

Mentha longifolia and *Citrus reticulata* Essential Oils: Potential wood-biofungicide and MNDO Quantum Chemical Studies

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Abstract

Essential oils (EOs) from aerial parts from *Mentha longifolia* (Saudi cultivar) and *Citrus reticulata* grown in Egypt, were analyzed by GC/MS to identify their chemical compounds. The EOs were tested for their antifungal activity against four common fungi *Aspergillus flavus*, *A. niger*, *A. fumigatus*, and *Fusarium culmorum*. The most abundant compounds identified in the EO from *M. longifolia* were menthone, and eucalyptol with percentages of 48.00%, and 21.66%, respectively. The main chemical compounds from *C. reticulata* EO were β -caryophyllene, β -caryophyllene oxide, and β -elemene with values of 15.57%, 7.04%, and 6.39%, respectively. At the concentrations of 500 and 250 μ L/mL, the EOs from *M. longifolia* and *C. reticulata* showed potent antifungal activity against *A. flavus* and *A. fumigatus* with 100% fungal mycelial inhibition growth (FMIG). FMIG with values of 98% and 95% were observed against *A. fumigatus* by *C. reticulata* and *M. longifolia* EOs, respectively, at 125 μ L/mL. At 500 and 250 μ L/mL levels of concentration from the EO of *M. longifolia*, potent antifungal activity found against *A. niger* with 100% FMIG. *F. culmorum* completely inhibited (100% FMIG) as EOs from *M. longifolia* and *C. reticulata* applied at 500 μ L/mL. *Pinus halepensis* wood treated with *M. longifolia* at 125 μ L/mL showed inhibition zone values of 7.33 mm, 21.33 mm *A. flavus* and *Aspergillus niger*, respectively. The results suggested the potential values of the EOs as potent antifungal activity against some common molds that grows over wood packaging during transportation of vegetables and fruits or can cause food spoilage.

Introduction

Fungi such as *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Alternaria tenuissima*, *Colletotrichum gloeosporioides*, *Fusarium culmorum*, *Penicillium chrysogenum*, *Rhizoctonia solani*, and, *Trichoderma harzianum* are capable of growing upon a wide range of organic substrates of wood, lignocellulosic materials, food, led to causes deterioration of stored food materials or discoloration of wood or paper substances.^{1–11} Essential oils (EOs), the aromatic substances, can be obtained from different plant parts such as leaves, flowers, seeds, fruits, bark, wood and roots by the extraction using steam or hydro distillation. Some plant EOs have recently been proven to be successful as eco-friendly bio-control agent as antibacterial, antifungal, antioxidant, insecticidal and antiviral,^{12–21} as well as for their potential uses as natural additives and in the food industry,^{22–25} or as wood-biofungicides.^{2–4,6,7}

Mentha longifolia (L.) Huds, or *M. lavandulacea* Willd., or *M. sylvestris* L., has a multi-purposes uses of antimicrobial, antioxidant and insecticidal activities.^{26,27} Piperitone oxide (63.58%) and 1,8-cineole (12.03%), the oxygenated monoterpenes, were found as main compounds in the EO of *M. longifolia*,²⁷ with strong antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica*. *cis*-Piperitone epoxide, piperitenone oxide and pulegone were the main components in *M. longifolia* L. *ssp. longifolia* and the essential oil showed strong antimicrobial activity against 30 microorganisms.²⁶ Carvone, limonene, 1,6-dihydrocarveol, 1,8-cineole, trans-dihydrocarvone, β -bourbonene, germacrene D, β -caryophyllene, and bicyclosesquiphellandrene were found as main compounds from *M. longifolia* EO collected from five regions of Saudi.²⁸ *M. longifolia* oils from different regions of South Africa showed good antibacterial activity against *S. aureus* and *E. coli*.²⁹

In fact, EOs from *M. longifolia* have shown widely variable antimicrobials against *S. aureus*, *Salmonella typhimurium*, *E. coli*, *Fusarium oxysporum*, *Aspergillus flavus*, *A. niger*, *Microsporium canis* and *Mucor ramamnianus* *Salmonella enteritidis* and *Klebsiella pneumonia*.^{30–32} In addition, *M. longifolia* EOs exhibited significant antibacterial activity against multi-resistant bacteria (*E. coli*, *S. enterica* and *P. aeruginosa*).³³ Furthermore, the EO from the dried herb shown potent antibacterial activity against *Bacillus subtilis*, *Micrococcus luteus* and *Enterococcus faecalis*.³⁴ Very strong antibacterial activity against *E. coli*, *Shigella sonnei* and *Micrococcus flavus* and significant fungistatic against

Trichophyton tonsurans and *Candida albicans* was found as the application of three *Mentha* species EO including *M. longifolia*.³⁵ The EO of *M. longifolia* was observed higher antibacterial and antifungal activities than tested commercial substances.³⁵ The main compounds of *M. Longifolia* EO were piperitenone oxide (70%), piperitenone (18.7%) and 1,8-cineole (2.2%), and this oil suppressed the growth of *A. flavus*.³⁶ Significant antifungal and antioxidant activities of *M. longifolia* EO where the main compounds were *trans*-dihydrocarvone (23.64%) and piperitone (17.33%).³¹ Recently, leaf EO with piperitone and eucalyptol as main compounds showed potent antibacterial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*.³⁷

Citrus reticulata L., belongs to the Rutaceae family, is an important fruit citrus-bearing plant with an excellent source of EO.³⁸ Peels of *C. reticulata* Blanco with its main compounds of tangeretin, nobiletin, 5-demethylnobiletin, tetramethyl-o-scutellarein, tetramethyl-o-isoscutellarein, pentamethoxyflavone and sinensetin observed potent inhibition growth of *A. niger*.³⁹ Limonene and other compounds were found to be the major in the EOs extracted from peels, also, other constituents such as sabinene, linalool, γ -terpinene and methyl *N*-methylantranilate were identified in leaf EO.⁴⁰ Generally, EOs from *C. reticulata* is regarded as safe with excellent antifungal, antibacterial and antioxidative properties and the usefulness in food and medicine.⁴¹ β -Caryophyllene oxide, the epoxide derivative from β -caryophyllene, is a component of many EOs, especially *C. reticulata*.⁴²

Therefore, the aim of the present study was to evaluate the bioactivity of essential oils from *M. longifolia* aerial parts and *C. reticulata* leaves against some common molds, and to identify the chemical composition of these oils using GC/MS with the semi-empirical calculations of molecules for the main compounds. Also, study the effects of those oils as wood-biofungicides.

Materials And Methods

Extraction of Essential Oils and identification the compounds using GC/MS

Aerial parts of *Mentha longifolia* (L.) Huds (Saudi cultivar) and leaves of *Citrus reticulata* L. (collected during March 2019, from Alexandria, Egypt), were used for the extraction. About 150 g of air-dried plant material from *M. longifolia* and another 150 g of fresh leaves from *C. reticulata* were extracted by hydrodistillation method using a Clevenger apparatus.²¹ The plant materials were inserted in 2-L flask that was contained of 1.5-L of distilled water, and heated for 3 h under refluxing.⁴³ The essential oils collected and measured to the respective amount of plant materials where the percentages were 2.5% and 1.12%, from *M. longifolia* aerial parts, and *C. reticulata* leaves, respectively.

GC/MS Analysis of Essential Oils

The chemical constituents of the essential oils were analyzed using Focus G C-DSQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA). The apparatus equipped with a direct capillary column TG-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness). The initial temperature of the column oven was held at 45 $^{\circ}$ C, and then increased to 200 $^{\circ}$ C by 5 $^{\circ}$ C/min and hold for 5 min. The temperature was then increased to 300 $^{\circ}$ C, with 30 increments of 5 $^{\circ}$ C/min.⁴⁴ All the compounds were identified using their retention times and mass spectra by matching them with those from WILEY 09 and NIST 11 mass spectral database. Standard Index and Reverse Standard Index with Xcalibur 3.0 data system of GC/MS, where the value $\geq$ 650 is acceptable to confirm the compounds,^{6,7,15,45} were measured for confirmation of the compounds.

Fungal isolates, antifungal activity method, and application on wood

Fungi of *Aspergillus flavus* AFI375, *Aspergillus niger* FC24771, *Aspergillus fumigatus* Afu694, and *Fusarium culmorum* CBS 128537 with their accession numbers of MH355958, MH355955, MH355959, and MH355954, were used for the bioassay evaluation. All the fungal isolates were previously identified through the molecular protocols and partial ITS gene.⁹ The EOs were prepared at the concentrations of 500, 250, 125, and 65 $\mu\text{L}/\text{mL}$ by the dilution in 10% dimethyl sulfoxide (DMSO) with few drops of Tween80 were added. Petri dishes (9-cm) were pouring with warm PDA medium (40 to 45 °C) that contain the prepared concentrations of the EOs. For comparisons, the standard antibiotic of Sertaconazol 3 g/L as a positive and 10% DMSO with Tween80 as negative controls were used. Each treatment was evaluated in triplicate. A seven-day-old colony from each fungus with 9 mm were put in the centre of the treated PDA dishes and controls and incubated at 26 ± 1 °C for 14 days. When the mycelial fungus growth completely full the Petri dish in control treatment (negative), the fungal mycelial inhibition growth (FMIG) percentage was calculated as follow; $\text{FMIG}\% = [(A_C - A_T)/A_C] \times 100$, where A_C and A_T represent the average diameters of the fungal colony of control and treatment, respectively.^{46,47} The lowest two concentrations were used for the application on wood samples of *Pinus halepensis* to show their activity as wood biofungicides using vapor treatment.^{48–50}

Computation Method

Based on semi-empirical calculations, the geometry optimization of the studied molecules has been done using the molecular modeling program Hyperchem7.5 (W.Thiel 2003, HyperChemTM, Release 7.5 Pro 2002). Semi-empirical calculations were carried out using the routine MNDO and Polak–Ribiere conjugated gradient algorithm as shown in previous works.^{18,51,52}

Statistical Analysis

The mycelial inhibition growth percentage values were statistically analyzed using two factors (EO type and EO concentration) using analysis of variance, SAS system.⁵³ Differences among means were measured using Duncan's Multiple Range Test at $\text{Alpha} < 0.05$.

Results

Essential oils composition

Figure 1a and b shows the GC/MS chromatograms of the separated chemical compounds in the essential oils (EOs) from aerial parts of *Mentha longifolia* and *Citrus reticulata*, respectively. Table 1 presents the chemical composition of the EO from *M. longifolia* aerial parts, where which 8 compounds were identified. The main constituents were menthone (48.00%), eucalyptol (21.66%) and pulegone (12.09%). Table 2 presents the chemical compounds of the EO from *C. reticulata* aerial parts, which composed 48 compounds, where the main compounds were β -caryophyllene (15.57%), β -caryophyllene oxide (7.04%), β -elemene (6.39%), γ -elemene (5.62%), β -bisabolene (4.86%), spathulenol (4.74%), α -caryophyllene (4.53%), longifolene (4.40%), γ -gurjunene (3.74%), geranyl acetate (3.34%), α -bergamotene (3.19%), linalyl acetate (2.96%), germacrene D (2.28%), nerol (2.24%), D-limonene (2.14%), and geraniol (2.00%).

Table 1
Chemical composition of the essential oil from *Mentha longifolia* aerial parts

Compound name	Percentage in the oil
Eucalyptol or 1,8-cineole	21.66 (946–947)
Menthone	48.00 (946–969)
Borneol	2.10 (885–910)
Pulegone	12.09 (917–934)
β -Caryophyllene	5.57 (910–938)
2-Methylene-5 α -cholestan-3 β -ol	4.89 (812–845)
1-Heptatriacotanol	5.69 (767–777)

Table 2
Chemical composition of the essential from *Citrus reticulata* aerial parts

Compound name	Percentage in the oil
Δ -3-Carene	0.41 (922–937)
D-Limonene	2.14 (923–925)
Linalool	1.12 (943–954)
Citronellal	0.50 (883–902)
Terpinen-4-ol	0.17 (879–882)
α -Terpineol	1.70 (938–940)
Nerol	2.24 (862–887)
Linalyl acetate	2.96 (885–889)
Geraniol	2.00 (929–935)
Citral	1.41 (888–901)
Δ -Elemene	1.65 (854–897)
Lsopulegol acetate	0.89 (782–843)
γ -Muurolene	0.24 (836–855)
Neryl acetate	1.63 (926–932)
α -Himachalene	0.17 (870–887)
Geranyl acetate	3.34 (902–936)
β -Elemene	6.39 (934–938)
Longifolene	4.40 (958–958)
β -Caryophyllene	15.57 (945–946)
γ -Elemene	5.62 (945–947)
α -Bergamotene	3.19 (933–955)
Nerolidol	0.40 (760–777)
α -Caryophyllene	4.53 (935–945)
Ylangene	0.23 (838–862)
Germacrene D	2.28 (924–935)
β -Selinene	1.34 (928–959)
α -Selinene	1.88 (943–958)
α -Farnesene	0.69 (917–945)
β -Bisabolene	4.86 (928–942)
Selina-3,7(11)-diene	0.25 (859–881)

Compound name	Percentage in the oil
<i>cis-Z-α</i> -Bisabolene epoxide	0.36 (810–817)
<i>trans</i> -Longipinocarveol	1.86 (802–810)
Caryophyllene oxide	0.43 (856–901)
γ -Gurjunene	3.74 (895–911)
β -Caryophyllene oxide	7.04 (954–956)
Isoaromadendrene epoxide	0.17 (798–847)
Humulene oxide II	0.85 (818–901)
Alloaromadendrene	0.12 (799–860)
Nerolidol-epoxyacetate	0.17 (799–853)
Spathulenol	4.74 (883–884)
Globulol	1.46 (839–871)
Guaiene	0.91 (831–842)
2-Methylene-5 α -cholestan-3 β -ol	0.47 (789–811)
Ledene oxide-(II)	0.34 (824–842)
Squalene	0.30 (708–716)
Urs-12-en-28-ol	1.13 (736–787)

Antifungal activity of the essential oils

Figures 2, 3, 4, and 5 presents the visual observations of the inoculated plats with *Aspergillus flavus*, *A. niger*, *A. fumigatus* and *Fusarium culmorum*, respectively, that was previously treated with the concentrated essential oils (EOs) from *M. longifolia* and *C. reticulata*. It can be observed that, with increasing the concentration of the EO, the inhibition of the fungal diameter growth was shown.

Table 3. Inhibition percentage of the diameter growth of *A. flavus*, *A. niger*, *A. terreus*, and *F. culmorum* as affected by essential oils from *Mentha longifolia* and *Citrus reticulata*

Oil source	Concentration ($\mu\text{L/mL}$)	Inhibition Percentage of Diameter Growth (%)			
		<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	<i>Fusarium culmorum</i>
<i>Mentha longifolia</i>	65	48 $\pm$ 3.46	70 $\pm$ 2.64	63 $\pm$ 2	46.66 $\pm$ 1.15
	125	74.33 $\pm$ 1.52	95 $\pm$ 1	73.33 $\pm$ 1.52	70 $\pm$ 1
	250	100	100	100	73.33 $\pm$ 3.21
	500	100	100	100	100
<i>Citrus reticulata</i>	65	86.33 $\pm$ 0.57	68.66 $\pm$ 3.05	65.33 $\pm$ 1.52	65.66 $\pm$ 1.15
	125	91 $\pm$ 3.61	98 $\pm$ 3.46	93.66 $\pm$ 0.57	81 $\pm$ 3
	250	100	100	97 $\pm$ 2.64	85.66 $\pm$ 0.57
	500	100	100	100	100
Negative control (DMSO)	10%	0.00	0.00	0.00	0.00
Sertaconazol	3 g/L	91 $\pm$ 1	88.66 $\pm$ 1.15	87 $\pm$ 1	88.33 $\pm$ 1.52
P value		**	**	**	**
Values are means $\pm$ SE, **: Highly significant at 0.01 level of probability.					

Table 3 shows the antifungal activity of essential oils from *M. longifolia* and *C. reticulata* against the growth of *A. flavus*, *A. niger*, *A. terreus*, and *F. culmorum*. *M. longifolia* and *C. reticulata* EO at the concentrations of 500 and 250 $\mu\text{L/mL}$ observed potent antifungal activity against *A. flavus* and *A. fumigatus* with 100% fungal mycelial inhibition growth (FMIG), which higher than those from Sertaconazol (91% and 88.66%, respectively). *C. reticulata* and *M. longifolia* EOs at 125 $\mu\text{L/mL}$ showed good activity against the growth of *A. fumigatus* with FMIG values of 98% and 95%, respectively. Potent activity of *M. longifolia* EO at 500 and 250 $\mu\text{L/mL}$ was observed against *A. niger* with 100% FMIG, while *C. reticulata* EO showed FMIG percentages of 100% and 97%, at 500 and 250 $\mu\text{L/mL}$, respectively, which higher than from Sertaconazol (87%). The EOs from *M. longifolia* and *C. reticulata* completely inhibited the growth of *F. culmorum* with 100% FMIG at the concentration of 500 $\mu\text{L/mL}$, which higher than those from Sertaconazol (88.33%), also, at the concentration of 250 $\mu\text{L/mL}$, EO from *C. reticulata* showed good activity against *F. culmorum* (FMIG value of 85.66%).

Application of oils on wood samples

As we can see from the Fig. 6 that both oils from *M. longifolia* and *C. reticulata* especially at 125 $\mu\text{L/mL}$ showed inhibition zone values around the treated wood samples compared to control treatment (wood without oils), where huge growth of fungi was observed. Table 4 presents the antifungal activity of both oils as wood-biofungicides with measuring the growth on wood samples and the inhibition zones (IZ) around the wood. *M. longifolia* applied to wood at 125 $\mu\text{L/mL}$ showed IZ value of 7.33 mm against the growth of *A. flavus*. IZ value of 15.33 mm was shown as wood treated with *C. reticulata* oil at 125 $\mu\text{L/mL}$ against *A. fumigatus*. *M. longifolia* at 65 and 125 $\mu\text{L/mL}$ observed potent antifungal activity against *Aspergillus niger* when applied to wood samples with IZ values of 8 and 21.33 mm, respectively. Wood treated with *C. reticulata* and *M. longifolia* at 125 $\mu\text{L/mL}$ showed IZ values of 3.66 and 2.33 mm, respectively, against the growth of *F. culmorum*.

Table 4

Growth on wood sample** and inhibition zone** (mm) of *A. flavus*, *A. niger*, *A. terreus*, and *F. culmorum* as affected by essential oils from *Mentha longifolia* and *Citrus reticulata*

Oil source	<i>Aspergillus flavus</i>			<i>Aspergillus fumigatus</i>		<i>Aspergillus niger</i>		<i>Fusarium culmorum</i>	
	Con. $\mu\text{L/mL}$	Growth on sample (mm)	Inhibition zone (mm)	Growth on sample (mm)	Inhibition zone (mm)	Growth on sample (mm)	Inhibition zone (mm)	Growth on sample (mm)	Inhibition zone (mm)
Control	0	20 $\pm$ 0.00	0.00	20	0.00	20	0.00	18.33 $\pm$ 0.88	0.00
<i>C. reticulata</i>	65	0.00	0.33 $\pm$ 0.33	5.66 $\pm$ 1.20	0.00	0.00	2.33 $\pm$ 0.33	0.00	1 $\pm$ 0.57
	125	0.00	0.66 $\pm$ 0.33	0.00	15.33 $\pm$ 3.75	0.00	5.33 $\pm$ 0.88	0.00	3.66 $\pm$ 0.88
<i>M. longifolia</i>	65	0.00	0.66 $\pm$ 0.33	2 $\pm$ 0.57	0.00	0.00	8.00 $\pm$ 2.00	0.00	1 $\pm$ 0.57
	125	0.00	7.33 $\pm$ 1.20	0.0000	3.33 $\pm$ 0.88	0.00	21.33 $\pm$ 5.69	0.00	2.33 $\pm$ 1.45
Values are means $\pm$ SE, **: Highly significant at 0.01 level of probability.									

Thermodynamic data for the most abundant essential oil compounds

In this study, the main components in the two studied samples were divided to two groups, the first group (I) are the compounds contain oxygen atom in single or double bond in there structures namely; eucalyptol, menthone, pulegone and β -caryophyllene oxide. The second group (II) the compounds contains no oxygen atom in there structures including β -caryophyllene and γ -elemene. Table 5 show all thermodynamic data calculated using MNDO semi-empirical calculation. From the calculated data of the studied molecule (Table 5), one can observe that the negative values of the heat of formations $\Delta F(M)$ and total energy for group (I) (eucalyptol, menthone, pulegone and caryophyllene oxide) neutral molecules have negative values that mean these molecules are stable and the Menthone, molecule is the most stable. This is due to the presence of the oxygen atom (single or double bond) in their structures, while group (II) β -caryophyllene, and γ -elemene have the positive values of heat of formations (12, 47 Kcal/mol, respectively). From these values, the second group is relatively less stable than the first group, which has oxygen atom in their structures. This is confirmed by the values of dipole moment, hence the first group has the higher values of dipole moment (1.384, 2.446, 2.497, and 1.684) in comparison with the second group (0.133 and 0.158). In addition, one can observed that group (I) have the higher values of ionization energies (9.4, 9.5, 9.0 and 8.8 e.V) in comparison with group (II) (8.7 and 8.8 e.V). These due to higher stability of the group (I). The same were observed in electron affinities values, group (I) have higher values of EA as shown in Table 5.

Table 5

Thermodynamic data of the studied molecules calculated within the modified neglect of diatomic overlap (MNDO) framework

compound	Total energy (TE) (Kcal/mol)	$\Delta H_f(M)$ (Kcal/mol)	$\Delta H_f(M)^{+*}$ (Kcal/mol)	$\Delta_f(M)^{-1}$ (Kcal/mol)	Dipole Moment (Debye)	Ionization energy(I.E)* e.V	Electron affinity (E.A)** e.V
Eucalyptol	-42819	-52	166	-31	1.384	9.4	0.91
Menthone	-42831	-64	156	-67	2.446	9.5	0.13
Pulegone	-42164	-50	156	-71	2.497	9.0	0.91
β -Caryophyllene oxide	-59481	-5	199	-3	1.684	8.8	0.08
β -Caryophyllene	-52074	12	213	11	0.133	8.7	0.04
γ -Elemene	-52039	47	252	49	0.158	8.8	0.08
*The value of the ionization energies (IE) was calculated with the following equation, $IE [M] = \Delta H_f [M]^{+*} - \Delta H_f [M]$; where $\Delta H_f [M]^{+*}$ and $\Delta H_f [M]$ are the heats of formation of the molecular ion and neutral molecule, respectively. ***The value of the electron affinity (EA) was calculated with the following equation, $EA [M] = \Delta H_f [M] - \Delta H_f [M]^{-1}$; where $\Delta H_f [M]^{-1}$ and $\Delta H_f [M]$ are the heats of formation of the anion and neutral molecule, respectively.							

Discussion

The essential oil (EO) from *Mentha longifolia* aerial parts showed the presence menthone, eucalyptol (1,8-cineole) and pulegone as the main compounds. Chemical composition of the EOs of many *M. longifolia* grown around the world showed different chymotypes. Menthone and iso-menthone were found in the range amount of (2.8-15.05%) and (0.96–43.79%), respectively, from the plant grown in Iran-Asia.⁵⁴ Menthone (10.7%), pulegone (47.15%), and 1,8-cineole (11.54%) from the plant grown in Tunisia,⁵⁵ while menthol (19.4–32.5%), menthone (20.7–28.8%), 1,8-cineole (5.6–10.8%), terpineol-4 (3.1–4.9%) and pulegone (7.8–17.8%) from the plant grown in Southern Africa.²⁹ In addition, the plant growing in Serbia, menthone (11.2%) and piperitone (8.8%) were identified as main compounds.³⁵ The main compounds from the plant growing in South Africa were menthone, 1,8-cineole, and pulegone.⁵⁶ Piperitone as the major one (30.77%), followed by eucalyptol (14.85%) and caryophellene (5.58%) were observed in the EO of leaves from Saudi *M. longifolia*,³⁷ while Desam et al.⁵⁷ reported that the menthone (39.55%), isopulegone (30.49%), eucalyptol (10.38%), and α -terpineol (3.15%) were major components from the aerial parts that were air-dried under shade with promising antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cereus* and antifungal activity against *Aspergillus flavus*, *A. fumigates*, *Alternaria alternaria*, *Fusarium oxysporum*, and *Penicillium* spp.

1,8-cineole found in 21.66% in *M. longifolia* oil, was also reported to be one of the major compound in *Mentha* species EO, which ranged from 1.6–15.58% from Iran,^{58–60} Tunisia,^{55,61} and Italy.⁶² Pulegone, 1,8-cineole, and L-menthone with percentages of 26.92%, 21.3%, and 10.66%, respectively, were found as main compounds in the EO of the plant

grown in the winter season, while the main compounds found in the EO from the plant grown in spring were pulegone, oleic acid and palmitic acid with percentages of 38.2%, 23.79% and 15.26%, respectively.³²

Other chymotypes were piperitenone oxide and piperitone oxide in plants growing in the Mediterranean region.^{27,59,62–64} The plant growing in Iran,⁶⁵ and Sudan,⁶⁶ are rich in carvone type, while the EOs from Jordan,⁶⁴ and Tunisia,⁶¹ are rich in pulegone type. Also, plants grown in Iran are contains 1,8-cineole rich-chemotype,⁵⁸ or rich in carveol.⁶⁷ *M. longifolia* EO grown in Croatia showed the presence of piperitenone oxide, β -caryophyllene, carvone, and limonene as main compounds.⁶⁸ The EO from *M. longifolia* flowers collected from Zlatar, Belgrade, Serbia, showed the presence of trans and cis-dihydrocarvone, piperitone, 1,8-cineole and neoisodihydrocarveol, as main compounds with 23.64%, 15.68%, 17.33%, 8.18% and 7.87%, respectively.³¹ Piperitone oxide with high amount and piperitenon oxide were found in *M. longifolia* EO from Morocco.⁶⁹ While cis-carveol was the dominant compound in *M. longifolia* from Iran with percentage ranged from 53 to 78%.⁷⁰ Piperitone oxide, and piperitenone oxide were found as main compounds from the EO from the plant grown in Turkey.⁷¹ From Iran, they were piperitone, limonene and trans-piperitol,⁷² and from France, were carvone, 1,8-cineole and limonene.⁷³ The EO of *M. longifolia* growing wild in the Bahcesaray area (Van Province, Turkey) showed the presence of menthone (19.31%), pulegone (12.42%), piperitone (11.05%), dihydrocarvon (8.32%), limonene (6.1%), 3-terpinolenone (5.66%), 1,8-cineole (4.37%), germacrene D (3.38%) and caryophyllene (3.19%) as main components.⁷⁴

The plant grown in India in different locations showed the presence of piperitenone oxide, cis/trans-piperitone oxide, 1,8-cineole, piperitenone, dl-limonene, piperitone, 4-hydroxy piperitone, β -caryophyllene.^{58,75–79} EOs with their main compounds (carvone, limonene and 1,6-dihydrocarveol) from plants grown from five regions (Saudi Arabia), showed moderate antifungal activity against *A. niger*, *A. flavus* and *Fusarium solani*.²⁸ At 250 ppm EO of *M. longifolia* inhibited the growth of *F. oxysporum* (92%) followed by *Sclerotium rolfsii* (70.66%) and *Rhizoctonia solani* (57.04%).⁷⁵ The EO from *M. longifolia* at the 10 μ l/ml observed potent fungicidal activity against *Aspergillus niger*, *A. ochraceus*, *A. flavus*, *A. versicolor*, *F. tricinctum*, *F. sporotrichioides*, *Penicillium funiculosum* and *Trichoderma viride*.³¹

In the present study, some bioactive compounds were found in the EO from *C. reticulata* such as β -caryophyllene, β -caryophyllene oxide, β -elemene, γ -elemene, β -bisabolene, spathulenol, α -caryophyllene, longifolene, γ -gurjunene, geranyl acetate, α -bergamotene, linalyl acetate, germacrene D, nerol, D-limonene, and geraniol. Among some citrus EO (*Citrus lemon*, *C. reticulata*, *C. paradisi* and *C. sinensis*), the EO from *C. reticulata* showed the lowest activity against *Lactobacillus curvatus*, *L. sakei*, *Staphylococcus carnosus*, *S. xylosus*, *Enterobacter gergoviae* and *E. amnigenus*.²² Hydrocarbons and linalool were mostly found in leaf EO from mandarin (*C. reticulata*), while thymol and/or terpinen-4-ol, were found leaf EO of some varieties.^{80,81} In 41 mandarin cultivars, γ -terpinene, sabinene, linalool, limonene, p-cymene, (E)- β -ocimene, β -pinene and terpinen-4-ol were found in the range of (0.2–61.3%), (0.2–59.4%), (0.2–54.3%), (1.5–44.3%), (tr-20.4%), (0.6–13.7%), (0.1–10.7%) and (0.1–10.6%), respectively, in the EO from leaves.⁴⁰

The EO from peel of fully matured ripen fruits of *C. reticulata* Blanco with its main compounds of limonene, geranial, neral, geranyl acetate, geraniol, β -caryophyllene, nerol, and neryl acetate showed good activity against some pathogenic fungi *Alternaria alternata*, *Rhizoctonia solani*, *Curvularia lunata*, *Fusarium oxysporum* and *Helminthosporium oryzae*.⁴¹ Mature fruits EO of *C. reticulata* showed the presence of citronellol, octanal, decanal, nonanal, β -pinene, limonene, citral, γ -terpinene, linalool, and α -terpineol with high antifungal activity against the growth of *Penicillium italicum* and *P. digitatum*.⁸² Leaves EO from six cultivars of *C. reticulata* Blanco from Nigeria showed the presence of sabinene, γ -terpinene, p-cymene, δ -3-carene and (E)- β -ocimene, while other constituents include linalool, myrcene, terpinen-4-ol and cis-sabinenehydrate. In addition, limonene, terpinolene, β -pinene, α -pinene,

β -sinensal and α -sinensal were detected and isolated.³⁸ Geranial, neryl acetate, geranyl acetate, β -pinene, myrcene, neral, and β -caryophyllene have been identified in the leaf oil of *C. limon*.⁸³

The applied EOs from *M. longifolia* and *C. reticulata* to *Pinus halepensis* wood showed good activity against the growth of *A. flavus*, *A. niger*, *A. terreus*, and *F. culmorum*. Previously, EOs and extracts have been used as wood-biofungicides and showed some potential antifungal activity, i.e., treated *Acacia saligna* wood with *Maclura pomifera* bark methanol against *Alternaria tenuissima*,² *A. saligna* wood treated with of *Cupressus sempervirens* methanolic extract against *Trichoderma harzianum* infestation,³ treated wood samples from *Pinus sylvestris*, *P. rigida* and *Fagus sylvatica* with two EOs of *P. rigida* (wood) or *Eucalyptus camaldulensis* (leaves) showed promising antifungal activity against five molds (*A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride*),⁵⁰ treated wood samples from *A. saligna*, *F. sylvatica*, *Juglans nigra* and *P. rigida* the oil from *Origanum* showed good antifungal activity against *T. harzianum* and *A. niger* without changing the wood structures,⁸⁴ *Leucaena leucocephala* wood treated with *Acer saccharum* var. *saccharum* extract from inner or outer bark extract in combination with citric acid showed bioactivity against the growth of *T. viride*, *F. subglutinans*, and *A. niger*,⁶ *Melia azedarach* wood treated with *E. camaldulensis* or *V. agenus-castus* n-hexane oily extracts showed potential antifungal against *F. culmorum*, *Rhizoctonia solani* and *Penicillium chrysogenum*,⁷ *M. azedarach* wood samples treated with *A. saligna* flowers extract showed promising antifungal activity against *P. chrysogenum*,⁴ *M. azedarach* wood treated with *Musa paradisiaca* extract showed good bioactivity against *F. culmorum* and *R. solani*,⁵ treated-chinaberry wood blocks with *E. camaldulensis* bark extract showed potential antifungal activity against *F. culmorum* and *Botrytis cinerea*,⁸⁵ and *M. azedarach* wood treated with whole plant extract *Haplophyllum tuberculatum* observed good antifungal activity against *F. culmorum* and *R. solani*.⁸⁶ *Corymbia citriodora* EOs from leaves (the main compounds were citronellal, citronellol and isopulegol) and fruits (α -pinene, eudesmol, limonene, γ -terpinene as main compounds) applied to wood samples at the amounts of 100, 50 and 25 μ L showed of 100% inhibition against *Fusarium culmorum*.¹⁸ Recovered oil dissolved in *n*-hexane solvent as partitioned from the distillate residue of hydro distillation of fresh flowers from *Matricaria chamomilla* showed have potent bioactivity activity against *A. niger* and *A. terreus*.⁴³

The EOs altered the hyphal morphology by causing loss of cytoplasm, the release of cell constituents, and the total lipid content of molds. In addition, the EOs could generate the cytotoxicity in fungi by disrupting the integrity of cell membrane and causing the leakage of cell components.^{82,87}

Conclusions

Findings of the present research confirmed the potent antifungal activities of essential oils from *Mentha longifolia* (Saudi cultivar) and *Citrus reticulata* (Egyptian growing plant). Essential oils from *M. longifolia* and *C. reticulata* showed a great interest for their antifungal activities against some common molds *Aspergillus flavus*, *A. niger*, *A. fumigatus*, and *Fusarium culmorum*. The oils showed good wood-biofungicide when applied to wood samples with vapor method. These activities suggesting their possible use as natural preservative additives and in the food industry.

Declarations

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Figures

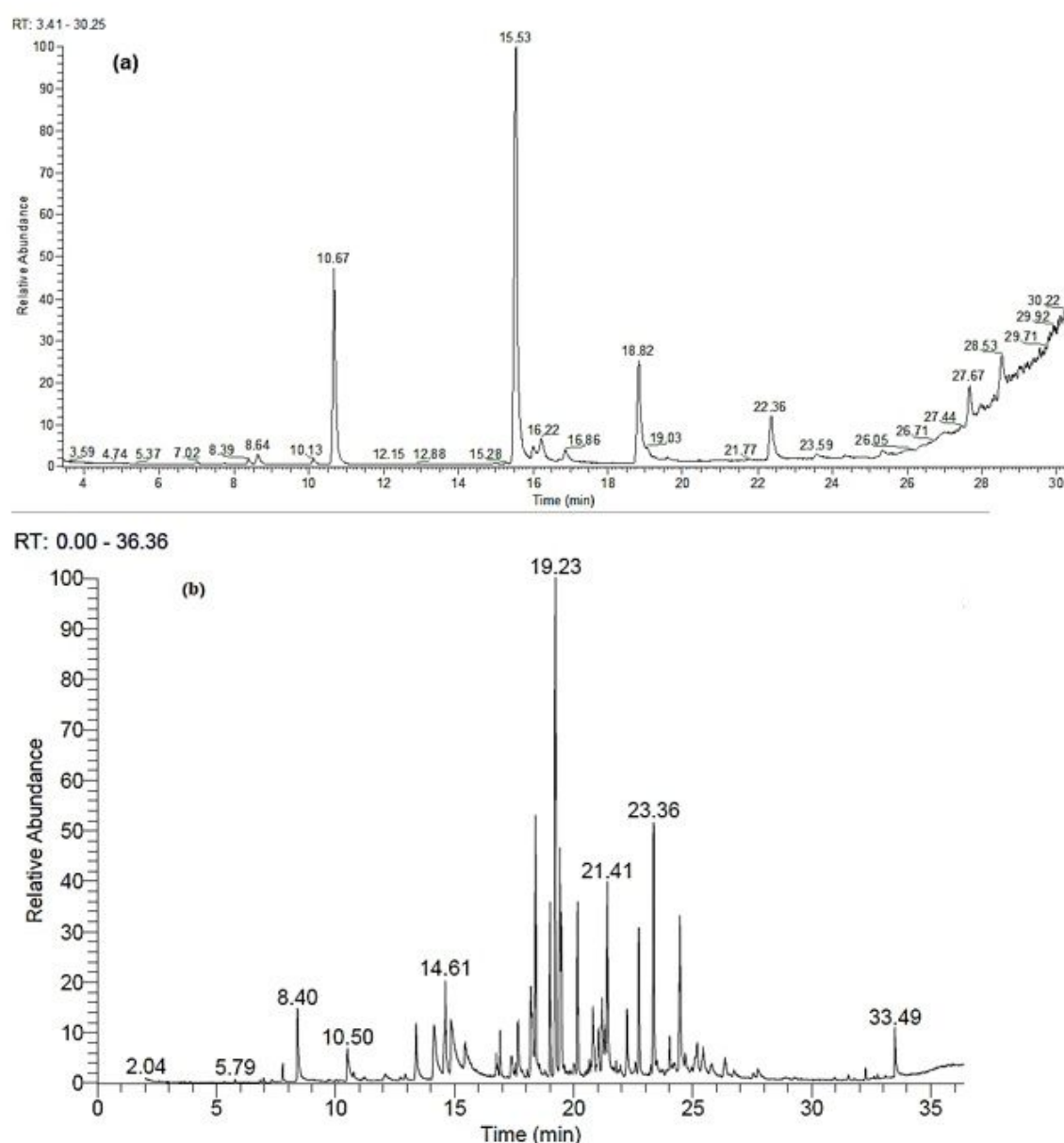


Figure 1

GC/MS separation chromatograms for the essential oils from aerial parts of (a) *Mentha longifolia* and (b) *Citrus reticulata*

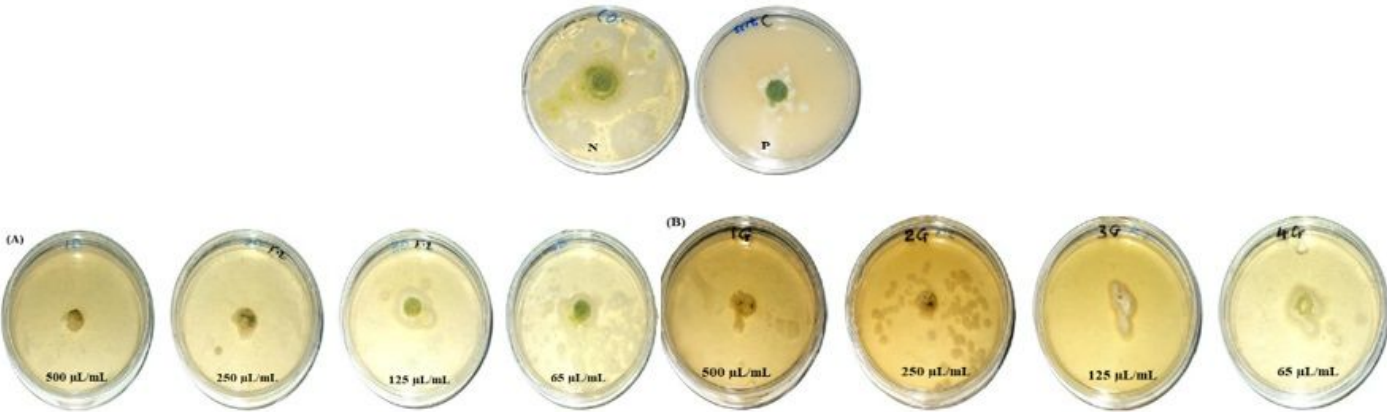


Figure 2

Visual observation of *Aspergillus flavus* growth inhibition as affected by the EOs from (A) *Mentha longifolia*; (B) *Citrus reticulata*; (P) Positive control (Sertaconazol 3 g/L); (N) Negative control (DMSO 10%).

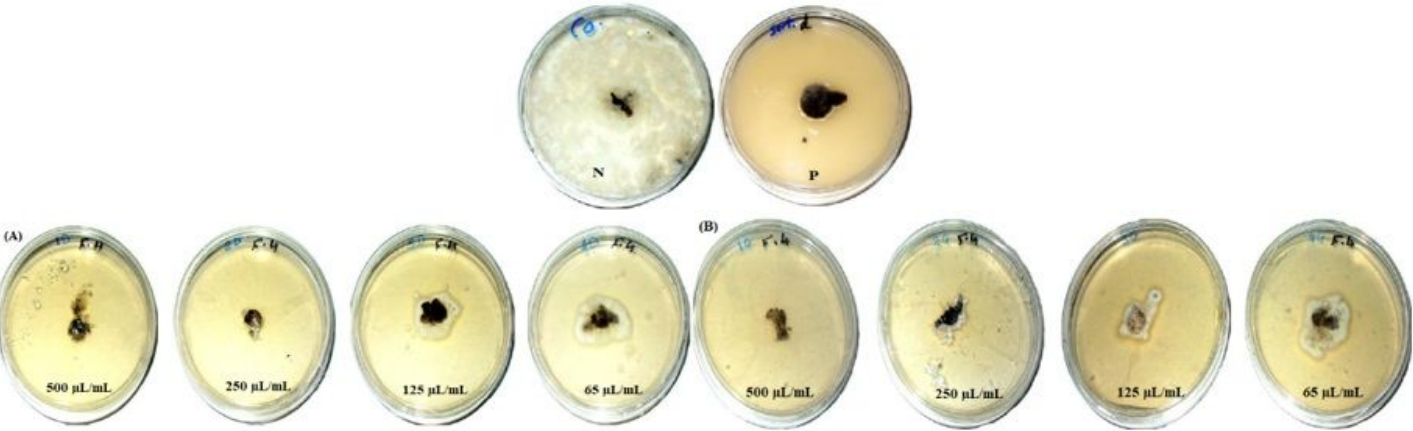


Figure 3

Visual observation of *Aspergillus niger* growth inhibition as affected by the EOs from (A) *Mentha longifolia*; (B) *Citrus reticulata*; (P) Positive control (Sertaconazol 3 g/L); (N) Negative control (DMSO 10%).

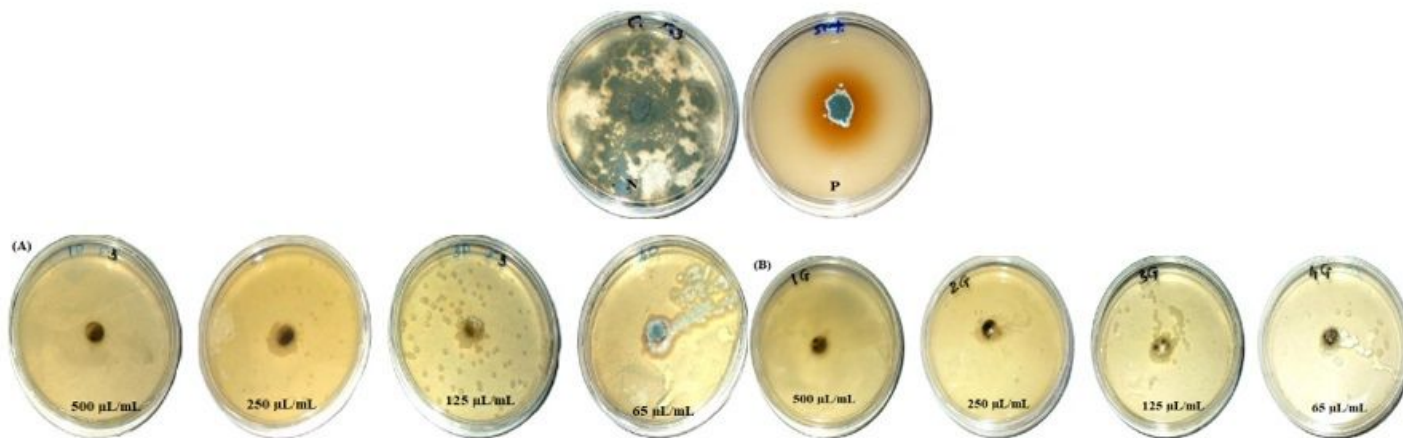


Figure 4

Visual observation of *Aspergillus fumigatus* growth inhibition as affected by the EOs from (A) *Mentha longifolia*; (B) *Citrus reticulata*; (P) Positive control (Sertaconazol 3 g/L); (N) Negative control (DMSO 10%).

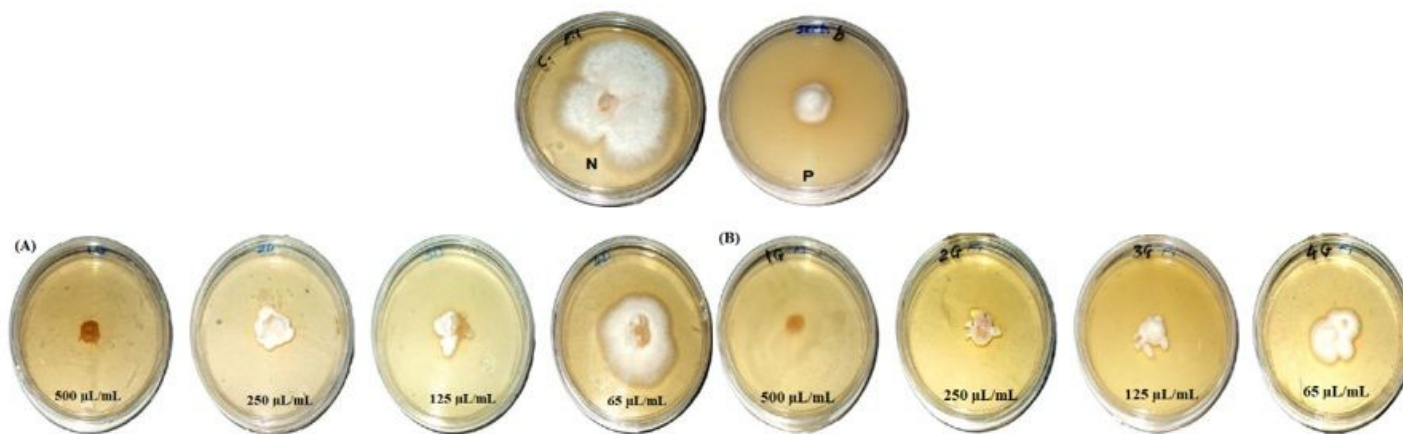


Figure 5

Visual observation of *Fusarium culmorum* growth inhibition as affected by the EOs from (A) *Mentha longifolia*; (B) *Citrus reticulata*; (P) Positive control (Sertaconazol 3 g/L); (N) Negative control (DMSO 10%).

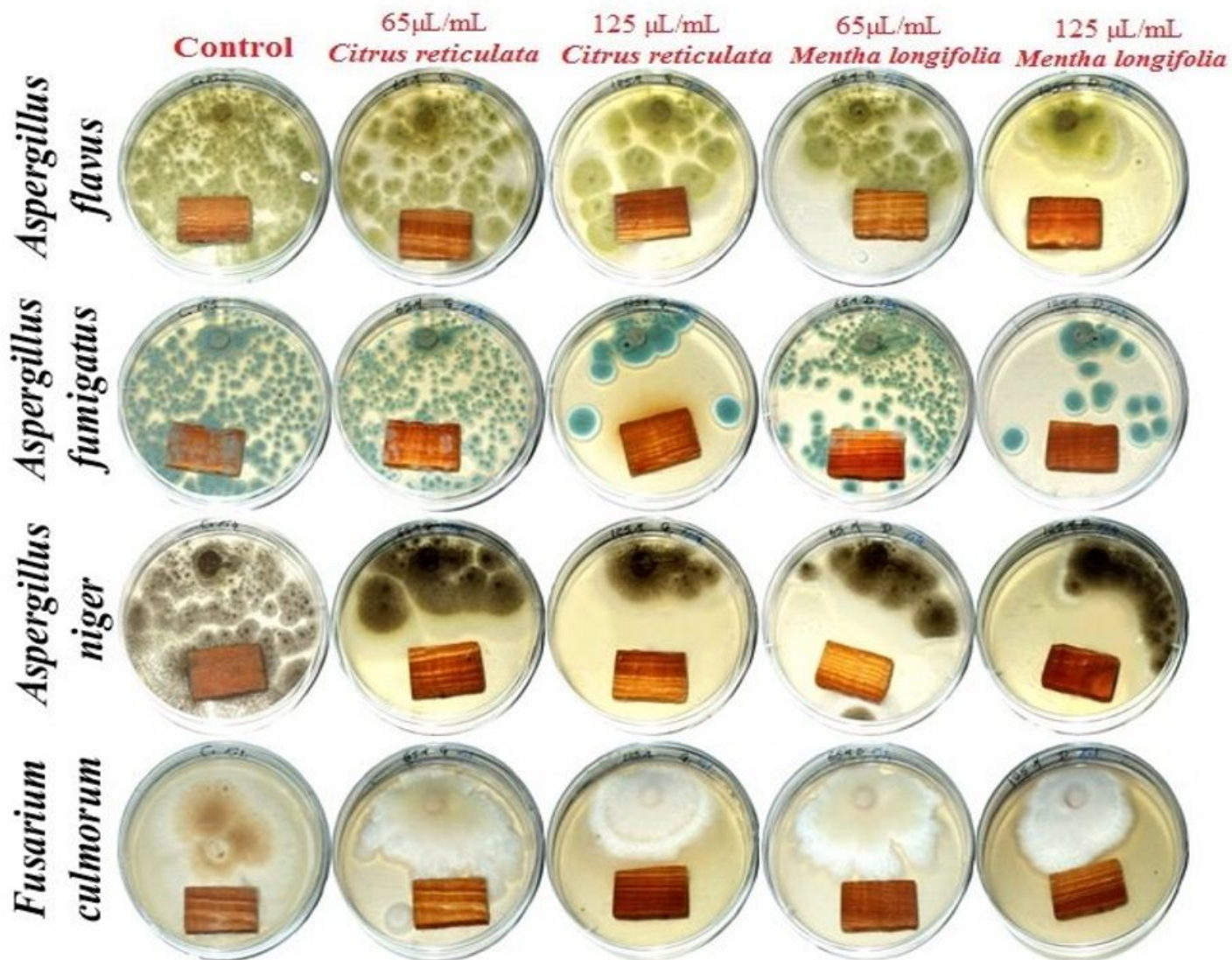


Figure 6

Experimental application of the essential oils from *Mentha longifolia* and *Citrus reticulata* to *Pinus halepensis* wood and the visual observation of growth of *A. flavus*, *A. niger*, *A. terreus*, and *F. culmorum*.