ANTIFUNGAL ACTIVITY OF SELECTED ESSENTIAL OILS AGAINST RHIZOPUS STOLONIFER

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INTRODUCTION

Consumption of fresh fruit and vegetables has significantly increased over the last decade. This change can relate to growing popularity of healthy eating habits and lifestyles (James and Zikankuba 2017; Yahaya and Mardiyya, 2019). Fruit and vegetables are highly valued in the human diet mainly for their minerals and vitamins content, which provide several benefits for human health, especially as a prevention of incidence of various diseases (Cooper et al., 2015). Despite the considerable benefits associated with the consumption of them, fruit and vegetables belong to highly perishable commodities, which leads to negative changes in their nutritional and sensory properties. Postharvest decay is one of the major causes for the postharvest loss of horticultural fresh produce during the supply chain (Matrose et al., 2021). Post – harvest losses of these commodities are mainly due to diseases caused by microorganisms (Spadaro and Droby, 2016). However, it is well known that the among microbial contaminants, most of the post – harvest loss occurs due to fungal pathogen (Singh and Sharma, 2018). The fungal genus Rhizopus is considered among the most devastating fungi during the storage of various horticultural commodities. Rhizopus stolonifer is one of the most common and fastest-growing species of this genus, particularly in wet conditions, and therefore, considered one of the most destructive (Bautista-Banos et al., 2014). R. stolonifer mainly penetrates the host commodity through external scratches during the period of harvesting, transportation, and sale. After that the mycelium grows on the fruit surface and produces long mycelial stolons, the mycelium grows on the fruit surface and produces long mycelial stolons, the mycelium grows on the fruit surface and produces long mycelial stolons, the mycelium grows on the fruit surface and produces long mycelial stolons.

MATERIAL AND METHODS

Fungal strains

Three identified strains of Rhizopus stolonifer (KM524; KM511 and KM510), obtained from the Collection of Microorganisms of the Department of Microbiology of the Slovak Agricultural University in Nitra were used in this study. The used strains of the genus Rhizopus were previously isolated from infected fruit and vegetable: R. stolonifer KM510 (strawberry), R. stolonifer KM511 (GenBank IDK1354577.1) (nectarine) and R. stolonifer KM524 (GenBank AM93354.6) (cherry tomatoes).

Plant essential oils

Totally 12 essential oils were used in this study. The EOs were obtained from commercial suppliers – Hanus Nitra (www.hanus.sk) and Calendula a.s. (Nová Lúbovňa, Slovakia). The essential oils used in this study were namely: angelica (Angelica archangelica L.), anise (Pimpinella anisum L.), fennel (Foeniculum vulgare Miller), camphore (Cinnamomum camphorum Nees & Eberm), litsea (Litsea decanensis L.), cumin (Cuminum cyminum L.), dill (Anethum graveolens L.), thyme (Thymus vulgaris L.), mint (Mentha piperita L.), laurel (Laurus nobilis L.), cinnamon (Cinnamomum zeylanicum L.).

Antifungal activity of essential oils

The antifungal activity of selected essential oils against Rhizopus stolonifer strains was investigated by gas diffusion method following the method (Cisarova et al., 2020). The test was performed in sterile plastic Petri dishes (90 mm) containing 15 mL of potato dextrose agar (PDA). Essential oils were firstly tested in highest concentration (625 μL/L of air). A round sterile filter paper (1 x 1 cm) was placed in the lid of Petri dish and EOs were added to the paper by micropipette.

Keywords: antifungal activity; essential oils; infected fruit and vegetables; Rhizopus stolonifer.
The results shown in Tables 1, 2 and 3 were evaluated by using STATGRAPHIC Centurion XVI (version 16.1.11) (The Plains, Virginia, USA) (analysis of variance – single factor and multifactor ANOVA (p < 0.05), and the homogeneity groups based on the efficiency of tested essential oils were found (95% Tukey HSD test, p < 0.05). The results shown in Table 4 were calculated using MS Excel program and expressed by percent of growth inhibition in comparison with the control sets. The results shown in Table 5 presented the MIC50 and MIC90 of EOs and were obtained by the probit analysis using the STATGRAPHIC Centurion XVI (version 16.1.11) (The Plains, Virginia, USA) program (p < 0.05).

RESULTS AND DISCUSSION

Antifungal activity of essential oils

Postharvest fungal diseases of fruit and vegetables are one of the major causes for the postharvest loss of horticultural fresh produce, with a great negative impact on economic. Therefore, the fresh produce industry is dependent on the use of synthetic fungicides. On the other hand, consumers prefer purchasing fruit that is not treated with pesticides, so it is important to find an alternative and effective solution to postharvest fungicide applications (Sivakumar and Bautista – Baños, 2014). Natural plant protectants, such as EOs and their major components represent an ideal option, which could replace the synthetic pesticides. In this study the activity of volatile components of selected EOs (Angelica archangelica L.), anise (Pimpinella anisum L.), fennel (Foeniculum vulgare Miller), camphore (Cinnamomum camphorum Nees & Eberm), litsea (Litsea deccanensis L.), cumin (Carum carvi L.), dill (Anethum graveolens L.), thyme (Thymus vulgaris L.), mint (citrata) (Mentha citrata L.), mint (piperita) (Mentha piperita L.), laurel (Laurus nobilis L.), cinnamon (Cinnamomum zeylanicum L.) on the growth of three Rhizopus stolonifer isolates from mouldy plant sources were determined. Also, the growth curves for each isolate of the genus Rhizopus treated by EOs were constructed. EOs that showed full inhibitory activity are shown in the Figures 1 – 3 without the points in one singular line. The results showed that six tested essential oils, namely litsea, mint citrata, mint piperita, cumin, thyme and cinnamon totally inhibited the growth of all tested fungi (100%) during all days (7 days) of cultivation. The Rhizopus stolonifer KM524 was least sensitive from all tested strains to effect of fennel (68.74 ± 31.92 mm), laurel (65.41 ± 36.89 mm), anise (58.88 ± 29.87 mm), camphore (53.27 ± 29.79 mm) and dill (40.05 ± 39.70 mm) EOs (Table 1).

Table 1 Summary statistic for antifungal activity of Rhizopus stolonifer KM524 treated by essential oils (625 µL/L of air) after 7 days of cultivation at 25 ± 1 °C in the dark (Tukey HSD test 95%, p < 0.05)

| Essential oil       | Average of colony (mm ± SD) | Coeff. of variation (%) | Min. | Max. |
|---------------------|-----------------------------|-------------------------|------|------|
| Angelica            | 90 ± 0                      | 0                       | 90   | 90   |
| Anise               | 58.88 ± 29.87               |                         | 20   | 90   |
| Fennel              | 68.74 ± 31.92               |                         | 22.65| 90   |
| Camphore            | 53.27 ± 29.79               |                         | 21.19| 90   |
| Dill                | 40.05 ± 39.70               |                         | 0    | 90   |
| Litsea              | 0 ± 0                       |                         | 0    | 0    |
| Mint (citrata)      | 0 ± 0                       |                         | 0    | 0    |
| Mint (piperita)     | 0 ± 0                       |                         | 0    | 0    |
| Cumin               | 0 ± 0                       |                         | 0    | 0    |
| Thyme               | 0 ± 0                       |                         | 0    | 0    |
| Laurel              | 65.41 ± 36.89               |                         | 15.96| 90   |
| Cinnamon            | 0 ± 0                       |                         | 0    | 0    |
| Control             | 90 ± 0                      |                         | 0    | 90   |

Legend: Data in the column followed by different letters are significantly different in 95.0 % Tukey HSD test. p < 0.05, KM524 – strain identity, SD – standard deviation

The 100 % inhibition effect of thyme, red thyme, mint, and savory EOs (625 µL/L of air) against Rhizopus spp. also reported Tančinová et al. (2018). Similar results for these EOs have been shown in study of Tančinová et al. (2019b) against strains of Penicillium commune and in study of Cisarová et al. (2020), where EOs completely inhibited the growth Aspergillus spp. during all days (14 days) of cultivation. Growth inhibition of Rhizopus stolonifer by thyme essential oil also recorded authors Bosque–Molina et al. (2010) and Taheri et al. (2018). Our results also agree with the authors Abdollahi et al. (2011). They evaluated the antifungal activity of EOs (sweet basil, fennel, summer savory and thyme) against R. stolonifer and Penicillium digitatum. Fennel EO showed the lowest antifungal activity against these pathogens. The growth curves of R. stolonifer KM524 during cultivation days (2º, 4º, and 7º) days showed Figure 1. Even though fennel and camphore were not considered as EOs with strong antifungal activity, were able to inhibit the growth of fungi until the second day of cultivation in comparison to the control. In contrast, the results of Tulio et al. (2006) showed that fennel belonged to the EOs with good antifungal activity.
The fungal strains *R. stolonifer* KMi510 and KMi511 showed similar results after the treatment with tested EOs (Table 2 and 3). Dill EO completely inhibited the growth of both strains *R. stolonifer* (KMi510 and KMi511) and on isolate KMi524 had only partial effect (40.05 ± 39.70 mm) (Table 1). However, the authors *Hlebová et al.* (2021a) reported that dill and other EOs (jasmine, fennel, laurel, tea tree, ginger, black pepper, cardamom, and camphore) had no inhibitory effect on the growth of the tested strains (*A. flavus, A. fumigatus, A. terreus, and A. niger*). In our study displayed moderate to good antifungal effects anise (44.61 ± 34.37 mm for KMi511, 29.46 ± 24.93 mm for KMi510) and fennel (63.02 ± 31.04 mm for KMi511, 48.69 ± 31.84 mm for KMi510) EOs. The main difference in the growth of the strains (KMi511 and KMi510) was antifungal effect of camphore and laurel EOs. In the case of *R. stolonifer* KMi511 camphore EO had good antifungal activity (30.96 ± 7.60 mm) and laurel oil had no inhibition effect (90 ± 0 mm) after 7 days of cultivation. No inhibition effect of laurel EO was obtained also by other authors *Massa et al.*, 2018; *Foltínová et al.*, 2019a. Remarkable efficacy of laurel EO showed study of Belasli et al. (2020), where EO provided protection against growth of *A. flavus* in fumigated wheat grains from 51.5% to 76.7% during 6–month of storage. For strain *R. stolonifer* KMi510, the opposite inhibition results were obtained. Camphore EO had no effect on growth of this strain (90 ± 0 mm) and laurel EO was able to inhibit its growth significantly (35.86 ± 40.40 mm) during all cultivation days.

### Table 2 Summary statistic for antifungal activity of *Rhizopus stolonifer* KMi511 treated by tested essential oils (625 µL/L of air) after 7 days of cultivation at 25 ± 1 °C in the dark (Tukey HSD test 95%, *p<0.05*).

| Tested essential oils | Average of colony (mm±SD) | Coeff. of variation (%) | Min. | Max. |
|-----------------------|---------------------------|-------------------------|------|------|
| Angelica              | 90 ± 0<sup>a</sup>        | 0                       | 90   | 90   |
| Anise                 | 44.61 ± 34.37<sup>bc</sup> | 77.04                   | 15.90| 90   |
| Fennel                | 63.02 ± 31.04<sup>c</sup>  | 49.26                   | 21.74| 90   |
| Camphore              | 30.96 ± 7.60<sup>d</sup>   | 24.58                   | 20.73| 90   |
| Dill                  | 0 ± 0<sup>c</sup>         | 0                       | 0    | 90   |
| Litsea                | 0 ± 0<sup>c</sup>         | 0                       | 0    | 0    |
| Mint (citrata)        | 0 ± 0<sup>c</sup>         | 0                       | 0    | 0    |
| Mint (piperita)       | 0 ± 0<sup>c</sup>         | 0                       | 0    | 0    |
| Cumin                 | 0 ± 0<sup>b</sup>         | 0                       | 0    | 0    |
| Thyme                 | 0 ± 0<sup>b</sup>         | 0                       | 0    | 0    |
| Laurel                | 90 ± 0<sup>d</sup>        | 0                       | 90   | 90   |
| Cinnamon              | 0 ± 0<sup>b</sup>         | 0                       | 0    | 0    |
| Control               | 90 ± 0<sup>d</sup>        | 0                       | 90   | 90   |

**Legend:** Data in the column followed by different letters are significantly different in 95.0 % Tukey HSD test, *p<0.05*, KMi511<sup>−</sup> – strain identity, SD – standard deviation.

### Table 3 Summary statistic for antifungal activity of *Rhizopus stolonifer* KMi510 treated by tested essential oils (625 µL/L of air) after 7 days of cultivation at 25 ± 1 °C in the dark (Tukey HSD test 95%, *p<0.05*).

| Tested essential oils | Average of colony (mm±SD) | Coeff. of variation (%) | Min. | Max. |
|-----------------------|---------------------------|-------------------------|------|------|
| Angelica              | 90 ± 0<sup>d</sup>        | 0                       | 90   | 90   |
| Anise                 | 29.46 ± 24.93<sup>a</sup> | 84.62                   | 8.35 | 64.40|
| Fennel                | 48.69 ± 31.84<sup>d</sup>  | 65.40                   | 18.79| 90   |
| Camphore              | 90 ± 0<sup>d</sup>        | 0                       | 90   | 90   |
| Dill                  | 0 ± 0<sup>d</sup>         | 0                       | 0    | 0    |
| Litsea                | 0 ± 0<sup>d</sup>         | 0                       | 0    | 0    |
| Mint (citrata)        | 0 ± 0<sup>d</sup>         | 0                       | 0    | 0    |
| Mint (piperita)       | 0 ± 0<sup>c</sup>         | 0                       | 0    | 0    |
| Cumin                 | 0 ± 0<sup>b</sup>         | 0                       | 0    | 0    |
| Thyme                 | 0 ± 0<sup>b</sup>         | 0                       | 0    | 0    |
| Laurel                | 35.86 ± 40.40<sup>b</sup> | 106.77                  | 90   | 90   |
| Cinnamon              | 0 ± 0<sup>b</sup>         | 0                       | 0    | 0    |
| Control               | 90 ± 0<sup>b</sup>        | 0                       | 90   | 90   |

**Legend:** Data in the column followed by different letters are significantly different in 95.0 % Tukey HSD test, *p<0.05*, KMi510<sup>−</sup> – strain identity, SD–standard deviation.

The antifungal activity of EOs against *R. stolonifer* KMi511 and *R. stolonifer* KMi510 during all days of cultivation (2<sup>nd</sup>, 4<sup>th</sup>, and 7<sup>th</sup> days) are shown in Figure 2 and 3.

![Figure 2 Antifungal effects of essential oils (n=12) (625 µL/L of air) on the growth of *R. stolonifer* KMi511 during 7 days of cultivation (in mm)](image)

![Figure 3 Antifungal effects of essential oils (n=12) (625 µL/L of air) on the growth of *R. stolonifer* KMi510 during 7 days of cultivation (in mm)](image)

### Mycelial growth Inhibition determination

Based on the effect of individual EOs on the growth of microscopic fungal strains, the percentage of mycelial growth inhibition was calculated (Table 4). Angelica EO had no effect (0%) on the growth of any tested isolates during all days (7 days) of cultivation. In study of *Fraternelle et al*. (2014) Angelica archangelica L. EO showed in vitro antifungal activity against some species of the genus *Fusarium* spp., *Botrytis cinerea*, and *Alternaria solani*. In our study the strongest...
The MIC₉₀ values after 7 days of cultivation were determined for thyme (99.81 µL/L) and cumin (194.30 µL/L) for strain KMi524, followed by mint (piperita) and litsea (194.30 µL/L) for strain KM515 < cinnamon (319.97 µL/L) for strain KM511 < mint (citrata) (500.00 µL/L) all strains < cumin (510.18 µL/L) for strains KM524 and KM511 < dill (562.12 µL/L) for strains KM515 and KM510. According to the probit analysis, the best MID₉₀ values after 7 and 14 days of cultivation was recorded for thyme EO 153.17 µL/L and 153.63 µL/L against KM524. Thyme EO has been used in study of Císarová et al. (2020) as Antispargillus spp. They reported a strong inhibition activity for thyme EO with MID₉₀ values range from 430.52 µL/L to 587.27 µL/L after 14 days of cultivation. The highest MID₉₀ were determined for strain KM510 (581.95 µL/L of air) in treatment with cumin and dill EOs on the 7th and 14th day of cultivation. Hlebová et al. (2021a) reported the highest MIC₉₀ values in treatments with cumin at the concentration of 207.57 µL/L for A. niger.

Chemical characterization of essential oils

Chemically, EOs are a rich mixture of many bioactive chemical components, which compositional analysis is usually the first step in many studies. Chemical components are the main factors that affect the various biological properties of EOs (Bazdar et al. 2018; Shukla et al., 2019). Based on the above, in this study the qualitative and quantitative analysis of the EOs were determined. The results of GC – MS + GSFID analysis of tested EOs are listed in Table 6 and Table 7. EOs with the higher antifungal activity against Rhizopus spp. were follows: litsea (Litsea decamennsis L.), cumin (Cuminum cylinum L.), dill (Anethum graveolens L.), thyme (Thymus vulgaris L.), mint (Mentha citrata L.), mint (Mentha piperita L.) cinnamon (Cinnamomum zeylanicum L.) (Table 6).

As the major components of effective EOs were determined: α-citral (38.1%) and β-citral (31.4%) (litsea), carvone (49.5%) and p-cymene (32.4%) (cumin), carvone (40.2%) and R(+)-limonene (37.1%) (dill), thymol (43.1%) and p-cymene (39.1%) (thyme), geraniol (42.1%) and linalool (37.2%) (mint citrata), carvone (74%) (mint piperita), cinnamaldehyde (67.00%) (cinnamon). Our results agree with other authors (Fotilasová et al., 2019b, Tančinová et al., 2019a, Tančinová et al., 2019b, Císarová et al., 2020, She et al., 2020, Hlebová et al., 2021a; Hlebová et al., 2021b). Obtained results showed that in essential oils with the most pronounced antifungal activity, components such as α- and β-citral (litsea EO), cinnamaldehyde (cinnamon EO) or carvone (cumin, mint piperita and dill EO) were the most represented. These components are characterized by capability disrupt fungal mycelia, damaging mitochondria or reduced stability or permeability of cell membrane (Zhou et al., 2014; Tao et al., 2014; Zheng et al., 2015). Component of thyme EO, thymol is also characterized by markedly antifungal activity. The excellent antifungal activity of thymol recorded authors de Lira Mota et al. (2012) too. In their study tested antifungal activity of the Thymus vulgarsi EO and its major components, thymol (46.6%) and p-cymene (38.9%) against Rhizopus oryzae. They reported that the fungidal and/or fungistic activity of this EO can be connected mainly with thymol, its principal constituent, especially with the hydroxyl group of this compound. The second major component, p-cymene (benzene), does not possess substantial antifungal activity. In our study mint citrate EO was the only one that contained a higher content of geraniol (42.1%), but its mechanism of action is least understood. However, the authors Tang et al. (2018) demonstrated, that geraniol displayed inhibitory effectiveness against A. flavus by inducing the intracellular ROS accumulation and showed toxicity against A. ochraceus by changing permeability of cell membrane. The major components of EOs with low or no inhibition activity were dymbrocoumarin (53.2%) (angelica), 1,8-cineole (52.5%) (laurel), trans-anethol.

Similar results obtained Tančinová et al. (2021), who tested the antifungal activity of anise EO against Rhizopus spp. In their study anise EO had the best antifungal activity on the 2nd day of cultivation (85% – 100%), too. According to our results, camphore EO display the good antifungal activity against KM524 (76%) and KM511 (76%); on 2nd day of cultivation but against growth of strain KM510 no inhibitory effect of camphore oil was observed (0%). After 7 days of cultivation, no effect of camphore EO against strains KM524 and KM515 was observed (0%). Only tested strain KM511 (57%) was the most sensitive to the effect of camphore EO after all days of cultivation. Satyal et al. (2013) tested antifungal activity of three cinnamon EOs (C. camphora, C. tamala, and C. glaucescens). Only EO of C. camphora showed significant antifungal activity against A. niger. This fact could by explain by different compounds and its content in chemical composition of these EOs.

Minimum inhibitory doses (MIDs) determination

Six EOs in our study, concretely: litsea, mint (citrata), mint (piperita), cumin, thyme, and cinnamon inhibited growth of the Rhizopus stolonifer strains during all days of cultivation completely. Therefore, lower concentrations (500 – 15.625 µL/L of air) of these EOs were used to determine the minimum inhibitory doses (MIDs) on the tested fungi. Using probit analysis, predicted MIDs₉₀ and MIDs₉₀ were calculated (Table 5).
Bipolaris maydis, Botryodiplodia theobromae, Fusarium graminearum, F. oxysporum, and trans–anethol showed a significant inhibitory effect against the growth of all tested strains of the genus Rhizopus spp. However, these oils did not show significant inhibition against Alternaria solani, Bipolaris maydis, Botryodiplodia theobromae, Fusarium graminearum, F. oxysporum, and trans–anethol was a major component of two EOs, anise (93.3%) and fennel (79.9%). However, these oils did not show significant antifungal properties.

Similar results were obtained by the authors Hlebová et al. (2021a) and Tančinová et al. (2021). Trans–anethol was a major component of two EOs, anise (93.3%) and fennel (79.9%). However, these oils did not show significant antifungal properties. On the contrary Huang et al. (2010) in their study exhibited strong inhibitory effect of this components against all test fungi (Alternaria solani, Bipolaris maydis, Botryodiplodia theobromae, Fusarium graminearum, F. oxysporum f. sp. cucumerinum, F. oxysporum f. sp. lycopersici, F. oxysporum f. sp. vasinfectum, Magnaporthe oryzae, Pythium aphanidermatum, Rhizoctonia cerealis, and R. solani). One of the explanations of the different antifungal activities of some EOs components and in composition of EOs is the content of the minor components, which could increase or decrease the inhibition activity of predominant components (Hlebová et al., 2021a).

### Table 6 Chemical composition (GC – MS + GS/FID) of essential oils with the higher antifungal activity against tested strains of Rhizopus spp.

| No. | Component | Cinnamon | Litsea | Cumin | Dill | Thyme | Mp. | Mc. |
|-----|-----------|----------|--------|-------|------|-------|-----|-----|
| 1   | α-thujene* | 6.8      |        |       |      |       |     |     |
| 2   | α-pinene*  | 1.5      | 2.4    | 2.5   |      |       |     |     |
| 3   | benzenaldehyde | 1.0 |       |       |      |       |     |     |
| 4   | β-Pinene*  | 1.1      | 1.4    |       |      |       |     |     |
| 5   | β-myrcene  |         |       |       | 1.0  |       |     |     |
| 6   | α-phellandrene | 9.4 |       |       |      |       |     |     |
| 7   | β-phellandrene | 1.6 |       |       |      |       |     |     |
| 8   | (R)-(+)-limonene* | 14.6 | 1.7 | 37.1 |      | 15.2  | 1.1 |
| 9   | 1,8-cineole* | 1.6    | 1.0    |       |      |       |     |     |
| 10  | γ-terpinene* |         |       |       |      |       |     |     |
| 11  | linalool*(+) | 1.1    | 5.1    | 37.2  |      |       |     |     |
| 12  | thujone     |         | 1.7    |       |      |       |     |     |
| 13  | β-terpinen  |         | 1.7    |       |      |       |     |     |
| 14  | menthol     |         |       |       |      |       |     |     |
| 15  | (+) fenchone* | 4.6    | 1.6    | 2.8   |      |       |     |     |
| 16  | α-terpineol |         | 1.1    | 1.3   |      |       |     |     |
| 17  | D-dihydrocarvon* | 1.3 | 1.0   |       | 1.2  |       |     |     |
| 18  | 4-carvomenthol* | 31.4  |      |       |      |       |     |     |
| 19  | β-citral    |         | 49.5   | 40.2  | 74.6 |       |     |     |
| 20  | carvone*    | 32.4    | 2.9    | 39.1  |      |       |     |     |
| 21  | p-cymene*   |         | 42.1   |       |      |       |     |     |
| 22  | geraniol    | 76.0    | 38.0   |       |      |       |     |     |
| 23  | cinnamaldehyde* |     |       |       |      |       |     |     |
| 24  | α-citral*   |         |       |       |      |       |     |     |
| 25  | thymol      |         |       |       |      |       |     |     |
| 26  | neryl acetate |       |       |       |      |       |     |     |
| 27  | geranyl acetate* |       |       |       |      |       |     |     |
| 28  | β-caryophyllene* | 1.0  |       |       | 1.3  |       |     |     |
| 29  | cumarine    |         | 2.4    |       |      |       |     |     |
| 30  | citronellyl propionate* | 4.2 | 1.1   | 11.6  |      |       |     |     |
| 31  | α-methoxycinnamaldehyde | 1.7 |       |       |      |       |     |     |
| 32  | asaron      |         |       |       |      |       |     |     |
|     | Total content | 94.8  | 96.0   | 96.9  | 96.5 | 97.7  | 98.6 | 96.3 |

**Legend:** No. – component peak number, * – authentic standard, Mp. – Mint (peperita), Mc. – Mint (citrata)

### Table 7 Chemical composition (GC–MS+GS/FID) of essential oils without a significant antifungal activity against tested strains of Rhizopus spp.

| No. | Component | Angelica | Laurel | Anise | Camphore | Fennel |
|-----|-----------|----------|--------|-------|----------|--------|
| 1   | α-pinene* | 1.1      | 5.4    | 2.1   |          |        |
| 2   | β-Pinene* | 4.0      |        | 2.1   |          |        |
| 3   | α-terpinene | 9.8    |        | 0.4   |          |        |
| 4   | β-phellandrene | 9.7   |        | 0.4   |          |        |
| 5   | (R)-(+)-limonene* | 9.7  | 27.6   | 5.1   |          |        |
| 6   | 1,8-cineole* | 1.3   | 52.5   | 30.2  |          |        |
| 7   | γ-terpinene* | 9.4    |        | 0.4   |          |        |
| 8   | terpinolene | 2.6    |        | 4.5   |          |        |
| 9   | menthol*  | 2.6      |        | 4.5   |          |        |
| 10  | α-terpineol | 1.5     |        | 4.4   |          |        |
| 11  | 4-allylanisole |     |        | 4.4   |          |        |
| 12  | estragol  |          |        | 4.4   |          |        |
| 13  | p-cymene* | 2.2      |        | 4.4   |          |        |
| 14  | geraniol  |          | 1.3    | 4.4   |          |        |
| 15  | trans-anethol* |       |        | 4.4   |          |        |
| 16  | α-terpineol acetate | 12.2  |        | 79.9  |          |        |
| 17  | citronellol acetate | 3.7 |        | 79.9  |          |        |
| 18  | β-caryophyllene* | 2.6   |        | 79.9  |          |        |
| 19  | (+)-ledene | 1.7     |        | 79.9  |          |        |
| 20  | myristicin | 1.1      |        | 79.9  |          |        |
| 21  | (−)-spathulenol | 1.5 |        | 79.9  |          |        |
| 22  | asaron    | 1.1      |        | 79.9  |          |        |
| 23  | dihydrocoumarin | 53.2  |        | 79.9  |          |        |
|     | Total content | 97.3   | 96.9   | 96.8  | 96.2   | 99.9  |

**Legend:** No. – component peak number, * – authentic standard, Mp. – Mint (peperita), Mc. – Mint (citrata)

### CONCLUSION

In this study, the antifungal activities of 12 EOs, namely: angelica, anise, fennel, camphore, litsea, cumin, dill, thyme, mint (citrate), mint (piperita), laurel, and cinnamon were determined. Six tested EOs: litsea, mint (citrate), mint (piperita), cumin, thyme and cinnamon completely inhibited the growth (100%) of all tested strains of the genus Rhizopus throughout all cultivation days (7 days) at concentration 625 μL/L. Only angelica EO had no effect (0%) on the growth of all
tested strains during whole cultivation period. EOs that completely inhibit the growth of all strains were used for their minimum inhibitory doses (MIDs) determination. According to probit analysis, the most effective tested EO was thyme and the least effective was cumin. The lowest MIDs were determined for thyme EO strain Km5124 (153.63 µL/L) in treatment with thyme EO after 14 days of cultivation. The highest MIDs were determined for strain Km5150 (581.95 µL/L) in treatment with cumin EO after 14 days of cultivation. The lowest MIDs were for the highest MIDs also for EOs of thyme, cinnamon and litsea. As conclusion can be canclude that EOs show promising antifungal activities and may be provide environmentally safer and more acceptable antifungal agents for fruit and vegetables and other natural compounds as alternatives for post-harvest management of fungal pathogens: A review. Food Biosciences, 41, 100840.

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