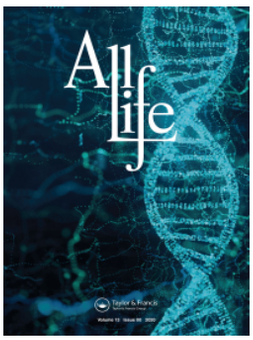

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Chemical composition of essential oils of three *Mentha* species and their antifungal activity against selected phytopathogenic and post-harvest fungi

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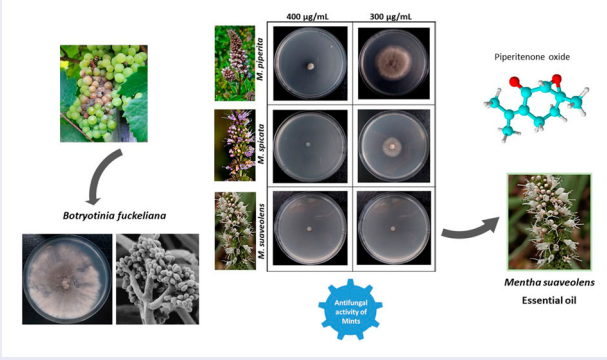
ABSTRACT

The postharvest life of most fruit, vegetables and cereals is limited by fungal proliferation. The chemical composition of *Mentha piperita*, *M. spicata* and *M. suaveolens* essential oils (EO), and the antifungal activity against four pathogenic and post-harvest fungi isolated from food, were herein investigated to evaluate their potential as natural food preservatives. The EO were obtained by hydrodistillation of aerial parts leaves, stems and inflorescences (except for peppermint oil, which was purchased in a specialized store) and submitted to GC-MS and GC-FID analysis. Regarding the EO composition, carvone (41.1%) and limonene (14.1%) were the major compounds in *M. spicata*, menthol (47.0%) and menthone (23.1%), as well as other menthol derivatives (neomenthol -3.6%- and menthofurane -3.7%-) in *M. piperita*, and piperitone oxide (40.2%) and piperitenone oxide (31.4%) in *M. suaveolens*.

Botryotinia fuckeliana was the most sensitive fungus. The three studied EO inhibited growth by 92–100%. The highest dose of *M. suaveolens* EO, 400 µg/mL, produced 100% MGI in all the studied fungi, except *Fusarium oxysporum* with 94.21%. The *M. suaveolens* EO can be considered to develop a low-risk enviro-friendly botanical biofungicide.



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Antifungal activity; essential oils; carvone; piperitenone oxide; phytopathogenic fungi

Introduction

Lamiaceae is a family with over 232 genera that include shrubby or sufruticosas plants, or perennial or annual herbs, which are sometimes ephemeral and often aromatic. Many aromatic plants used in medicine, food and pharmaceutical industries belong to the *Lamiaceae* family. *Mentha* is a well-known genus of this family and includes numerous species that generally grow in temperate areas worldwide, particularly in Europe, Asia, Africa, Australia and North America. *Mentha* spp. includes plants that exhibit relevant biological activities. It has a wide morphological variability and very high chemical diversity in relation to its their essential oils (Tucker and Naczi 2006; Stringaro et al. 2018).

Mentha essential oils (EO) have been used as a popular remedy for respiratory diseases like bronchitis, sinusitis, tuberculosis and the common cold (Peixoto et al. 2009). The *Mentha* chemistry is complex and extremely variable, and each species

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has a characteristic main compound. Additionally, the chemical composition of essential oils can vary depending on the stage of the life cycle, plant organs, geographic location, soil, and ecological factors (Llorens-Molina et al. 2017; Valadares et al. 2018; Silva et al. 2019; Diasa et al. 2020). Among the properties of the genus *Mentha* the following have been reported: antiviral, antibacterial, antifungal, high antioxidant, cytotoxic, contraceptive, anti-inflammatory and antiallergic (Sharma et al. 2014; Chávez-González et al. 2016).

The taxonomy of the genus *Mentha* is controversial and several classifications are proposed due to frequent hybridisation occurring in both wild populations and cultivation (El-Kashoury et al. 2014). Tucker (2006) describes 42 species, 15 hybrids and hundreds of subspecies, varieties and cultivars distributed in five sections. The genus is of much gastronomical, pharmacological, chemical and agricultural interest.

The EO of the species belonging to the genus *Mentha* show high chemodiversity according to their chemotypes and ecotypes (Lawrence 2006). Thus *Mentha* x *piperita* (peppermint oil) is characterised by its high menthol proportion and its derivatives, such as menthona, menthyl acetate, etc., and their isomers. Significant proportions of 1,8-cineole and menthofuran have sometimes been reported. Other compounds containing the menthane skeleton are the main components in other species: piperitone, piperitenone and their oxides (*M. suaveolens* and some chemotypes of *M. spicata, M. longifolia,* and others); carvone and related compounds like carveol and carvyl esters (*M. spicata);* pulegone, a major compound in many different *Mentha* specie, such as *M. pulegium, M. rotundofolia, M. arvensis, M. piperita*, etc. (Baser et al. 1998). It is worth mentioning that all these compounds can be found in a higher or lower proportion in many chemotypes of a consdierable number of species. It is also notworthy that the presence of certain monoterpenic compounds, such as limonene or 1,8-cineole, can also be found in high proportions.

Society's tendency to eliminate chemicals to treat diseases caused by different biological agents, consumer consumers and food safety agencies worldwide have led researchers to consider natural alternatives to control these diseases. *Mentha* sp. as antimicrobial agents contain several EO that are odorous and volatile products with a complex variable composition that is rich in biologically active molecules displaying antibacterial, antifungal, antiviral and other biological activities (Stringaro et al. 2018; Luković et al. 2019; Oliveira Filho et al. 2019; Karpiński 2020).

The postharvest life of most fruit and vegetables is limited by fungal proliferation occurring when these products are stored, and fungal appearance implies mass losses of harvest quality and yield. Fungal food contamination causes both economic and human health problems. The objectives of this study were to: determinate the composition of *Mentha piperita*, *M. spicata* and *M. Suaveolens*; evaluate its antifungal potential against four important phytopathogenic and post-harvest fungi *Verticillium dahliae*, *Fusarium oxysporum*, *Curvularia hawaiiensis* and *Botryotinia fuckeliana.*

Materials and methods

Essential oils

The essential oils of *M. spicata* and *M. suaveolens* were obtained from the plant material taken from the experimental field at the Universitat Politécnica de València. For this purpose, 300 g of air-dried (room temperature) aerial parts of both species collected in the full flowering stage were submitted to hydrodistillation with 3 L of distilled water using a Clevenger-type apparatus (Vidra Foc, SA) for 3 h. After drying with anhydrous sodium sulphate (Sigma-Aldrich), the essential oil was diluted to 2% (v/v) in dichloromethane (Sigma-Aldrich, capillary GC grade) and stored in glass vials at −18°C in the absence of light until the GC analysis.

The peppermint essential oil was purchased from Sigma-Aldrich (steam distillation). It was diluted and stored in the same way.

Gas chromatography (GC/FID)

Gas Chromatography (GC) was performed using a Perkin-Elmer Clarus 500GC apparatus equipped with a flame ionization detector (FID), and a Hewlett-Packard HP-1 (cross-linked methyl silicone) capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The column temperature programme was 60°C for 5 min, with 3°C/min increases to 180°C, and then 20°C/min increases to 280°C, which was maintained for 10 min. The carrier gas was helium applied at a flow rate of 1 mL/min. Both the FID detector and injector port temperature were 250 and 220°C,

respectively. Data were expressed as % peak normalised areas.

Gas chromatography and mass spectrometry (GC-MS)

The GC-MS analysis was carried out on a Varian Saturn 2000 equipped with a Varian C.S VA-5MS with the same capillary column. The same working conditions used for GC and split mode injection (ratio 1:25) were employed. Mass spectra were taken over the m/z 28–400 range with an ionising voltage of 70 eV. Linear retention indices were calculated using co-chromatographed standard hydrocarbons. Individual compounds were identified by MS and their identity was confirmed by comparing their LRIs to C_8-C_{25} n-alkanes, and by comparing their mass spectra and retention times with those of authentic samples or with data already available in the NIST 2005 Mass Spectral Library and the literature (Adams 2007). For quantification purpose, relative peak areas percentages from GC/FID analysis were used without considering response factors.

Fungal species

The fungal strain herein employed were:

Fusarium oxysporum lycopersici (FO) CECT 2715 isolated from tomato, *Botryotinia fuckeliana* (BF) CECT 2100 isolated from grape wine, and *Verticillium dahliae* (VD) CECT 2694 isolated from olive, were supplied by the Spanish Type Culture Collection (CECT).

Curvularia hawaiiensis (CH) (CECT 20934), which was isolated in the Botany Laboratory in the Department of Agroforest Ecosystems from the rice samples collected from the 'La Albufera' rice-producing Mediterranean Region in Valencia (Spain). The fungal species was morphologically and molecularly identified and then deposited in the CECT.

Antifungal activity

The bioassay was performed in Petri dishes (90 × 15 mm and 150 × 20 mm) with dissolving depending on mint 200, 300 and 400 µg/mL (Tween 20, 0.1%) of EO in previously sterilised Potato Dextrose Agar (PDA-Pronodisa) growth medium flasks at 45–50°C, while the medium was still liquid to be distributed in Petri dishes. Petri dishes were inoculated with an 8 mm-diameter disk of a 7-day old colony on the PDA of each tested fungi. Plates were incubated in the dark at 25°C for 7 days. Fungal growth was evaluated by measuring the colony diameter in two perpendicular directions daily. Five replicate dishes were used for each EO, dose and fungus, it was done 3 times. The control Petri dishes contained only PDA/Tween 20 (0.1%) and the analysed fungus. Mycelial growth was evaluated by measuring the perpendicular colony diameters after 7 days. Mycelial growth inhibition was determined by the following formula (Albuquerque et al. 2006):

$$\mathrm{MGI} = \frac{\mathrm{DC} - \mathrm{DO}}{\mathrm{DC}} \times 100$$

Where DC is the average of the colonies in the control dishes, DO is the average of the colonies' diameter in the dishes with oil.

Statistical analysis

The fungal growth results were submitted to an analysis of variance (ANOVA). The HSD Tukey intervals were represented to compare species and treatment, with significant values at $P < 0.05$. The data analysis was performed by the Statgraphics Centurion XVII software (Stat Point, Inc., Herndon, Virginia, USA).

Results and discussion

Chemical composition of commercial essential oils

According to classes of terpenic compounds, the composition of oils from *M. spicata*, *M. piperita* and *M. suaveolens* were, respectively: 63.6%, 92.0% and 77.3% for oxygenated monoterpenes (the major fraction) and 25.4%, 4.0% and 7.3% for hydrocarbon monoterpenes. Sesquiterpenic compounds (both hydrocarbon and oxygenated) accounted for 7.9%, 3.1% and 6.6%, respectively (Table 1).

The major compounds in *M. piperita* were menthol (47.0%) and its derivatives: menthone (23.1%), menthyl acetate (5.2%), menthofurane (3.7%) and neomenthol (3.6%). It is worth noting the relatively large amount of 1,8-cineole (5%) as a very similar composition has been reported by De Oliveira et al. (2017) and Desam et al. (2019). Peppermint oil generally exhibits a composition in which menthol isomers and their esters, as well as derivatives menthone, isomenthone

Table 1. Chemical composition of the *Mentha spicata*, *M. piperita* and *M. suaveolens* essential oils (EOs).

					% Peak area in FID Chromatogram		
Compounds[a]	RT[b] (min.)	LRI exp	LRI lit.[c]	Id. Met.[d]	*M. spicata*	*M. piperita*	*M. suaveolens*
α-Thujene	6.863	924	924	MS,LRI	0.3	0.2	0.1
α-Pinene	7.133	930	932	MS,LRI, St	1.1	0.8	0.8
Camphene	7.978	952	946	MS,LRI, St	t[e]	nd[f]	0.1
Sabinene	8.718	970	969	MS,LRI	1.2	0.1	0.5
β-Pinene	8.825	973	974	MS,LRI, St	1.5	0.8	0.9
Octen-3-ol	9.044	978	974	MS,LRI	t	0.1	0.3
Myrcene	9.478	989	988	MS,LRI, St	1.1	0.1	1.2
3-Octanol	9.732	996	988	MS,LRI	nd	0.2	0.1
Mentha-1(7),8-diene (meta)	9.967	1001	1000	MS,LRI	t	nd	t
α-Phellandrene	10.022	1003	1002	MS,LRI	1.4	nd	nd
α-Terpinene	10.618	1015	1014	MS,LRI, St	t	nd	0.1
p-Cymene	10.985	1023	1020	MS,LRI, St	t	0.6	0.1
Limonene	11.209	1028	1024	MS,LRI, St	14.4	1.2	3.0
1,8-Cineole	11.231	1029	1026	MS,LRI, St	3.3	5.0	0.3
(*Z*)-β-Ocimene	11.628	1037	1032	MS,LRI	t	t	0.2
(*E*)-β-Ocimene	12.065	1047	1044	MS,LRI	0.1	0.1	nd
γ-Terpinene	12.557	1057	1054	MS,LRI, St	2.5	nd	0.3
(*Z*)-Sabinene hydrate	13.012	1067	1065	MS,LRI	4.7	1.2	2.1
Terpinolene	13.881	1086	1086	MS,LRI, St	t	t	0.1
p-Mentha-2,4(8)-diene	13.892	1086	1085	MS,LRI	1.8	nd	nd
(*E*)-Sabinene hydrate	14.378	1096	1098	MS,LRI	0.6	nd	nd
Linalool	14.567	1100	1095	MS,LRI, St	0.8	0.3	nd
2- Methylbuthyl 2-Methylbutanoate	14.756	1104	1100	MS,LRI	0.1	0.1	nd
1-Octen-3-yl acetate	15.145	1113	1110	MS,LRI	t	nd	t
(*Z*)-*p*-Menth-2-en-1-ol	15.444	1119	1118	MS,LRI	t	nd	nd
3-Octyl acetate	15.662	1124	1120	MS,LRI	0.5	nd	t
(*E*)-*p*-Menth-2-en-1-ol	16.322	1138	1136	MS,LRI	0.1	nd	nd
Camphor	16.736	1146	1141	MS,LRI, St	t	nd	nd
Menthone	17.284	1158	1148	MS,LRI	nd	23.1	0.1
Menthofurane	17.633	1165	1159	MS,LRI	nd	3.7	nd
Neomenthol	17.698	1167	1161	MS,LRI	nd	3.6	nd
Terpinen-4-ol	18.185	1177	1174	MS,LRI, St	5.7	nd	0.6
Menthol	18.62	1186	1167	MS,LRI	nd	47.0	nd
Neoisomenthol	18.686	1188	1184	MS,LRI	nd	0.6	nd
α-Terpineol	18.805	1190	1186	MS,LRI, St	4.8	0.3	t
(*E*)-Dihydrocarvone	19.373	1202	1200	MS,LRI	0.2	nd	nd
Linalyl formiate	20.067	1217	1214	MS,LRI	t	nd	nd
(*Z*)-Carveol	20.495	1227	1226	MS,LRI	t	nd	0.4
(*Z*)-Hex-3-enyl-3-methylbutanoate	20.727	1232	1232	MS,LRI	t	nd	nd
Pulegone	21.005	1238	1233	MS,LRI	0.3	1.1	0.5
Carvone	21.658	1252	1239	MS,LRI	41.1	nd	0.3
Piperitone	21.713	1254	1249	MS,LRI	nd	0.5	nd
Piperitone oxide	21.765	1255	1250	MS,LRI	nd	nd	40.2
(*Z*)-Carvone oxide	22.301	1266	1259	MS,LRI	t	nd	nd
(*E*)-Carvone oxide	22.595	1273	1273	MS,LRI	0.1	0.2	nd
Neomenthyl acetate	22.707	1275	1273	MS,LRI	t	nd	nd
Bornyl acetate	23.268	1288	1287	MS,LRI	nd	nd	0.7
Dihydroedulan I	23.624	1295	1293	MS,LRI	nd	nd	0.4
Menthyl acetate	23.633	1296	1294	MS,LRI	nd	5.2	nd
Isomenthyl acetate	24.133	1307	1304	MS,LRI	nd	0.2	nd
Dihydrocarveol acetate	25.059	1328	1326	MS,LRI	nd	nd	0.1
(*E*)- Carvyl acetate	25.463	1338	1339	MS,LRI	0.2	t	nd
Piperitenone	25.502	1339	1340	MS,LRI	nd	nd	0.2
(*Z*)-Carvyl acetate	26.572	1363	1365	MS,LRI	1.7	nd	nd
Piperitenone oxide	26.658	1365	1366	MS,LRI	nd	nd	31.4
α-Bourbonene	27.102	1376	1376	MS,LRI	nd	0.3	0.1
β-Bourbonene	27.435	1383	1387	MS,LRI	0.1	0.1	t
(*E*)-Jasmone	27.621	1388	1390	MS,LRI	0.2	nd	t
β-Elemene	27.731	1390	1389	MS,LRI	0.1	nd	0.6
(*Z*)-Jasmone	28.069	1398	1392	MS,LRI	nd	nd	0.1
α-Gurgujene	28.673	1413	1409	MS,LRI	nd	nd	t
Unknow[g]	28.755	1415			nd	nd	6.1
(*E*)-Caryophyllene	28.838	1417	1417	MS,LRI, St	2.1	1.2	1.0
β-Copaene	29.213	1426	1430	MS,LRI	0.2	nd	0.2
β-Gurgujene	29.407	1430	1431	MS,LRI	0.2	nd	nd
(*Z*)-Muurola-3,5-diene	29.944	1444	1448	MS,LRI	0.3	0.1	0.2
α-Humulene	30.201	1450	1452	MS,LRI	0.2	0.1	0.6
(*E*)-β-Farnesene	30.485	1457	1454	MS,LRI	0.5	nd	0.4

(*continued*).

Table 1. Continued.

Compounds[a]	RT[b] (min.)	LRI exp	LRI lit.[c]	Id. Met.[d]	% Peak area in FID Chromatogram		
					M. spicata	*M. piperita*	*M. suaveolens*
Alloaromadendrene	30.491	1457	1458	MS,LRI	nd	nd	0.3
(*Z*)-Cadina-1(6),4-diene	30.603	1460	1461	MS,LRI	t	nd	nd
(*Z*)-Muurola-4(14),5-diene	30.737	1463	1465	MS,LRI	t	nd	t
(*E*)-Cadina-1(6),4-diene	31.245	1475	1475	MS,LRI	0.1	nd	nd
Germacrene D	31.387	1479	1484	MS,LRI	2.7	0.2	2.9
Bicyclogermacrene	32.002	1494	1500	MS,LRI	0.1	0.4	t
α-Muurolene	32.197	1498	1500	MS,LRI	0.1	nd	nd
Germacrene A	32.282	1501	1508	MS,LRI	nd	nd	t
γ-Cadinene	32.705	1511	1513	MS,LRI	0.4	0.1	0.1
(*E*)-Calamenene	33.001	1519	1521	MS,LRI	t	nd	t
δ-Cadinene	33.102	1522	1522	MS,LRI	0.1	0.2	nd
α-Cadinene	33.627	1535	1537	MS,LRI	t	nd	t
Spathulenol	35.127	1574	1577	MS,LRI	0.3	0.6	nd
Germacrene D-4-ol	35.161	1575	1574	MS,LRI	nd	nd	t
Caryophyllene oxide	35.270	1578	1582	MS,LRI	nd	0.2	0.1
Globulol	35.731	1589	1590	MS,LRI	nd	t	nd
Viridiflorol	35.879	1593	1592	MS,LRI	nd	nd	t
Cubenol < 1,10-di-epi >	36.559	1611	1618	MS,LRI	0.2	nd	t
epi-α-Cadinol	37.644	1641	1638	MS,LRI	nd	nd	t
α-Cadinol	38.002	1650	1652	MS,LRI	0.2	nd	t
(*Z,Z*)-Farnesol	39.555	1693	1698	MS,LRI	nd	nd	t
Eudesm-7(11)-en-4-ol	39.841	1700	1700	MS,LRI	nd	nd	t
Hydrocarbon monoterpenes					**25.4**	**4.0**	**7.3**
Oxygenated monoterpenes					**63.6**	**92.0**	**77.3**
Hydrocarbon sesquiterpenes					**7.2**	**2.3**	**6.5**
Oxygenated sesquiterpenes					**0.7**	**0.8**	**0.1**
Other compounds					**0.6**	**0.4**	**6.6**
Total identified					**97.5**	**99.5**	**97.8**

[a]Compounds listed by elution order in a ZB5 column.
[b]Retention time according GC/MS analysis.
[c]Linear retention indices from Adams, J.P. (2007), except for those referenced in the footnotes.
[d]Identification method: MS: Comparing with mass spectra from NIST 2.0; LRI: linear retention indices based on C_8-C_{25} alkanes; St: Co-injection of pure standards.
[e]Traces (% < 0.05 in FID and identified by GC/MS).
[f]No detected.
[g]Mass spectra (E.I. 70 eV). Relative intensity of the 10 most abundant ions: 43 (999), 81 (294), 41 (258), 112 (198), 55 (112), 127 (89), 109 (88), 154 (73), 53 (67), 69 (65).

and menthofurane, are major compounds. Nevertheless as reported by Desam et al. (2019), some samples show noticeably different profiles in which hydrocarbon monoterpenes, such as limonene, α-terpinene or oxygenated monoterpenes like linalool, pulegone or carvone are major compounds.

Carvone (41.1%) and limonene (14.4%) were the major components in *M. spicata* (spearmint), together with some noticeable amounts of oxygenated monoterpenes in: terpinen-4-ol (5.7%), α-terpineol (4.8%) and (*Z*)-sabinene hydrate (4.7%). This profile closely coincided with that reported by Bardaweel et al. (2018) with carvone (49.5%) and limonene (16.1%), but with different other oxygenated monoterpenes, namely: 1,8-cineole (8.7%) and (*Z*)-dihydrocarvone (3.9%). Carvone and limonene have generally been reported as major compounds in spearmint: Brahmi et al. (2017): 20.8% and 48.5%; Nikšić et al. (2018): 56.4% and 16.2%, of these components, respectively.

Regarding *M. suaveolens*, the oil herein applied showed a balanced profile between the two of the most representative compounds in this species: piperitone oxide (40.2%) and piperitenone oxide (31.4%), and small amounts of other monoterpenic compounds likes limonene (3.0%) or (*Z*)-sabinene hydrate (2.1%). *M. suaveolens* oil generally shows a profile characterised by the dominating menthane skeleton compounds oxygenated in C3: piperitone, piperitenone, and their epoxides (Bouyahya et al. 2019), as well as pulegone and oxygenated compounds in C2 like carvone and dihydrocarvone (El-Kashoury et al. 2012). Other compositions have been reported: such as (*Z*)-piperitol and 1,8-cineol chemotypes (Lawrence 2006), or oils rich in cinerone, β-caryophyllene, terpinen-4-ol and other monoterpenes (Ed-Dra et al. 2019).

Table 2. Mean growth (mm) and standard deviation values calculation for each fungus species grown on PDA (control), PDA-*M. piperita* EO, PDA-*M. spicata* EO and PDA-*M. suaveolens* EO at different concentrations.

		M. piperita		*M. spicata*		*M. suaveolens*		
Species	Control	300 µg/mL	400 µg/mL	300 µg/mL	400 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL
VD	21.75 ± 0.13	13.95 ± 0.14	7.15 ± 0.15	14.50 ± 0.19	9.45 ± 0.19	14.55 ± 0.28	1.95 ± 0.11	0.00 ± 0.00
FO	74.30 ± 0.54	41.85 ± 0.42	32.45 ± 0.37	38.50 ± 0.16	19.28 ± 0.30	48.65 ± 0.17	7.55 ± 0.28	4.30 ± 0.08
CH	24.35 ± 0.31	10.55 ± 0.17	5.65 ± 0.07	17.85 ± 0.36	13.57 ± 0.25	22.20 ± 0.30	6.35 ± 0.53	0.00 ± 0.00
BF	105.50 ± 0.85	67.35 ± 0.70	8.31 ± 0.45	43.88 ± 1.55	0.10 ± 0.36	48.00 ± 1.01	0.00 ± 0.00	0.00 ± 0.00

VD: *Verticillium dahliae*, FO: *Fusarium oxysporum lycopersici*, CH: *Curvularia hawaiiensis*, BF: *Botryotinia fuckeliana*.

Table 3. Mycelial Growth Inhibition (MGI) percentage for each fungus grown on PDA- *M. piperita* EO, PDA-*M. spicata* EO and PDA-*M. suaveolens* EO at different ferent doses.

	M. piperita		*M. spicata*		*M. suaveolens*		
Species	300 µg/mL	400 µg/mL	300 µg/mL	400 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL
VD	35.86	67.13	33.33	61.10	33.10	91.03	100
FO	43.67	56.33	48.18	74.05	34.52	89.84	94.21
CH	56.67	76.80	26.69	44.27	8.83	73.92	100
BF	36.16	92.12	58.41	99.91	54.50	100	100

VD: *Verticillium dahliae*, FO: *Fusarium oxysporum lycopersici*, CH: *Curvularia hawaiiensis*, BF: *Botryotinia fuckeliana*.

Antifungal activity. Mycelial growth inhibition

The fungus *Botryotinia fuckeliana* was the most sensitive species to the effect of the three tested EO: *M. piperita*, *M. spicata* and *M. suaveolens* (Tables 2 and 3). *B. fuckeliana* was completely or practically inhibited, at the 400 µg/mL, in all trials, and also at 300 µg/mL in *M. suaveolens*. The three studied EO inhibited its growth with 92–100% mycelial growth inhibition (MGI).

The EO of *Mentha suaveolens* obtained the best antifungal activity results. The highest dose, 400 µg/mL, produced 100% MGI in all the studied fungi, except for *Fusarium oxysporum* with 94.21%. At 300 the µg/mL dose, effectiveness slightly decreased, but the results were still very satisfactory with 74% MGI for *C. hawaiiensis*, 89.94% for *F. oxysporum*, 91.03% for *V. dahliae* and up to 100% for *B. fuckeliana*. When lowering the dose to 200 µg/mL in the *M. suaveolens* EO, considerably low MGI values were obtained, with a major difference in the MGI percentages compared to the results of the immediately higher dose (300 µg/mL), except for BF that exceeded 50%. The behaviour of the *V. dahliae* fungus in *M. piperita* and *M. spicata* led to

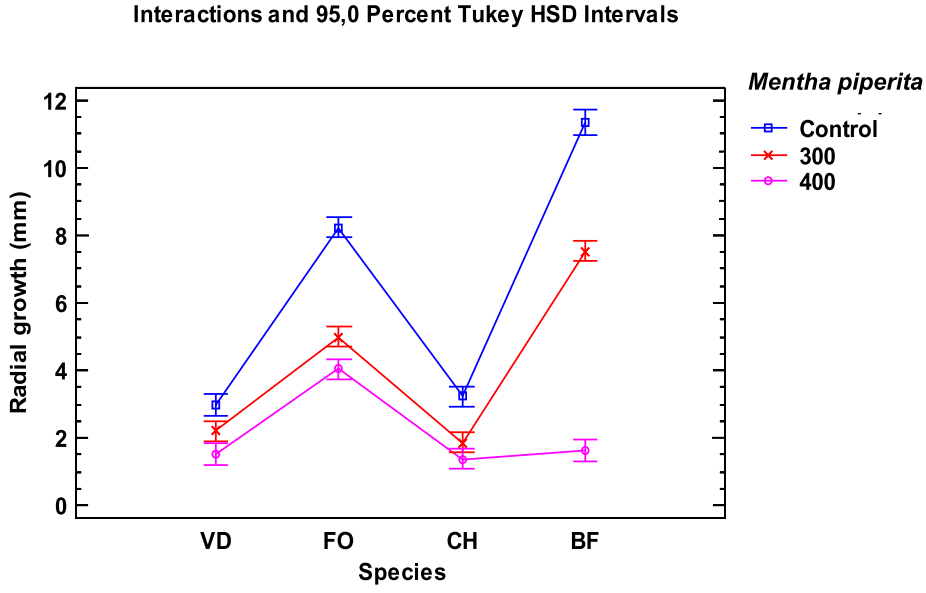

Figure 1. Interaction plot, mean growth, species, concentration 300 and 400 µg/mL of *Mentha piperita* essential oil against *Verticillium dahliae* (VD), *Fusarium oxysporum lycopersici* (FO), *Curvularia hawaiiensis* (CH) and *Botryotinia fuckeliana* (BF). n (30) observations per treatment were used in the statistical analysis.

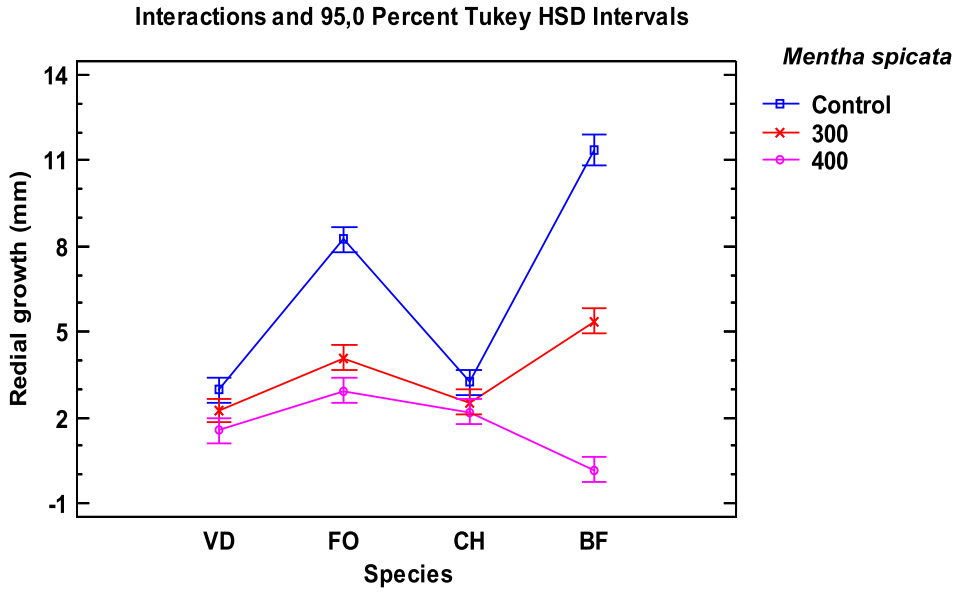

Figure 2. Interaction plot, mean growth, species, concentration 300 and 400 µg/mL of *Mentha spicata* essential oil against *Verticillium dahliae* (VD), *Fusarium oxysporum lycopersici* (FO), *Curvularia hawaiiensis* (CH) and *Botryotinia fuckeliana* (BF). n (30) observations per treatment were used in the statistical analysis.

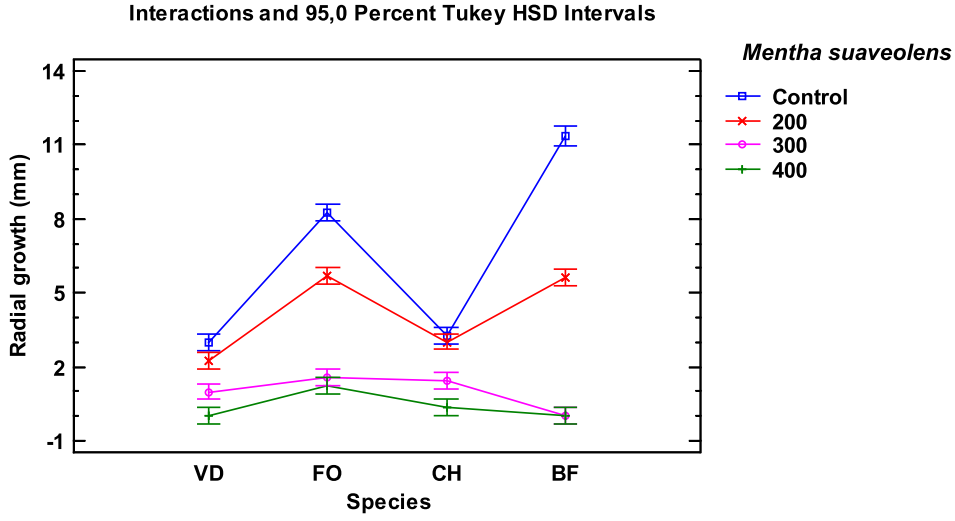

Figure 3. Interaction plot, mean growth, species, concentration 200, 300 and 400 µg/mL of *Menta suaveolens* essential oil against *Verticillium dahliae* (VD), *Fusarium oxysporum lycopersici* (FO), *Curvularia hawaiiensis* (CH) and *Botryotinia fuckeliana* (BF). n (30) observations per treatment were used in the statistical analysis.

similar results. The Tukey HSD intervals (Figures 1–3) showed the significance of the results for fungal growth under the tested conditions. *Botryotinia fuckeliana* was the most sensitive fungus to treatment with the EO of the three mints. No significant differences appeared in the growth of BF and *F. oxysporum* at the 400 µg/mL and 300 µg/mL doses, respectively, in the treatment with the *M. suaveolens* EO. This the treatment was equally effective. Significant differences in *V. dahliae* growth were observed at both doses.

The antifungal activity of the EO from different *Mentha* species has been tested by many researchers (Peixinho et al. 2017; Stringaro et al. 2018; Oliveira Filho et al. 2019).

The results obtained in this work with the *M. piperita* EO against the four pathogenic fungi are similar to those obtained against other fungal species. The *M. piperita* mint oil was evaluated with the spore germination of *Colletotrichum gloeosporioides* and its mycelial growth *in vitro* and with papaya fruit

at differents concentrations. The results revealed a strong inhibitory effect on both spore germination and mycelial growth *in vitro* and *in vivo* (Andrade and Vieira 2016). Fialho et al. (2015) tested the same EO against *Phakopsora euvitis* and other pathogenic fungi and obtained good results. However, Behidj et al. (2018) and Rachitha et al. (2017), reported limited antifungal effectiveness for *M. piperita* on the species of the genus *Fusarium*, which also happened in our research.

In this study, the most inhibition recorded under the tested conditions was found for the fungus *B. fuckeliana*. Lorenzetti et al. (2011) evaluated the mycelial growth, conidia production and germination of this *B. fuckeliana* species isolated from strawberry by incorporating oil into the culture medium (125–1.000 ppm), and obtained the different assayed EO (lemon grass, palmrose, citronella, clove, cinnamon, lavender, tangerine, eucalyptus, tea tree, rosemary, orange) with the best results for the *M. piperita*. In a recent study, Oliveira Filho et al. (2019) demonstrated that *M. spicata* may be a potentially efficient and safe alternative to be used as a potential fumigant to control *B. cinerea* in stored fresh products. Bayan and Küsek (2018) investigated the chemical composition and the antifungal and antibacterial activity of volatile oil from *Mentha spicata*. The main component was carvone (56.94%), followed by limonene (11.63%), sabinene hydrate (7.04%) and caryophyllene (4.06%). Antifungal activity was determined against *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Rhizoctonia solani Alternaria solani* and *Verticillium dahliae*. Volatile oil displayed marked antifungal activity against plant pathogenic fungi, and an oil concentration was used that doubled our concentration in this work. Volatile oil also exhibited remarkable activity against *Xanthomona*s spp. 'Furthermore, Dias et al. (2020) studied the antifungal activity of limonene against *Sclerotinia sclerotium*, obtaining excellent results at 300 µL. This monoterpene showed to be highly active by inhibiting 100% of fungal growth'.

M. suaveolens was the most effective EO in our study. In a study by El-Kashoury et al. (2014), oil displayed strong antifungal activity on *Aspergillus niger*. The biological activities of *M. suaveolens*, after paying special attention to its most representative compound piperitenone oxide, have been extensively described by Božović et al. (2015) and Spagnoletti et al. (2016) for its antifungal and antioxidant activities. In addition, due to its low risk, it is used as food additive and flavouring agent.

Conclusion

Carvone and limonene were the major compounds in *Mentha spicata* (spearmint), menthol and menthone in *M. piperita*, and piperitone oxide and piperitenone oxide in *M. suaveolens*.

Botryotinia fuckeliana was the most sensitive of all the tested fungi, the three essential oils studied inhibited its growth. The *Mentha suaveolens* essential oil obtained the best antifungal activity results. The highest dose, 400 µg/mL, produced 100% MGI in all the studied fungi, except for *Fusarium oxysporum* with 94.21%. *Mentha suaveolens* might be an alternative for controlling *Botryotinia fuckeliana*, *Curvularia hawaiiensis*, *Verticillium dahliae* and *Fusarium oxysporum* in food and might, thus, extend their shelf life. Essential oil can be considered to develop a low-risk enviro-friendly botanical biofungicide.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability

The authors confirm that the data supporting the findings of this study are available in the Mendeley repository, Mendeley Data, (https://data.mendeley.com/datasets/98prxvbk8s/1).

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