



# Antifungal and Insecticidal Activities of Essential Oils of Four *Mentha* Species

Kenza Mejdoub<sup>1</sup>, Fatima Zahra Benomari<sup>2</sup>, Nassim Djabou<sup>2</sup>, Mohammed El Amine Dib<sup>3,\*</sup>, Nassira Gaouar Benyelles<sup>1</sup>, Jean Costa<sup>4</sup> and Alain Muselli<sup>4</sup>

<sup>1</sup>Laboratory of Ecology and Management of Natural Ecosystems, Faculty of Natural Sciences, Life and Science of the Earth and the Universe, University of Tlemcen, Tlemcen, Algeria

<sup>2</sup>Laboratory of Organic Chemistry, Natural Substances and Analyses (COSNA), University of Tlemcen, Tlemcen, Algeria

<sup>3</sup>Laboratory of Natural and Bioactive Substances (LASNABIO), University of Tlemcen, Tlemcen, Algeria

<sup>4</sup>Laboratoire de Chimie des Produits Naturels, Université de Corse, Campus Grimaldi, Corte, France

\*Corresponding author: Laboratory of Natural and Bioactive Substances (LASNABIO), University of Tlemcen, BP 119, 13000, Tlemcen, Algeria. Email: a\_dibdz@yahoo.fr

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## Abstract

**Background:** *Mentha* species are commonly used in traditional medicine for their several pharmacological properties. *Mentha* species are also used as spice and are known for their bactericidal, antiviral and fungicidal properties.

**Objectives:** The main objective of this work was to evaluate the antifungal activity and fumigation toxicity of essential oils of *Mentha* spicata, *M. pulegium*, *M. piperita* and *M. rotundifolia* against fungi and *Bactrocera oleae* insect responsible for olive rot.

**Methods:** Essential oils of the four *Mentha* species were extracted by a Clevenger-type apparatus. Their antifungal activity was tested using radial growth technique, and their insecticidal activity was examined by fumigant test.

**Results:** Oxygenated monoterpenes were the main components of the four *Mentha* essential oils. All the essential oils presented antifungal activity against *Aspergillus flavus*, *A. niger*, *Alternaria* spp. and *Penicillium* spp. At the highest concentration (15  $\mu$ L/mL air), essential oil of *M. pulegium* caused 100% mortality after 1.5 h of exposure. However, for *M. piperita* and *M. rotundifolia* essential oils, 25  $\mu$ L/mL air was required to have mortality of 100%.

**Conclusions:** The essential oils could act as antifungal agents and fumigants against *B. oleae*.

**Keywords:** Antifungal, Insecticidal Activities, *Mentha* Species

## 1. Background

In Algeria, olive oil production is a developing industry. Olives are infected with several soilborne fungal pathogens such as *Alternaria*, *Aspergillus* and *Penicillium* (1). *A. niger* causes many diseases called black mold on fruits and vegetables and produces potent mycotoxins called ochratoxins that can be harmful to human beings. On the other hand, *A. flavus*, *Alternaria* spp. and *Penicillium* spp. are the most dominant fungal species during postharvest storage condition (2). It is known that fungal strains that occur most frequently at mild and cold temperatures affect fruits. Furthermore, many olives are attacked mainly by *Bactrocera oleae* insect that is considered to be a serious threat to olive production in the Mediterranean region.

Chemical fungicides are widely used to control phytopathogenic fungi; nevertheless, the use of these types of compounds represents a concern associated with the risk of exposure and environmental hazards; therefore, new alternatives are needed (3). The genus *Mentha* belongs to

Lamiaceae family and includes 25 species of herbaceous perennials. Mints are distributed predominantly in the temperate regions of the world and have varied growth characteristics, and aromas. Many *Mentha* species are used in traditional folk medicine for its stimulant, carminative, antispasmodic, stomachic and diuretic proprieties (4).

Many mint species are grown for commercial purposes such as their use in food flavors, cosmetics and pharmaceuticals (4, 5). Numerous studies have been carried out on the fungicidal and insecticidal activities of mint species (6-12).

## 2. Objectives

The main objective of this study was to assess (I) the antifungal activity of four mint essential oils against several phytopathogens responsible for olive diseases, such as *A. flavus*, *A. niger*, *Alternaria* spp. and *Penicillium* spp. and (II) insecticidal activity of these four oils against *B. oleae* insect responsible for olive rot.

### 3. Methods

#### 3.1. Plant Material

The plant materials of *M. spicata*, *M. pulegium*, *M. piperita* and *M. rotundifolia* were collected from Tlemcen region (Algeria) in July 2014 during full bloom stage.

Each mint specimen was identified by Professor Noury Benabadji of University of Tlemcen (Algeria) and deposited in the Herbarium of the University with voucher specimens (*M. spicata*: MSP-0714; *M. pulegium*: MPU-0715; *M. piperita*: MPI-716 and *M. rotundifolia*: MRO-0716).

#### 3.2. Essential Oils Isolation

The aerial parts were stored at 18°C after harvest, and 550-600 g of each species was subjected to a Clevenger-type apparatus (13) for 5 h. The yields of the oils were 0.5% for *M. spicata*, 0.7% for *M. pulegium*, 0.67% for *M. piperita*, and 0.9% for *M. rotundifolia*. Before chromatographic analysis, the essential oils were dried over sodium sulfate and stored in sterilized amber glass flasks.

#### 3.3. Gas Chromatography

The gas chromatography (GC) apparatus used for the determination of retention indices was a Perkin Elmer Clarus 600 GC equipped with two flame ionization detectors (FIDs) and 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). Program conditions were temperature of 60 to 230°C at 2°C.min<sup>-1</sup> and then held isothermal at 230°C (30 min); the carrier gas was hydrogen (0.7 mL.min<sup>-1</sup>). Injector and detector temperatures were held at 280°C. Injected volume was 0.1 μL.

#### 3.4. Gas Chromatography-Mass Spectrometry

The essential oils were investigated using a Perkin Elmer TurboMass quadrupole apparatus, 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), with the same program as GC described above. Ion source temperature was 150°C and energy ionization was 70 eV; electron ionization mass spectra were acquired with a mass range of 35 - 350 Da and scan mass of 1 s. The injected oil volume was 0.1 μL.

#### 3.5. Component Identification

The different components of essential oils were identified by comparison of GC retention indices (RI), determined from retention time of a series of n alkanes with linear interpolation, with those of authentic compounds (14, 15). For this purpose, computer matching with commercial mass spectral libraries and comparison of the spectra with those of the in-house laboratory library were performed (16).

#### 3.6. Pathogenic Fungi

*Aspergillus flavus*, *A. niger*, *Alternaria* spp. and *Penicillium* spp., the four fungal isolates causing olive rot, were isolated directly from rotten olive harvested from orchards of Remchi, Ain Temouchent (Algeria). The four fungal species were transferred to sterilized Petri dishes, and 20% of lactic acid was added to the middle to stop the growth of bacteria. The plates were incubated at 25 ± 2°C for eight days away from light. Strains identification was firstly based on morphological characters and secondly on microscopic observations according the following references (17, 18).

#### 3.7. In Vitro Antifungal Activity

The radial growth technique was used for testing the antifungal activity of essential oils (18). The concentrations varying from 0.1 to 300 mL/L used in the in vitro tests were obtained from stock solutions. For this purpose, appropriate volumes of essential oils were dissolved in dimethyl sulfoxide (DMSO) and added to Potato Dextrose Agar (PDA) medium immediately before it was poured into the Petri dishes of 9.0 cm diameter at 40°C - 45°C. The controls were prepared with DMSO mixed with PDA (without essential oils). The mycelial discs were filled with plant pathogenic fungi taken from 7-day-old cultures on PDA plates, and then they were transferred aseptically to the center of Petri dishes and incubated. This process was performed in triplicate.

The treatments were incubated at 27°C in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. The half maximal inhibitory concentration (IC<sub>50</sub>) and the minimum inhibitory concentration (MIC) were determined at 95% confidence intervals (19) using Probit analysis.

#### 3.8. Fumigation Toxicity of Essential Oils Against *Bactrocera oleae*

To determine the fumigant toxicity of essential oils, appropriate concentrations were applied separately on filter papers (Whatman No. 1, 2 cm diameter) to achieve the concentrations of 8 to 65 mL/L air without using any solvent, and the filter papers were attached to the under surface of plastic jar lids at 50-ml volumes. The control sets received no oil. The lids were screwed tightly on the jars containing 15 insects each, all of the same age. These were kept at a temperature of 25 - 26°C and in 80% - 85% relative humidity (19). Mortality was checked 24 h after commencement of exposure. The mortality of insects was expressed in % and calculated by using the Abbott correction formula:

$$\text{Corrected mortality} = (\text{OMT} - \text{OMC}/100 - \text{CM}) \times 100$$

OMT, observed mortality in treatment; OMC, observed mortality in control; CM, control mortality.

$$\text{Percentage mortality} = (\text{NDI}/\text{NII}) \times 100$$

NDI, number of dead insect; NI, number of insect introduced.

### 3.9. Statistical Analysis

Statistical analysis was performed by ANOVA using the SAS software. The means were separated using the least significant difference test at  $P \leq 0.05$ . All the tests were performed in triplicate.

## 4. Results

### 4.1. Chemical Composition of the Four Mint Species Essential Oils

A total of 29, 18, 35 and 47 compounds were identified in essential oils of *M. spicata*, *M. pulegium*, *M. piperita* and *M. rotundifolia* that accounted for 98.1%, 98.5%, 98.8% and 98.9% of the oils, respectively (Table 1). Components identification was performed by comparison of IR and GC-MS with pur compounds of Arômes library (Table 1). In the GC-MS analysis of *M. spicata* essential oil, the most prominent compounds were carvone (54.1%) and limonene (21.9%). The main compounds found in *M. pulegium* were pulegone (77.3%) and menthone (10.8%). The chemical composition of *M. piperita* essential oil was dominated by linalool (40.4%) and linalyl acetate (32.6%). Therefore, *M. rotundifolia* essential oil was characterized by an appreciable amount of menthone (28.5%) and neo-menthol (10.4%).

### 4.2. In Vitro Antifungal Activity of the Four Mint Essential Oils Against Plant Fungi

Essential oils' minimum and medium inhibitory concentrations (MIC and MIC<sub>50</sub>, respectively), as well as inhibition of the four fungi amended with the estimated MIC and MIC<sub>50</sub> of each essential oil are presented in Table 2. All the essential oils presented antifungal activity against *A. flavus*, *A. niger*, *Alternaria* spp. and *Penicillium* spp. The lowest activity was observed with essential oils of *M. piperita* and *M. rotundifolia* with MIC<sub>50</sub>s ranging from 80 to 300 mL/L and MICs from 1.2 to 25.2 mL/L. *M. spicata* and *M. pulegium* essential oils exhibited good activities compared to *M. piperita* and *M. rotundifolia* essential oils. Essential oil of *M. pulegium* was active against *A. flavus*, *A. niger*, *Alternaria* spp. and *Penicillium* spp. with IC<sub>50</sub>s of 4.2, 1.1, 1.3, and 1.1 mL/L and MICs of 0.1, 0.2, 0.08, and 0.08 mL/L, respectively. However, essential oil of *M. spicata* was more active against *Alternaria* spp. and *Penicillium* spp. with IC<sub>50</sub>s of 1.5 and 0.8 mL/L and MICs of 0.1 and 0.08 mL/L, respectively. However, essential oil exhibited moderate activity against *A. flavus* and *A. niger* with IC<sub>50</sub>s of 45 and 50 mL/L and MICs of 0.2 and 1.2 mL/L, respectively.

### 4.3. Fumigation Toxicity

The results regarding fumigation toxicity of mint essential oils against *Bactrocera oleae* are summarized in Table 3. The efficacy of essential oils varied with their concentrations. At the concentration of 10  $\mu\text{L}/\text{mL}$  air, the essential oils of *M. pulegium*, *M. piperita* and *M. rotundifolia* caused over 46% mortality after 24 h of exposure. However, *M. spicata* essential oil showed no efficacy at this concentration. At the highest concentration (15  $\mu\text{L}/\text{mL}$  air), *M. pulegium* essential oil caused 100% mortality after 1.5 h of exposure (Table 3). Nonetheless, for the *M. piperita* and *M. rotundifolia* essential oils, a concentration of 25  $\mu\text{L}/\text{mL}$  air was required to have 100% mortality.

## 5. Discussion

Chemical analysis of the four *Mentha* species essential oils showed that *M. piperita* mostly contains oxygenated monoterpenes principally dominated by monoterpene ketones such as pulegone, carvone, menthone and iso-menthone, and appreciable amounts of monoterpene alcohols such as linalool and neo-menthol. However, the chemical composition of *M. spicata* essential oil was characterized by appreciable amounts of monoterpene hydrocarbons, such as limonene and myrcene.

Essential oils from plants have attracted increasing interest as ecologically safe alternatives to fungicides and insecticides. The in vitro evaluation of antifungal properties of essential oils was performed in the present study, which showed that essential oils of the four *Mentha* species have good antifungal activity against *A. flavus*, *A. niger*, *Alternaria* spp. and *Penicillium* spp. Furthermore, in review of the fumigant toxicity results of essential oils of the four mints, it can be noticed that oils show very interesting activities. Essential oils are complex volatile mixtures. Monoterpenes and sesquiterpenes are usually the main groups of compounds that are responsible for many of their biological activities. On the basis of these results, we suggest that antifungal activity and fumigant toxicity of *Mentha* essential oils was due to their major components such as linalool, carvone, pulegone, menthone and linalyl acetate with percentages exceeding 28%.

Carvone is abundantly found in cumin, dill and spearmint. It is a natural product with strong antiseptic properties used as a mosquito repellent (20). It has been demonstrated that carvone has strong fungicidal activity against different mycotoxigenic fungi involved in several plant diseases (20). Naigre et al. (21) and Flamini et al. (22) also found that pulegone, limonene, carvone and menthone showed biocidal activity. We found that *M. pulegium* essential oil is rich in pulegone and *M. spicata* is rich in carvone and that they have significant insect antifeedant (*M. pulegium*) and nematocidal (*M. spicata*) effects (11).

**Table 2.** Minimum (MIC) and Medium (IC<sub>50</sub>) Inhibitory Concentration Values Against Radial Growth of Fungal Species Determined After Seven Days of Incubation on PDA + Tween Amended with the Essential Oils<sup>a</sup>

Treatment (mL/L)	<i>A. flavus</i>		<i>A. niger</i>		<i>Alternaria Spp.</i>		<i>Penicillium Spp.</i>	
	CMI	IC <sub>50</sub>	CMI	IC <sub>50</sub>	CMI	IC <sub>50</sub>	CMI	IC <sub>50</sub>
<i>M. spicata</i>	0.2 <sup>A</sup>	45 <sup>B</sup>	1.2 <sup>B</sup>	50 <sup>B</sup>	0.1 <sup>A</sup>	1.5 <sup>A</sup>	0.08 <sup>A</sup>	0.8 <sup>A</sup>
<i>M. pulegium</i>	0.1 <sup>A</sup>	4.2 <sup>A</sup>	0.2 <sup>A</sup>	1.1 <sup>A</sup>	0.08 <sup>A</sup>	1.3 <sup>A</sup>	0.08 <sup>A</sup>	1.1 <sup>A</sup>
<i>M. piperita</i>	1.5 <sup>B</sup>	150 <sup>D</sup>	1.2 <sup>B</sup>	150 <sup>C</sup>	1.3 <sup>B</sup>	80 <sup>B</sup>	1.2 <sup>B</sup>	150 <sup>C</sup>
<i>M. rotundifolia</i>	1.3 <sup>B</sup>	90 <sup>C</sup>	12.5 <sup>C</sup>	250 <sup>D</sup>	25.2 <sup>C</sup>	300 <sup>C</sup>	1.2 <sup>B</sup>	100 <sup>B</sup>

<sup>a</sup> Values are means from the three experiments. Different letters within a column represent significant differences (P < 0.05).

**Table 3.** Larvicidal Efficacy of *Mentha* Species Essential Oils Against *Bactrocera oleae*<sup>a</sup>

Concentrations (μL/mL air)	% Mortality ± SE			
	<i>M. spicata</i>	<i>M. pulegium</i>	<i>M. piperita</i>	<i>M. rotundifolia</i>
8	-	16.6 ± 1.2	20.2 ± 1.6	0.0 ± 0.0
10	0.0 ± 0.0	50.0 ± 2.1	66.6 ± 3.2	46.6 ± 3.2
15	40.3 ± 4.2	100.0 ± 0.0	86.5 ± 4.2	76.6 ± 5.6
25	53.3 ± 5.3	-	100.0 ± 0.0	100.0 ± 0.0
45	76.6 ± 3.5	-	-	-
65	86.6 ± 6.6	-	-	-
LC50 (μL/L air)	0.22		0.27	
LC90 (μL/L air)	0.33		0.45	

<sup>a</sup> The results are expressed as mean ± standard deviation.

We demonstrated in this study that the essential oils could act as antifungal agents and fumigants against *Bactrocera oleae*. Thus, due to their antifungal and insecticidal effects, these essential oils could be used as in fungicides and insecticides to prevent the infestation of olive products. However, further trials are necessary to devise a method for the application of essential oils in fungicides against *Bactrocera oleae*.

**Footnotes**

**Authors' Contribution:** Kenza Mejdoub: Identification of antifungal and insecticidal activities; Fatima Zohra Benomari: Analysis of essential oils and harvesting plants ; Mohammed El Amine Dib: Writing the manuscript and identifying the chemical composition of the four mint species; Nassim Djabou: Analysis of essential oils and harvesting plants; Nassira Gaouar Benyelles: Harvesting and identifying plants; Jean Costa: Director of laboratory of CPN; Alain Muselli: Identification of the chemical composition of the four mint species and correction of the manuscript.

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Table 1. *Mentha* Species Essential Oils

Compounds	IRI <sub>a</sub>	RI <sub>a</sub>	RI <sub>p</sub>	<i>M. spicata</i>	<i>M. pulegium</i>	<i>M. piperita</i>	<i>M. rotundifolia</i>
1. (E)-hex-3-en-1-ol	812	810	1360				tr
2. Ethyl-2-methyl butyrate	829	829	1016				0.1
3. (E)-2-hexenal	830	830	1210	tr		0.1	0.1
4. (Z)-hex-3-en-1-ol	831	832	1375				0.1
5. (Z)-2-hexenol	851	848	1400				tr
6. 1-hexenol	852	851	1414				tr
7. $\alpha$ -thujene	922	923	1021	0.4	0.1	tr	0.2
8. $\alpha$ -pinene	931	932	1023	0.7	0.5	0.2	0.4
9. Camphene	943	944	1066				tr
10. Oct-1-en-3-ol	959	962	1440		0.8		0.5
11. Sabinene	964	966	1118				0.2
12. $\beta$ -pinene	970	972	1108	0.7	0.2	0.3	0.4
13. Myrcene	976	982	1159	3.3	tr	1.2	1.3
14. 3-octanol	982	982	1350		0.8		0.2
15. $\gamma$ -phellandrene	997	998	1164	0.1			
16. $\alpha$ -terpinene	1008	1010	1175	0.3			0.1
17. P-cymene	1010	1012	1259	0.1			1.0
18. Limonene	1020	1021	1195	21.9	1.1		0.3
19. 1,8-cineole	1020	1021	1205		0.6	3.8	0.2
20. (Z)- $\beta$ -ocimene	1024	1025	1225			0.4	0.2
21. (E)- $\beta$ -ocimene	1034	1036	1241	0.4		0.4	tr
22. $\gamma$ -terpinene	1047	1049	1237	0.7	0.1	0.2	0.3
23. Trans-hydrate sabinene	1051	1054	1444	1.7			3.0
24. Terpinolene	1078	1080	1247	0.1		0.1	0.5
25. Linalool	1078	1075	1280	0.2	tr	40.4	
26. Cis-sabinene hydrate	1083	1082	1535	0.5			0.1
27. 1-oct-3-enyl acetate	1093	1087	1390		tr		0.1
28. 2-methyl-butyl isovalerate	1098	1096	1274			0.4	
29. Cis-p-menth-2-en-1-ol	1108	1110	1600			tr	0.1
30. 3-octyl acetate	1111	1110	1315			0.2	
31. Trans-p-menth-2-en-1-ol	1123	1126	1612			tr	tr
32. Menthone	1134	1135	1456		10.8		28.5
33. P-menth-3-en-8-ol	1135	1135	1590				3.1
34. Iso-menthone	1143	1142	1490		0.7		19.0
35. Borneol	1148	1150	1690		-		0.1
36. Neo-menthol	1156	1157	1637	0.2	1.6		10.4
37. Terpinene-4-ol	1161	1162	1583	1.3	-		2.7
38. Menthol	1164	1163	1629		tr		1.4
39. Iso-menthol	1174	1173	1660		tr		2.1
40. Z-dihydro carvone	1175	1174	1601	2.6			
41. Dihydro carveol	1178	1174	1723	tr			
42. $\alpha$ -terpineol	1179	1177	1688		tr	6.4	2.9
43. E-dihydro carvone	1180	1180	1626	3.1			
44. $\alpha$ -campholenol	1186	1188	1782				tr
45. Nerol	1211	1213	1799			1.1	
46. Pulegone	1213	1216	1640		77.3	0.1	5.6
47. Carvone	1222	1226	1739	54.1			
48. Piperitone	1232	1229	1727		0.3		1.3
49. Geraniol	1232	1234	1844			2.4	
50. Linalyl acetate	1240	1237	1557		tr	32.6	

51. Geranial	1244	1243	1731			0.2	
52. Neryl formate	1263	1266	1647			0.1	
53. Neo-menthyl acetate	1263	1268	1548		0.1		5.0
54. Bornyl acetate	1269	1268	1475				tr
55. Lavandulyl acetate	1270	1273	1593			0.1	
56. Menthyl acetate	1282	1285	1578				2.1
57. Iso-menthyl acetate	1294	1295	1594		0.1		1.8
58. Dihydro carvyl acetate	1311	1312	1661	2.2			
59. Piperitenone	1315	1313	1900	tr	2.7		1.8
60. Piperitenone oxide	1333	1335	1945	0.3			
61. $\alpha$ -terpenyl acetate	1336	1336	1678			0.1	0.1
62. Neryl acetate	1342	1345	1725	1.7		2.7	
63. Geranyl acetate	1361	1364	1725			2.5	
64. $\alpha$ -copaene	1379	1379	1475				0.1
65. $\beta$ -bourbonene	1385	1385	1515	0.3		0.1	tr
66. E- $\beta$ -caryophyllene	1424	1418	1583	0.6	0.3	0.8	0.4
67. E- $\beta$ -farnesene	1448	1447	1660			0.1	0.2
68. $\alpha$ -humulene	1456	1456	1665	0.2	0.4		
69. $\gamma$ -muurolene	1471	1469	1679			0.1	0.2
70. Germacrene D	1480	1474	1692	0.1		0.1	0.1
71. $\alpha$ -muurolene	1496	1492	1709				0.1
72. $\gamma$ -cadinene	1507	1506	1750	0.1	tr	0.2	0.2
73. Trans-calamenene	1512	1510	1810	0.1		0.2	0.1
74. $\delta$ -cadinene	1516	1515	1748	0.1	tr	0.2	0.1
75. Cadina-1,4-diene	1523	1520	1763			0.1	
76. $\alpha$ -calacorene	1531	1528	1890				0.1
77. $\alpha$ -cadinene	1535	1530	1740		tr	0.1	tr
78. $\beta$ -calacorene	1548	1546	1936				tr
79. Caryophyllene oxide	1578	1580	1980			0.3	
80. Globulol	1580	1582	2074			0.5	
<b>Total identification %</b>				98.1	98.5	98.8	98.9
<b>Hydrocarbon compounds</b>				2.7	4.8	6.5	
Monoterpene hydrocarbons				2.0	2.8	4.9	
Sesquiterpene hydrocarbons				0.7	2.0	1.6	
<b>Oxygenated compounds</b>				95.8	94.0	92.4	
Oxygenated monoterpenes				94.2	92.5	91.3	
Oxygenated sesquiterpenes				-	0.8	-	
Non-terpenic oxygenated compounds				1.6	0.7	1.1	