Antifungal/Antibacterial Activity of Peppermint Oil and Cornmint Oil

Subjects: Agronomy

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The genus mint (Mentha) belongs to the Lamiaceae family and includes 42 species, 15 hybrids, and hundreds of subspecies, varieties, and cultivars, which potentially crossbreed when in proximity. Different mints are known for a reasonably high content of essential oils (EO), which are deposited in the glandular trichomes, mostly located on the adaxial surface of their leaves. There are two well-known, so-called menthol mints in cultivation: Mentha x piperita L. (Hudson): peppermint—MP, and Mentha arvensis L., (syn. M. canadensis L., Japanese mint): cornmint—MA.

Keywords: botanical pesticides ; chemical composition ; agriculture

1. Introduction

The genus mint (*Mentha*) belongs to the Lamiaceae family and includes 42 species, 15 hybrids, and hundreds of subspecies, varieties, and cultivars [1], which potentially crossbreed when in proximity. Different mints are known for a reasonably high content of essential oils (EO), which are deposited in the glandular trichomes, mostly located on the adaxial surface of their leaves [2]. There are two well-known, so-called menthol mints in cultivation: *Mentha x piperita* L. (Hudson): peppermint—MP, and *Mentha arvensis* L., (syn. *M. canadensis* L., Japanese mint): cornmint—MA [3].

MP originates from the Mediterranean region and is a natural hybrid between *M. viridis* (*M. longifolia* x *M. rotundifolia*) and *M. aquatica* [4]. It has higher yields in the temperate climate regimes of higher precipitation levels. A widely cultivated botanical form of MP is *Mentha x piperita* L. var. officinalis Sole f. rubescens (Camus), called black or English MP, which has violet stems and leaves [5][6]. MP can be grown as a sole crop or intercropped with other species [7][8][9]. A recent study showed that the inclusion of MP in crop rotation can negatively affect a succeeding maize, which may result from the allelopathic interactions of MP [10], possibly due to changes in the quantitative and qualitative profiles of EO during its decomposition in the soil [11].

MA originates from the temperate climates of Europe and western and central Asia. It has higher yields under the subtropical conditions of Asia [12][13]. MA is usually included in the crop rotation with different crop species as it reacts well to intercropping and green manuring [14].

The popularity of MP and MA cultivation results from a wide application of both herbs and essential oils. Due to the biological quality of the raw material obtained from plantations, and its use for medicinal purposes, ecological cultivations of both menthol mints are recommended [15][16][17][18][19][20].

The menthol mints contain many biologically active compounds, with EOs being a significant part of them. The biological interactions of the menthol mints with the other components of agrobiocenoses, i.e., weeds or insect pests, have been observed for a long time. Recently, the investigation of the menthol mints EOs as natural (aka botanical) pesticides is being carried out. Peppermint essential oil is already exempt from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as a pesticide formulation, alone or in combination with other ingredients [21].

Nowadays in agriculture there is a growing interest in botanical pesticides with the active ingredient composed of natural compounds, among them EOs [22][23]. This is due to two major factors. Firstly, the misuse of synthetic pesticides has resulted in the rise of a number of pesticide-resistant organisms, which are also posing a significant threat to the diversity of ecosystems [24][25].

2. Content and Chemical Composition of Peppermint Oil and Cornmint Oil

Essential oils are multicomponent mixtures of secondary plant volatiles produced by steam- or hydrodistillation of different plant parts, with the exception of citrus peel oils, which are produced by expression. The main constituents of essential oils belong to the mono- and sesquiterpenes, which are classified into hydrocarbons, alcohols, aldehydes, ketones, esters, and ethers. Essential oils are limpid, oily liquids that dissolve well in ethanol, unpolar organic solvents, and lipids, and are insoluble in water.

MPEO and MAEO containing the same major constituents, namely menthol and menthone, are among the most produced and marketed essential oils all over the world. The main producers of MPEO are India, the USA, and China, and of MAEO China, India, Brazil, and Japan [26][27]. The oils are obtained by hydrodistillation of the fresh or partly dried flowering herb with a yield of 0.3–0.7%. In both EOs about 300 constituents were identified. The main constituents of MPEO are menthol (20–60%), menthone (5–35%), menthyl acetate (1–20%), and menthofuran (0.1–15%). MAEO is dominated by menthol (above 60%) and menthone (4–18%). Menthol is separated from this oil by crystallization and the remaining oil has an appearance and odor resembling MPEO. The dementholized MAEO is used as a cheap alternative to MPEO, but it is easily recognized organoleptically because of its harsh flavor.

Both menthol-rich mint oils have monographs in the *European Pharmacopoeia* 5 (*EP* 5) [28] as Peppermint oil and Mint oil partly dementholized, respectively. EP 5 defines mint oils as colorless, pale yellow, or pale greenish-yellow liquids with a characteristic odor. EP 5 establishes the limits of 10 key components in peppermint oil determined by GC analysis: menthol (30.0–55.0%), menthone (14.0–32.0%), isomenthone (1.5–10.0%), menthyl acetate (2.8–10.0%), menthofuran (1.0–9.0%), 1,8-cineole (3.5–4.0%), limonene (1.0–5.0%), isopulegol (max. 0.2%), pulegone (max. 4.0%), and carvone (max. 1.0%). The limits of these compounds in dementholized cornmint oil are similar: menthol (30–50%), menthone (17–35%), isomenthone (5.0–13.0%), menthyl acetate (1.5–7.0%), 1,8-cineole (max. 1.5%), limonene (1.5–7.0%), isopulegol (1–3%), pulegone (max. 2.0%), and carvone (max. 2.0%). The structures of the main constituents of menthol mint oils are presented in **Figure 1**.

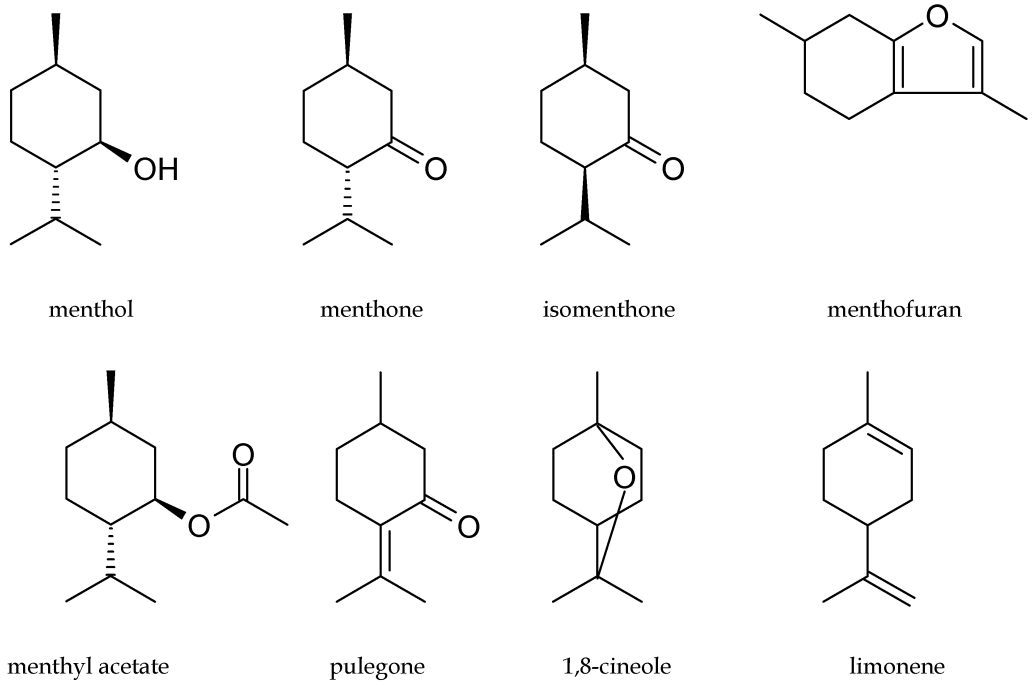

Figure 1. Structures of the main components of menthol mint essential oils.

The yield as well as the qualitative and quantitative composition of MPEO differs in relation to cultivar, geographic origin, and condition of cultivation (temperature, water, fertilizers), and strongly depend on the time of harvest. At the beginning of ontogenesis, the herb contains menthone (40–55%) as a main compound and lower amounts of menthol (20%). During shoot growth, the menthol content starts to increase, reaching more than 40%, while the menthone content decreases. At the flowering stage, the content of two other constituents, namely menthofuran and pulegone, which adversely influence peppermint oil quality, increases and diminishes after flowering, while the content of menthol and menthyl acetate increases to levels higher than 50% and 7%, respectively [29]. The conditions of growth of MP in cultivation may additionally affect the quality of MPEO. For example, organic fertilizers promote the production of EO of a higher amount of menthol and a decreasing amount of menthofuran and pulegone [30][31]. The amount of menthol can also be increased as a result of microbial activity in the MP's rhizosphere. For example, an increase in the number of native rhizospheric

strains of bacteria *Pseudomonas putida* and their microbial volatile organic compounds stimulated MP's shoot growth and reduced the content of menthofuran in the EO, with a simultaneous induction of menthol production [32]. A similar effect brought about the arbuscular mycorrhizal inoculation of soil with *Funneliformis mosseae*, which, when applied alongside foliar-sprayed natural humic substances, promoted the biochemical activity of MP plants [33].

The rhizosphere of MA is also rich in several different strains of mycorrhizal fungi, which positively influence the production of MAEO, specifically the menthol content. Interestingly, the highest production of menthol was achieved when MA plants were inoculated with *Trichoderma viride* [34].

Menthol is a monoterpene alcohol with three chiral carbon atoms and occurs in eight stereoisomes. In both mint oils, (−)-(1*R*,3*S*,4*S*)-menthol, called menthol, is dominant. Three dextrorotatory menthol isomers, (+)-(1*S*,3*R*,4*S*)-isomenthol, (+)-(1*R*,3*R*,4*R*)-neomenthol, and (+)-(1*R*,3*R*,4*S*)-neoisomenthol, are present in the oils in smaller amounts. Out of four stereoisomers of appropriate ketone, (−)-(1*R*,4*S*)-menthone dominated over (+)-(1*R*,4*R*)-isomenthone.

In a recent review on the genus *Mentha*, previous literature data on MPEO and MAEO composition were reported [1]. Only five MPEOs met the requirements of *EP* 5 in respect of the main constituents' percentages. Five oils have components from the *EP* 5 list as the main constituents, but in different proportions. The other oils were composed of totally different compounds. In two of them, carvone and limonene were the main constituents, as in *M. spicata* oil, while in three oils linalool and linalyl acetate dominated. Similarly, among the three MAEOs there were oils dominated by menthol/isomenthone, menthol/pulegone, or linalool/linalyl acetate [1].

3. Biological Activity and Application of Peppermint Oil and Cornmint Oil

MPEO is the most important of the mint oils because of its exceptional properties [26][35][36]. It is also the most extensively used oil in therapy, both internally and externally, being recommended for the treatment of acute and chronic gastritis and enteritis, in disorders of the respiratory tract, and for inflammation of the oral mucosa [26]. The biological activity of menthol mint oils is due to the content of their main constituent menthol, which is used as an individual phytochemical in the treatment of respiratory pathologies. Both MPEO and menthol are ingredients in numerous medications.

MPEO possesses a fresh, minty flavor and cooling effect. Due to these properties and its antimicrobial activity, it is also widely used in chewing gums, toothpastes, and mouthwashes, and as a fragrance in perfumes, soaps, and air refreshers, where it is often replaced by a cheaper, dementholized MAEO.

4. Antifungal and Antibacterial Activity of Peppermint Oil and Cornmint Oil against Phytopathogens

The wide spectrum of therapeutic properties of peppermint oil includes antibacterial and antifungal activities. Due to these activities, MPEO and MAEO are also used for controlling microorganisms in other areas. In the last few decades, the use of EOs in agriculture, as agents protecting crops from bacterial and fungal diseases, has been extensively researched.

Two basic techniques are used for the in vitro assessment of antibacterial and antifungal activities of EOs. In the agar diffusion method, agar broth is inoculated with microorganisms and EO or EO solution is placed on a paper disc or in a well. After incubation, the diameter of the inhibition zone is measured. In the serial dilution agar or liquid broth method, EO is added to the broth, which is inoculated with microorganisms. In fungi this method, called a poisoned food technique, is used for the assessment of mycelial growth inhibition at specified EO concentrations. In both variants, the activity of EOs can be assessed in a vapor phase. The results are presented in terms of the growth inhibition as a percentage ratio to the control or as the minimal inhibitory concentration (MIC) restraining microorganism growth. Sometimes the bactericidal (MBC) or fungicidal (MFC) concentration is also assessed. A negative control without EO and positive control with standard antibiotics for bacteria and fungicide for fungi should be included in the experiment. It should be mentioned that the results obtained in different laboratories are hardly comparable because of a high number of factors influencing the final result. Among them, the origin and susceptibility of microorganisms, i.e., environmental fungi and bacteria are more resistant than collection strains, and conditions of assessment, i.e., method, solvent, MIC definition, and different units of EO concentration, are the most important [37][38]. To a broad extent, MIC values can be compared between laboratories. On the contrary, inhibition zones measured by disc diffusion method are incomparable because of the varying EO amounts used.

The results of MPEO and MAEO antimicrobial activity investigated by in vitro methods against phytopathogenic fungi and bacteria are presented in **Table 1**, with an emphasis on the results obtained by the dilution method. In the majority of

studies, several EOs were assessed in one study. For comparison purposes, the data for the most active EO are also presented. Different units used for the EO concentration (mg/mL, µL/mL, µL/L, ppm, etc.) were converted to the same unit, µg/mL, on the assumption that EO density amounts to 1 g/mL. In fact, it is ca. 0.9 g/mL [28].

Table 1. In vitro antifungal and antibacterial activity of peppermint oil and cornmint oil against phytopathogens.

Fungi/Bacteria (B)	MIC or Total Inhibition Concentration	No. of Essential Oils Mint Oil Composition [%]	Methods Results for the Most Active Essential Oil	Ref.
Alternaria alternata *Alternaria solani* *Aspergillus flavus* *Aspergillus niger* *Fusarium solani* *Rhizopus solani* *Rhizopus* spp.	117.0/57.9µg/mL[1] 127.1/129.0 122.0/110.7 49.5/63.5 130.7/89.8 44.11/63.9 149.7/137.1	4 EOs, 4 compounds MPEO menthone 28.1, menthol 4.8, menthyl acetate 9.5, limonene 7.1 MAEO, menthol 78.9, menthone 6.4	broth microdilution, agar disc diffusion (15 µL), positive control: fluconazole 30 µg *M. spicata* and *M. longifolia* similar results as MPEO	[39]
Alternaria brassicae *Botrytis cinerea* *Cladobotryum mycophilum* *Fusarium oxysporum* *Phytophthora parasitica* *Pythium aphanidermatum* *Sclerotinia sclerotiorum*isolated from vegetables and mushrooms	16.2%[2] - 7.4% 15.8% 5.7% - 6%	12 EOs MPEO menthol 42.0, menthone 28.8, 1,8-cineole 7.1	disc diffusion 8 µL of 5–30% EO solution MPEO oil belonged to four most effective	[40]
Alternaria citrii *Aspergillus fumigatus* *Aspergillus oryzae* *Fusarium oxysporum* *Fusarium solani* *Helminthosporium compactum* *Macrophomina phaseolina* *Sclerotium rolfsii*	0.25 µL/mL 1.0 1.0 3.0 2.0 0.5 2.0 0.25	4 EOs MPEO no data	disc diffusion, 5 µL, agar dilution 0.16–20 µg/mL MPEO less active than other three	[41]

Fungi/Bacteria (B)	MIC or Total Inhibition Concentration	No. of Essential Oils Mint Oil Composition [%]	Methods Results for the Most Active Essential Oil	Ref.
Alternaria citrii *Botrytis cinerea* *Colletotrichum gloeosporioides* *Lasiodiplodia theobromae* *Penicillium digitatum* isolated from fruits	3000 µL/L 3000 3000 >3000 2000	18 EOs MPEO menthol 40.7, menthone 21.7	agar dilution thyme 500–1000 µL/L (3000 *P. digitatum*)	[42]
Aspergillus ochraceus	2000 µg/L (broth) 1500 µg/L (vapor)	5 EOs, 5 compounds MPEO menthol 50	broth dilution/vapor phase cinnamon oil and cinnamaldehyde: 250–500 µg/L (broth), 150–250 µg/L (vapor), ochratoxin A production inhibited at 200 µg/L	[43]
Aspergillus ochraceus	1000 ppm	4 EOs MAEO, no data	broth dilution MAEO and oregano oil were the most effective in inhibition of fungal growth and ochratoxin A production	[44]
Aspergillus flavus *Aspergillus niger* *Aspergillus parasiticus* *Penicillium chrysogenum*	10000 ppm 5000 2500 1250	8 EOs and EOs combinations MPEO menthol, menthone	broth dilution, vapor phase MPEO less active than thyme (312.5–1250 ppm) and oregano oils, similar activity to cinnamon oil, more active than other four oils	[45]
Aspergillus flavus *Aspergillus niger* *Fusarium oxysporum* *Mucor* spp. *Penicillium digitatum*	1.13/2.25 mg/mL[3] 1.13/2.25 1.13/2.25 1.13/2.25 2.25/4.5	MPEO	agar dilution (MIC), broth dilution (MFC), well diffusion, vapor phase	[46]

Fungi/Bacteria (B)	MIC or Total Inhibition Concentration	No. of Essential Oils Mint Oil Composition [%]	Methods Results for the Most Active Essential Oil	Ref.
Aspergillus flavus *Aspergillus fumigatus* *Aspergillus niger* *Botryodiplodia theobromae* *Cladosporium cladosporioides* *Fusarium oxysporum* *Helminthosporium oryzae* *Macrophomina* sp. *Sclerotium rolfsii*	0.1 mg/mL 0.1 <0.5 0.1 0.1	18 EOs MAEO menthol 73, menthone 6.1	agar dilution, positive control: four synthetic fungicides MAEO was the most efficient of EOs and more efficient than synthetic fungicides at 0.1 mg/mL four fungi were inhibited totally, other 72–100% inhibition aflatoxin B1 production by *A. flavus* inhibited at 0.05 mg/mL	[47]
Alternaria alternata *Aspergillus fumigatus* *Aspergillus candidus* *Aspergillus nidulans* *Aspergillus versicolor* *Cladosporium cladosporioides* *Curvularia lunata* *Fusarium nivale* *Fusarium oxysporum* *Fusarium roseum* *Penicillium* sp. *Monilia* sp. *Trichoderma viride*	400 µg/L	18 EOs MAEO no data	agar dilution, positive control: nine synthetic fungicides MAEO was the most efficient of EOs and more efficient than all synthetic fungicides at 400 µg/L 11 fungi were inhibited totally, other two >84%	[48]
Botrytis cinerea *Geotrichum citri-aurantii* *Phytophthora citrophthora* *Penicillium digitatum*	no inhibition at 250 ppm	19 EOs MPEO menthol 50, menthone 30, menthyl acetate 10	radial growth on plate at different concentration, positive control: four synthetic fungicides *Chrysanthemum viscidehirtum* total inhibition at 150 ppm, synthetic fungicides at 50 ppm	[49]

Fungi/Bacteria (B)	MIC or Total Inhibition Concentration	No. of Essential Oils Mint Oil Composition [%]	Methods Results for the Most Active Essential Oil	Ref.
Phytophthora cinnamomi *Pyrenochaeta lycoprsici* *Verticillium dahliae*	800 ppm 400 800	8 EOs MPEO menthol 39.0, menthone 21.0, menthofuran 19.5, 1,8-cineole 7.0	agar dilution oregano 200, 50, 50 ppm, resp.	[50]
Dreschlera spicifera *Fusarium oxysporum* f.sp. *ciceris Macrophomina phaseolina*	1600 ppm >1600 800	MPEO menthol 25.2, menthone 30.6	agar dilution	[51]
Colletotrichum gloeosporioides isolated from fruits	2.0 mg/mL	28 EOs MPEO, no data	agar microdilution, positive control: amphotericin B 5–60 µL/mL coriander leaf, two lemongrass sp. 0.25 mg/mL (lemongrass oil evaluated on passion fruit)	[52]
Fusarium spp. *Penicillium* spp. *Phythium* spp. isolated from corn seeds	1000 µL/L 1000 >1000	18 EOs MPEO, no data	agar dilution oregano MIC 100–200 µL/L	[53]
Mucor sp. *Rhizopus stolonifer* *Sclerotinia sclerotiorum*	30 µL/400 mL air	2 EOs, 4 compounds MPEO menthol 33.3, menthone 29.5, 1,8-cineole 7.0	vapor phase sweet basil and menthol 30 µL/400 mL air, menthone not active	[54]
Rhizoctonia botaticola *Sclerotium rolfsii*	1000 µg/mL	20 EOs MAEO, no data	agar dilution 6 EOs totally inhibited both fungi's growth at 1000 µg/mL	[55]
Lecanicillium fungicola var. *fungicola*	750–1000 µL/L	11 EOs MPEO menthol 39.2, menthyl acetate 20.4, menthone 15.3	broth dilution mushroom *Agaricus bisporus* MPEO was similarly active against mushroom and its pest, savory and thyme oils showed the best selectivity index	[56]

Fungi/Bacteria (B)	MIC or Total Inhibition Concentration	No. of Essential Oils Mint Oil Composition [%]	Methods Results for the Most Active Essential Oil	Ref.
Aspergillus niger	11.4 µg/mL	9 EOs MPEO linalool 41.4 linalyl ac 39.5	agar dilution *Thymus letrobotrys* 2.7 and 2.2 µg/mL	[57]
Penicillium funiculosum	11.4			
Alternaria alternata	1.50 µg/mL	MPEO menthol 36.0, isomenthone 23.5, menthone 24.6, menthyl acetate 9.0, menthofuran 6.9	disc diffusion 10 µL, broth microdilution, positive control: amphotericin B MIC 1–5 µg/mL menthol, menthone MIC against *P. syringae* 2.0, 1.0 µg/mL *X. campestris* 2.0, 2.0 µg/mL	[58]
Aspergillus flavus	10.0			
Aspergillus fumigatus	0.50			
Cladosporium herbarum	1.50			
Fusarium oxysporum	1.50			
Aspergillus veriscolor	10.0			
Fusarium acuminatum	2.50			
Fusarium solani	10.0			
Fusarium tabacinum	1.50			
Monilinia fructicola	5.50			
Penicilliumspp.	1.50			
Rhizoctonia solani	1.50			
Sclerotinia minor	10.0			
Sclerotinia sclerotiorum	10.0			
(B) *Pseudomonas syringae*	2.50			
(B) *Xanthomonas campestris*	80.0			

Fungi/Bacteria (B)	MIC or Total Inhibition Concentration	No. of Essential Oils Mint Oil Composition [%]	Methods Results for the Most Active Essential Oil	Ref.
Alternaria alternata *Aspergillus flavus* *Aspergillus niger* *Aspergillus ochraceus* *Aspergillus terreus* *Aspergillus versicolor* *Cladosporium cladosporioides* *Fusarium tricinctum* *Penicillium funiculosum* *Penicillium ochrochloron*	1.5–3.0 µL/mL in ethanol 1.0–2.5 µL/mL in Tween	4 EOs MPEO menthol 37.4, menthone 12.7, limonene 6.9, menthofuran 6.8	agar macro- (in ethanol) and micro- (in Tween) dilution, positive control: bifonazol MIC 10–15 µL/mL thyme oil 0.125–0.5 µL/mL in ethanol, 0.05–0.25 in Tween menthol 0.25–1.5 µL/mL in ethanol, 0.05–1.0 µL/mL in Tween	[59]
Trichoderma harzianum *Verticillium fungicola* (B) *Pseudomonas tolaasii*	3–4 µL/mL	10 EOs, 10 compounds MPEO menthol 37.4, menthyl acetate 17.4, menthone 12.7	microdilution, macrodilution, disc diffusion, vapor phase, positive control: bifonazol and prochloraz (fungi), streptomycin + penicillin (bacteria) oregano and thyme 1.5–2.0 µL/mL	[60]
(B) *Agrobacterium tumefaciens* (B) *Erwinia carotovora*		13 EOs, 14 compounds MPEO, no data	agar diffusion, 50 µL solution MPEO moderately active at 200 mg/mL 6 EOs were effective, MPEO showed weak activity	[61]

Fungi/Bacteria (B)	MIC or Total Inhibition Concentration	No. of Essential Oils Mint Oil Composition [%]	Methods Results for the Most Active Essential Oil	Ref.
Aspergillus flavus	-	four MPEO menthol 27.5–42.3, menthone 18.4–27.9	fungi: agar diffusion, 50 µL, weak activity bacteria: microdilution menthol 0.07–1.25 mg/mL menthone 1.25–2.5 mg/mL	[62]
Aspergillus parasiticus	-			
Fusarium solani	-			
Sclerotium rolfsii	-			
(B) *Pseudomonas syringae pv. phaseolicola*	-			
(B) *Pseudomonas syringae pv. tomato*	0.07–0.625 mg/mL 0.156–0.312			
(B) *Pseudomonas syringae pv. syringae*	0.156–0.312 0.312–0.625			
(B) *Xanthomonas campestris pv. campestris*	0.625–2.5			
(B) *Xanthomonas campestris pv. phaseoli*				

[1] MPEO/MAEO; [2] ED_{50} concentration of 8 µL EO solution that inhibited mycelial growth by 50%; not determined; [3] MIC/MFC.

The antimicrobial effectiveness of MPEO was assessed more often, and the spectrum of tested plant-pathogenic microorganisms was broader than that of MAEO. The research applied to antifungal activity predominated over bacterial activity.

The antifungal and antibacterial activity of MPEO and MAEO, expressed as the MIC value, was, in the majority of studies presented in **Table 1**, in the range of 0.25–3 µL/mL 250–3000 µg/mL). However, in some cases the MIC was about 10 times lower, at 44–149 µg/mL [39][47], or even hundreds of times lower, 0.5–10 µg/mL [58]. In the latter research, the MIC values of MPEO were lower for five fungi, the same for two, and for others higher than that of synthetic fungicide amphotericin [58]. MPEO and MAEO were additionally proven to reveal antimicrobial activity in numerous disc diffusion tests.

In research in which series of EOs were investigated, menthol mint oils usually belonged to the group of highly or moderately effective oils. Among 32 essential oils, only MPEO and basil oils were effective in a disc diffusion assay at 20 and 50 µL, respectively, against the *Acidovorax citrulli* bacterium that caused fruit blotch in watermelon [63]. Similarly, MAEO was the most effective against nine fungi out of 18 EOs. The oil at 0.1 mg/mL (100 µg/mL) totally inhibited the growth of four fungi and showed 72–100% inhibition of five others. The highly sensitive fungi were *Aspergillus flavus*, *Helmithosporium oryzae*, and *Sclerotium rolfsii*, with MIC 0.1 mg/mL (100 µg/mL). MPEO was more effective against two toxigenic *A. flavus* strains than four synthetic fungicides [47]. In other research, MAEO was the only one out of 18 EOs that totally inhibited 11 fungal strains at 1000 µg/L (1 µg/mL), with an MIC at 400 µg/L (0.4 µg/mL) toward nine strains being more efficient against *A. flavus* than 10 synthetic fungicides that had the MICs in a range 500–2000 µg/L (0.5–2 µg/mL) [48]. From 105 samples of essential oils representing 53 plant species, MPEOs (20 samples) were among the 18 species exhibiting the highest antifungal activity. When introduced at 1 and 10 µL/mL (1000 and 10,000 µg/mL) to the broth, MPEOs caused a 70–98% reduction of *Aspergillus niger* and *A. ochraceus* and a 47–85% reduction of *Fusarium culmorum* mycelial growth [64]. In an activity assessment of eight EOs against three plant-pathogenic fungi, *Phytophthora cinnamomi, Pyrenochaeta lycoprsici,* and *Verticillium dahliae*, only oregano and thyme oil were more active than MPEO, while the other five oils showed lower activity [50]. Among the 10 EOs assessed against mushroom pathogens, the fungi *Trichoderma harzianum* and *Verticillium fungicola* and the bacterium *Pseudomonas tolaasii*, only the thyme and oregano oils (MIC 1.5–2.0 µL/mL = 1500–2000 µg/mL) were more effective than MPEO (MIC 3–4 µL/L = 3000–4000 µg/mL), which

showed better activity than bifonazole against fungi and almost the same activity as the streptomycin and penicillin mixtures against *P. tolasii* [60]. Similarly, among 18 EOs only three were more efficient than MPEO against five fungal strains isolated from fruits [42]. MPEO was in the group of moderate activity among the 45 EOs researched against three fungi and eight bacteria strains by the disc diffusion method [65]. On the other hand, MPEO appeared the least active out of four EOs against *Fusarium moniliforme* [66] and showed poor efficacy against two plant-pathogenic bacteria, *Agrobacterium tumefaciens* and *Erwinia carotovora* [61].

In spite of quite good antifungal activity against *Lecanicillium fungicola* var. *fungicola*, a fungus that causes dry bubble disease in the mushroom *Agaricus bisporus*, MPEO was not suitable for mushroom protection because of similar activity against fungi, MIC 750–1000 µL/L (750–1000 µg/mL). Among 11 EOs, activity toward mushrooms and pest mycelial growth were assessed in a broth dilution assay, savory (carvacrol 38%) and thyme (carvacrol 46.1%, thymol 30.4%) oils showed the best selectivity index, i.e., were more inhibitive to the growth of the pathogen (MIC 200–250 µg/mL) in comparison to the mushroom (MIC 400 µg/mL) [56].

Fungal toxins are common contaminants in grains, fruits, and vegetables during storage. EOs play a role not only in the reduction of fungal growth, but also in the inhibition of toxin production. MAEO at 1000 ppm completely inhibited the fungal growth of *A. ochraceus* and ochratoxin A production for up to 21 days [44]. Hua et al. [43], in research on five EOs and five compounds against *A. ochraceus* growth and ochratoxin production, showed that cinnamon oil and cinnamaldehyde were the most effective. They did not investigate ochratoxin production in the presence of MPEO. However, they proved that MPEO inhibited fungal growth at 1500 µL/L and the decrease in ochratoxin production by other oils was proportional to the decrease in fungal biomass and correlated with ergosterol inhibition. In other research, MAEO completely inhibited aflatoxin B1 production by the toxigenic strain of *A. flavus* at 0.05 mg/mL, while the radial mycelial growth of this strain was stopped by 0.1 mg/mL [47].

Four MPEOs of different origin and small differences in quantitative composition (main components in accordance with *EP* 5 demands) showed weak antifungal activity in an agar diffusion test. On the other side, the oils strongly inhibited plant-pathogenic bacteria in a dilution test. Pathovars of *Pseudomonas syringae* and *Xanthomonas campestris* differed in terms of their susceptibility to the oil. For some bacterial strains, correlations were found between the oil activity and menthol and menthone percentages [62].

Hussain et al. [39] investigated the content, composition, and antimicrobial activity of four mint species EOs in two harvesting seasons, summer and winter. The authors observed variation in all aspects. However, they stated that, along with the changes in EOs composition depending on the planting time and mineral fertilization, the oils showed a different degree of inhibition: the oils from crops planted and fertilized in the spring were more active against some bacteria. The authors concluded that MAEO exhibited the highest antifungal and antibacterial activity in both tested methods (disc diffusion and broth microdilution), while MPEO, *M. longifolia*, and *M. spicata* oils revealed a similar efficacy [39].

The antifungal and antibacterial activity of MAEO (78.9% menthol) was assessed by the disc diffusion method and compared with the activity of fractions obtained from this oil: dementholized EO (DMAEO, 28.1% menthol), monoterpenes (mainly α- and β-pinene, limonene, and myrcene), menthol, menthone, and isomenthone. At a dose of 5 µL per disc, MAEO and monoterpene fraction showed the highest activity against *A. fumigatus* and *A. niger* (IZ 12–15 mm), followed by DMAEO (IZ 7–11 mm). Similarly, the highest activity against 12 bacterial strains was observed for monoterpenes, MAEO, and DMAEO [67].

In general, the most antimicrobial EOs are oregano, thyme, and savory oils. In the presented research these EOs were shown to be more effective than both menthol mint oils [39][42][50][56][60]. The activity of any EO is strictly connected with its composition. Thyme, oregano, and savory oils contained, as their main constituents, monoterpene phenols, carvacrol, and thymol, which showed higher activity against fungi [68][69] and bacteria strains [61] than menthol. However, there are exceptions to this rule. In nine foodborne fungal pathogens, menthol was more effective to *Penicillium citrinum* than both phenols and similarly effective to *A. ochraceus* (MIC 100 µg/L, MFC 125) [70]. Among 10 monoterpenes, the efficacy of menthol against three fungal pathogens of mushroom was the same as that of thymol and carvacrol, and better than that of other compounds [60].

The antimicrobial activity of MPEO and MAEO is definitively attributed to the presence of menthol, which in all studies was shown to be more effective than menthone. When 22 compounds were tested against *Botrytis cinerea* and *Monilinia fructicola* conidial germination and mycelial growth in broth culture, thymol and carvacrol showed total inhibition at 100 µg/mL, while menthol at 250 µg/mL showed 96% and 97% inhibition and menthone 45% and 8% inhibition of conidial germination of *B. cinerea and M. fructicola*, respectively. At 100 µg/mL, menthol was effective against *M. fructicola* (95%

inhibition) and less effective against the mycelial growth of *B. cinerea* (47% inhibition) [69]. Menthol belonged to a group of the eight most active compounds in the set of 21 EO constituents assessed by the disc diffusion method toward 10 Gram+ and 20 Gram− bacterial strains. The most susceptible were *Aerococcus viridans*, *Clavibacter michiganense*, *Kocuria varians*, two of seven *P. syringae* pathovars, two of four *Erwinia* spp., three *Xanthomonas* taxa, *Neisseria subflava*, and *Agrobacterium tumefaciens*. None of the compounds was effective against all strains. Menthol inhibited the growth of 16 strains but menthone of two strains only [71]. The antimicrobial activity of the main mint oil constituents against seven plant-pathogenic fungi strains was compared with the activity of the standard drug fuconazole in a microdilution assessment The menthol activity (MIC 30.8–107.7 µg/mL) was similar to that of fluconazole (MIC 10.4–100 µg/mL). Menthone, carvone, and piperitenone oxide showed lower activity [39]. According to these reports, it seems that the higher antimicrobial effectiveness of MAEO as compared to MPEO could be attributed to a higher content of menthol, which is more active than menthone.

Chirality is an important aspect of EO compounds because enantiomers may possess different biological activity. According to recent research, in the case of antimicrobial activity, the essential oil constituents' chirality seems to be insignificant. Only a few studies have been performed on that topic. No differences were observed in the activity against three bacteria strains between (−)- and (+)-menthol. However, (+)-menthol was significantly more active than its enantiomer against *Aspergillus brasiliensis* [72].

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