Research Article

Use of *Mentha spicata* essential oil for prolonging postharvest life of fresh vegetables

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Mentha spicata L. (Lamiaceae), commonly called spearmint, is cultivated worldwide for its remarkable aroma and commercial value. Antimicrobial effectiveness of essential oils against many foodborne microorganisms when applied directly has been extensively demonstrated. The antimicrobial potential of *Mentha spicata* essential oil in the vapor phase against different microorganisms (*Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, *Enterococcus faecalis* CCM 4224, *Staphylococcus aureus* subsp. *aureus* CCM 2461, *Candida albicans* CCM 8186, *C. glabrata* CCM 8270, *C. krusei* CCM 8271, *C. tropicalis* CCM 8223) was determined by *in situ* method on vegetable model (carrot, radish, potatoes, and kohlrabi). The vapor phase was determined for seven days in Petri dishes with four concentrations (500, 250, 125, 62.5 µL.L⁻¹) of *M. spicata* essential oil on the food models. *M. spicata* essential oil against *Yersinia enterocolitica* on carrot, potato, and kohlrabi model in concentration of 500 µL.L⁻¹ was the most effective. *M. spicata* essential oil shows good potential as preservative and shelf-life prolongation of vegetables.

Keywords: microorganisms, Mentha spicata, Candida, fresh vegetables, vapor phase, shelf-life prolongation

1 Introduction

There is a growing interest in the use of essential oils because of their positive impact on human health, and most of them are considered safe. They save the environment because they are easily degradable and non-phytotoxic (Da Cruz Cabral et al., 2013). Several studies have examined essential oils under *in vitro* conditions and the results report that the essential oils in the liquid phase have high efficacy against foodborne pathogens and microorganisms. It is generally reported that a higher concentration is required to achieve the same effect on food than under *in vivo* conditions. Should essential oils be used as a natural preservative, the sensory properties of such foods may be considered to alter the taste and aroma (Gutierrez et al., 2008).

To minimize the concentrations of essential oils, which are needed to inhibit microorganisms, various studies have been carried out and alternatives have been sought. Use of essential oil in its volatile vapor phase to reduce the amount needed to provide an antimicrobial effect is one example (Nadjib et al., 2014). The use of essential oil in the vapor phase is an interesting alternative that could potentially be used as an alternative method for the preservation of the stored fresh produce. Promising results demonstrating antimicrobial activity, especially in the case of bacteria and fungi, have already been achieved by several studies (Moreira et al., 2005; Murbach Teles Andrade et al., 2014; Sacchetti et al., 2005).

Various research organizations around the world have focused on research on the biological activity of essential oils (EOs) as well as on the biological activity of essential oil from different *Mentha* species and their constituents. It has been reported that 40–50% of the loss of the stored food is due to diseases caused by bacteria and fungi (Pandey et al., 2017).

Plant-infecting bacteria cause diseases in agricultural crops that seriously affect consumer safety as well as safety of post-harvest commodities worldwide (Vidhyasekaran, 2002). A 30–40% yield loss due to bacterial diseases is reported. *Pseudomonas, Xanthomonas* and *Erwinia* are the bacteria responsible for causing plant diseases

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in most cases (Agrios, 2005). Many pesticides are commonly available against these pathogens, but they can be a risk to human health and also have side effects on plants (Louws et al., 2001). There are also bacterial pathogens that are already resistant to the commonly used pesticides. Scientists working on plant bacteria claim that plant pathogenic bacteria often acquire resistance to the copper bactericides and streptomycin (Cuppels & Elmhirst, 1999; Singh & Pandey, 2018). Therefore, alternatives are being sought to help solve the problem of resistance, which would not be harmful but at the same time would be sufficiently effective against bacterial pathogens. Essential oils and their constituents have great potential in combating several plant pathogenic bacteria applying various methods which have been tested by several techniques such as agar and broth dilution, agar wells, and disk diffusion (Perricone et al., 2015). The research on Mentha essential oils and their distillation has not provided sufficient data yet. Mentha piperita, which was collected from different provinces such as Yakima, Turkey and which the essential oil was prepared from, showed a strong antibacterial activity against P. syringae pv. phaseolicola, Xanthomonas campestris pv. campestris, X. campestris pv. phaseoli, P. syringae, Pseudomonas syringae pv. tomato (İşcan et al., 2002).

The aim of this study is to examine the antimicrobial activity of spearmint essential oil (*Mentha spicata* var. *crispa*) in the vapor phase against the growth of microorganisms inoculated on carrot, radish, potato, and kohlrabi (an *in situ* experiment).

2 Material and methods

2.1 Sample

Mentha spicata var. crispa essential oil (MSEO) from company Hanus s. r. o. (Nitra, Slovakia) was used for the study. MSEO was prepared by steam distillation of flowering herb from China. The main components of the essential oil marked by the producer were carvone min., 55%, limonene. The sample was stored in the cold (4 °C) and in the dark throughout the analyses. MSEO was stored in a refrigerator (4 °C) protected from light, in glass vessels.

2.2 Microorganisms tested

In our study, Gram-negative (G⁻) bacteria (*Salmonella* enterica susp. enterica CCM 3807, Yersinia enterocolitica CCM 5671), Gram-positive (G⁺) bacteria (*Enterococcus* faecalis CCM 4224, Staphylococcus aureus subsp. aureus CCM 2461) and yeasts (*Candida albicans* CCM 8186, *C. glabrata* CCM 8270, *C. krusei* CCM 8271, *C. tropicalis*

CCM 8223) were used. The tested microorganisms were obtained from the Czech Collection of Microorganisms (CCM; Brno, Czech Republic).

2.3 In situ antimicrobial activity

The evaluation of the antimicrobial activity of MSEO against eight microbial species on vegetable models (carrot, radish, potato, and kohlrabi) was performed according to the method described by Kačániová et al. (2022). Briefly, 5 mm thick slices of vegetables were placed on solidified Mueller Hinton agar for PD (\emptyset = 60 mm) and a microbial inoculum (0.5 McFarland) was applied.

Then, diluted MSEO (100 μ L) in ethyl acetate at 4 dilution levels (500, 250, 125 and 62.5 μ L.L⁻¹) was applied to a disc of sterile filter paper. Hermetically sealed Petri dishes were incubated for 7 days at the temperature suitable for the analysed microorganisms. An equivalent volume of ethyl acetate was used as a negative control. The percentage of inhibitory activity was calculated in ImageJ by stereological method.

Bulk density was calculated according to the formula:

$$V_{\nu} = P_{1}/p \times 100 \tag{1}$$

where: P_1 – the stereological lattice of the colonies; p – the substrate

Growth inhibition was expressed as:

$$GI = [(C_1 - T_2)/C] \times 100$$
 (2)

where: C₁ – the growth density of the control group; T₂ – the growth density in the group containing MSEO (Kačániová et al., 2022)

2.4 Statistical analyses

All measurements and analyses were carried out in triplicate. Mean and standard deviation (SD) were calculated using Microsoft Excel software. One-way analysis of variance (ANOVA) was performed using Prism 8.0.1 (GraphPad Software, San Diego, CA, USA).

3 Results and discussion

The antimicrobial activity of essential oil in the vapor phase tends to be lower compared to the *in vitro* assays for reasons that have not been elucidated yet and thus remain unclear (Lee et al., 2018). Since the antimicrobial effects of the essential oils in the vapour phase against microorganisms are still being studied, there is little information to suggest that they could be used in the food industry. However, results show a positive effect in the vapor phase in active packaging (Nielsen & Rios, 2000; Serrano et al., 2005; Skandamis & Nychas, 2002).

Good antibacterial activity against *Rhizobium leguminosarum, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* is exhibited by the essential oil of *M. spicata* and its major constituents (Sivropoulou et al., 1995). *Mentha (M. spicata)* exhibits a high antibacterial and antioxidant activity, but it also has a high mineral content. Compounds such as linalool, piperitone oxide, menthol, R-carvone and menthone contained in the *Mentha* plant have an antibacterial and antifungal effect and therefore can protect food against spoilage and contamination (Edris & Farrag, 2003; Rasooli & Rezaei, 2002; Regnier et al., 2010; Soković & van Griensven, 2006; Ziedan & Farrag, 2008).

The results of the antibacterial activity of the vapor phase of MSEO on carrot are presented in Table 1. Intensity of the bacterial inhibition by MSEO has increased with the increasing concentration of MSEO in assays across all the tested bacteria. MSEO has the most effective influence on carrot model against bacteria *Yersinia enterocolitica* in the concentration of 500 μ L.L⁻¹ with the value of 64.88%. Intensity of the yeast growth inhibition by MSEO has increased with the increasing concentration of MSEO in assays across all the tested yeasts. MSEO applied on the carrot model shows the best antimicrobial activity against yeasts with the testing of *C. albicans* in the concentration of 500 μ L.L⁻¹

Carrot				
Bacterial growth inhibition (%)	bacteria			
Spearmint EO (μL.L ⁻¹)	S. enterica	E. faecalis	Y. enterocolitica	S. aureus
62.5	15.04 ±0.57ª	12.71 ±0.96ª	24.77 ±2.00ª	9.56 ±0.69ª
125	24.92 ±1.68 ^b	25.87 ±0.96 ^b	32.96 ±2.12 ^b	25.15 ±1.64 ^b
250	46.85 ±1.52°	35.18 ±1.44 ^c	54.48 ±1.47°	42.74 ±0.97 ^c
500	56.46 ±1.33 ^d	53.26 ± 1.43^{d}	64.88 ± 1.64^{d}	53.44 ±2.07 ^d
Mycelial growth inhibition (%)	yeast			
Spearmint EO (µL.L ⁻¹)	C. albicans	C. glabrata	C. krusei	C. tropicalis
62.5	23.48 ±2.11ª	16.48 ±1.13ª	15.70 ±2.21ª	14.80 ±2.10ª
125	45.31 ±1.99⁵	32.96 ±2.20 ^b	33.48 ±2.07 ^b	25.98 ±3.47 ^b
250	64.56 ±2.46 ^c	52.77 ±1.94 ^c	54.14 ±2.46 ^c	45.37 ±2.71°
500	83.55 ±1.49 ^d	75.02 ±3.09 ^d	76.37 ±2.90 ^d	54.56 ±3.06 ^d

 Table 1
 In situ analysis of the antibacterial activity of the vapor phase of MSEO on carrot

one-eay ANOVA; individual letters (a–d) as superscripts indicate the statistical differences between the concentrations; $p \le 0.05$

Table 2 In situ analysis of the antibacterial activity of the vapor phase of MSEO on radish

Radish				
Bacterial growth inhibition (%)	bacteria			
Spearmint EO (µL.L ⁻¹)	S. enterica	E. faecalis	Y. enterocolitica	S. aureus
62.5	12.92 ±1.41ª	13.29 ±2.16ª	10.69 ±1.69ª	16.03 ±2.22ª
125	26.83 ±1.05 ^b	34.13 ±2.61 ^b	$25.80 \pm 1.80^{\text{b}}$	33.80 ±2.07 ^b
250	43.76 ±1.65°	54.14 ±2.02°	44.37 ±3.03 ^c	53.96 ±1.91°
500	52.29 ±5.77 ^d	72.82 ± 0.56^{d}	65.89 ±1.39 ^d	74.07 ±2.36 ^d
Mycelial growth inhibition (%)	yeast			
Spearmint EO (µL.L ⁻¹)	C. albicans	C. glabrata	C. krusei	C. tropicalis
62.5	16.17 ±3.08ª	24.77 ±2.81ª	23.04 ±2.47ª	13.79 ±1.45ª
125	35.27 ±3.51 ^b	45.20 ±2.31 ^b	42.41 ±1.09 ^b	35.91 ±2.70 ^b
250	54.78 ±2.83°	53.80 ±2.69°	56.25 ±3.11°	55.14 ±2.56°
500	74.21 ±0.40 ^d	76.96 ±1.32 ^d	82.47 ±0.63 ^d	83.56 ±2.36 ^d

one-way ANOVA; individual letters (a–d) as superscripts indicate the statistical differences between the concentrations; $p \leq 0.05$

with the value of 83.55%. MSEO was the most effective against the inoculated yeasts.

Table 2 shows the antimicrobial effect of MSEO in the vapor phase on radish. The best antimicrobial activity against the tested bacteria was found against *Staphylococcus aureus* in the concentration of 500 μ L.L⁻¹ with the value of 74.07%. The same table shows results of the anti-candida activity of MSEO on radish. The best anti-candida activity was found against *C. tropicalis* in the concentration of 500 μ L.L⁻¹ with the value of 83.56%.

Studies report that essential oils have good preservative properties for dairy products, meat, fruit, and other products. Essential oils are natural volatile compounds which are responsible for antimicrobial activity, and this makes them potential fumigants that can be used as protection for storing various commodities. They can extend the shelf life of food by inhibiting or slowing down the growth of pathogens in food (Bhat et al., 2012). The results of studies show that the spearmint essential oil can inhibit the growth of *Phytobacterium phosphoreum*, *Salmonella enteritids* in foods that are low in fat (Tassou et al., 1995). Moreover, studies report that *Mentha* essential oil may be effective against pathogens that are resistant to commonly used antibiotics.

Furthermore, research reports that the essential oil is effective in fighting fungi, bacteria, and viruses (Jeyakumar et al., 2011). The essential oil of *M. spicata* exhibits fungitoxic activity namely against *Pyricularia oryzae, Aspergillus ochraceus* and *Penicillium digitatum*. According to studies, the essential oil of the *Mentha* plant retains its activity up to 80 °C, and even when stored at room temperature, and remains active for 24 months. *Aspergillus ochraceus* is sensitive to the essential oil and shows inhibition within 30 min, *Penicillium oryzae* within 15 min. *Aspergillus alternata* and *Penicillium digitatum* show growth inhibition within 10 min and *Mentha* essential oil shows maximum toxicity to the fungi with pH values of 4.5–7.5. The essential oil was in the vapor phase, i.e. the volatile vapor that was released also showed fungitoxic effects. Results from the study of Yadav et al. (2006) show that *M. spicata* has fungitoxic effects at doses ranging from 1,100–2,200 ppm.

Table 3 shows antimicrobial effect of MSEO in the vapor phase on potato. The best antimicrobial activity against the tested bacteria was found in the case of *Yersinia enterocolitica* in the concentration of 500 μ L.L⁻¹ with the value of 83.99%. Table 4 shows also the results of the anti-candida activity of MSEO on potato. The best anti-candida activity was found against *C. albicans* in the concentration of 500 μ L.L⁻¹ with the value of 84.73%.

Phenolic compounds inhibit the growth of bacterial pathogens by forming complexes with bacterial enzymes and proteins (Rhouma et al., 2009). These phenolic compounds dissolve the bacterial membrane and when they interact with the cellular metabolism, they cause damage of the plasma membrane. This subsequently leads to disruption of the permeability of the cell. Depolarization of the membrane occurs, too, which then leads to bacterial death (Oussalah et al., 2006; Xu et al., 2008).

Fungi are considered to be the major plant pathogen which infects various field crops and causes complications for the stored food commodities (Sharifi-Rad et al., 2018). Fungi produce mycotoxins and various toxic metabolites which are hazardous to human and animal health, and affect the nutritional value, thereby rendering the food

Table 3In situ analysis of the antibacterial activity of the vapor phase of MSEO on potato

Potato				
Bacterial growth inhibition (%)	bacteria			
Spearmint EO (µL.L ⁻¹)	S. enterica	E. faecalis	Y. enterocolitica	S. aureus
62.5	14.40 ± 1.62^{a}	12.63 ±1.18ª	13.34 ±3.11ª	14.40 ±1.55ª
125	25.40 ±2.78 ^b	24.96 ±1.59 ^b	35.60 ±2.71 ^b	34.85 ± 3.00^{b}
250	44.05 ±2.53°	45.41 ±1.65°	53.41 ±1.48°	51.01 ±1.52°
500	78.88 ± 2.94^{d}	74.69 ± 3.60^{d}	83.99 ±2.67 ^d	80.13 ± 1.39^{d}
Mycelial growth inhibition (%)	yeast			
Spearmint EO (µL.L ⁻¹)	C. albicans	C. glabrata	C. krusei	C. tropicalis
62.5	25.87 ±2.73ª	17.44 ±2.17ª	17.14 ±1.49ª	26.04 ±3.25 ^b
125	44.91 ±2.69 ^b	34.24 ± 3.26^{b}	34.25 ±3.27 ^b	15.20 ±3.19ª
250	64.58 ±3.00°	55.70 ±3.93°	44.12 ±4.46 ^c	35.10 ±3.75°
500	84.73 ±3.00 ^d	75.28 ±3.55 ^d	76.19 ±0.81 ^d	65.24 ±3.36 ^d

one-way ANOVA; individual letters (a–d) as superscripts indicate the statistical differences between the concentrations; $p \leq 0.05$

unhealthy for human consumption (Paranagama et al., 2003; Sonker et al., 2015). The genera that are often responsible for plant diseases are Aspergillus, Fusarium, Cladosporium, Alternaria, Penicillium, Macrophomina, Colletotrichum, Rhizoctonia, and Botrytis (Pandey et al., 2017). It is believed that they are responsible for 40–50% of the losses. Commercially available synthetic fungicides such as captan, mancozeb, and carbendazim are suitable, although they cause several side effects. There is also a complication that many pathogenic fungi become resistant (Chang et al., 2007; Price et al., 2015) a devastating disease that can cause total crop loss. To assess the effect of repeated fungicide application on disease progress, strobilurin fungicides, primarily alternating pyraclostrobin and azoxystrobin treatments, were applied up to five times per year in each of 2 yr. A single application or two early applications reduced blight severity. A third application resulted in additional benefits in 1 of 2 yr, but additional applications did not reduce severity further. To monitor for fungicide tolerance in populations of A. rabiei, 66 single- spore isolates were collected and grown on growth media amended with chlorothalonil, mancozeb, or pyraclostrobin. Insensitivity to one or more of the fungicides was detected in 49 (74%). Therefore, research on finding environmentally friendly and renewable alternatives to synthetic fungicides has begun. In the last decade, essential oils which could have been effective fungicides against pathogens including Mentha essential oil showed promising results (Goudjil et al., 2016; Moreira et al., 2005; Teixeira et al., 2012).

The results of the antibacterial activity of the vapor phase of MSEO on kohlrabi are presented in Table 4. Intensity of the bacterial inhibition by MSEO has increased with the increasing concentration of MSEO in assays across all the tested bacteria. MSEO has the most effective influence on the kohlrabi model against bacteria *Yersinia enterocolitica* in the concentration of 500 μ L.L⁻¹ with the value of 84.96%. Intensity of the yeast growth inhibition by MSEO has increased with the increasing concentration of MSEO in assays across all the tested yeasts. MSEO applied on the kohlrabi model shows the best antimicrobial activity against yeasts with the testing of *C. krusei* in the concentration 500 μ L.L⁻¹ with the value of 75.40%.

In the study by Du et al. (2009), the antimicrobial activity of the essential oil of allspice, oregano, and garlic against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* has been tested. The results show that growth of the selected microorganisms was inhibited and in a concentration-dependent manner in tomatoes wrapped in foil impregnated with the essential oil. The study concludes that tomatoes wrapped in foil impregnated with the essential oil can produce vapor that has potential to provide consumers with multiple benefits.

In another study, the essential oil of orange and bergamot has been tested for its antifungal effect in the vapor phase on tomatoes and grains. The results show that the essential oil acts as an antifungal agent, and at the same time, the application of essential oil does not affect the sensory properties (Phillips et al., 2012). Likewise, tea tree essential oil was tested in the vapor phase on strawberry to inhibit the growth of *Rhizopus stolonifera* and *Botrytis cinerea*. The results show that the vapor phase can inhibit the growth of fungi and thus reduce fruit rot (Shao et al., 2013).

The antifungal activity of clove and mustard essential oil on strawberry in the steam phase against grey mould (*Botrytis cinerea*) was also examined, both individually

Table 4In situ analysis of the antimicrobial activity of the vapor phase of MSEO on kohlrabi

Kohlrabi				
Bacterial growth inhibition (%)	bacteria			
Spearmint EO (µL.L ⁻¹)	S. enterica	E. faecalis	Y. enterocolitica	S. aureus
62.5	15.23 ±2.31ª	13.32 ±2.05ª	15.05 ±1.55ª	13.75 ±1.57ª
125	26.20 ±2.07 ^b	26.43 ±1.50 ^b	34.72 ± 3.06^{b}	26.42 ±2.53 ^b
250	45.79 ±3.08°	43.78 ±1.95°	54.82 ±2.63°	37.14 ±2.14 ^c
500	63.77 ±1.96 ^d	65.11 ±2.53 ^d	84.96 ±3.48 ^d	65.70 ±2.05 ^d
Mycelial growth inhibition (%)	yeast			
Spearmint EO (µL.L ⁻¹)	C. albicans	C. glabrata	C. krusei	C. tropicalis
62.5	14.62 ±3.81ª	16.00 ±4.32ª	14.63 ±2.90ª	15.70 ±2.96ª
125	24.74 ±4.34 ^b	35.66 ±2.63 ^b	35.08 ±3.41 ^b	24.00 ±1.69 ^b
250	45.80 ±3.77°	63.85 ±2.00 ^c	54.69 ±3.58°	34.45 ±4.03°
500	74.28 ±2.47 ^d	84.69 ±4.49 ^d	75.40 ±4.00 ^d	54.23 ±2.19 ^d

one-way ANOVA; individual letters (a–d) as superscripts indicate the statistical differences between the concentrations; $p \leq 0.05$

and in combination. The results suggest that the major constituents present in the essential oils, namely eugenol in clove essential oil, and allyl isothiocyanate in mustard essential oil, are responsible for the inhibitory activity of these essential oils. Combinations of these essential oils have a synergistic effect, suggesting that the combined use may be more effective than the individual application (Aguilar-González et al., 2015).

In the study by Aguilar-González et al. (2017)a sensory evaluation was also performed among treated tomatoes and compared with nontreated ones. Minimum inhibitory concentration (MIC, researchers demonstrate the antifungal activity of the vapor phase of mustard essential oil against *A. niger* in both *in vitro* conditions and tomato fruits. Based on the results, they conclude that this mustard essential oil has inhibitory effects due to its volatile compounds, and thus it could be an alternative to traditional synthetic antimicrobial agents.

Lee et al. (2018) tested the essential oil on the surface of radish sprouts against *L. monocytogenes*. The incidence of *L. monocytogenes* was significantly reduced after the application of the essential oil, and the results under laboratory conditions were even more effective.

4 Conclusions

Foodborne diseases caused by microorganisms that infect food commodities are a major problem in both developing and developed countries. Some plant extracts or essential oils exhibit antimicrobial activity and can inhibit the growth of many microorganisms, thereby suppressing the formation of toxins in food.

Essential oils used in the vapor phase have great potential as they have an antimicrobial effect and thus could be used for protecting food against the growth of foodborne pathogens, even at relatively lower concentrations. In our study, *M. spicata* showed the best antimicrobial activity against *Y. enterocolitica* and a very good antimicrobial effect against all tested *Candida* species. Based on the above mentioned data, our findings demonstrate that the essential oil of *M. spicata* in the vapor phase can be considered a potential agent for the prevention of the microbially mediated food spoilage. *M. spicata* has the potential to be used as a preservative and to assist in extending the shelf life of raw and processed foods, although further testing and studies under the *in vivo* conditions are still required.

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