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CHEMISTRY, ACTIVITY AND IMPACT OF PLANT BIOCONTROL PRODUCTS

Essential oils from Algerian species of *Mentha* as new bio-control agents against phytopathogen strains

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Abstract Chemical composition and antifungal activity of essential oils of Algerian *Mentha* species were studied. Chemical compositions of different *Mentha* species oils (*Mentha rotundifolia*, *M. spicata*, *M. pulegium*, and *M. piperita*) were investigated by capillary GC and GC/MS, and their antifungal activities were evaluated by means of paper disc diffusion method and minimum inhibitory concentration (MIC) assays. In total, 98 components from all *Mentha* species were identified. All oils were rich in monoterpene-oxygenated components. In addition, we reported fumigant antifungal activity of Algerian *Mentha* essential oils against four fungi: *Botrytis cinerea*, *Penicillium expansum*, *Monilinia laxa*, and *M. fructigena*. All oils demonstrated very good inhibition especially against *B. cinerea*, *M. laxa*, and *M. fructigena*. Both *Monilinia* fungi were extremely sensitive to all Algerian *Mentha* oils, which suggests that *Mentha* essential oils have the potential to be used as bio-pesticides to protect fruit trees, such as apple and pear trees, and provides an alternative to chemical pesticides.

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Introduction

Aromatic plants are increasingly used in agro-alimentary storage and bio-agriculture pest control. Essential oils and volatile constituents extracted from these plants are widely used as new bio-control alternative agents against microbial strains and insect pests, because of their specificity of action, biodegradable nature, and potential for commercial application (Kerdchoechuen et al. 2010; Park et al. 2003).

The Taxa of genus *Mentha* (*Lamiaceae* family) includes 25 species of herbaceous perennial (rarely annual) plants (Celenk et al. 2008; Fenwick and Ward 2001; Gobert et al. 2002; Khanuja et al. 2000; Krasnyanski et al. 1998; Shasany et al. 2005). Mints are distributed predominantly in the temperate region of the world and show substantial variation in terms of their natural habitats, growth characteristics, and aromas (Celenk et al. 2008; Shasany et al. 2005).

The genus *Mentha* consists of some of the most frequently cultivated spice plants in the world. Many species of *Mentha* are used in traditional folk medicine for many of its proprieties. *Mentha* EOs were known to be rich in oxygenated monoterpenes (Rösch et al. 2002). Depending on the nature of major components, they were used in several commercial and industrial fields. Recently, many works has shown strong activities of *Mentha* EOs as bio-control agents and possible substitute of traditional chemical fungicides, pesticides, and insecticides (Al-Bayati 2009; Al Yousef 2013; Mahboubi and Haghi 2008; Mohammadi et al. 2015; Odeyemi et al. 2008; Santana-Méridas et al. (2014, 2017); Soković et al. 2009).

In Algeria, the literature reported the occurrence of six species of *Mentha* genus: *M. rotundifolia*, *M. spicata*, *M. pulegium*, *M. piperita*, *M. longifolia*, and *M. aquatica*, and also three hybrids of these species: *M. durandoana*, *M. niliaca*, and *M. schultzii* (Quezel and Santa 1963). The same reference reported that *M. longifolia* and *M. aquatica* were very rare at that time and probably decimated now.

In the context of our characterization of Algerian aromatic plants, we investigated the chemical composition of four species of *Mentha*: *M. rotundifolia*, *M. spicata*, *M. pulegium*, and *M. piperita* from western Algeria, by analyzing their essential oils. A comparative analysis was performed between chemical compositions of all species. A chemical analysis was performed using a combination of capillary GC-RI and GC/MS after fractionation using column chromatography. Lastly, we report the bio-control effect for all essential oils against four fungal pathologies (*M. fructigina*, *M. laxa*, *Penicillium expansum*, and *Botrytis cinerea*) through the use of a fumigant antifungal assay.

Materials and methodologies

Plant material and oil isolation

The aerial parts of all *Mentha* from western Algeria were collected at the flowering stage (July 2014 to September 2014) from 11 location areas: 4 locations from *M. rotundifulia* (**MRO1–MRO4**), 2 locations from *M. spicata* (**MSP1, MSP2**), 3 locations from *M. pulegium* (**MPU1-MPU3**), and 2 locations from *M. piperita* (**MPI1, MPI2**) (Fig. 1). From each location, many samples were collected to poll collective oils from the same species. For all species, voucher specimens were deposited in the Herbarium of the University of Tlemcen. Essential oils were obtained from the fresh aerial parts of all stations by hydrodistillation for 4 h using a Clevenger-type apparatus according to the *European Pharmacopoeia* (Council of Europe 1997) and yielded (*w*/*w*) 0.78–0.96% from *M. rotundifulia*, 0.50–0.56% from *M. spicata*, 0.70–0.75% from *M. pulegium*, and 0.67–0.72% from *M. piperita* (see Fig. 1).

Gas chromatography

GC analyses were carried out using a Perkin Elmer Clarus 600 GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FIDs). The apparatus was used for simultaneous sampling of two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Temperature program 60 to 230 °C at 2 °C min^{-1} and then held isothermal 230 °C (30 min). Carrier gas was hydrogen (0.7 mL min^{-1}). Injector and detector temperatures were held at 280 °C. Split injection was conducted with a split ratio of 1:80. Injected volume was 0.1 μL.

Gas chromatography-mass spectrometry

The oils and the fractions obtained by CC were investigated using a Perkin Elmer TurboMass quadrupole analyzer, directly coupled with a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the same as described above. Ion source temperature was

Mentha species		Species[a]	N° Voucher[b]	Localities[c]	GPS Coordinates[d]	Yields[e]	Alt.[f]
	rotundifulia	MRO1	MRO-0714-KA1	Oulhassa1	35°11'50"N ; 1°29'24"O	0.78	476
		MRO2	MRO-0714-KA2	Oulhassa2	35°12'46"N ; 1°29'41"O	0.91	269
		MRO3	MRO-0714-KA3	Lakhmis	34°38'03"N ; 1°33'56"O	0.96	845
		MRO4	MRO-0714-KA4	Ain kebira	35°01'51"N ; 1°41'30"O	0.90	507
	spicata	MSP1	MSP-0714-KA1	Oudjlida	34°55'21"N ; 1°19'43"O	0.56	616
		MSP2	MSP-0714-KA2	Ouchba	34°52'11"N ; 1°10'52"O	0.50	779
	pulegium	MPU1	MPU-0714-KA1	Maaziz	34°54'35"N ; 1°48'28"O	0.70	526
		MPU2	MPU-0714-KA2	Ghazaouet	35°04'56"N ; 1°51'05"O	0.73	48
		MPU3	MPU-0714-KA3	Djbala	34°58'16"N ; 1°45'10"O	0.75	949
	piperita	MPI1	MPI-0714-KA1	Ouzidene	34°57'00"N ; 1°17'33"O	0.72	456
		MPI2	MPI-0714-KA2	Ourite	34°52'02"N ; 1°16'00"O	0.67	734

[a] Sampler codes
[b] Voucher codent
[c] Harvest localities
[d] Sample positioning
[e] Yields % (w/w)
[f] Altitudes (m)

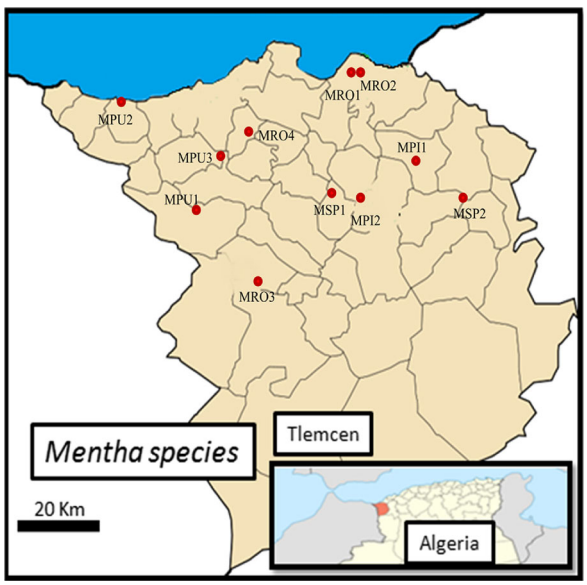

Fig. 1 Geographical distribution of *Mentha* species from western Algeria

Table 1 Chemical compositions of *Mentha* essential oils from Algeria

					Mentha species[e]											
					Rotundifulia				*Spicata*		*Pulegium*			*Piperita*		
No.[a]	*Composés*	lRI_a[b]	RI_a[c]	RI_p[d]	*MRO1*	*MRO2*	*MRO3*	*MRO4*	*MSP1*	*MSP2*	*MPU1*	*MPU2*	*MPU3*	*MPI1*	*MPI2*	*Identification*
1	(E)-Hex-3-en-1-ol	812	810	1360			tr	tr								RI, MS
2	Ethyl-2-methyl butyrate	829	829	1016	tr	0.4	0.3	0.1								RI, MS
3	(E)-2-hexenal	830	830	1210	0.1	0.2	0.3	0.1	tr	tr				0.1	0.1	RI, MS
4	(Z)-hex-3-en-1-ol	831	832	1375	tr	tr	0.1	0.1								RI, MS
5	(Z)-2-hexenol	851	848	1400	tr	tr	0.1	tr								RI, MS
6	1-Hexenol	852	851	1414	0.1	tr	0.1	tr								RI, MS
7	α-Thujene	922	923	1021	0.4	0.6	0.2	0.2	0.1	0.4	0.1	tr	tr	0.1	tr	RI, MS
8	α-Pinene	931	932	1023	0.7	1.2	0.8	0.4	0.3	0.7	0.5	0.2	0.1	0.2	0.2	RI, MS
9	Camphene	943	944	1066	0.5	0.5	0.3	tr								RI, MS
10	Oct-1-en-3-ol	959	962	1440	1.8	1.6	1.8	0.5			0.8	0.4	0.2			RI, MS
11	Sabinene	964	966	1118	0.2	1	0.3	0.2								RI, MS
12	β-Pinene	970	972	1108	0.6	0.6	0.9	0.4	0.4	0.7	0.2	0.1	0.1	0.5	0.3	RI, MS
13	Myrcene	976	982	1159	0.9	1.2	0.9	1.3	0.7	3.3	tr	tr	tr	1.9	1.2	RI, MS
14	3-octanol	982	982	1350	0.1	0.5	0.1	0.2			0.8	0.5	0.3			RI, MS
15	γ-Phellandrene	997	998	1164	0.2	0.3			0.1	0.1						RI, MS
16	α-Terpinene	1008	1010	1175	1.5	0.3	0.2	0.1	0.1	0.3						RI, MS
17	p-Cymene	1010	1012	1259	0.7	0.4	0.8	1.0	tr	0.1						RI, MS
18	**Limonene**	1020	1021	1195	1	1.9	1.3	0.3	**8.4**	**21.9**	1.1	1	1.5			RI, MS
19	1,8-cineole	1020	1021	1205	0.5	0.3	0.4	0.2			0.6	1.0	0.4	5.4	3.8	RI, MS
20	(Z)-β-ocimene	1024	1025	1225	0.8	1.8	0.7	0.2						0.4	0.4	RI, MS
21	(E)-β-ocimene	1034	1036	1241	0.1	0.2	0.1	tr	tr	0.4				0.2	0.4	RI, MS
22	γ-Terpinene	1047	1049	1237	3.2	0.9	0.4	0.3	0.1	0.7	0.1	0.4	0.2	0.3	0.2	RI, MS
23	Trans-hydrate sabinene	1051	1054	1444	3.9	2.8	0.9	3.0	tr	1.7						RI, MS
24	Terpinolene	1078	1080	1247	0.8	0.2	0.2	0.5	0.1	0.1				0.1	0.1	RI, MS
25	**Linalool**	1078	1075	1280					0.6	0.2	tr	tr	tr	**47.6**	**40.4**	RI, MS
26	Nonanal	1083	1082	1394	0.2	0.4										RI, MS
27	Cis-sabinene hydrate	1083	1082	1535	0.9	1.2	tr	0.1	0.1	0.5						RI, MS
28	**1-Oct-3-enyl acetate**	1093	1087	1390	**6.5**	**3.2**	**0.7**	**0.1**			tr	0.1	tr			RI, MS
29	2-Methyl-butyl isovalerate	1098	1096	1274										0.4	0.4	RI, MS
30	Cis-p-menth-2-en-1-ol	1108	1110	1600	0.6	0.4	tr	0.1						tr	tr	RI, MS, Ref
31	3-Octyl acetate	1111	1110	1315										0.2	0.2	RI, MS
32	Trans-p-menth-2-en-1-ol	1123	1126	1612	0.3	0.4	0.1	tr						0.1	tr	RI, MS, Ref
33	**Menthone**	1134	1135	1456			**16.8**	**28.5**			**10.8**	**38.3**	**40.5**			RI, MS

Table 1 (continued)

					Mentha species[e]										
34	p-Menth-3-en-8-ol	1135	1135	1590			0.7	3.1							RI, MS
35	**Iso-menthone**	1143	1142	1490			**5.5**	**19.0**			0.7	1.7	0.6		RI, MS
36	Borneol	1148	1150	1690	1.7	1.4	1.9	0.1							RI, MS
37	Cis-iso-pulegone	1152	1151	1569			0.1								RI, MS
38	Trans-iso-pulegone	1152	1151	1580			tr								RI, MS
39	**Neo-Menthol**	1156	1157	1637			**2.4**	**10.4**	0.1	0.2	**1.6**	**9.1**	**42.3**		RI, MS
40	p-Cymen-8-ol	1161	1162	1833	0.4	0.7									RI, MS
41	**Terpinene-4-ol**	1161	1162	1583	**10.4**	**3.4**	**0.1**	**2.7**	2.7	1.3					RI, MS
42	Menthol	1164	1163	1629			0.1	1.4			tr	tr	0.1		RI, MS
43	Iso-menthol	1174	1173	1660			0.2	2.1			tr	0.1	0.1		RI, MS
44	Z-dihydro carvone	1175	1174	1601					2.3	2.6					RI, MS
45	Dihydro carveol	1178	1174	1723					tr	tr					RI, MS
46	**α-Terpineol**	1179	1177	1688	0.1	0.9	0.1	2.9			tr	0.2	0.1	**10.4**	**6.4** RI, MS
47	E-dihydro carvone	1180	1180	1626					0.9	3.1					RI, MS
48	α-Campholenol	1186	1188	1782	0.2	0.2	tr	tr							RI, MS
49	Nerol	1211	1213	1799										1.7	1.1 RI, MS
50	**Pulegone**	1213	1216	1640	**0.2**	**0.3**	**56.3**	**5.6**			**77.3**	**40.7**	**9.2**	0.3	0.1 RI, MS
51	**Carvone**	1222	1226	1739					**79.3**	**54.1**					RI, MS
52	**Z-piperitone oxide**	1230	1229	1700		**11.7**									RI, MS
53	**E-piperitone oxide**	1230	1229	1722		**10.6**									RI, MS
54	Piperitone	1232	1229	1727		tr	0.1	1.3			0.3	2.3	0.9		RI, MS
55	**Geraniol**	1232	1234	1844										**5**	**2.4** RI, MS
56	**Linalyl acetate**	1240	1237	1557							tr	tr	tr	**12**	**32.6** RI, MS
57	Geranial	1244	1243	1731										0.5	0.2 RI, MS
58	Lyratyl acetate	1256	1258	1630		0.1									RI, MS
59	Pulegyl acetate	1260	1258	2113			0.1								RI, MS
60	Neryl formate	1263	1266	1647										tr	0.1 RI, MS
61	Neo-menthyl acetate	1263	1268	1548			0.3	5.0			0.1	0.9	0.5		RI, MS, Ref
62	Bornyl acetate	1269	1268	1475	0.9	1.4	0.1	tr							RI, MS
63	Lavandulyl acetate	1270	1273	1593										tr	0.1 RI, MS
64	Menthyl acetate	1282	1285	1578			0.1	2.1							RI, MS, Ref
65	Iso-menthyl acetate	1294	1295	1594			0.1	1.8			0.1	tr	tr		RI, MS, Ref
66	Dihydro carvyl acetate	1311	1312	1661					0.4	2.2					RI, MS
67	Piperitenone	1315	1313	1900	0.2	0.2	0.6	1.8	0.1	tr	2.7	1	0.9		RI, MS
68	**Piperitenone oxide**	1333	1335	1945	**34**	**36.1**			0.3	0.3					RI, MS

Table 1 (continued)

					Mentha species[e]											
69	α-Terpenyl acetate	1336	1336	1678	1	1.2	tr	0.1						0.1	0.1	RI, MS
70	Neryl acetate	1342	1345	1725					tr	1.7				2.7	2.7	RI, MS
71	**Geranyl acetate**	1361	1364	1725										**5.3**	**2.5**	RI, MS
72	E-jasmone	1363	1366	1889	0.9	0.3										RI, MS
73	α-Copaene	1379	1379	1475	0.8	0.3	tr	0.1								RI, MS
74	β-Bourbonene	1385	1385	1515	0.1	0.5	tr	tr	0.8	0.3				0.1	0.1	RI, MS
75	**E-β-caryophyllene**	1424	1418	1583	**7.5**	**0.5**	**0.4**	**0.4**	0.2	0.6	0.3	0.2	0.1	0.7	0.8	RI, MS
76	β-Copaene	1431	1430	1581	0.4	tr										RI, MS
77	E-β-farnesene	1448	1447	1660	0.2	0.8	0.1	0.2						0.1	0.1	RI, MS
78	α-Humulene	1456	1456	1665	0.8	0.4			0.1	0.2	0.4	0.3	0.2			RI, MS
79	2-Phenyl ethyl isovalerate	1465	1465	1980	0.1	0.1										RI, MS
80	γ-Muurolene	1471	1469	1679	0.9	0.5	tr	0.2						0.1	0.1	RI, MS
81	Germacrene D	1480	1474	1692	1.3	0.2	0.2	0.1	tr	0.1				tr	0.1	RI, MS
82	4-epi-cubebol	1487	1486	1870	0.3	0.3										RI, MS
83	α-Muurolene	1496	1492	1709	0.6	tr	tr	0.1								RI, MS
84	Epizonarene	1499	1496	1701			tr	tr								RI, MS, Ref
85	γ-Cadinene	1507	1506	1750	0.7	0.6	0.1	0.2	0.1	0.1	tr	0.1	tr	tr	0.2	RI, MS
86	trans-Calamenene	1512	1510	1810	0.8	0.5	0.1	0.1	0.2	0.1				0.1	0.2	RI, MS
87	δ-Cadinene	1516	1515	1748	1.2	1	0.1	0.1	tr	0.1	tr	0.1	0.1	tr	0.2	RI, MS
88	Cadina-1,4-diene	1523	1520	1763										tr	0.1	RI, MS
89	α-Calacorene	1531	1528	1890			tr	0.1								RI, MS
90	α-Cadinene	1535	1530	1740	0.5	0.3	tr	tr			tr	tr	tr	tr	0.1	RI, MS
91	β-Calacorene	1548	1546	1936			tr	tr								RI, MS
92	Caryophyllene oxide	1578	1580	1980	1.2	0.4								0.8	0.3	RI, MS
93	Globulol	1580	1582	2074										0.5	0.5	RI, MS
94	1,10-di-epi-cubenol	1608	1605	2031	1.3	0.3										RI, MS
95	Cadin-4-en-7-ol	1627	1626	2096	0.5	0.2										RI, MS
96	Tau-cadinol	1632	1630	2169	0.5	0.5										RI, MS
97	Tau-muurolol	1634	1630	2143	0.6	0.5										RI, MS
98	α-Cadinol	1645	1642	2231	1.3	0.3										RI, MS
	Total identification %				**98.2**	**99.2**	**98.6**	**98.9**	**98.5**	**98.1**	**98.5**	**98.7**	**98.4**	**97.9**	**98.8**	
	Yields % (w/w)				0.78	0.91	0.96	0.90	0.56	0.50	0.70	0.73	0.75	0.72	0.67	
	Hydrocarbon compounds				**27.4**	**16.7**	**8.1**	**6.5**	**11.7**	**30.2**	**2.7**	**2.4**	**2.3**	**4.8**	**4.8**	
	Monoterpene hydrocarbons				11.6	11.1	7.1	4.9	10.3	28.7	2	1.7	1.9	3.7	2.8	
	Sesquiterpene hydrocarbons				15.8	5.6	1	1.6	1.4	1.5	0.7	0.7	0.4	1.1	2	

Table 1 (continued)

	Mentha species[e]										
Oxygenated compounds	**70.8**	**82.5**	**90.5**	**92.4**	**86.8**	**67.9**	**95.8**	**96.3**	**96.1**	**93.1**	**94**
Oxygenated monoterpenes	55.3	73.3	87	91.3	86.8	67.9	94.2	95.3	95.6	91.1	92.5
Oxygenated sesquiterpenes	5.7	2.5	0	0	0	0	0	0	0	1.3	0.8
Non-terpenic oxygenated compounds	9.8	6.7	3.5	1.1	0	0	1.6	1	0.5	0.7	0.7

The writing in bold denotes the major components

RI retention indices, *MS* mass spectra in electronic impact mode, *Ref.* compounds identified from literature data: Konig et al. 2001 (**30**, **32**, **61**, **64**, **65**, and **84**)

[a] Order of elution is given on apolar column (Rtx-1)

[b] Retention indices of literature on the apolar column (RI_A) reported from Konig et al. (2001)

[c] Retention indices on the apolar Rtx-1 column (RI_A)

[d] Retention indices on the polar Rtx-Wax column (RI_P)

[e] Algerian *Mentha* species, normalized % abundance: *M. rotundifolia* (**MRO**), *M. spicata* (**MSP**), *M. pulegium* (**MPU**), and *M. piperita* (**MPI**)

150 °C and energy ionization 70 eV; electron ionization mass spectra were acquired with a mass range of 35–350 Da and scan mass of 1 s. Oil injected volume was 0.1 μL and fraction injected volume of 0.2 μL.

Component identification

Identification of the components was based (i) on the comparison of their GC retention indices (RI) on non-polar and polar columns, determined to the retention time of a series of *n*-alkanes with linear interpolation, with those of authentic compounds for our laboratory library from University of Corsica and literatures data (Jennings and Shibamoto 1980; Konig et al. 2001), and (ii) on computer matching with mass spectral of our laboratory library and commercial mass spectral libraries (McLafferty and Stauffer 1988).

Component quantification

The quantification of the essential oil components was performed using the methodology reported by Bicchi et al. (2008) and adapted in laboratory of UMR CNRS 6134 from University of Corsica (Djabou et al. 2013). Component quantification was carried out using peak normalization, including FID response factors (RFs) relative to tridecane (0.7 g/100 g), used as an internal standard and expressed as normalized % abundance.

Statistical analysis

Data analyses were performed using principal component analysis (PCA) (Brereton 2003). This method aims to reduce the multivariate space in which objects (oil samples) are distributed but are complementary in their ability to present results (Massart 1998). Indeed, PCA provides the data in which both objects (oil samples) and variables (oil major components and/or antibacterial inhibition) are plotted while canonical analysis informs a classification tree in which objects (sample locations) are gathered. PCA was carried out using function "PCA" from the statistical R software (factominR package). The variables (major components and/or antibacterial inhibition) have been selected using function from the statistical software (compounds > 1% were selected for the analysis).

Microbial strains and growth conditions

B. cinerea and *P. expansum* strains were obtained from *Institut Pasteur*, Paris, France (IP 1854.89, batch 185489; IP 1405.82, batch 050291 and IP 1350.82, batch 230992). *M. laxa* and *M. fructigena* strains were obtained from Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (CBS 127258, batch 127258 and CBS 101499, batch 101499). They were grown

on Sabouraud Glucose Agar (Sigma-Aldrich, Saint-Louis, Missouri, USA) and incubated at 23 °C in darkness.

Fumigant antifungal activity

Essential oils were evaluated for fumigant antifungal activity based on their ability to inhibit mycelial growth. Six-millimeter-diameter mycelial plugs of each fungal strain from a 7-day-old culture were placed into a Petri dish. Essential oils were introduced onto a 6-mm cellulose disc, placed on the agar-free lid of the Petri dish. A negative control (cellulose disc without essential oil) was performed in the same way. Petri dishes were then sealed with parafilm and incubated at 23 °C in the dark.

Mycelial radial growth was measured after 3 to 7 days of incubation, and the antifungal index was calculated with the formula as follows:

$$Antifungal\ index\ (\%) = (1-\mathrm{Dex}/\mathrm{Dc}) \times 100$$

where Dex is the diameter of growth zone in the experimental plate (mm) and Dc is the diameter of growth zone in the control plate (mm).

After a preliminary assay to determine antifungal index, Minimal Inhibitory Concentration (MIC) was determined for more active essential oils. In each treatment, the MIC was determined, with two replicates for preliminary assays and three replicates for the determination of MIC.

Results and discussion

Composition of the essential oils

Preliminary analysis of 11 Algerian oil sample areas showed that the GC chromatograms of all samples from the same species were qualitatively similar but differed by abundances of their major components from the same species of *Mentha* studied, except for *M. rotundifulia* species where we found

Table 2 Normalized percentage abundances from class compounds and function of Algerian *Mentha* species essential oils

		Mentha species[a]										
Class		*Rotundifulia*				*Spicata*		*Pulegium*			*Piperita*	
compounds		MRO1	MRO2	MRO3	MRO4	MSP1	MSP2	MPU1	MPU2	MPU3	MPI1	MPI2
Hydrocarbon compounds	Σ	27	28	28	28	18	18	11	11	11	18	18
	%	**27.4**	**16.7**	8.1	6.5	**13.7**	**29.2**	2.7	2.4	2.3	4.8	4.8
MH	Σ	14	15	14	14	11	11	6	6	6	8	8
	%	**11.6**	**11.1**	7.1	4.9	**12.3**	**27.7**	2	1.7	1.9	3.7	2.8
SH	Σ	13	13	14	14	7	7	5	5	5	10	10
	%	**15.8**	5.6	1	1.6	1.4	1.5	0.7	0.7	0.4	1.1	2
Oxygenated compounds	Σ	33	36	34	31	14	14	17	17	17	20	20
	%	**70.8**	**82.5**	**90.5**	**92.4**	**84.1**	**67.9**	**95.8**	**96.3**	**95.1**	**93.1**	**94**
MO	Σ	15	18	25	22	13	13	14	14	14	15	16
	%	**55.3**	**73.3**	**87**	**91.3**	**84.1**	**67.9**	**94.2**	**95.3**	**94.6**	**91.1**	**92.5**
SO	Σ	7	7	0	0	0	0	0	0	0	2	2
	%	5.7	2.5	0	0	0	0	0	0	0	1.3	0.8
NTO	Σ	11	11	9	9	1	1	3	3	3	3	3
	%	9.8	6.7	3.5	1.1	tr	tr	1.6	1	0.5	0.7	0.7
Alcohols	Σ	20	20	18	18	6	6	7	7	7	7	7
	%	**25**	**17.6**	8.7	**26.7**	2.5	3.9	3.2	10.3	**42.1**	**65.3**	**50.8**
Aldehydes and cetones	Σ	5	5	8	6	5	5	5	5	5	3	3
	%	1.6	1.4	**79.7**	**56.3**	**80.9**	**59.8**	**91.8**	**84**	**52.1**	0.9	0.4
Esters	Σ	5	6	8	7	2	2	4	4	4	8	8
	%	8.5	6.4	1.7	9.2	0.4	3.9	0.2	1	0.5	**20.7**	**38.7**
Oxides	Σ	3	4	1	1	1	1	1	1	1	2	2
	%	**35.7**	**57.1**	0.4	0.2	0.3	0.3	0.6	1	0.4	6.2	4.1

The bold corresponded to the important values

MH monoterpene hydrocarbons, *SH* sesquiterpene hydrocarbons, *MO* monoterpenes oxygenated, *SO* sesquiterpenes oxygenated, *NTO* non-terpenic oxygenated, % normalized % abundances, *tr* trace, ∑ total number of specified components

[a] Algerian *Mentha* species: *M. rotundifolia* (**MRO**), *M. spicata* (**MSP**), *M. pulegium* (**MPU**) and *M. piperita* (**MPI**)

qualitative differences between major components in different areas of the same species (see Table 1). In the 11 oils from four *Mentha* species, 98 compounds were identified, (14 monoterpene hydrocarbons, 17 sesquiterpene hydrocarbons, 45 oxygenated monoterpenes, 8 oxygenated sesquiterpenes, and 14 non-tearpenic oxygenated compounds). In Table 1, the compositions from all samples of each species are presented. In total, we identified 80 compounds in *M. rotundifulia* samples (60 compounds in **MRO1** simple representing 98.2% of the total number of compounds identified, 64 compounds in **MRO2** sample representing 99.2% of total, 62 compounds in **MRO3** simple representing 98.6% of total, and 59 compounds in **MRO4** simple representing 98.9% of total), among them **MRO1** (14 monoterpene hydrocarbons, 13 sesquiterpene hydrocarbons, 15 oxygenated monoterpenes, 7 oxygenated sesquiterpenes, and 11 non-terpenic oxygenated compounds), **MRO2** (15 monoterpene hydrocarbons, 13 sesquiterpene hydrocarbons, 18 oxygenated monoterpenes, 7 oxygenated sesquiterpenes, and 11 non-terpenic oxygenated compounds), **MRO3** (14 monoterpene hydrocarbons, 14 sesquiterpene hydrocarbons, 25 oxygenated monoterpenes, and 9 non-terpenic oxygenated compounds), and **MRO4** (14 monoterpene hydrocarbons, 14 sesquiterpene hydrocarbons, 22 oxygenated monoterpenes, and 9 non-terpenic oxygenated compounds) (see Table 2). Regarding *M. spicata*, we identified 32 compounds (in both **MSP1** and **MSP2** samples representing 98.5 and 98.1% of total respectively from **MSP1** and **MSP2**), among them 11 monoterpene hydrocarbons, 7 sesquiterpene hydrocarbons, 13 oxygenated monoterpenes, and 1 non-terpenic oxygenated compound (see Table 2). About *M. pulegium*, we reported 28 compounds in different stations (**MPU1**, **MPU2**, and **MPU3** samples representing 98.5, 98.7, and 98.4% of total respectively from **MPU1**, **MPU2**, and **MPU3**), among them 6 monoterpene hydrocarbons, 5 sesquiterpene hydrocarbons, 14 oxygenated monoterpenes, and 3 non-terpenic oxygenated compounds (see Table 2). Finely, we reported 38 compounds in both *M. piperita* essential oil samples (**MPI1** and **MPI2** representing 97.9 and 98.8% of total respectively from **MPI1** and **MPI2**), among them 8 monoterpene hydrocarbons, 10 sesquiterpene hydrocarbons, 15 oxygenated monoterpenes, 2 oxygenated sesquiterpenes, and 3 non-terpenic oxygenated compounds) (see Table 2).

From all species analyzed, 92 components were verified by comparing their EI-MS and retention indices with those in our laboratory library (UMR CNRS 6134 from University of Corsica). Six components were identified by comparing their EI-MS and apolar retention indices with those of literature libraries under the same analytical conditions as ours (see Table 1),

In *M. rotundifulia* (**MRO**) essential oils, four different types of oils were shown to be different both qualitatively and quantitatively in their compositions. **MRO1** was characterized by the abundance of piperitenone oxide (34%), terpinene-4-ol (10.4%), E-β-caryophyllene (7.5%), and 1-oct-3-enyl acetate (6.5%). The oil was dominated by oxygenated compounds (70.8%), especially oxygenated

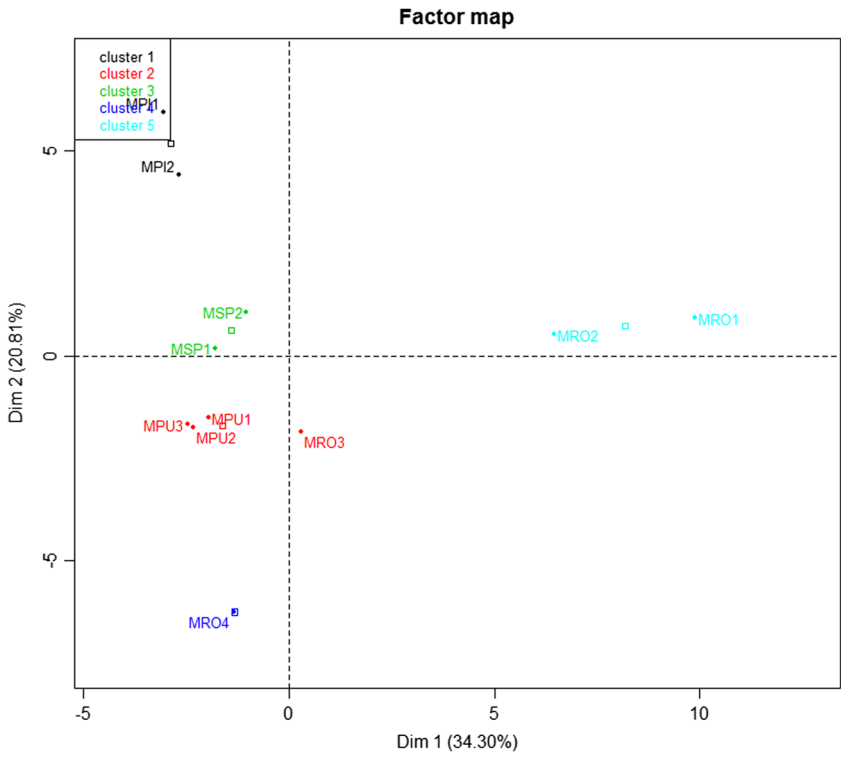

Fig. 2 Individual factor map (principal component analysis)

monoterpenes (55.3%). **MRO2** was characterized by the abundance of piperitenone oxide (36.1%), Z-piperitone oxide (11.7%), and E-piperitone oxide (10.6%). The oil was dominated by oxygenated compounds (82.5%), especially oxygenated monoterpenes (73.3%). **MRO3** was characterized by the abundance of pulegone (56.3%), menthone (16.8%), and iso-menthone (5.5%). The oil was dominated by oxygenated compounds (90.5%), especially oxygenated monoterpenes (87%). Finely **MRO4** was characterized by the abundance of menthone (28.5%), iso-menthone (19%), neo-menthol (10.4%), pulegone (5.6%), and neo-menthyl acetate (5%). The oil was dominated by oxygenated compounds (92.4%), especially oxygenated monoterpenes (91.3%) (see Table 1).

In *M. spicata* (**MSP**) essential oils, two oils were identified, with only qualitative differences in the abundance of their major components. **MSP1** and **MSP2** were characterized respectively by the abundance of carvone (79.3 and 54.1%), limonene (8.4 and 21.9%), and myrcene (0.7 and 3.3%). Both oils were dominated by oxygenated monoterpene (86.8 and 67.9%) and monoterpene hydrocarbons (10.3 and 28.7%) respectively from **MSP1** and **MSP2** (see Table 1).

In *M. pulegium* (**MPU**) essential oils, tree oils were characterized and differenced only quantitatively by the abundance of their major components. **MPU1**, **MPU2**, and **MPU3** were characterized respectively by the abundance of pulegone (77.3, 40.7, and 9.2%), neo-menthol (1.6, 9.1, and 42.3%), and menthone (10.8, 38.3, and 40.5%). All oils were dominated respectively by oxygenated monoterpene (94.2, 96.3, and 96.1%) (see Table 1).

In *M. piperita* (**MPI**) essential oils, both oils were qualitatively similar but differed by the abundance of their major components. **MPI1** and **MPI2** were respectively dominated by linalool (47.6 and 40.4%), linalyl acetate (12 and 32.6%), α-Terpineol (10.4 and 6.4%), 1,8-Cineole (5.4 and 3.8%), geranyl acetate (5.3 and 2.5%), and geraniol (5 and 2.4%). Both oils were dominated by oxygenated monoterpene (91.1 and 92.5%) respectively in **MP1** and **MP2** (see Table 1).

As presented in Table 2, chemical composition of essential oils of *Mentha* species were characterized by the high amount of oxygenated compounds (70.8 to 96.3%) in all samples, especially monoterpene-oxygenated compounds (55.3 to 95.3%), in accordance with literature. Only some samples of *M. rotundifulia* and *M. spicata* exhibited a significant level of hydrocarbon compounds. Finally, *M. spicata*, *M. piperita*, and *M. rotundifulia* (**MRO3** and **MRO4**) were characterized by high amounts of aldehydes and ketones (52.1 to 91.8%). *M. piperita* were characterized by the presence of alcohols (50.8 to 65.3%) and esters (20.7 to 38.7%), and finally, **MRO1** and **MRO2** from *M. rotundifulia* were dominated by oxides (35.7 to 57.1%) and alcohols (17.6 to 25%) (see Table 2).

To identify possible correlation between the chemical oil compositions of Algerian *Mentha* species, principal component analysis (PCA) was applied to matrix linking essential oil components to species identities. PCA (Figs. 2 and 3) confirmed our interpretation that there are similarities between *M. pulegium* (**MPU1**, **MPU2**, and **MPU3**) and **MRO3** and **MRO4** from *M. rotundifulia* especially **MRO3**. Both simples (**MSP1** and **MSP2**) of *M. spicata* were also close to *M. pulegium*. The most important differences were found in *M. piperita* (**MPI1** and **MPI2**) and in the **MRO1** and **MRO2** from *M. rotundifulia*, which were quite different from the others.

Fumigant antifungal activity

The antifungal activity of essential oils of Algerian *Mentha* species was tested against for fungi. Results of fumigant antifungal activity are shown in Tables 3 (inhibition rate) and 4 (MIC against more sensitive fungal strains). *P. expensum* is less sensitive than are other strains and for this reason, MICs were only identified for *Monilinia sp.* and *B. cinerea* strains.

Essential oils of *M. rotundifulia* are less active than the essential oils from other species of *Mentha*, except for **MRO3**,

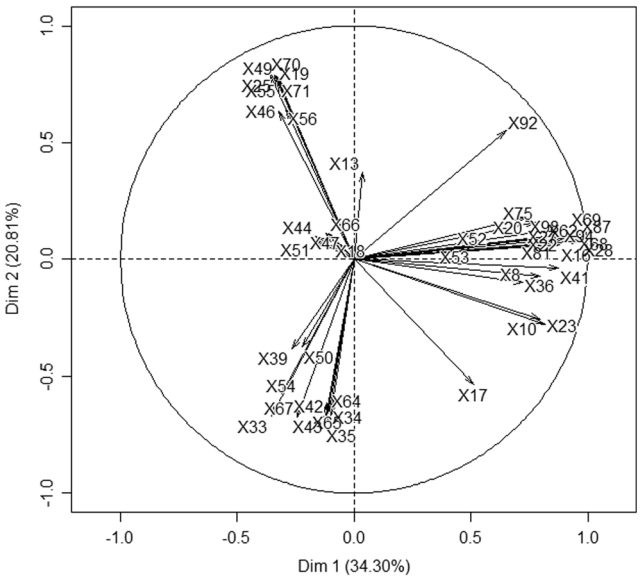

Fig. 3 Variable factor map (principal component analysis). X8 = α-Pinene; X10 = Oct-1-en-3-ol; X13 = myrcene; X16 = α-terpinene; X17 = p-cymene; X18 = limonene; X19 = 1,8-cineole; X20 = (Z)-α-ocimene; X22 = α-terpinene; X23 = trans-hydrate sabinene; X25 = linalool; X27 = cis-sabinene hydrate; X28 = 1-oct-3-enyl acetate; X33 = menthone; X34 = p-menth-3-en-8-ol; X35 = iso-menthone; X36 = borneol; X39 = neo-menthol; X41 = terpinene-4-ol; X42 = menthol; X43 = iso-menthol; X44 = Z-dihydro carvone; X46 = α-terpineol; X47 = E-dihydro carvone; X49 = Nerol; X50 = pulegone; X51 = carvone; X52 = Z-piperitone oxide; X53 = E-piperitone oxide; X54 = piperitone; X55 = geraniol; X56 = linalyl acetate; X62 = bornyl acetate; X64 = menthyl acetate; X65 = iso-menthyl acetate; X66 = dihydro carvyl acetate; X67 = piperitenone; X68 = piperitenone oxide; X69 = α-terpenyl acetate; X70 = neryl acetate; X71 = geranyl acetate; X75 = E-α-caryophyllen; X81 = germacrene D; X87 = α -cadinene; X92 = caryophyllene oxide; X94 = 1,10-di-epi-cubenol; X98 = cadinol

Table 3 Fumigant antifungal activity of *Mentha* essential oils from Algeria

Mentha species[a]		Volume (μl)	Concentration ($\times 10^{-3}$ μl/ml air)	Inhibition rate (mean ± SD)			
				Botrytis cinerea (3 days)[b]	*Penicillium expansum* (3 days)	*Monilinia fructigena* (3 days)	*Monilinia laxa* (7 days)
Mentha rotundifulia	*MRO1*	5	71	40.7	0	89.7 ± 12.1	80.8
		10	142	58.3 ± 17.0	38.1 ± 1.1	100	100
	MRO2	5	71	14.8 ± 10.4	19.4 ± 15.5	52.5 ± 17.1	87.2
		10	142	52.7 ± 9.1	39.8 ± 1.1	100	100
	MRO3	5	71	100	0	100	100
		10	142	100	43.2 ± 3.5	100	100
	MRO4	5	71	51.8 ± 20.9	38.9 ± 9.5	92.3 ± 14.4	93.6
		10	142	66.6 ± 5.23	50.8 ± 2.3	100	100
Mentha spicata	*MSP1*	5	71	100	14.4 ± 10.7	100	96.8 ± 3
		10	142	100	55.9	100	100
	MSP2	5	71	100	20.3	100	100
		10	142	100	20.3 ± 2.3	100	100
Mentha pulegium	*MPU1*	5	71	100	9.3 ± 20.3	100	100
		10	142	100	61.0 ± 2.3	100	100
	MPU2	5	71	100	27.1 ± 16.7	100	100
		10	142	100	38.1 ± 3.5	100	100
	MPU3	5	71	100	46.6 ± 5.9	100	100
		10	142	100	71.1 ± 7.1	100	100
Mentha piperita	*MPI1*	5	71	100	29.6 ± 1.1	84.1 ± 17.7	74.4
		10	142	100	36.4 ± 8.3	100	100
	MPI2	5	71	66.6	5.9 ± 10.7	100	100
		10	142	25.9	4.2 ± 32.3	100	100

[a] *M. rotundifolia* (MRO), *M. spicata* (MSP), *M. pulegium* (MPU), and *M. piperita* (MPI)

[b] Duration of growth

which has an MIC of 36 × 103 μl/ml air against *B. cinerea*. We observed an MIC between 36 and 142 × 103 μl/ml air for *M. spicata*, *M. pulegium*, and *M. piperita*. These results can be correlated with both their chemical compositions and statistical results. Oils dominated by alcohols (**MPI1** and **MPI2** and **MPU3**), aldehydes and ketones (**MRO3**, **MSP1**, **MSP2**,

Table 4 Minimal inhibitory concentration of *Mentha* essential oils from Algeria

Mentha species[a]		Minimal inhibitory concentration ($\times 10^3$ μl/ml air)		
		Botrytis cinerea	*Monilinia fructigena*	*Monilinia laxa*
Mentha rotundifulia	*MRO1*	> 142	142	142
	MRO2	> 142	142	142
	MRO3	36	142	142
	MRO4	> 142	142	142
Mentha spicata	*MSP1*	71	71	142
	MSP2	71	71	71
Mentha pulegium	*MPU1*	36	71	71
	MPU2	71	71	71
	MPU3	36	71	71
Mentha piperita	*MPI1*	36	142	142
	MPI2	36	71	71

[a] *M. rotundifolia* (MRO), *M. spicata* (MSP), *M. pulegium* (MPU) and *M. piperita* (MPI)

MPU1, **MPU2**, and **MPU3**) exhibited an interesting inhibition again the fungi tested, in contrast to **MRO1** and **MRO2** which were rich in oxide compounds and were less active. On the basis of these results, we suggest that the most interesting activity of *Mentha* essential oils was due to alcohol, aldehyde, and ketone compounds, like linalool in *M. piperita*; carvone in *M. spicata*; and pulegone, menthone, and neo-menthol in both *M. pulegium* and *M. rotundifulia*. Those results were in accordance with the results published recently and demonstrated strong activities of molecules like iso-menthose, pulegone, carvone, piperitone, piperitone oxide, and piperitenone oxide present in essential oils of *M. spicata* and *M. pulegium*, as good activities against insect pests (*Leptinotarsa decemlineata*, *Spodoptera littoralis*, and *Myzus persicae*), root-knot nematodes (*Meloydogine javanica*), and plants (*Lactuca sativa*, *Lolium perenne*, *Solanum lycopersicum*) (Santana-Méridas et al. 2017). In addition, we suggest that these molecules can significantly inhibit the growth of fungi, especially *Molilinia sp.* and *B. cinerea*, and to a lesser degree *P. expansum*.

Conclusions

The aerial parts of the Algerian *Mentha* species produce one type of essential oil dominated by oxygenated compounds; however, the oils can be classed in three different groups. The most important group is characterized by ketone compounds like carvone, menthone, and pulegone and the second by alcohol compounds like linalool and neo-menthol. Finally, the third group was characterized by oxide compounds like piperitenone oxide and piperitone oxide.

The *Mentha* essential oil activity was very interesting and almost all the oils tested demonstrated a strong inhibition against the fungi studied except for *P. expensum* where the activity was moderate. Thus, the *Mentha* essential oils could be considered as a good bio-control agent to protect fruit trees, such as apple and pear trees, which are known targets for fungi like *Molilinia sp.* and *B. cinerea*, and as an alternative to chemical pesticides, to treat trees infected with these fungi.

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References

Al Yousef S (2013) Antifungal activity of volatiles from lemongrass (*Cymbopogon citratus*) and peppermint (*Mentha piperita*) oils against some respiratory pathogenic species of Aspergillus. Int J Curr Microbiol App Sci 2:261–272

Al-Bayati FA (2009) Isolation and identification of antimicrobial compound from *Mentha longifolia* L. leaves grown wild in Iraq. Ann Clin Microbiol Antimicrob 8:20

Bicchi C, Liberto E, Matteodo M, Sgorbini B, Mondello L, Zellner BA, Costa R, Rubiolo P (2008) Quantitative analysis of essential oils: a complex task. Flavour Frag J 23:382–391

Brereton RG (2003) Chemometrics: data analysis for the laboratory and chemical plant, first edn. Wiley Interscience, New-York

Celenk S, Tarimcilar G, Bicakci A, Kaynak G, Malyer H (2008) A palynological study of the genus Mentha L. (Lamiaceae). Bot J Linn Soc 157:141–154

Council of Europe (1997) European Pharmacopoeia, First edn. Council of Europe, Strasbourg

Djabou N, Andreani S, Varesi L, Tomi F, Costa J, Muselli A (2013) Analysis of volatile fraction of Teucrium marum L. Flavour Frag J 28:14–24

Fenwick AL, Ward SM (2001) Use of random amplified polymorphic DNA markers for cultivar identification in mint. Hort Sci 36:761–764

Gobert V, Moja S, Colson M, Taberlet P (2002) Hybridization in the section Mentha (Lamiaceae) inferred from AFLP markers. Am J Bot 89:2017–2023

Jennings W, Shibamoto T (1980) In: Jovanovich HB (ed) Qualitative analysis of flavour and fragrance volatiles by glass-capillary gas chromatography, Academic Press, New York

Kerdchoechuen O, Laohakunjit N, Singkornard S, Matta FB (2010) Essential oils from six herbal plants for bicontrol of the maize weevil. Hortscience 45(4):592–598

Khanuja SPS, Shasany AK, Srivastava A, Kumar S (2000) Assessment of genetic relationships in Mentha species. Euphytica 111:121–125

Konig WA, Hochmuth DH, Joulain D (2001) Terpenoids and related constituents of essential oils library of mass finder 2.1. Ed. Institute of Organic Chemistry, Hamburg

Krasnyanski S, Ball TM, Sink KC (1998) Somatic hybridization in mint: identification and characterization of Mentha piperita M. spicata hybrid plants. Theor Appl Genet 96:683–687

Mahboubi M, Haghi G (2008) Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. J Ethnopharmacol 119: 325–327

Massart DL (1998) Chemometrics: a textbook. Elsevier Science Ltd, New-York

McLafferty FW, Stauffer DB (1988) The Wiley/NBS registry of mass spectra data. Wiley-Interscience. Ed, New-York

Mohammadi A, Hashemi M, Masoud Hosseini S (2015) Comparison of antifungal activities of various essential oils on the *Phytophthora drechsleri*, the causal agent of fruits decay. Iran J Microbiol 7(1): 31–37

Odeyemi OO, Masika P, Afolayan AJ (2008) Insecticidal activities of essential oil from the leaves of Mentha longifolia L. subsp. capensis against Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae). Afr Entomol 16:220–225

Park IK, Lee SG, Choi DH, Park JD, Ahn YJ (2003) Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinesis* (L.) and *Sitophilus oryzea* (L.) J. Stored Prod Res 39:375–384

Quezel P, Santa S (1963) Nouvelle Flore de l'Algérie. Centre National de la Recherche Scientifique, Paris

Rösch P, Kiefer W, Popp J (2002) Chemotaxonomy of mints of genus *Mentha* by applying Raman spectroscopy. Biopolymers 67(6):358–361

Santana-Méridas O, Fe Andrés M, Sanz J, Errahmani N, Abdeslam L, González-Coloma A (2014) Valorization of essential oils from Moroccan aromatic plants. Nat Prod Commun 9(8):1109–1114

Santana-Méridas O, González-Coloma A, Fe Andrés M, Vidali VP, Polissiou MG, Kimbaris AC (2017) Biocidal compounds from Mentha sp essential oils and their structure-activity relationships. Chem Biodivers. https://doi.org/10.1002/cbdv.201600270

Shasany AK, Darokar MP, Dhawan S, Gupta AK, Gupta S, Shukla AK, Patra NK, Khanuja SPS (2005) Use RAPD and AFLP markers to identify inter- and intraspecific hybrids of Mentha. J Hered 96(5): 542–549

Soković MD, Vukojević J, Marin PD, Brkić DD, Vajs V, Van Griensven LJ (2009) Chemical composition of essential oils of thymus and Mentha species and their antifungal activities. Molecules 14:238–249