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Antifungal potential of essential oil of *Mentha spicata* (L.) in the biological control against phytopathogenic fungi

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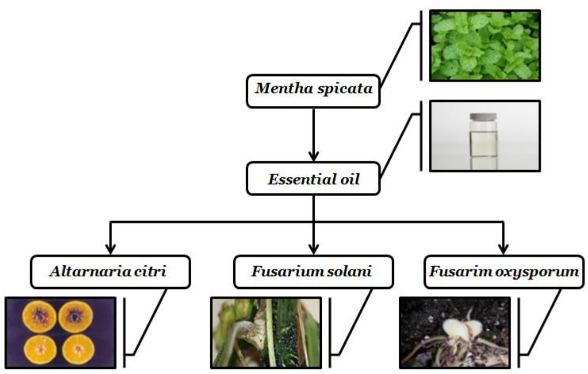
Graphical abstract



Abstract

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

The objective of this study is to evaluate the antifungal activity of Mentha spicata essential oil, against fungal strains isolated from infested plants, namely Altarnaria citri, Fusarium solani and Fusarim oxysporum.

The results obtained illustrate the inhibitory effect of Mentha spicata essential oil on the mycelial growth of fungal strains with inhibition rates ranging from 90% to 100%. The phytopathogen Fusarium solani turns out to be the most sensitive strain with an IC50 of around 8.70 µL/L.

Keywords: Mentha spicata, essential oil, antifungal activity, biological control

1. Introduction

There are more than 4000 species in 200 genera in the Lamiaceae family. This family contains many genera of medicinal plants that are used in both raw and cooked food preparation and the treatment of human illnesses. Essential oils found in several Lamiaceous species exhibit biological activity against a wide range of bacterial and fungal diseases (Snoussi *et al*. 2015; Fattahpour *et al*. 2024).

From year to year, the pressure of fungal diseases increases, especially with the change climate which has a direct effect on pathogens. We also observe their mutation geographically to previously unaffected areas. These diseases have multiple impacts: quantitative, on production yields, qualitative, on humans and animals, through the production of mycotoxins for example (Seidel *et al*. 2024).

Chemical control is the most effective means of reducing the incidence of diseases caused by moulds. However, new regulations encourage the reduction of synthetic fungicide intake due to the appearance of new resistant strains (Avenot *et al*. 2008), potential risks for human health or environmental pollution (Krieger 2001; Unnikrishnan and Nath 2002).

Awareness of the environmental cost and consumer fears of the danger that pesticide residues can pose to human health are giving rise to growing interest in alternative control methods for crop protection. Among the solutions considered, the use of aromatic and medicinal plants represents an inestimable potential for the search for new substances with antifungal or antimicrobial power. Thus essential oils and organic extracts are attracting growing interest as a potential source of natural bioactive molecules that can be used as alternatives to certain synthetic substances (Bruneton 1999; Isman 2000, Soković *et al*. 2009; Boughendjioua 2019, Piras *et al*. 2021; Djekoun *et al*. 2022).

In order to validate the use of plant species of Mentha genus and to seek alternatives to synthetic chemical substances, our work revolved around the evaluation of the antifungal properties of Mentha spicata. Our choice was based essentially on its abundance and its frequent use in local culinary and medicinal traditions.

2. Materials and methods

2.1. Plant materials

The selected species *Mentha spicata* was collected during March and April 2022, in its natural habitat in the commune of Ben Mehidi, located in the state of El Tarf (North-East of Algeria),

(Geographical coordinates: 36° 46' 2.23"N**,** 7° 54' 13.16"E). The taxonomic identification of *Mentha spicata* was made by Pr. Hicham Boughendjioua from the Department of Natural Sciences, Higher School of Professors for Technological Education, Skikda, Algeria, and voucher specimen was deposited in the herbarium of Plant Physiology Laboratory, (PPL 03/2022).

The aerial parts of the plant are cleaned of soil and other contaminating grasses than dried in the shade at room temperature.

2.2. Essential oil extraction

The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus. A 50 g sample is subjected to steam distillation for 3 hours at a temperature of 100°C (Chanthaphon *et al.* 2008). The oil-laden vapors pass through a cooler, condense and separate into two liquid phases; an aqueous phase (aromatic water) and an organic phase consisting of the essential oil. At the end of the hydrodistillation, the yellow essential oil is collected in an airtight container and stored at 4°C away from light until use.

2.3. Fungal strainwaA

The fungal strains were generously provided to us by the National Institute for Plant Protection (INPV) of El Kous in the commune of Ben Mehidi (El Tarf - Algeria). The strains come from a survey carried out in the eastern region of the country. They were obtained from infected lemon roots (*Alternaria citri* and *Fusarium solani*) and peas (*Fuasrium oxysporum*).

2.4. Antifungal test

The antifungal activity of *Mentha spicata* essential oil was investigated using agar incorporation method (De Billerbeck *et al.* 2002; Angaman *et al.* 2018). Because of their hydrophobic nature, the essentiel oil was diluted in the in DMSO, and four concentrations were selected: 8, 12, 16 and 20 µL/mL. The mixture essential oil-DMSO is incorporated aseptically into the Potato Dextrose Agar medium (PDA) maintained under supercooling (40 to 45°C). The solvent DMSO was used as a negative control. After homogenization, this mixture was poured into Petri dishes. The inoculation was carried out on the surface, in form of deposits of a mycelial fragment about 5–6 mm aged of 7-day old placed at the center of the Petri dish. Than dishes were incubated at 25°C for 7 days. Three repetitions were performed for each concentration. The average diameter of colonies is estimated from 2 perpendicular diameters to calculate inhibition rates according to the formula:

$$I (\%) = [(D_c - D_t) / D_c] \times 100$$

D_c: mycelial growth in the control plate,

D_t: mycelial growth in the inserted plate.

2.5. Determination of IC_{50}

The IC_{50} is calculated graphically by linear regression. It corresponds to the concentration of the essential oil that inhibits mycelial growth by 50%.

2.6. Nature of the fungitoxicity

This method is based on the transfer of a mycelial fragment from the Petri dish where the inhibition by essential oil is total, in a new PDA medium devoid of essential oil. The latter is considered fungistatic if growth of the fungal strain resumes again and is fungicidal or lethal if there is no growth (Yakhlef *et al.* 2020).

2.7. Data analysis

Data entries were expressed as means ± standard error (SE) for triplicate results and graphs were accomplished by using Microsoft Excel 2013. One-way ANOVA was conducted using XLSTAT 2016 at confidence level 95%, i.e. at 5% significance level. The IC_{50} was calculated by the linear equation of the binary logistic regression with the probit model.

3. Results and discussion

3.1. Yield and chemical composition

Essential oil content and composition differences may be attributed to factors related to ecotype, phenophases, temperature, relative humidity, photoperiod, irradiance, genotype, and agronomic conditions (harvesting time, plantage, and crop density) (Snoussi *et al.* 2015), generally, the yields of *Mentha spicata* essential oil varied between 2.41-2.74% (w/w) with the main components of pulegone (26.7-29.6%), piperitone (22.2-28.2%), limonene (3.2-5.2%), α-phellandrene (1.3-2.6%), trans-caryophyllene (5.2-8.0%) and germacrene D (3.08-5.32%) (Mahboubi 2021).

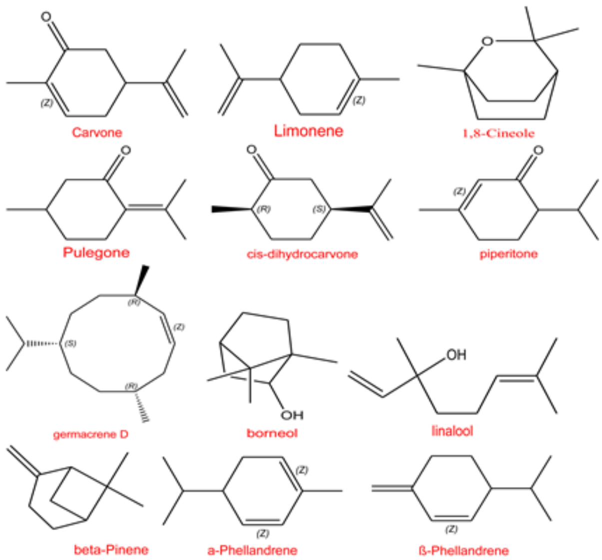


Figure 1. The chemical structures of main components of Mentha spicata essential oils (Mahboubi 2021)

In Algeria several studies have aimed at the yield and chemical composition of *Mentha spicata* essential oil (Table 1).

Table 1. Chemical composition *Mentha spicata* essential oil growing in different regions in Algeria

Author's	Region	Yield (%)	Major constituent
Laggoune *et al*. 2016	Ghardaïa	2.1	cis-carvone oxide (44.06%), 1,8-cineole (15.32%), cis-dihydrocarvone (8.85%) and limonene (5.80%).
Selles *et al*. 2018	Tiaret	1.97	carvone (63.59%). limonene (5.85%), dihydrocarvyl acetate (4.17%), 1,8-cineole + β-phellandrene (3.75%), trans-thujanol (2.74%), cis-dihydrocarvone (1.68%), β-caryophyllene (1.37%),terpinen-4-ol (1.23%), borneol (1.11%) and cis-carvyl acetate (1.07%).
Bardaweel *et al*. 2018	Laghouat	1,04	carvone (49.5%), limonene (16.1%), 1,8-cineole (8.7%), cis-dihydrocarvone (3.9%), β-caryophyllene (2.7%), germacrene D (2.1%) and β-pinene (1.1%).
Mejdoub *et al*. 2019	Tlemcen	0.7%	carvone (54.1%) and limonene (21.9%).
Kehili *et al*. 2000	Tipaza	Not reported	carvone (52.60%), limonene (24.99%), 1.8-cineole (7.22%) and trans-caryophyllene (3.38%).
Benchohra *et al*. 2022	El Bayadh	Not reported	carvone (42.23%), limonene (29.57%), 1,8-cineole (5.31%), β-pinene (3.54%) β-caryophyllene (2.18%), α-Pinene (1.85%) and germacrene D (1.66%).
Brahmi *et al*. 2023	Bejaia	1.1	carvone (48.5%), limonene (20.7%), and 1,8-cineole (5.4%).
Mekhelfi *et al*. 2023	Ouargla	1.5	D-carvone (28.24%), eucalyptol (19.63%), D-limonene (09.20%), beta.-Myrcene (07.36%), and Cyclohexanone, 2-methyl-5-(1-methyl ethenyl) (05.33%).

3.2. Antifungal activity

Our results show a gradual decrease in colony diameters (Figures 2, 3 and 4). The species *Alternaria citri* seems to be the most sensitive to treatment with essential oil of *Mentha spicata*. Indeed, mycelial growth is totally inhibited at higher concentration (20 µL/mL). As for the colonies of the *Fusarium solan*i and *Fusarium oxysporum* species, the diameter reaches 0.8 cm for the 20 µL/mL concentration, compared to the control colonies which are around 8 and 8.5 cm respectively.

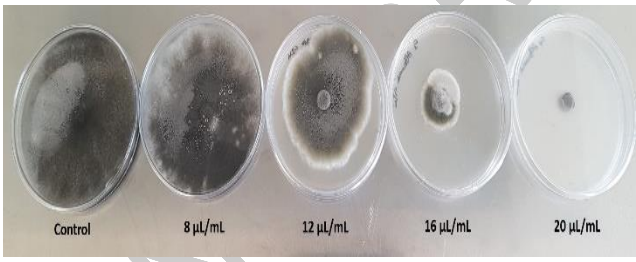


Figure 2. Size colonies evolution of Alternaria citri strain exposed to increasing concentrations of the essential oil of Mentha spicata

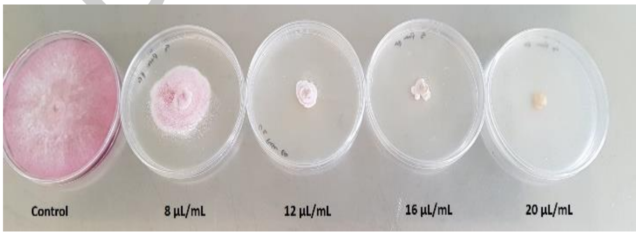


Figure 3. Size colonies evolution of Fusarium solani strain exposed to increasing concentrations of the essential oil of Mentha spicata

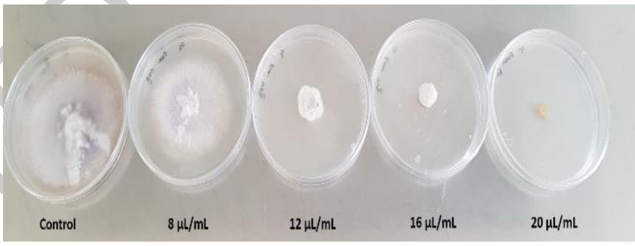


Figure 4. Size colonies evolution of Fusarium oxysporum strain exposed to increasing concentrations of the essential oil of Mentha spicata

The concentrations tested therefore allowed to appreciate a dose-dependent and proportional inhibitory effect. The exposure of phytopathogens to the essential oil of *Mentha spicata* generates inhibition percentages of the order of 90% in *Fusarium solani* and *Fusarium oxysporum* and 100% in *Alternaria citri* when these are exposed to a concentration of 20 µL/mL (Figures 5, 6 and 7).

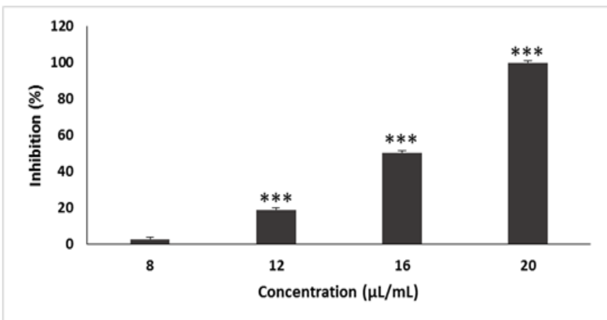


Figure 5. Inhibition percentages of Alternaria citri strain exposed to increasing concentrations of Mentha spicata essential oil

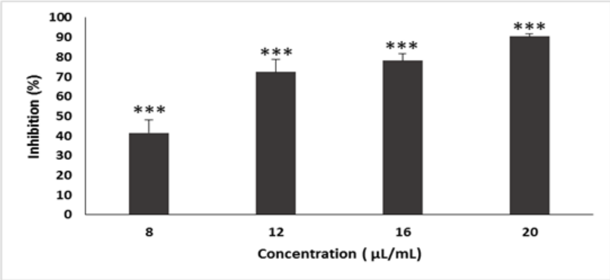


Figure 6. Inhibition percentages of Fusarium solani strain exposed to increasing concentrations of Mentha spicata essential oil

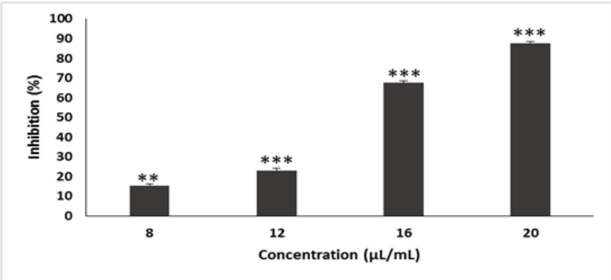


Figure 7. Inhibition percentages of Fusarium oxysporumi strain exposed to increasing concentrations of Mentha spicata essential oil

The main finding of the ANOVA analysis is a significant effect on the overall fungal growth inhibition compared to control ($P < 0.0001$) except for *Alternaria citri* treated with 8 µL/mL, the concentration of the oil used showed no significant effect.

The IC_{50} recorded in this study are between 8 and 15 µL/mL. They show a sensitivity of the three phytopathogens to the action of the essential oil of *Mentha spicata*. The phytopathogen *Fusarium solani* is the most sensitive strain with an IC_{50} of the order of 8.70 followed by *Fusarium oxysporum* and *Alternaria citri* with respective values of 13.18 and 15.58 µL/mL.

The nature of the fungitoxicity of the essential oil was studied by the transfer of the mycelial fragment, whose inhibition of growth is complete, on a new PDA medium without essential oil. In fact, the results obtained showed that mycelial growth of the three fungal strains is relaunched after the incubation period, thus suggesting a fungistatic effect of the essential oil of *Mentha spicata* against the phytopathogens *Alternaria citri*, *Fusarium solani* and *Fusarium soxysporum*.

Many recent studies on plants of the genus Mentha, in the form of extracts or oils, have shown the antimicrobial potential of various species. Indeed Hmiri *et al.* (2011) and Yakhlef *et al.* (2020) demonstrated the inhibitory effects of extracts of *Mentha pulegium* on fungal strains of *Alternaria alternata*, *Botrytis cinerea*, *Penicillium expansum* and *Fusarium culmorum*, responsible for many diseases in post-fruit harvest and the withering of wheat.

Our results corroborate those of other research which clearly demonstrated the notable antifungal power of mint essential oils and which showed very significant fungitoxicity on several fungal species (Lahlou *et al.* 2005; Ouraïni *et al.* 2007, Hajlaoui *et al.* 2009).

According to (McEwan 1994), the inhibition of the growth of filaments in the tested strains could be explained by an impediment of germination of the conidia by the volatile compounds of the oil. The germination of conidia is the first essential step leading to the establishment of a germ tube and a hypha, thereafter he process begins with hydration followed by the action of lytic enzymes such as chitinase and α and β-glucanases. This breaks down the thickened conidial cell wall to allow the initial tube to appear. Once this event takes place, there is a balance between the lytic and synthetic enzyme systems necessary for the normal extension of hyphae. An imbalance in either enzyme system leads to growth inhibition and/or suppression.

The antimicrobial activities of essential oils are difficult to attribute to a specific compound because of their complexity and variability. In general, the antimicrobial activity is mainly explained by the presence of mono and sesquiterpenes with aromatic rings and phenolic hydroxyl groups capable of forming hydrogen bonds with the active sites of target enzymes (Rojas *et al.* 2007).

The antifungal effect of *Mentha spicata* essential oil could be attributed to its chemical composition rich in oxygenated monoterpenes (Boukhebti *et al.* 2011). These compounds are known for their ability to inhibit mycelial growth. In their study, Marei and Abdelgalei, (2018) evaluated the inhibitory effects of six monoterpenes on the mycelial growth of eight phytopathogenic fungal species. All monoterpenes tested caused inhibition of mycelial growth. However, the antifungal effect depended on the specific compound and fungal species tested.

The essential oil's antifungal activity could also be due to the presence of caryophyllene oxide (oxygenated sesquiterpene) (Boukhebti *et al.* 2011). In their study conducted to assess the sensitivity of certain strains of molds to essential oils of *Pinus halepensis*, of which caryophyllene oxide is the main constituent (52%), Abi-Ayad *et al.* (2011) observed antifungal activity against strains of *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopus stolonifera.*

The amplitude of effects of the components of essential oils depends only on their concentration when they were tested alone or included in essential oils (Hajlaoui *et al.* 2009). However, it is possible that other minor molecules modulate the activity of the main components.

The mechanisms by which essential oils can inhibit microorganisms involve different modes of action and in part perhaps due to their hydrophobic character. As a result, they become embedded in the lipid bilayer of the cell membrane, making them more permeable, which leads to leakage of vital cell contents. Impairment of bacterial enzyme systems may also be a potential mechanism of action (Edris 2007).

The antifungal activity is not general for all types of moulds, some of them can consume terpenes as a carbon source, degrade or transform them, which may explain the ineffectiveness of some of these molecules (Heyen and Harder 1995). In addition, some fungal species are more resistant than others. Millon *et al.* (2002) attributes this to the fact that these strains develop mechanisms of resistance to the antifungal molecules present in the extract of essential oils. Among these mechanisms is the ability to switch or change phenotype "phenotypic switch".

4. Conclusion

The use of plants represents an inestimable potential for the search for new substances with antimicrobial power. Thus natural compounds are attracting growing interest as a potential source of natural bioactive molecules that can be used as an alternative to certain synthetic substances in pest management.

The confrontation of fungal isolates with increasing concentrations of the essential oil revealed that *Mentha spicata* exhibits an inhibitory effect on mycelial growth, displaying inhibition rates ranging from 90% to 100% for the highest concentration.

Mentha spicata essential oil could be an interesting alternative to synthetic products. However, it still requires further studies to develop biocide treatments that can be used on an industrial scale.

Conflict of interest

The authors declare no conflicts of interest.

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