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Chemical composition of healthy and infected leaves by *Forda riccobonii* and *Geoica swirskii of Pistacia atlantica* Desf. collected from Maaziz region in Morocco

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Abstract: The Atlas Pistachio, *Pistacia atlantica* Desf., from the family of Anacardiaceae is a widespread species in Morocco and widely present in traditional pharmacopoeia [1]. This species is frequently infected by *Forda riccobonii and Geoica swirskii* that are foliar galls-inducing aphids.

This study aimed to compare the chemical composition of essential oils (EO) of the infected and healthy leaves of *Pistacia atlantica*. The extraction of essential oils was performed by hydro-distillation using Clevenger, phytochemical screening was realized by revelations tests, and chemical composition was identified by gas chromatography coupled with mass spectrometry (GC-MS).

Phytochemical analysis showed the presence of some biologically active chemical compounds such as tannins, flavonoids, sterols and triterpenes. The infected leaves were richer in tannins than healthy ones, especially in gallic tannins.

Also, the results revealed that the EO yield of leaves with galls was higher (0.71%) than that of healthy ones (0.11%); also, the first type of leaves was predominated by α-Pinene (19.76%) followed by Terpinen-4-ol, Spathulenol and Sabinene with percentages of 11.94%, 8.90% and 7.63%, respectively. Whereas, healthy leaves were predominated by α-Amorphene (15.05%) followed by Terpinen-4-ol, Spathulenol and α-Muurolene with percentages of 7.17%, 6.46% and 6.20%, respectively.

So, we arrived to conclude that infected leaves have a higher level of monoterpenes hydrocarbon than healthy ones that contain interesting molecules useful in different domains, especially in the health field.

Keywords: *Pistacia atlantica* Desf.; Leaves; Galls, Essentia1 Oil; Phytochemistry; Chemical composition.

1. Introduction

The Atlas Pistachio, *Pistacia atlantica* Desf., from the family of Anacardiaceae and known under the vernacular name "Betoum" or "Btem" [1]. It extends spontaneously, from the margins of the Sahara to the plains, low and medium mountains, rarely in pure stands, often in mixture with Thuya «*Tetraclinis articulata* (Vahl) Masters» [2], in arid bioclimate, semi-arid mild, semi-arid cold and subhumid [1].

Although three other pistachios meet in Morocco, *P. terebinthus* L. « Ij », *P. lentiscus* L. « Drou » in the wild state and *P.vera* L. « edible pistachios » in culture [1,]. only Atlas pistachio is infected by Forda riccobonii and Geoica swirskii aphids that cause leaf galls.

According to Álvarez and *al*. (2009) [4], galls develop on plants in response to an induction caused by several organisms: microorganisms, nematodes and mites. Also, there are more than 15,000 species of gallic insects. The galls can be found on many organs of the host plant, and a specific organ of the plant can support different types of galls. In the Pistacia genus, we find two subfamilies of insect inducing gall formation: *Pemphiginae* and *Fordinea*.

Generally when a plant receives an external attack will have morphological transformations [5], After the leaf injury at the tea tree (*Melaleuca alternifolia* Cheel.) the chemical composition of gasoline is also affected in all leaves irrespective of the initial gasoline composition. Some terpenes do increase their concentration [6].

In this context, the evaluation of the variation in the chemical composition of infected leaves and healthy

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ones of *Pistacia atlantica* Desf. essential oils has been the main objective of the present work.

2. Materials and methods

2.1. Harvest location

Maaziz (Figure 1) is a rural Moroccan town located in Rabat-Salé-Kenitra region about 35 km west from Khemisset city via N6 R404 road.

Figure 1. Harvest location area (Maaziz region); **33°38'44.6"N 6°17'58.9"W**

2.2. Plant material

The Atlas Pistachio, *Pistacia atlantica* Desf. **(Herbarium specimen: 1810/IS/N°38)**, is a tree can reach up to 20 m in high, with a well-individualized trunk [7]. Leaves, imparipinnate with finely winged rachis, are composed of 7-9 broad leaflets, soft, glabrous and deciduous in autumn [1]. The species is dioecious, some monoecious feet have been observed in eastern part of Morocco in Debdou region [8], however, no hermaphroditism was observed [8]**.** the inflorescence is a compound cluster, with apetalous flowers; flowering takes place in the spring-summer and the fruit is a little fleshy drupe, bluish when ripe [1], commonly called Goddime or Tikouaouche and often consumed by residents [8].

The harvest of Atlas Pistachio was carried out on May 03, 2019 in the Maaziz region. The individuals from whom the leaf samples were taken were randomly selected; each time the healthy leaves were carefully separated from those bearing galls.

We took two samples: healthy leaves, leaves infected, the samples were dried in the shade for about 15 days.

Figure 2. **a-**Leaves infested by aphids (Photo by *HAROUAK, 2019*);
b-Healthy leaves of *Pistacia atlantica* Desf.

2.3. Essential oil extraction of *Pistacia atlantica* Desf.

Healthy or infected leaves of Atlas Pistachio were immersed in a 2-liter balloon equipped with a Clevenger type apparatus and a ballon cooler. This mixture was boiled for three hours, using a balloon heater to produce the steam that brings the Essential Oils, the steam produced condenses through a refrigerant. The condensates (EO + Water) were separated by decantation, then dried over Magnesium sulphate and kept at a temperature of 4 ° C, in opaque glass flasks, hermetically closed to preserve them from air, light and variations of temperature which are major degradation agents.

2.4. Phytochemical screening

in order to highlight the different families of secondary metabolites present in the extracts of studied plants (decocted, infused and macerated) , the main way of putting these compounds was the phytochemical screening which focused either on the formation of a colored complex using color reactions, or on the formation of insoluble complexes using precipitation reactions. to achieve this characterization we have followed the experimental protocols of [9,10].

2.5. Chromatographic analysis of *Pistacia atlantica* Desf. essential oil

Chromatographic analysis of essential oils was performed at CNRST Rabat using a THERMO ELECTRON Trace MS system (THERMO ELECTRON: Trace Ultra GC; Polaris Q MS) gas chromatograph. The fragmentation is effected by electron impact intensity of 70 eV. The capillary column is DB-5 MS (5% phenyl-methyl-siloxane) (30m x 0.25mm, film thickness: 0.25µm). The temperature of the column increases from 50 to 200°C. at a rate of 4°C./min. Helium is used as a carrier gas with a flow rate of 1.5 ml/min. The injection is made in split mode (leak report: 1/70). The listed masses fall in the range of 30 to 500 m / z. The apparatus is connected to a computer system managing a library of NIST 98 mass spectra.

A standard hydrocarbon (C7-C40) is injected under the same conditions as the essential oils in order to calculate retention indices (Kovats index) [11] necessary for the identification of the essential oil compounds.

After receiving the spectra (chromatography and spectrometry), the essential oil compounds are identified by comparing the calculated retention indices for each of the eluted compounds (on the basis of the retention times of a hydrocarbon standard (C7-C40) with those contained in the available databases: Adams, 2007 [12] and the National Institute of Standards and Technology (NIST) The mass spectra of each of the compounds are also compared with those of the previously cited databases.

3. Results and discussions

3.1. Yields of *Pistacia atlantica* Desf. essential oils

Hydrodistilation of leaves of Atlas Pistachio permitted us to obtain an essential oil with a yield of 0.71% for infected leaves and 0.11% for healthy ones. Some works in differents Algerian localities, about male and female leaves with galls, show the values of the yield (0.08–1.89) [13] and (0.46%-0.53%) [14]. Others authors reported that the yield for male leaves were (0.02–0.12%) [15], and (0.08-0.17%) for different samples leaves [16].

So, our results are almost compatible in terms of values with those research; Face to a predator or a parasite attacks, the plant reacts by increasing its secondary metabolites production.

3.2. Phytochemical screening of infected and healthy leaves of *Pistacia atlantica* Desf.

Table 1. Phytochemical screening of infected and healthy leaves of *Pistacia atlantica* Desf.

	Healthy leaves	Infected leaves by FR
Tannins	++	+++
Gallic tannins	+	+
Catechic tannins	+	+
Alkaloids	0	0
Flavone compounds:		
Flavones	+	+
Anthocyanes	0	0
Genins	+	+
Leucoanthocyanes	+	+
Sterols and triterpenes	+	+
Oses and holosides	+	+
Reducing compounds	+	+
Mucilages	+	++
Free anthracene	0	0
Combined Anthracene		
C- heterosides	+	+
O- heterosides	+	+
Saponosides	0	0

+ + + : Very positive reaction; + + : Moderately positive reaction; + : lowly positive reaction; 0 : Total absence

According to the Table 1 we have detected the presence of some biologically active chemical groups such as tannins, flavonoids, sterols and tri terpenes, the two types of leaves of *Pistacia atlantica* are rich in tannins and flavonoids.

On the other hand, we noticed that leaves infected by *Forda riccobonii* are richer in tannins than healthy ones, this difference in the content of individual phenolic compounds is frequently correlated with a resistance level of plants to infection [17]. So, the therapeutic power of tannins producing species would correlate with their content of metabolites, especially with their high content of tannins [18]. It was reported also that hydrolysable tannins from crude drugs showed a strong inhibitory effect against Glucan Synthesis by Glucosyltransferase from *Streptococcus mutans* causal agent of tooth caries [19].

We have also detected that infected leaves contain more mucilages, the therapeutic value of this compounds has been extended to wound healing, diabetes, immunostimulation, cancer, angiotensin-converting enzyme inhibition, stomachic, and antioxidant properties, mucilages have been proposed to be one of the most useful materials to modulate drug delivery [20]. Also, the infected leaves and their richness in tannins and mucilages increase their medicinal potency compared to healthy ones.

3.3. Chemical composition of infected and healthy leaves of *Pistacia atlantica* Desf. essential oil

The essential oil analysis results of healthy and infected leaves of *Pistacia atlantica* Desf. Are presented on the chromatogram below (Figure 3 and 4).

Table 2 shows the identification of 54 compounds for infected leaves and 83 compounds for healthy ones representing respectively 99.29% and 98.69% of the chemical composition.

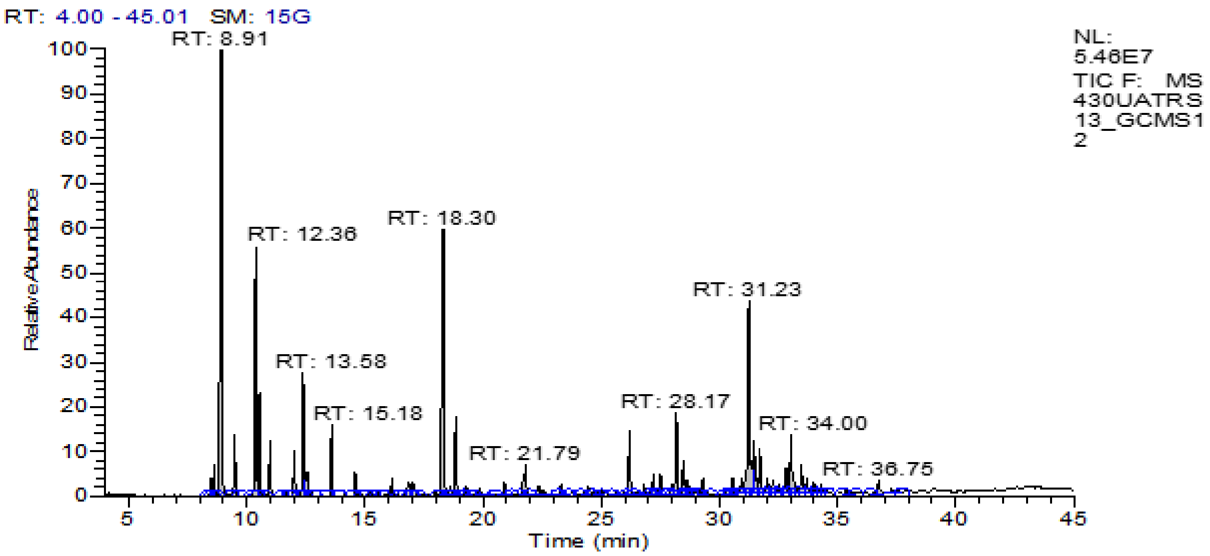

Figure 3. Essential oil chromatogram from the infected leaves by galls of *Pistacia atlantica* Desf.

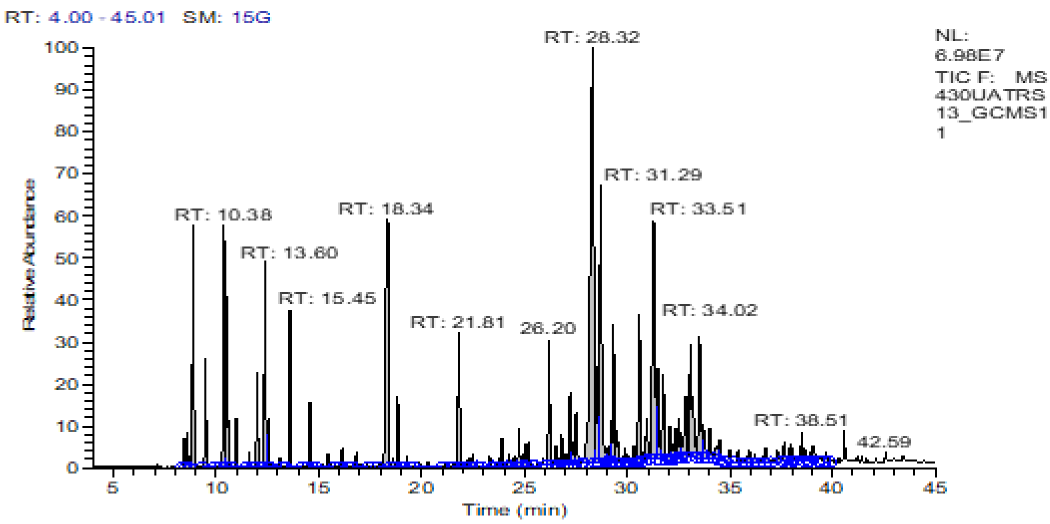

Figure 4. Essential oil chromatogram from the healthy leaves of *Pistacia atlantica* Desf.

Table 2. Chemical Composition of the essential oil of infected and healthy leaves of *Pistacia atlantica* Desf.

Compound	IK (Adams)	Weight molecular	brute Formula	Area (%) of infected leaves	Area (%) of healthy leaves
Tricyclene	926	136	C10 H16	0.47	0.38
α-Thujene	930	136	C10 H16	0.89	0.44
α-Pinene	939	136	C10 H16	**19.76**	**3.70**
Camphene	954	136	C10 H16	1.67	1.39
Sabinene	975	136	C10 H16	**7.63**	**3.81**
β-Pinene	979	136	C10 H16	**2.87**	**2.31**
Myrcene	990	136	C10 H16	1.56	0.66
α-Phellandrene	1002	136	C10 H16	0.20	0.20
α-Terpinene	1017	136	C10 H16	1.27	1.23
ρ-Cymene	1024	134	C10 H14	**3.51**	**3.47**
β-Phellandrene	1029	136	C10 H16	1.28	0.62
(Z)-β-Ocimene	1037	136	C10 H16	-	0.20
(E)-β-Ocimene	1050	136	C10 H16	**2.00**	**2.22**
γ-Terpinene	1059	136	C10 H16	-	0.89
cis-Sabinene hydrate	1070	154	C10 H18 O	0.70	0.27
cis-Linalool oxide	1072	170	C10 H18 O2	0.29	-
trans-Sabinene hydrate	1098	154	C10 H18 O	0.62	0.45
α-Pinene oxide	1099	152	C10 H16 O	0.39	-
1-Undecyne	1125	152	C11 H20	-	0.31
α-Campholenal	1126	152	C10 H16 O	0.40	-
Terpinen-4-ol	1177	154	C10 H18 O	**11.94**	**7.17**
ρ-Cymen-8-ol	1182	150	C10 H14 O	0.35	0.17
α-Terpineol	1188	154	C10 H18 O	**2.47**	1.08
cis-Piperitol	1196	154	C10 H18 O	0.30	0.17
cis-Carveol	1229	152	C10 H16 O	-	0.34
trans-Piperitone epoxide	1256	168	C10 H16 O	0.86	-
2-Ethyl menthone	1282	182	C12 H22 O	0.44	0.28
Isobornyl acetate	1285	196	C12 H20 O2	1.55	**2.44**
Thymol	1290	150	C10 H14 O	-	0.11
trans-Verbenyl acetate	1292	194	C12 H18 O2	0.84	0.14
Carvacrol	1299	150	C10 H14 O	-	0.24
Undec-(9E)-en-1-al	1312	168	C11 H20 O	0.49	-
neoiso-Isopulegyl acetate	1313	196	C10 H16	-	0.45
α-Cubebene	1351	204	C15 H24	-	0.12
1,2-dihydro-1,1,6-trimethylnaphthalene	1355	172	C13 H16	-	0.18
α-Copaene	1376	204	C15 H24	0.40	0.75
β-Bourbonene	1388	204	C15 H24	-	0.33
β-Elemene	1390	204	C15 H24	-	0.55
(Z)-Caryophyllene	1408	204	C15 H24	1.93	-
α-Gurjunene	1409	204	C15 H24	-	0.22
(E)-Caryophyllene	1419	204	C15 H24	-	1.93
β-Copaene	1432	204	C15 H24	-	0.37
γ-Elemene	1436	204	C15 H24	0.61	-
β-Humulene	1438	204	C15 H24	0.23	0.35
Aromadendrene	1441	204	C15 H24	0.61	0.11
α-neo-Clovene	1454	204	C15 H24	0.67	0.91
allo-Aromadendrene	1460	204	C15 H24	-	0.47
cis-Cadina-1(6),4-diene	1463	204	C15 H24	0.30	-
Ishwarane	1466	204	C15 H24	-	0.19
cis-Muurola-4(14),5-diene	1466	204	C15 H24	**2.77**	-
4,5-di-epi-Aristolochene	1473	204	C15 H24	1.15	-
γ-Gurjunene	1477	204	C15 H24	-	1.23
Widdra-2,4(14)-diene	1482	204	C15 H24	0.73	-
α-Amorphene	1484	204	C15 H24	-	**15.05**

Viridiflorene	1496	204	C15 H24	-	1.69
α-Muurolene	1500	204	C15 H24	-	**6.20**
Cuparene	1504	202	C15 H22	0.27	-
Germacrene A	1509	204	C15 H24	-	0.20
α-Bulnesene	1509	204	C15 H24	0.75	-
δ-Amorphene	1512	204	C15 H24	-	0.56
γ-Cadinene	1513	204	C15 H24	0.80	**2.07**
trans-Cycloisolongifol-5-ol	1513	220	C15 H24 O	-	0.58
7-epi-α-Selinene	1522	204	C15 H24	-	0.11
trans-Cadina-1,4-diene	1534	204	C15 H24	-	0.22
α-Cadinene	1538	204	C15 H24	-	0.09
α-Calacorene	1545	204	C15 H20	-	0.09
Hedycaryol	1548	222	C15 H26 O	-	0.34
Elemol	1549	222	C15 H26 O	-	**2.67**
trans-Dauca-4(11),7-diene	1557	204	C15 H24	-	0.13
Germacrene B	1561	204	C15 H24	0.56	0.76
Maaliol	1567	222	C15 H26 O	**2.15**	1.68
Spathulenol	1578	220	C15 H24 O	**8.90**	**6.46**
Globulol	1590	222	C15 H26 O	1.91	1.83
Cubeban-11-ol	1595	222	C15 H26 O	0.52	0.63
cis-dihydro-Mayurone	1595	206	C14 H22 O	-	0.29
β-Biotol	1613	220	C15 H24 O	-	0.50
1-epi-Cubenol	1628	222	C15 H26 O	0.69	-
epi-α-Muurolol	1642	222	C15 H26 O	0.38	0.72
Selina-3,11-dien-6-α-ol	1644	220	C15 H24 O	0.87	-
α-Cadinol	1654	222	C15 H26 O	1.23	-
Valerianol	1658	222	C15 H26 O	-	1.80
14-hydroxy-9-epi-(E)-Caryophyllene	1669	220	C15 H24 O	0.54	0.53
Eudesm-7(11)-en-4-ol	1700	222	C15 H26 O	**-**	**2.64**
Nootkatol	1715	220	C15 H24 O	**2.54**	**3.35**
(E)-Nuciferal	1728	216	C15 H20 O	0.43	0.68
Khusimol	1742	220	C15 H24 O	-	0.36
β-(Z)-Curcumen-12-ol	1756	220	C15 H24 O	-	0.36
7,14-anhydro-Amorpha-4,9-diene	1756	218	C15 H22 O	-	0.25
Ambroxide	1757	236	C16 H28 O	0.55	-
8-α-11-Elemodiol	1747	238	C15 H26 O2	0.34	0.35
Benzyl benzoate	1760	212	C14 H12 O2	1.08	-
(E)-α-Atlantone	1778	218	C15 H22 O	-	0.10
(Z)-α-Santalol acetate	1778	262	C17 H26 O2	-	0.32
14-hydroxy-α-Muurolene	1780	220	C15 H24 O	-	0.22
7-acetoxy-Elema-1,3-dien-8-ol	1786	280	C17 H28 O3	-	0.19
14-hydroxy-δ-Cadinene	1803	220	C15 H24 O	-	0.35
Eudesm-11-en-4-α, 6-α-diol	1808	238	C15 H26 O2	-	0.29
2-Butyl chromone	1816	202	C13 H14 O2	-	0.30
α-Chenopodiol	1856	238	C15 H26 O2	-	0.51
Cubitene	1878	272	C20 H32	-	0.26
β-Chenopodiol-6-acetate	1890	280	C17 H28 O	-	0.11

Total (infected leaves) : 99.29%
Monoterpenes
Hydrocarbon : 43.31%
Oxygenated : 18.32 %
Sesquiterpenes
Hydrocarbon : 12.21 %
Oxygenated : 20.50 %

Total (healthy leaves) : 98.69%
Monoterpenes
Hydrocarbon : 21.97 %
Oxygenated : 10 %
Sesquiterpenes
Hydrocarbon : 34.70 %
Oxygenated : 27.20 %

It should be noted that for infected leaves, the hydrocarbon monoterpenes represent 43.31% followed by oxygenated monoterpenes 18.32% whereas the sesquiterpenes represent 32.71% of the total composition of the essential oil ; it is the opposite case concerning the healthy leaves which represent for the hydrocarbonated sesquiterpenes 34.70% followed by oxygenated sesquiterpenes 27.20% whereas for monoterpenes represents 31.97%.

The chemical profile of infected leaves of Atlas Pistachio is predominated by α-Pinene (19.76%) followed by Terpinen-4-ol, Spathulenol and Sabinene with percentages of 11.94%, 8.90% and 7.63%, respectively. Healthy leaves are predominated by α-Amorphene (15.05%) followed by Terpinen-4-ol, Spathulenol and α-Muurolene with percentages of 7.17%, 6.46% and 6.20%, respectively.

Several works have been devoted to chemical composition study of *P. atlantica* essential oils: The essential oils of the leaves have been studied by [21, 22, 16, 15], and the galls by [16, 14, 13].

Essential oils from leaves and galls of *P. atlantica*, analyzed by GC/FID, GC/MS and 13C-NMR, are dominated by monoterpene hydrocarbons, α-pinene (32.6–54.7%) and β-pinene (8.0–20.2%) being the major components. Sesquiterpenes accounted for 14.1–21.7% in leaf oils and 4.8% in gall oil [16].

Concerning healthy leaves, oxygenated monoterpenes and sesquiterpenes predominated in the essential oil of *Pistacia atlantica* Desf. collected in July 2000 from Marrakesh region, of which terpinene-4-ol (21.7%) and elemol (20.0%) were the most abundant components [21].

The Atlas Pistachio from Greek East Aegean islands (Kalimnos and Lesvos) collected during 2002 have myrcene (17.8%, 24.8%) and terpinen-4-ol (11.6%, 6.0%) in the essential oil from the female plants. In the Kalimnos and Lesvos samples, respectively, while in the leaf oil from the male plants terpinen-4-ol (17.3% Kalimnos) and p-mentha-1(7),8-diene (41.1%, Lesvos) were the dominant constituents [22].

The essential oil of leaves collected from Male trees of Pistacia atlantica Desf. (34 samples) Randomly from four different locations in Algeria are rich in monoterpenes and oxygenated sesquiterpenes. Forty-seven compounds were identified; the main compounds were not the same in all analysed samples. The percentages ranges of the major components identified from the different locations were α-pinene + α-thujene (5.54–66.61%), camphene (0.75–20.85%), β-pinene (1.09–13.12%), p-cymene (0.39–10.19%), terpinen-4,ol (0.42–15.97%) and spathulenol (0.46–32.64%) [15].

In the present work spathulenol and terpinen-4-ol found in healthy leaves as a secondary and tertiary compound are also present in other plants from different locations, it is the first time that **α-Amorphene** chemotype is revealed. Concerning infected leaves, the essential oils (EOs) of unripe galls (from male and female plants) of a total number of 52 samples of *Pistacia atlantica* collected from different regions in Algeria were analysed by GC/MS and GC. The results of both methods of principal component analysis and hierarchical ascendant classification revealed the presence of two different chemotypes: α-pinene chemotype and α-pinene/sabinene/ terpinen-4-ol chemotype [13].

The essential oils obtained by hydrodistillation of unripe galls of *Pistacia atlantica* and collected from both male and female trees from Algeria showing the occurrence of a new 3-Carene rich chemotype showing content of 75.34%. The second chemotype was α-pinene/β-pinene, which is rich in α-pinene (59.01%) and β-pinene (13.26%) [14]. Also, α-pinene/sabinene/ terpinen-4-ol chemotypes were found by these researchers; this is in agreement with our results, especially for **α-pinene** as a first major compound. Also, we must notice a new 3-Carene found by Gourine *in Algeria*.

So we observe that infected leaves contain more monoterpenes hydrocarbon responsible for various pharmacological properties including antifungal, antibacterial, antioxidant, anticancer, antispasmodic, hypotensive, and vasorelaxant [23]. The significant volatile constituent α-pinene present in our infected leaves has interesting antibacterial properties on various bacterial strains [24].

Hence, it appears that the profile of the Pistacia essential oils varies depending on leaves health, the gender of the tree, geographical area, and the harvest period. These differences in the chemical composition are generally responsible for the diversity of medicinal uses, which differs from tradition to tradition and from country to another.

This study gives a better idea about volatile compounds contained in the infected and healthy leaves of *P. atlantica*, which grows in Morocco and plays an important role in the daily life populations (fodder for livestock, wood for the industry, medicinal uses). Also, the difference between the two types of leaves allows us to conclude that the attack of plants by a predator or a parasite modifies its chemical composition and could let to the appearance of new chemotypes.

Conclusion

The work we did led us to conclude that essential oils of leaves with galls differ in yield and composition in comparison with healthy ones; so, *Pistacia atlantica* Desf . responds to the aphids aggression by increasing essential oil production; also, there was a difference between chemical composition as infected leaves were predominated by α-Pinene for and instead of α-Amorphene observed in healthy leaves. Moreover, infected leaves reduce their total number of compounds but increase the monoterpenes hydrocarbon class, which contains new molecules useful in industrial and pharmaceutical domains.

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