Chemical composition and *in vitro* antimicrobial, insecticidal and antioxidant activities of the essential oils of *Mentha pulegium* L. and *Mentha rotundifolia* (L.) Huds growing in Algeria

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ABSTRACT

The essential oils of *Mentha pulegium* L. (MPE) and *Mentha rotundifolia* (L.) Huds (MRE) which are growing in Algeria were prepared by hydrodistillation and their chemical compositions investigated by GC/MS. The oils were tested for their antimicrobial activity using disc-diffusion and spot assays, antioxidant activity using 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) test and Kit Radicaux Libres® (KRL) biological assay. Also, contact toxicity, fumigant toxicity and repellency tests of these essential oils were evaluated against adults of *Rhyzopertha dominica* (F.) (the principal pest of wheat). The major components found in MPE are pulegone (70.4%), neo-menthol (13.4%), neo-menthol acetate (3.5%) and menthone (2.7%). On the other hand, MRE provided *trans*-piperitone epoxide (30.2%), piperitone oxide (8.7%), thymol (4.5%), germacrene D (3.5%) and terpinen-4-ol (2.7%) as major ingredients. MRE exhibited stronger antimicrobial effect and antioxidant activity in the KRL test than MPE. In the contact assay, DL50 values of MRE and MPE were 3.3 and 6.9 μL/mL, respectively. Fumigant toxicity assay of MPE and MRE showed mortality ratio of 39.2 and 44.3%, respectively at the dose of 2 μL/mL. Moreover, at this dose and after 30 min exposure time, the repellent effect showed death rates of 46.03% and 47.54% for MPE and MRE, respectively. As conclusion, MPE and MRE are potential alternatives to chemical additives in food and pharmaceutical industries.

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1. Introduction

The species of the genus *Mentha* are cultivated in several countries of the world for essential oil production (Sutour et al., 2010; Ladjel et al., 2011). According to the flora of Algeria, this genus is represented by five major species: *Mirabilis rotundifolia*, *Mentha longifolia*, *Mentha* spicata, *Mentha aquatica* and *Mentha pulegium* (Quezel and Santa, 1962).

M. pulegium L. (MP) (Pennyroyal) known in Algeria as *Fliou*, is one of the most frequently used herb. The essential oils and aerial

parts of the plant are widely used in traditional medicine mainly for the treatment of various digestive tract diseases such as flatulence, dyspepsia and intestinal colic. Besides, the plant is used in gastronomy as culinary herb, in fragrance and pharmaceutical industries (Brahmi et al., 2014a).

M. rotundifolia (L.) Huds (MR) commonly known as 'apple mint' is a wild growing perennial, herbaceous plant. It is widely distributed in North Algeria in sub-humid areas, along rivers in plains and mountains where it is known as "Timija or Timarssat" (Brada et al., 2006). Although according to Lawrence (2007) *Mentha suaveolens* Ehrh. is the synonym of *Mentha rotundifolia* (L.) Huds, the latter is nowadays known as a hybrid of *M. longifolia* and *M. suaveolens* (Sutour et al., 2010).
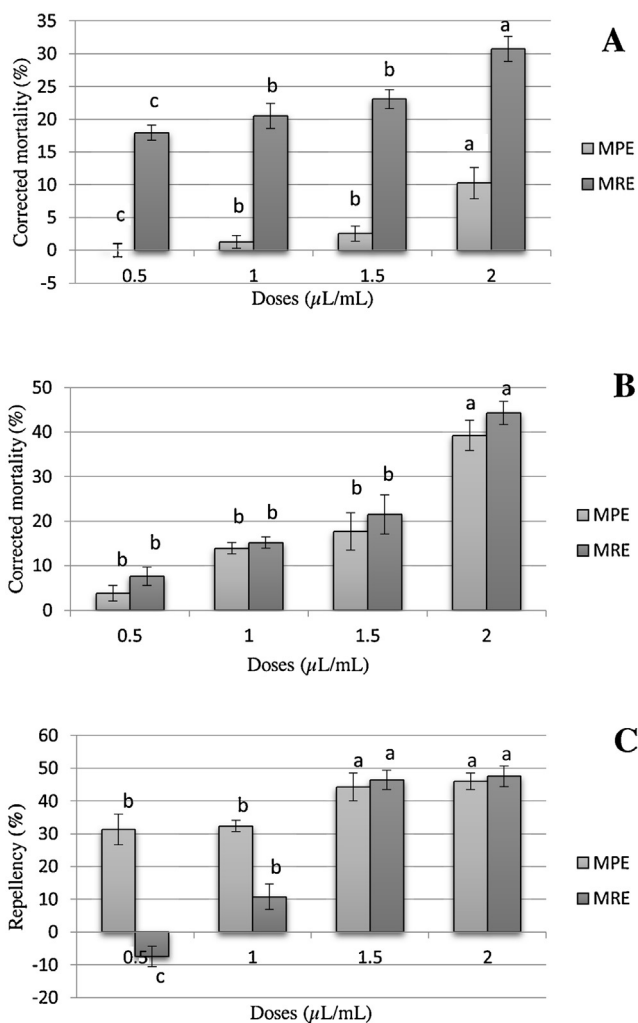
Fig. 1. Insecticidal activity of *M. pulegium* essential oil (MPE) and *M.rotundifolia* essential oil (MRE) against *Rhyzopertha dominica* (F.) adults. Percentage mortality after 96 h of treatment (A) in contact (B) fumigation and (C) repellency bioassays. Each data point represents mean ± SD of four replicates, each comprising 20 adult insects. Data was analyzed using one-way ANOVA (analysis of variance). Different letters above the bars for the same concentration indicates significant differences among means of treatments ($P < 0.05$).

In Algeria *M. rotundifolia* is widely used e.g. leaf decoction is made for topical application to treat furunculosis and abscesses, to reduce fever and as a mouthwash for dental pains (Brahmi et al., 2014b). In addition, the plant is reported to treat bronchitis, cough, and ulcerative colitis. It is also taken as tonic, used as stimulant, stomachic, carminative, analgesic, choleritic, antispasmodic, sedative, and hypotensive as well as a common spice (Ladjel et al., 2011).

Scientific studies on both MP and MR in different parts of the world focused mainly on their chemical composition. Moreover, reports on essential oils of Algerian *Mentha* species described particularly their composition (Brada et al., 2006, 2007; Beghidja et al., 2007; Seridi et al., 2007). Essential oil composition among species of the genus *Mentha* demonstrated chemical diversity due to the geographical environmental factors (Beghidja et al., 2007; Brada et al., 2007; Hussain et al., 2010; Kumar et al., 2011; Baser et al., 2012; Sitzmann et al., 2014; Kasrati et al., 2015).

Literature search reveals a few studies regarding biological activities of MPE and MRE conducted on plants collected outside Algeria. These include; antioxidant activity (Cherrat et al., 2014; Sitzmann et al., 2014; Ouakouak et al., 2015), antimicrobial properties (Cherrat et al., 2014), insecticidal and nematicidal activities (Oka et al., 2000; Kasrati et al., 2015; Zekri et al., 2015).

There is little information regarding the biological properties of Algerian MRE and MPE namely, the antibacterial effect (Mohamed and Eddine, 2010; Boukhebti et al., 2011; Ladjel et al., 2011), and the antioxidant activity using only DPPH assay of MPE collected from South East of Algeria (Ouakouak et al., 2015). Despite the fact that, plant essential oils possess insecticidal activity, both MPE and MRE had never been tested for this bioactivity.

Goals of this work were to chemically and biologically investigate the Algerian species *M. pulegium* L. and *M. rotundifolia* (L.) Huds collected from Kabylie mountains (Bejaia) regarding the following ; (i) the chemical composition of their essential oils using GC/MS, (ii) the *in vitro* antioxidant activity using two different methods viz., 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) test and Kit Radicaux Libres® (KRL) biological assay, (iii) antimicrobial effects against pathogenic microorganisms and, (iv) insecticidal activities against the lesser grain borer *Rhyzopertha dominica* (F.).

2. Material and methods

2.1. Plant material and extraction of essential oils

The leaves of *M. pulegium* L. (MP) and *M. rotundifolia* (L.) Huds (MR) were collected in March 2013 from Smaoun Algerian locality (Bejaia, Algeria). Prepared herbaria were identified by S. Bachir, a botanist at University of Bejaia, Algeria by comparing with voucher specimens previously deposited in the Herbarium of the

National Botanical Garden of Meise (Belgium) and referenced as BR 0000006946043 for *M. pulegium* L. and BR 000000 6946197 for *M. rotundifolia* (L.) Huds.

The leaves of MP and MR were shade dried at room temperature with ventilation then subjected to hydrodistillation with Clevenger-type apparatus. The obtained oils were stored in sealed glass vials in a refrigerator at 4 °C.

2.2. Essential oils analysis

Analytical GC was carried out in a gas chromatograph (Agilent, Model 7890A, PaloAlto, CA), equipped with a flame ionization detector (FID), an autosampler (Agilent, Model 7683B), Agilent HP5 fused silica column (5% phenyl-methylpolysiloxane), 30 m × 0.25 mm i.d., film thickness 0.25 μm and a Agilent ChemStation software system.

The analysis of the essential oils was done according to the method of Piras et al. (2013) employing the following GC conditions: programmed heating from 60 to 250 °C at 3 °C/min followed by 20 min under isothermal conditions. The injector was maintained at 250 °C. Helium was the carrier gas at 1.0 mL/min; the sample (1 μL) was injected in the split mode (1:10). The detectors temperature was 300 °C.

The GC was fitted with a quadrupole mass spectrometer, MS, Agilent model 5973 detector. MS conditions were as follows: ionization energy 70 eV, electronic impact ion source temperature 200 °C, quadrupole temperature 150 °C, scan rate 3.2 scan/s, mass range 30–480 u.

Software adopted to handle mass spectra and chromatograms was a ChemStation. Samples were run in hexane with a dilution ratio of 1:100. Compounds were identified by comparison of their mass spectra with those of NIST02 library data of the GC/MS system and Adams libraries spectra (NIST/EPA/NIH, 2002; Adams, 2007). The results were further confirmed by comparison with the compounds elution order with their retention indices on semi-polar phases reported in the literature (Adams, 2007). Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes (2 standard mix C8–C20 and C21–C40) (Sigma) with linear interpolation (Van Den Doul and Kratz, 1963).

Percentage of individual components was calculated based on GC peak areas without FID response factor correction.

2.3. Antioxidant activity

The antioxidant activity of the MRE and MPE using the radical scavenging capacity of the 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic) acid radical cation (ABTS•+) was determined as the reported method of Brahmi et al. (2015). An ABTS•+ reduction assay, is useful in assessing antioxidant activity of samples in different assay media, another advantage of ABTS+ radical method is the rapid sample reaction with ABTS+ (Jayaprakasha et al., 2008). 50% inhibition (IC$_{50}$) was calculated and the results were expressed as gallic acid or Trolox® equivalents. The antioxidant defense was studied by *in vitro* "Kit Radicaux Libres"® (KRL) biological assay as depicted by Brahmi et al. (2016). This assay allows the evaluation of red blood cell resistance against the free radicals induced by 2,2′-azobis (2-amidinopropane) hydrochloride (AAPH) that acts by producing peroxyl radicals, which induce lipid and protein peroxidations in the cell membrane (Rossi et al., 2009).

2.4. Antimicrobial activity

Evaluation of the antimicrobial effects of MPE and MRE was adopted according to the method previously described by Brahmi et al. (2016) using disc-diffusion (DM) and spot methods (SM). The nine tested microorganisms included six bacterial strains namely; Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *S. aureus* NCCB 9163, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeroginosa* ATCC 27853 and *Klebsiella pneumoniae* E47 and three fungi namely; *Aspergillus niger* 2CA 936, *Aspergillus flavus* NRRL 391 and *Candida albicans* ATCC 1024 strain.

2.5. Insecticidal activity

Insecticidal activity against the lesser grain borer *R. dominica* (F.) was accomplished by evaluation of the mortality effect with contact toxicity and fumigation bioassays (Brahmi et al., 2016). The essential oils were also tested for repellence on filter paper according to the method described by Conti et al. (2011). Based on the mean repellency value, the insecticidal activity was classified according the scale suggested by Juliana and Su (1983) i.e. class 0 (PR = 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (PR = 40.1–60%), class IV (PR = 60.1–80%) and class V (PR = 80.1–100%).

2.6. Statistical analysis

Dose-response relationship and the Lethal Dose (LD$_{50}$) values of the essential oils were determined by probit analysis (Finney, 1971). Mortality data were corrected using Abbott's formula (Abbott, 1925).

All experiments were conducted in triplicates and results expressed as mean ± standard deviation (SD). Data were subjected to analysis of variance using Statistica (Statsoft, 1998), and means were compared by least significant difference (LSD). Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

3.1. Chemical composition of the essential oils

Essential oil yield was higher for leaves of MP (1.14 g/100 g) compared to leaves of MR (0.49 g/100 g). The amount of essential oil obtained from MP was even higher compared to previous works i.e. 0.60% (Aghel et al., 2004), 0.65%, (Kamkar et al., 2010) and 0.27%. (Mahboubi and Haghi, 2008). However, aerial parts of MP collected from South East Algeria (Reguiba, El-Oued) yielded 2.34% (*v/w*) essential oil (Ouakouak et al., 2015). In case of MR, the yield of essential oils from leaves of other locations of Algeria (Rouina and Meliana) gave 1.6–1.8% (Brada et al. (2007), and MR harvested before flowering in France yielded essential oil of about 0.13%. Reported MRE and MPE yields of plants collected from Morocco were in higher amounts i.e. 4.33% and 2.33% respectively (Derwich et al., 2010), whereas the yield obtained from different wild Morrocan *M. suaveolens* ranged from 0.20 ± 0.02 to 1.17 ± 0.25% (*v/w*) (Kasrati et al., 2015). Another study reported yields of 4.33% for *M. rotundifolia* and 2.33% for *M. pulegium* (Benayad et al., 2012). Variation in the essential oil contents depends not only on temperature, relative humidity, but also on the duration of sunshine, air movement and rainfall (Boukhebti et al., 2011).

Water-distilled essential oils of *M. pulegium* (MPE) and *M. rotundifolia* (MRE) leaves were analyzed by GC and GC/MS. Analysis of MPE afforded twenty eight components constituting 98.5% of the total composition. A cyclic monoterpene, pulegone (70.4%) was identified as the major component. Other important compounds include; neo-menthol (13.4%), neo-menthol acetate (3.5%) and menthone (2.7%). Regarding the analysis of MRE, fifty three compounds were identified and the main constituents were *trans*-piperitone epoxide (30.2%), piperitone oxide (8.7%), thymol (4.5%), germacrene D (3.5%), terpinen-4-ol (2.7%), β-pinene (1.9%) and *trans*-calamenene (1.8%) (Tables 1 and 2).

Table 1
Chemical composition of *Mentha pulegium* L. essential oil from Algeria.

No.	I_R[a]	Tr (min)	Compound	%	Identification
1	932	5.2	α-Pinene	0.4	MS, I_R
2	972	6.2	Sabinene	0.1	MS, I_R
3	976	6.3	β-Pinene	0.2	MS, I_R
4	985	6.5	3-Octanone	0.3	MS, I_R
5	994	6.8	3-Octanol	0.7	MS, I_R
6	1027	7.9	Limonene	0.9	MS, I_R
7	1029	7.9	1,8-Cineol	0.1	MS, I_R
8	1068	9.3	*p*-Mentha-3,8-diene	0.1	MS, I_R
9	1123	11.3	3-Octanol acetate	0.1	MS, I_R
10	1146	12.2	n.i.	1.0	
11	1151	12.4	Menthone	**2.7**	MS, I_R
12	1154	12.5	iso-Menthone	0.5	MS, I_R
13	1164	12.9	Neo-Menthol	**13.4**	MS, I_R
14	1174	13.3	*trans*-Isopulegone	1.0	MS, I_R
15	1216	15.0	n.i.	0.2	MS, I_R
16	1237	15.9	Pulegone	**70.4**	MS, I_R
17	1253	16.5	Piperitone	0.2	MS, I_R
18	1274	17.4	Neomenthol acetate	**3.5**	MS, I_R
19	1288	18.0	n.i.	0.3	
20	1294	18.3	Menthyl acetate	0.1	MS, I_R
21	1307	18.8	iso-Methyl acetate	0.1	MS, I_R
22	1339	20.1	Piperitenone	0.2	MS, I_R
23	1417	23.3	β-Caryophyllene	0.5	MS, I_R
24	1451	24.7	α-Humulene	1.0	MS, I_R
25	1479	25.8	Germacrene D	0.2	MS, I_R
26	1579	29.8	Caryophyllene oxide	0.8	MS, I_R
27	1595	30.4	n.i.	0.1	
28	1606	30.7	Humulene epoxide II	1.1	MS, I_R
Grouped components (%)					
Total monoterpene hydrocarbons				1.7	
Total sesquiterpene hydrocarbons				1.7	
Total oxygenated monoterpenes				92.3	
Total oxygenated sesquiterpenes				1.9	
Other components				1.0	
Total identified				98.5	

N.i.: not identified.
The major compounds are marked in bold.

[a] I_R, correspond to Linear Retention Index as described by Van Den Dool and Kratz (1963).

Lawrence (2007) reported the main constituents of pennyroyal oil as pulegone and other 3-oxo-*p*-menthanes. According to Kumar et al. (2011), the essential oil of *M. pulegium* L. (pennyroyal oil) from various countries contains pulegone as the main constituent, and the percentage of which is ranging from 10 to 90%. Thus, previous studies on MP harvested in Turkey, Uruguay, Iran, Bulgaria, Egypt, Spain, Portugal, Tunisia, Greece, Morocco, Iran, India, Algeria (Setif), North Eastern of Morocco, North Morocco and South East Algeria (Reguiba, El-Oued) reported pulegone as the major component of the essential oil, but in different proportions (Baser et al., 1999; Lorenzo et al., 2002; Aghel et al., 2004; Agnihotri et al., 2005; Stoyanova et al., 2005; El-Ghorab, 2006; Diaz-Maroto et al., 2007; Mata et al., 2007; Hajlaoui et al., 2009; Petrakis et al., 2009; Kamkar et al., 2010; Boukhebti et al., 2011; Ait-Ouazzou et al., 2012; Cherrat et al., 2014; Ouakouak et al., 2015). The oil from MP grown in different areas of the East of Algeria studied by Beghidja et al. (2007) was mostly related to the pulegone type (43.3–87.3% yield of pulegone). Additionally, the studies on the essential oils of *Mentha* species from Marmara region of Turkey revealed that nine samples of MP had contained pulegone as the main constituent (Baser et al., 2012).

Pulegone is naturally occurring organic compound commonly found in the essential oil of *Mentha* species. It is used as flavouring agent, in perfumery and aromatherapy (Kumar et al., 2011). The essential oil of MP in our study had a composition similar to the previously analyzed samples; the main differences were in the relative quantities of individual compounds. Although pulegone is reported as a major constituent in many studies, of MPE analysis, some studies come out with different major constituents including; menthone (Teixeira et al., 2012), menthol (Marzouk et al., 2008),

piperitone (Zwaving and Smith, 1971), piperitenone (Kokkini et al., 2002) and isomenthone (Baser et al., 1999). Possible exaplanation for such differences could be associated with the geographical source collection, seasons or environmental growing conditions (Boukhebti et al., 2011).

The various chemotypes of MR are clearly identified and had shown an oxygenated menthane derivative as the main component present in almost all chemotypes (Lawrence, 2007). This fact is in agreement with the results obtained from various studies on MRE chemical compositions from different parts of the world with different principal constituents ; pulegone (El Arch et al., 2003; Sutour et al., 2008; Riahi et al., 2013), carvone (Koyalta et al., 1993), menthol (Derwich et al., 2010), piperitol (Perez-Raya et al., 1990), piperitenone oxide (Oumzil et al., 2002; Brada et al., 2006, 2007; Sutour et al., 2010; Baser et al., 2012; Sitzmann et al., 2014), *trans*-piperitone oxide (Umemoto et al., 1994), lippione (Koyalta et al., 1993), *cis-cis-p*-menthenolide (Sutour et al., 2008), 2,4(8), 6-*p*-menthatrien-2,3-diol and germacrene (Pino et al., 1999).

Kasrati et al. (2015) divided the oil samples of *M. suavelens* into four main groups. The essential oils of group I were further classified into three subgroups. The first subgroup was rich in piperitenone oxide, *trans*-piperitone epoxide and germacrene D. The second subgroup provided oil with high levels of piperitenone oxide, *cis*-piperitone epoxide and piperitenone. The third subgroup was characterized by an oil with high contents of piperitenone oxide, pulegone and menthone. In the essential oils of group II, the dominant compounds were *cis*-piperitone epoxide and germacrene D. The essential oils of group III were characterized by high contents of piperitone, piperitenone oxide and germacrene D. The

Table 2
Chemical composition of *Mentha rotundifolia* (L.) Huds essential oil from Algeria.

No.	R$_I$[a]	Tr (min)	Compound	%	Identification
1	925	5.1	α-Thujene	0.1	MS, I$_R$
2	932	5.2	α-Pinene	0.7	MS, I$_R$
3	947	5.6	Camphene	0.2	MS, I$_R$
4	972	6.2	Sabinene	0.2	MS, I$_R$
5	976	6.3	β-Pinene	1.9	MS, I$_R$
6	990	6.7	Myrcene	0.4	MS, I$_R$
7	996	6.8	3-Octanol	0.1	MS, I$_R$
8	1016	7.5	α-Terpinne	0.5	MS, I$_R$
9	1023	7.7	o-Cymene	0.1	MS, I$_R$
10	1027	7.9	Limonene	0.8	MS, I$_R$
11	1029	7.9	1,8-Cineole	0.1	MS, I$_R$
12	1035	8.1	(Z)-β-Ocimene	1.1	MS, I$_R$
13	1046	8.5	(E)-β-Ocimene	0.1	MS, I$_R$
14	1057	8.9	γ-Terpinene	0.8	MS, I$_R$
15	1065	9.1	cis-Sabinene hydrate	0.1	MS, I$_R$
16	1088	9.9	Terpinolene	0.2	MS, I$_R$
17	1100	10.3	Linalool	0.1	MS, I$_R$
18	1112	10.8	1-Octen-3-yl acetate	0.5	MS, I$_R$
19	1123	11.3	3-Octanol acetate	0.1	MS, I$_R$
20	1134	11.7	n.i.	0.1	
21	1163	12.9	Borneol	1.0	MS, I$_R$
22	1176	13.4	Terpinen-4-ol	**2.7**	MS, I$_R$
23	1184	13.7	p-Cymen-8-ol	0.2	MS, I$_R$
24	1190	13.9	α-Terpineol	0.1	MS, I$_R$
25	1215	15.0	Coahuilensol, methylether	0.3	MS, I$_R$
26	1227	15.5	n.i.	0.3	
27	1238	15.9	Pulegone	0.1	MS, I$_R$
28	1255	16.6	trans-Piperitone epoxide	**30.2**	MS, I$_R$
29	1261	16.9	n.i.	0.1	
30	1285	17.9	Bornylacetate	0.2	MS, I$_R$
31	1286	17.9	n.i.	0.2	
32	1292	18.2	Thymol	**4.5**	MS, I$_R$
33	1299	18.5	Carvacrol	0.6	MS, I$_R$
34	1302	18.6	n.i.	0.6	
35	1339	20.2	Piperitone	0.2	MS, I$_R$
36	1357	20.9	Eugenol	0.2	MS, I$_R$
37	1365	21.2	Piperitone oxide	**8.7**	MS, I$_R$
38	1374	21.6	α-Copaene	0.2	MS, I$_R$
39	1383	22.0	α-Bourbonene	0.1	MS, I$_R$
40	1391	22.3	β-Elemene		
41	1397	22.6	Z-Jasmone		
42	1404	22.8	n.i.		
43	1411	23.1	n.i.		
44	1417	23.4	β-Caryophyllene		
45	1434	24.0	n.i.	0.2	
46	1444	24.5	cis-Muurola-3,5-diene	0.4	MS, I$_R$
47	1451	24.7	α-Humulene	0.6	MS, I$_R$
48	1461	25.1	cis-Muurola-4(14), 5-diene	1.7	MS, I$_R$
49	1471	25.5	n.i.	5.9	
50	1475	25.7	γ-Muurolene	0.2	MS, I$_R$
51	1479	25.8	Germacrene D	3.5	MS, I$_R$
52	1512	27.1	γ-Cadinene	0.4	MS, I$_R$
53	1520	27.5	trans-Calamenene	1.8	MS, I$_R$
54	1535	28.1	α-Cadinene	0.3	MS, I$_R$
55	1552	28.7	n.i.	0.2	
56	1570	29.4	n.i.	2.3	
57	1574	29.6	Spathulenol	0.5	MS, I$_R$
58	1580	29.8	Caryophyllene oxide	1.3	MS, I$_R$
59	1588	30.1	Globulol	0.5	MS, I$_R$
60	1609	30.9	β-Atlantol	0.2	MS, I$_R$
61	1612	31.0	1,10-di-epi-Cubenol	1.2	MS, I$_R$
62	1638	31.9	n.i.	1.1	
63	1644	32.2	n.i.	0.2	
64	1652	32.4	t-Cadinol	1.3	MS, I$_R$
65	1676	33.3	n.i.	0.4	MS, I$_R$
66	1683	33.6	Eudesma-4(15), 7-dien-1-β-ol	0.6	MS, I$_R$

Grouped components (%)

Total monoterpene hydrocarbons	6,92
Total sesquiterpene hydrocarbons	12,88
Total oxygenated monoterpenes	49,78
Total oxygenated sesquiterpenes	5,68
Other hydrocarbons	1,69
Total identified	76,93

N.i.: not identified.

[a] I$_R$, correspond to Linear Retention Index as described by Van Den Dool and Kratz (1963).

Table 3
Antioxidant activities of Algerian *Mentha pulegium* and *Mentha rotundifolia* essential oils.

	KRL assay					
	Augmentation of control blood half-haemolysis time (%)		Equivalent Trolox® mg/g of oil		Equivalent gallic acid mg/g of oil	
Concentrations	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
MPE	–	–	–	–	–	–
MRE	PE	58.8 ± 1.7	PE	586.1 ± 17.4	PE	258.5 ± 7.4
	ABTS assay					
	IC_{50} (µg/mL)		Trolox® equivalent (%)		Gallic acid equivalent (%)	
MPE	57.4 ± 1.9		15.4 ± 0.5		5.2 ± 0.2	
MRE	138.2 ± 8.9		6.4 ± 0.4		2.2 ± 0.1	

MPE: *Mentha pulegium* essential oil; MRE: *Mentha rotundifolia* essential oil; PE: pro-oxidant effect, –: no activity.

fourth group had oils with a chemical composition dominated by menthone associated with pulegone and isomenthone.

In our study, the major compound of MRE was *trans*-piperitone epoxide (30.2%), this is not in agreement with the previous study of MRE from Meliana and Rouina (Algeria) that showed piperitenone oxide (23.5–38.6%) and *cis*-piperitone oxide (28.1–30.5%) as major constituents and less than 0.67% thymol (Brada et al., 2006) as opposed to 4.5% in the present study. This was expected since the composition of the essential oil varies with season, location, climate, soil type, age of the leaves, fertility regime and the method used for drying the plant material and oil extraction (Singh et al., 2012).

3.2. Antioxidant activity

The *in vitro* antioxidant activity of the essential oils of MP and MR was investigated using two different methods (i) an ABTS•+ reduction assay and (ii) the KRL "Kit Radicaux Libres"® biological test. A moderate antioxidant activity was obtained to scavenge the ABTS•+ radical cation (Table 3). MRE ($IC_{50} = 138.2 \pm 8.9$ µg/mL; 6.4 ± 0.4% Trolox® equivalents ; 2.2 ± 0.1% gallic acid equivalents) was less active than MPE ($IC_{50} = 57.4 \pm 1.9$ µg/mL; 15.4 ± 0.5% Trolox®equivalents ; 5.2 ± 0.2% gallic acid equivalents). The antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. The most powerful scavenging compounds were reported to be the monoterpene ketones menthone and isomenthone (Yadegarinia et al., 2006), and menthone was among the main components identified in MPE. In many cases, minor compounds of essential oils are likely to play a significant role for the antioxidant activity instead of the major compounds (Mukazayire et al., 2011).

To the best of our knowledge, there is no report on MPE antioxidant activity using ABTS assay but, a few studies have been conducted to investigate its antioxidant potential using other test methods including; the antioxidant activity of MPE from South East of Algeria that was evaluated by DPPH free radical scavenging bioassay giving the $IC_{50} = 157$ µg/mL (Ouakouak et al., 2015), DPPH and β-carotene-linoleic acid methods in Iran (Kamkar et al., 2010), and Ferric reducing antioxidant power (FRAP), reducing power and DPPH assays in Portugal that demostrated poor activity (Teixeira et al., 2012) or by β-carotene-linoleic acid (Mata et al., 2007).

The studies on the antioxidant activity of MRE are scarce in the literature. Riahi et al. (2013) studied the antioxidant effects of the Tunisian MRE using DPPH and FRAP tests. Among *Mentha* essential oils examined by Sitzmann et al. (2014) for their antioxidant capacity in the ABTS assay, *M. suaveolens* oil showed the highest antioxidant activity, which was ascribed to the components germacrene D, piperitone oxide and piperitenone oxide.

In the antioxidant activity by "Kit Radicaux Libres"® biological assay, MRE exhibited an antioxidant capacity at 50 µg/mL (Table 3) implying that, at this concentration MRE is capable to protect blood

cells of oxidative stress. This shows the ability of MRE to cross the plasma membrane of cells to reduce the damage caused by free radical attack on erythrocytes. However, at a concentration of 100 µg/mL, MRE presented a pro-oxidant behavior. This result indicated that a high concentration of essential oil act as pro-oxidant. The major components of the *Mentha* essential oils tested in the ABTS assay by Sitzmann et al. (2014) demonstrated potential pro-oxidant effects. This phenomenon was also observed for the phenolic compounds (Jayaprakasha et al., 2008). In the previous study similar observation was found where high level of vitamin E acted as pro-oxidant (Lee et al., 2008). There was no antioxidant activity of MPE in the KRL assay (Table 3), this could be explained by its high content of monoterpene compounds (94%) that are almost ineffective as reported by Yadegarinia et al. (2006). The constituents of essential oils, often mono- and sesquiterpenes hydrocarbons, are of low molecular sizes. Hence, they are unable to cross cell membranes, and walls to affect many biochemical processes (Mukazayire et al., 2011). Since *Mentha* species essential oils contain predominantly monocyclic terpenes are not likely to enter the erythrocytes membrane. To the best of our knowledge, KRL biological test has not been formerly tested for *Mentha* species essential oils investigated in this study.

3.3. Antimicrobial activity

The results obtained by the spots method (SM) showed better activity than those obtained by disc diffusion agar method (DM). However, the variation in antimicrobial activities of *Mentha* essential oils with respect to the used methods was not statistically significant ($P < 0.05$) (Table 4).

The MPE exhibited weak inhibitory effects toward most tested bacteria, fungi and yeast and the results are in agreement with the findings of the MPE from another location of Algeria (Setif) that exhibited weak antibacterial activity (Boukhebti et al., 2011). Nevertheless, the opposite effect was observed in other studies; strong activity against *S. aureus* and *B. subtilis* (Mahboubi and Haghi, 2008), strong antimicrobial activity against 16 tested microorganisms by MPE from Tunisian plants (Hajlaoui et al., 2009), a high antibacterial activity against all tested bacterial strains from MPE of Portugal origin (Teixeira et al., 2012), the best bacteriostatic and bactericidal effect are among tested Morrocan medicinal and aromatic plants (Ait-Ouazzou et al., 2012; Cherrat et al., 2014).

Such differences can be due to distinct plant origins determining essential oil chemical composition. In previous reports, the antibacterial activity of MP has been attributed to its major compounds (Mahboubi and Haghi, 2008; Hajlaoui et al., 2009). However, minor components appear to play a significant role and it is not well known which constituents/mixtures of them are responsible for their antimicrobial activity (Ait-Ouazzou et al., 2012).

The MRE was found to be more active than MPE (Table 4). So, the variation in these antimicrobial activities of *Mentha* essential oils

Table 4
Zones of growth inhibition (mm) showing antimicrobial activity of Algerian *Mentha pulegium* and *Mentha rotundifolia* essential oils.

Microorganisms	Samples	1/1 v/v	1/2 v/v	1/5 v/v	1/10 v/v	Penicillin	Streptomycin
Disc method							
E. coli	MPE	9.0 ± 0.0[a]	8.3 ± 0.6[a]	8.0 ± 0.0[a]	7.0 ± 0.0[a]	9.0 ± 1.0	nd
ATCC 25922	MRE	11.0 ± 1.0[a]	7.6 ± 0.6[a]	7.6 ± 1.1[a]	7.3 ± 0.6[a]	nd	nd
P. aeruginosa	MPE	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	nd	20.3 ± 0.6
ATCC27853	MRE	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	nd	nd
K. pneumoniae	MPE	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	nd	36.0 ± 3.5
E47	MRE	8.0 ± 0.0[a]	7.6 ± 0.6[a]	7.3 ± 0.6[a]	6.3 ± 0.6[a]	nd	nd
MRSA	MPE	7.3 ± 0.6[b]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	20.0 ± 1.0	23.6 ± 0.6
ATCC 43300	MRE	26.0 ± 1.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	nd	nd
S. aureus	MPE	12.0 ± 0.0[b]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	45.5 ± 0.7	21.0 ± 0.0
NCCB 9163	MRE	20.0 ± 1.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	nd	nd
B. subtilis	MPE	8.6 ± 1.1[b]	8.0 ± 0.0[a]	7.6 ± 0.6[a]	7.3 ± 0.6[a]	37.3 ± 0.6	nd
ATCC6633	MRE	19.6 ± 0.6[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	nd	nd
A. flavus	MPE	8.3 ± 0.6[b]	8.0 ± 1.0[b]	8.0 ± 1.0[b]	8.0 ± 1.0[b]	nd	nd
NRRL 391	MRE	49.0 ± 0.0[a]	41.0 ± 2.6[a]	30.3 ± 3.5[a]	19.0 ± 2.0[a]	nd	nd
A. niger	MPE	7.0 ± 1.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	nd	nd
2CA 936	MRE	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	nd	nd
C. albicans	MPE	6.0 ± 0.0[b]	6.0 ± 0.0[b]	6.0 ± 0.0[b]	6.0 ± 0.0[b]	nd	nd
ATCC 1024	MRE	41.0 ± 1.7[a]	20.6 ± 2.1[a]	14.6 ± 1.5[a]	8.6 ± 1.1[a]	nd	nd
Spots method							
E. coli	MPE	10.0 ± 1.0[b]	8.6 ± 0.6[a]	8.0 ± 0.0[a]	7.6 ± 0.6[a]		
ATCC 25922	MRE	15.0 ± 1.0[a]	9.6 ± 0.6[a]	9.6 ± 0.6[a]	9.6 ± 0.6[a]		
P. aeruginosa	MPE	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]		
ATCC27853	MRE	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]		
K. pneumoniae	MPE	10.3 ± 0.6[b]	9.6 ± 0.6[b]	7.6 ± 0.6[b]	6.6 ± 0.6[b]		
E47	MRE	15.3 ± 0.6[a]	13.6 ± 0.6[a]	13.3 ± 0.6[a]	11.0 ± 1.0[a]		
MRSA	MPE	7.3 ± 0.6[b]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]		
ATCC 43300	MRE	20.0 ± 1.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]		
S. aureus	MPE	13.3 ± 0.6[b]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]		
NCCB 9163	MRE	20.6 ± 0.6[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]	7.0 ± 0.0[a]		
B. subtilis	MPE	10.0 ± 0.0[b]	8.3 ± 0.6[b]	7.6 ± 0.6[a]	7.3 ± 0.6[a]		
ATCC6633	MRE	30.0 ± 7.9[a]	23.3 ± 1.1[a]	6.0 ± 0.0[a]	6.3 ± 0.6[a]		
A. flavus	MPE	6.6 ± 1.1[b]	6.6 ± 1.1[b]	6.6 ± 0.6[b]	6.3 ± 0.6[b]		
NRRL 391	MRE	48.0 ± 0.0[a]	47.3 ± 0.6[a]	36.0 ± 1.0[a]	27.0 ± 3.6[a]		
A. niger	MPE	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]		
2CA 936	MRE	7.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]	6.0 ± 0.0[a]		
C. albicans	MPE	6.0 ± 0.0[b]	6.0 ± 0.0[b]	6.0 ± 0.0[b]	6.0 ± 0.0[b]		
ATCC 1024	MRE	45.6 ± 1.1[a]	24.6 ± 0.6[a]	18.6 ± 2.5[a]	14.3 ± 1.1[a]		

Different concentrations of the studied oils, 1/1 v/v, undiluted oils; 1/2 v/v; 1/5 v/v; 1/10 v/v, dilutions of the oils in DMSO; *MRSA*: Methicillin-resistant *Staphylococcus aureus*; *S. aureus*: *Staphylococcus aureus*; *B. subtilis*: *Bacillus subtilis*; *E. coli*: *Escherichia coli*; *P. aeroginosa*: *Pseudomonas aeroginosa*; *K. pneumonia*: *Klebsiella pneumonia*; *A. niger*: *Aspergillus niger*; *A. flavus*: *Aspergillus flavus NRRL*; *C. albicans*: *Candida albicans*; SM: spots method; DM: disc method; nd: not determined. Results are means of three different assays; letters a and b designed significant difference at *P* < 0.05.

with respect to species was statistically significant (*P* < 0.05). MRE showed good to excellent antimicrobial activities against some microorganisms tested where, *A. flavus* being the most sensitive microorganism with the MCF value of 0.125 mg/mL followed by *C. albicans*. In accordance with our observation, is the high antifungal activity of MRE from Morocco (El Arch et al., 2003). Thus, MRE can be considered for the treatment of the infections caused by *C. albicans* and *A. flavus*.

The MRE is consisted mainly of oxygenated monoterpenes (49.78%), whereas monoterpene hydrocarbons were weakly represented (6.97%). It seems that the antifungal activity of essential oil is related to its chemical structure, where the oxygenated terpenes possess significant activity while the hydrocarbon monoterpenes had the lowest one (Sokovic et al., 2009). This is probably due to their limited hydrogen binding capacity and water solubility. MRE showed promising antifungal activity compared with that of MPE. This could be due to the difference of their major compounds. MRE contains thymol at a concentration of 4.5% which is known for its strong antifungal activity (Sokovic et al., 2009).

It was believed that the phenolic components of essential oils, such as thymol showed the strongest antimicrobial activity, followed by aldehyde, ketones and alcohols (Boukhebti et al., 2011). It seems that the phenol components may interfere with cell wall

enzymes such as chitin synthase/chitinase as well as with the α- and β-glucanases of the fungus (Sokovic et al., 2009).

MRE possess appreciable antibacterial activity against Gram-positive bacteria: MRSA (DM:26.0 ± 1.0, SM:20.6 ± 0.6 mm), *S. aureus* (20.0 ± 1.0 in the two methods) and *B. subtilis* (DM:19.6 ± 0.6, SM: 30.0 ± 7.9 mm). The growth inhibition was obtained by the undiluted essential oils. MRE inactivity against Gram-negative bacteria could be explained by the fact that, they have an external lipopolysaccharide wall surrounding the peptidoglycan cell wall, which limits access of these compounds (Riahi et al., 2013). On the contrary MRE of plants collected from South East of Algeria and Tunisia exhibited activity especially against *E. coli* (Ladjel et al., 2011; Riahi et al., 2013). The possible explanation for the activity differences could be associated with oil chemical compositions.

3.4. Insecticidal activity

The use of naturally occurring plant materials to protect agricultural products against various insect pests is an old-age practice in different countries of the world (Nikpay, 2006). The lesser grain borer *R. dominica* (F.) (Coleoptera : Bostrychidae) is one of the most destructive beetle species of stored grain world-wide. Interesting pesticidal activity of *Mentha* species essential oils includes, insec-

ticidal potential and nematocidal activity against the root-knot nematode (Benayad et al., 2012; Oka et al., 2000). Out team investigated on the fumigant and contact toxicities, and repellency of MPE and MRE against *R. dominica* (F.)

3.4.1. Contact toxicity

In the contact toxicity bioassay, MPE and MRE resulted in mortality of *R. dominica* within 96 h of exposure compared to untreated controls. Dead insects from oil treated grains showed signs of rapid immobilization with their legs flexed and clinging to either the grain or the surface of containers. MRE showed the strong activity in this assay. Differences in the observed activity against *R. dominica* are due to different composition of the oils used in the two studies. However, with the highest concentration used (2.0 μL/mL) the mortality percentage did not exceed 30.7% (Fig. 1A). The corresponding DL_{50} for MPE and MRE were 6.95 and 3.32 μL/mL, respectively, these are moderate activities compared to Morrocan oils, where 3 μL of MRE showed an acute toxicity causing the mortality of 85% in the first day of treatment to 100% in the second day (Benayad et al., 2012). Supporting our results is the interesting contact activity against *R. dominica* obtained from essential oil due to its major components; 1,8-cineole and carvone (Benyoussef et al., 2006).

Previous studies reported that MPE was very toxic in the first 24 h in a contact toxicity bioassay, against *R. dominica* (Benayad et al., 2012). Additionally, literature data shows the interesting potential of MPE in controlling *Mayetiola destructor*, the major pest of wheat in Morocco (Lamiri et al., 2001).

3.4.2. Fumigant toxicity

Fumigation is generally used in case of stored grain insects. Essential oils have shown interesting fumigant properties, due to high volatility and low toxicity to warm blood animals (Kumar et al., 2011). Our study demostrated that the fumigant activity variation is due to the plant species, oil concentration and exposure duration. MRE was more toxic at all tested concentrations. However, statistical analysis showed no significant difference at $P < 0.05$ between the two oils. There was also no difference between concentrations at 0.5, 1.0 and 1.5 μL/mL. The highest concentration (2.0 μL/mL) showed the best fumigant effect. After 96 h of treatment, MRE fumigant activity (44.3%) was slightly higher compared to MPE (39.2%) at the same dose (Fig. 1B).

To the best of our knowledge, this is the first report on the MRE and MPE fumigant effect against *R. dominica*. Although, fumigation activity of essential oils of *Mentha* species has been investigated against several storage pests such as efficacy of *M. arvensis* L. oil against *Sitophilus oryzae* (Lee et al., 2001), complete inhibition of *S. oryzae* and *Tribolium castaneum* (Varma and Dubey, 2001); activity of *M. × piperita* L. than *M. spicata* L. against *T. Castaneum* (Lee et al., 2002). Also, essential oil of *M. microphylla* K. Koch. gave remarkable fumigation activity against *T. castaneum* and *S. oryzae* (Mohamed and Abdelgaleil, 2008).

In another study toxicity of the essential oils extracted from the aerial parts collected from the ten MR populations against *T. castaneum* adults showed activity in variable degrees with the order of the mean insecticidal activity of the MRE samples correlating with their chemical composition. In both the contact and fumigation assays, the MRE samples rich in pulegone and menthone exhibited superior insecticidal activity. In the fumigation assay, the least efficiency was observed for the oil samples rich in *cis*-piperitone epoxide and germacrene D (Kasrati et al., 2015). Also, *M. suaveolens* hydrosol showed higher insecticidal activity than *M. pulegium* toward an insect pest of citrus, *Toxoptera aurantii* (Homoptera, Aphididae). There, the degree of insecticidal effect was dependent significantly on the applied doses and the age of tested aphids and this effect was associated with the chemical composition of the hydrosols (Zekri et al., 2015).

3.4.3. Repellency activity

Repellents are substances that act locally or at a distance, deterring insect/arthropod in general from flying to, landing on or biting human or animal skin. The use plants as insect repellents, particularly aromatic herbs and oils, has a long histotry in herbal folklore (Kumar et al., 2011).

In this study, repellent action was dependent upon oil concentration but there was no significant difference ($P < 0.05$) between MPE and MRE. In filter paper tests, the two essential oils at 2 μL/mL showed almost the same repellent activity against *R. dominica* adults after 30 min of exposure (Fig. 1C). Percentages of repellency, at this dose were 46.03% and 47.54% for MPE and MRE, respectively. These oils fall under repellency class III (i.e. Moderate Repulsive) according to Juliana and Su (1983). Our observations are in agreement with the repellent properties of essential oils and extracts of other *Mentha* species on other pests of Diptera species. For instance, 100% repellency of *M. longifolia* (L.) Huds essential oil was observed against *Sitophilus zeamais* (Odeyemi et al., 2008) and 85% repellency of *M. arvensis* L. oil against *C. chinensis* (Kumar et al., 2009).

The insecticidal properties of many essential oils are mainly attributed to monoterpenoids which are typically volatile and rather lipophilic compounds that can penetrate rapidly into the insects and interfere with their physiological functions (Bakkali et al., 2008). Due to high volatility, essential oils possess fumigant and gaseous action which are very important in controlling the stored-product insects.

At this juncture it is important to mention that, insecticidal activities of essential oils of *Mentha* species depend upon the type/nature of the constituents and individual concentration levels of the constituents (Kumar et al., 2011).

4. Conclusion

Of the two *Mentha* species namely; *M. pulegium* and *M. rotundifolia* collected in Bejaia from Algeria their oils were different in terms of composition and quantity. The *M. pulegium* essential oil was characterized by its high oxygenated monoterpenes (92.3%) content and the pulegone was the major component whereas, *trans*-piperitone epoxide, piperitone oxide and thymol were reported as major compounds of the essential oil of *M. rotundifolia*. The essential oils of the both plants have a moderate scavenging activity against $ABTS^{\bullet+}$ free radicals while only MRE showed activity in the KRL biological assay. *M. rotundifolia* essential oil is a potential candidate for drug development to prevent oxidative stress induced diseases.

Disc diffusion agar and spot assays adapted to evaluate antimicrobial activity showed weak inhibitory effects toward most tested bacteria, fungi and yeast for MPE compared to MRE that exhibited high activity against Gram-positive bacteria, fungi and yeast species and could serves as a drug source for treatment of several infectious diseases.

Algerian MRE and MPE demonstrated fumigant and contact toxicity properties against adults of *R. dominica* with MRE being more toxic in all assays. Both plant species are promising candidates for further investigation for developing new botanical insecticide particularly for stored-product insect pest control.

Algerian MP and MR as medicinal and aromatic plants offer interesting sources of biological active compounds such as antimicrobial and antioxidant agents, natural pesticides. To reach practical application for these essential oils in medicine and or different industries, further studies including human safety are needed.

Acknowledgments

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