ruscic, and M. Milos, "Chemical composition and antimicrobial activity of the essential oil of endemic dalmatian black pine (*Pinus nigra* ssp. dalmatica)," *Chemistry & Biodiversity*, vol. 8, no. 3, 2011.

[9] S. Rezzi, A. Bighelli, D. Mouillot, and J. Casanova, "Composition and chemical variability of the needle essential oil of *Pinus nigra* subsp. laricio from corsica," *Flavour and Fragrance Journal*, vol. 16, no. 5, pp. 379–383, 2001.

[10] F. Macchioni, P. L. Cioni, G. Flamini, I. Morelli, S. Maccioni, and M. Ansaldi, "Chemical composition of essential oils from needles, branches and cones of *Pinus pinea*, *P. halepensis*, *P. pinaster* and *P. nigra* from central Italy essential oils of *Pinus* Spp," *Flavour and Fragrance Journal*, vol. 16, pp. 379–383, 2003.

[11] A. Sezik, O. Ustun, B. Demirci, C. Husnu, and K. Baser, "Composition of the essential oils of *Pinus nigra* arnold from Turkey," *Turkish Journal of Chemistry*, vol. 34, pp. 313–325, 2010.

[12] I. Amri, M. Hanana, B. Jamoussi, and L. Hamrouni, "Essential oils of *Pinus nigra* arnold subsp. *laricio* maire: chemical composition and study of their herbicidal potential," *Arabian Journal of Chemistry*, vol. 10, pp. 3877–3882, 2017.

[13] I. Amri, S. Gargouri, L. Hamrouni, M. Hanana, T. Fezzani, and B. Jamoussi, "Chemical composition, phytotoxic and antifungal activities of *Pinus pinea* essential oil," *Journal of Pest Science*, vol. 10, pp. 3877–3882, 2012.

[14] L. Hamrouni, M. Hanana, I. Amri, A. E. Romane, S. Gargouri, and B. Jamoussi, "Allelopathic effects of essential oils of *Pinus halepensis* miller: chemical composition and study of their

antifungal and herbicidal activities," *Archives of Phytopathology And Plant Protection*, vol. 48, no. 2, pp. 145–158, 2015.

[15] G. Dogan and E. Bagci, "Chemical composition of essential oil of *Pinus nigra* subsp. pallasiana (pinaceae) twigs, from different regions of Turkey," *Journal of Essential Oil Bearing Plants*, vol. 21, no. 2, pp. 511–519, 2018.

[16] A. Bader, G. Flamini, P. L. Cioni, and I. Morelli, "Composition of the essential oils from leaves, branches and cones of *Pinus laricio* poiret collected in sicily, Italy," *Journal of Essential Oil Research*, vol. 12, no. 6, pp. 672–674, 2000.

[17] D. Jurc, S. Bojovic, and M. Jurc, "Influence of endogenous terpenes on growth of three endophytic fungi from the needles of *Pinus nigra* arnold," *Phyton Horn Austria*, vol. 39, pp. 225–229, 1999.

[18] E. Ormeño and C. Fernandez, "Effect of soil nutrient on production and diversity of volatile terpenoids from plants," *Current Bioactive Compounds*, vol. 8, no. 1, pp. 71–79, 2012.

[19] M. Staudt and L. Lhoutellier, "Monoterpene and sesquiterpene emissions from *Quercus coccifera* exhibit interacting responses to light and temperature," *Biogeosciences*, vol. 8, no. 9, pp. 2757–2771, 2011.

[20] E. Ormeño, J. P. Mévy, B. Vila et al., "Water deficit stress induces different monoterpene and sesquiterpene emission changes in mediterranean species. Relationship between terpene emissions and plant water potential," *Chemosphere*, vol. 67, no. 2, pp. 276–284, 2007.