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ANTIFUNGAL ACTIVITY OF SELECTED ESSENTIAL OILS AGAINST *RHIZOPUS STOLONIFER*

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ABSTRACT

Nowadays, it is very important to find out the protection of plant products as an alternative to synthetic fungicides. The promising alternative is the use of the essential oils (EOs). The aim of our research was to evaluate the antifungal effect of angelica (*Angelica archangelica* L.), anise (*Pimpinella anisum* L.), fennel (*Foeniculum vulgare* Miller.), camphore (*Cinnamonum camphorum* Nees & Eberm), litsea (*Litsea deccanensis* L.), cumin (*Carum carvi* L.), dill (*Anethum graveolens* L.), thyme (*Thymus vulgaris* L.), mint (citrate) (*Mentha citrata* L.), mint (piperita) (*Mentha piperita* L.), laurel (*Laurus nobilis* L.), cinnamon (*Cinnamomum zeylanicum* L.) EOs against three isolates of the genus *Rhizopus* obtained from moldy plants source the chemical composition of selected EOs was determined by gas chromatography coupled with mass spectrometry (GC – MS) and by gas chromatography with flame ionization detector (GC – FID). The antifungal activity of EOs against the growth of *Rhizopus* spp. was investigated by gas diffusion method (625 µL/L of air). The mycelium growth inhibition was measured on the 2nd, 4th, and 7th days of cultivation. Six EOs: litsea, mint (citrata), mint (piperita), cumin, thyme and cinnamon completely inhibited the growth of all tested fungi. For these EOs the minimum inhibitory doses were determined. According to probit analyses, the most effective tested EO was thyme and the least effective was cumin. Our results indicated that EOs are effective in the vapor phase and had a potential antifungal activity against *Rhizopus stolonifer* strains. So, they could be utilized in novel biological fungicide development.

Keywords: antifungal activity; essential oils; infected fruit and vegetables; *Rhizopus stolonifer*

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 growth, reproduction, and infection rate are very fast, and the spores can extend with airflow, once it invades the host, it will spread rapidly, and cause decay and softness within 1 – 2 days, which seriously affects the transportation and sales of fruit and vegetables **(Yan *et al.* , 2020).** Therefore, diseases caused by *R. stolonifer* is called as soft rot, black mould and *Rhizopus* rot. This fungus is often responsible for about 50% of loss of fruit that would be commercialized **(Bassetto *et al.*, 2007).** Synthetic fungicide treatment is currently the primary strategy for post–harvest disease management **(Romanazzi *et al.*, 2016).** However, their use is increasingly limited by the emergence of resistant fungal strains associated with their excessive application **(Feliziani *et al.*, 2013)**. In addition, today's consumers also demand high – quality, safe, and environmentally friendly products with little or no chemical residues. Promising option is using plant – based products essential oils (EOs) **(Nikkhah *et al.*, 2017).** EOs are natural, volatile, and aromatic liquids with a wide range of biological activities **(Falleh *et al.*, 2020).** Thanks to the abundant mixture of active components, EOs are endowed antimicrobial, antioxidant, and anti – pest activities, which make EOs possess great fresh – keeping effect on food products **(Pateiro *et al.*, 2018).**

The aim of the present research was to evaluate the antifungal effect of 12 essential oils to growth of three strains of *Rhizopus stolonifer* isolated from infected fruit and vegetable.

MATERIAL AND METHODS

Fungal strains

Three identified strains of *Rhizopus stolonifer* (KMi524; KMi511 and KMi510), 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* KMi510 (strawberry), *R. stolonifer* KMi511 (GenBank IDKU554577.1) (nectarine) and *R. stolonifer* KMi524 (GenBank ID AM933546.1) (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 Ľubovna, Slovakia). The essential oils used in this study were namely: angelica (*Angelica archangelica* L.), anise (*Pimpinella anisum* L.), fennel (*Foeniculum vulgare* Miller.), camphore (*Cinnamonum 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.). Essential oils were stored in airtight sealed glass bottles at 4±1 °C.

Antifungal activity of essential oils

The antifungal activity of selected essential oils against *Rhizopus stolonifer* strains was investigate by gas diffusion method following the method **Císarová *et al.* (2020).** The test was performed in sterile plastic Petri dishes (Ø 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. Petri

dishes were kept in inverted position. Filter paper discs impregnated with sterilized distilled water were used as a control to confirm no solvent effect of bioactivity. Each isolate was inoculated in the centre of Petri dishes with needle. Dishes were tightly sealed with parafilm and incubated for 7 days at 25 ± 1°C (three replicates per treatment were carried out). Diameters (Ø mm) of the growing colonies were measured at the 2nd, 4th, and 7th day with a digital capiler.

Minimum inhibitory doses (MIDs)

Essential oils that completely inhibit the growth of all strains of *R. stolonifer* were used to determine their minimum inhibitory doses (MIDs) according to **Císarová *et al.* (2016)** using a two–fold dilution (**Klouček *et al.*, 2012)** to give a final concentration range of 500 – 15.63 µL/L of air. For each fungal strain, a conidial spore suspension of 10^6 spore in mL^{-1} was prepared. The EVETM Automatic cell counter (NanoEnTek, Korea) was used to determine the number of spores. Petri dishes (Ø 90 mm, three – sector, six replicates) containing 15 ml of PDA were inoculated by 5 µL spore suspension. Cultivation was carried out at 25 ± 1°C and measured after 7. and 14. days. The MID (expressed as microliters of EOs per volume unit of the atmosphere above the organism growing on the agar surface) was defined as the lowest concentration of the oil which did not permit any visible growth after 7. or 14. days in comparison with control sets.

Chemical characterization of essential oils

The relative composition of essential oils was analysed by gas chromatography with mass spectrometry (GC – MS by the adopted method from **Klouček *et al.* (2012).** Essential oils were diluted in hexane to a concentration of l µL/mL. The analyses were performed in an Agilent 7890A GC coupled to an Agilent MSD 5975C MS detector (Agilent Technologies, Palo Alto, CA, USA) with an HP – 5MS column (30 m × 0.25mm, 0.25 mm film thickness). One microliter of the sample was injected in split mode 1:12, at an injector temperature of 250 °C and electron ionization energy of 70 eV. Analysis was measured in SCAN mode, the mass range was 40 – 400m/z. Starting at 60 °C, the oven temperature was increased at a rate of 3°C/min to a maximum of 231°C, where it was kept constant for 10 min. The identification of constituents was based on a comparison of their mass spectra and relative retention indices (RI) against the National Institute of Standards and Technology Library (NIST, USA), as well as authentic analytical standards and data from the literature **Adams (2007)**. Relatively proportion of EO components were assessed by Agilent 6890 GC – FID (Agilent Technologies, Palo Alto, CA, USA) with RTX5 column (Restek, Bellefonte, PA; 20 m × 0.18 mm, 0.2 µm film thickness) **(Božik *et al.*, 2017)**. Relative proportions were calculated by dividing individual peak area by total area of all peaks and confirmed by comparison their spectra with the authentic standards. Only compounds over 1% were included. The used standards are listed in Table 6 and 7.

Statistical Analysis

The results shown in Tables 1, 2 and 3 were evaluated by using STASTGRAPHIC 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 MIC_{50} and MIC_{90} of EOs and were obtained by the probity analysis using the STASTGRAPHIC 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 (*Angelica archangelica* L.), anise (*Pimpinella anisum* L.), fennel (*Foeniculum vulgare* Miller.), camphore (*Cinnamonum camphorum* Nees & Eberm), litsea (*Litsea deccanensis* L.), cumin (*Carum carvi* L.), dill (*Anethum graveolens* L.), thyme (*Thymus vulgaris* L.), mint (citrate) (*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* KMi524 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* KMi524* 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^{c}	0	90	90
Anise	58.88 ± 29.87^{bc}	50.73	20	90
Fennel	68.74 ± 31.92^{bc}	46.44	22.65	90
Camphore	53.27 ± 29.79^{b}	55.91	21.19	90
Dill	40.05 ± 39.70^{b}	99.14	0	90
Litsea	0 ± 0^{a}	0	0	0
Mint (citrata)	0 ± 0^{a}	0	0	0
Mint (piperita)	0 ± 0^{a}	0	0	0
Cumin	0 ± 0^{a}	0	0	0
Thyme	0 ± 0^{a}	0	0	0
Laurel	65.41 ± 36.89^{bc}	56.39	15.96	90
Cinnamon	0 ± 0^{a}	0	0	0
Control	90 ± 0^{c}	0	90	90

Legend: Data in the column followed by different letters are significantly different in 95.0 % Tukey HSD test, $p<0.05$, KMi524* – 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 **Císarová *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 **Bosquez–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* KMi524 during cultivation days (2nd, 4th, and 7th 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 fungus 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.

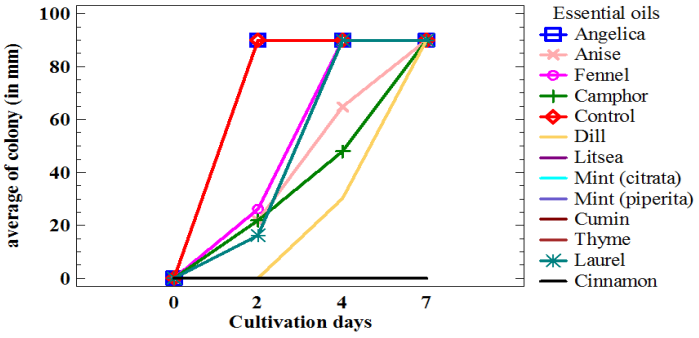

Figure 1 Antifungal effects of essential oils ($n=12$) (625 µL/L of air) on the growth of *R. stolonifer* KMi524 during 7 days of cultivation (in mm).

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* (KM510 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^{d}	0	90	90
Anise	44.61 ± 34.37^{bc}	77.04	15.90	90
Fennel	63.02 ± 31.04^{c}	49.26	21.74	90
Camphore	30.96 ± 7.60^{b}	24.58	20.73	90
Dill	0 ± 0^{a}	0	0	90
Litsea	0 ± 0^{a}	0	0	0
Mint (citrata)	0 ± 0^{a}	0	0	0
Mint (piperita)	0 ± 0^{a}	0	0	0
Cumin	0 ± 0^{a}	0	0	0
Thyme	0 ± 0^{a}	0	0	0
Laurel	90 ± 0^{d}	0	90	90
Cinnamon	0 ± 0^{a}	0	0	0
Control	90 ± 0^{d}	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* – 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^{d}	0	90	90
Anise	29.46 ± 24.93^{b}	84.62	8.35	64.40
Fennel	48.69 ± 31.84^{b}	65.40	18.79	90
Camphore	90 ± 0^{c}	0	90	90
Dill	0 ± 0^{a}	0	0	0
Litsea	0 ± 0^{a}	0	0	0
Mint (citrata)	0 ± 0^{a}	0	0	0
Mint (piperita)	0 ± 0^{a}	0	0	0
Cumin	0 ± 0^{a}	0	0	0
Thyme	0 ± 0^{a}	0	0	0
Laurel	35.86 ± 40.40^{b}	106.77	90	90
Cinnamon	0 ± 0^{a}	0	0	0
Control	90 ± 0^{c}	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* – strain identity, SD–standard deviation

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

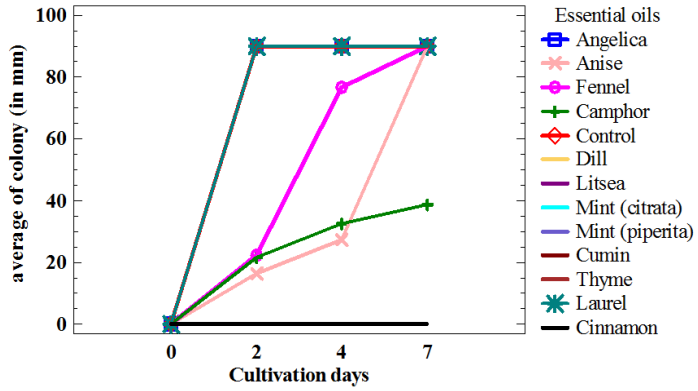

Figure 2 Antifungal effects of essential oils (*n=12*) (625 µL/L of air) on the growth of *R. stolonifer* KMi511 during 7 days of cultivation (in mm)

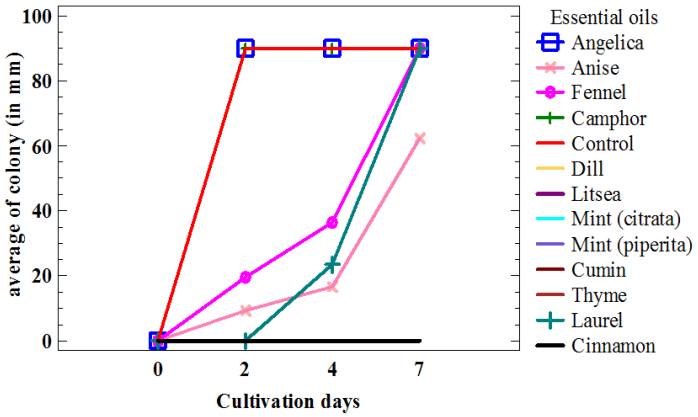

Figure 3 Antifungal effects of essential oils (*n=12*) (625 µL/L of air) on the growth of *R. stolonifer* KMi510 during 7 days of cultivation (in mm)

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 **Fraternale *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

inhibitory effect of anise oil was observed on the 2nd day for strains KMi510 (90%) > KMi511 (82%) > KMi524 (76%).

Table 4 Mycelial growth inhibition (%) of *Rhizopus stolonifer* strains by tested essential oils (625 µL/L of air) after the 2nd, 4th and 7th days of cultivation

Essential oils	Cultivation days	Tested strains		
		Mycelial growth inhibition (%)		
		RS KMi524	RS KMi511	RS KMi510
Fennel	2nd day	71	75	78
	4th day	0	15	59
	7th day	0	0	0
Dill	2nd day	100	100	100
	4th day	66	100	100
	7th day	0	100	100
Anise	2nd day	76	82	90
	4th day	28	70	82
	7th day	0	0	31
Laurel	2nd day	82	0	100
	4th day	0	0	74
	7th day	0	0	0
Angelica	2nd day	0	0	0
	4th day	0	0	0
	7th day	0	0	0
Camphore	2nd day	76	76	0
	4th day	47	64	0
	7th day	0	57	0

Similar results obtained **Tančinová *et al.* (2021),** who tested the antifungal activity of anise EO against *Rhizopus* spp. In their study anise oil 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 KMi524 (76%) and KMi511 (76%) on 2nd day of cultivation but against growth of strain KMi510 no inhibitory effect of camphore oil was observed (0%). After 7. days of cultivation, no effect of camphore EO against strains KMi524 and KMi510 was observed (0%). Only tested strain KMi511 (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 $MIDs_{90}$ and $MIDs_{50}$ were calculated (Table 5).

Table 5 The minimum inhibitory doses (MID_{50} and MID_{90}) of tested essential oils expressed as µL/L of air estimated by probit analysis for tested strains of *R. stolonifer* after 7. and 14. days of cultivation at 25 ± 1 °C in the dark

Tested essential oils	Days of cultivation	Tested strains					
		RS KMi524*		RS KMi511		RS KMi510	
		Minimum inhibitory doses (µL/L)					
		MID_{50}	MID_{90}	MID_{50}	MID_{90}	MID_{50}	MID_{90}
Thyme	**7.**	99.81	153.17	135.22	200.39	139.43	224.21
	14.	99.93	153.63	165.93	265.44		
Cinnamon	**7.**	408.77	569.62	319.97	554.56	487.90	523.89
	14.	408.77	569.62	351.85	619.04		
Mint (piperita)	**7.**	194.30	211.47	213.81	306.01	NA	NA
	14.	250.00	280.38	270.09	397.47		
Cumin	**7.**	510.18	540.45	510.18	540.45	520.78	548.30
	14.					562.12	581.95
Litsea	**7.**	194.30	211.47	261.08	294.04	364.95	392.43
	14.	250.00	280.38	277.58	314.11		
Mint (citrata)	**7.**	500.00	532.91	500.00	532.91	500.00	532.91
	14.	520.78	548.30	510.18	540.45		
Dill	**7.**	NA	NA	562.12	581.95	562.12	581.95
	14.						

Legend: *– strain identity; NA – not analyzed, MID_{50} – minimum inhibitory doses caused the death of 50 % cells, MID_{90} – minimum inhibitory doses caused the death of 90 % cells

The lowest MID_{50} values after 7. Days of cultivation was determined for thyme (99.81 µL/L of air) KMi524, followed by mint (piperita) and litsea (194.30 µL/L) for strain KMi524 < cinnamon (319.97 µL/L) for strain KMi511 < mint (citrata) (500.00 µL/L) all strains < cumin (510.18 µL/L) for strains KMi524 and KMi511 < dill (562.12 µL/L) for strains KMi511 and KMi510. According to the probit analysis, the best MID_{90}values after 7. and 14. days of cultivation was recorded for thyme EO 153.17 µL/L and 153.63 µL/L against KMi524. Thyme EO has been used in study of **Císarová *et al.* (2020)** against *Aspergillus* spp.. They recorded a strong inhibition activity for thyme oil with MID_{90} values range from 430.52 µL/L to 587.27 µL/L after 14. days of cultivation. The highest $MIDs_{90}$ were determined for strain KMi510 (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 MIC_{90} values in treatments with cumin at the concentration of 207.57 µLg/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 + GS/FID 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 deccanensis* L.), cumin (*Carum carvi* 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 detected: 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 (74.6%) (mint piperita), cinnamaldehyde (76.00%) (cinnamon). Our results agree with many other authors **(Foltínová *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 EOs, 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 vulgaris* EO and its major components, thymol (46.6%) and p–cymene (38.9%) against *Rhizopus oryzae*. They reported that the fungicidal and/or fungistatic 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 **w**as 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 dihydrocoumarin (53.2%) (angelica), 1,8–cineole (52.5%) (laurel), trans–anethol

(93.3%) (anise), 1,8–cineole (30.2%) (camphore) and trans–anethol (79.9%) (fennel) (Table 7).

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**[a]			6.8				
2	**α–pinene**[a]		1.5	2.4		2.5		
3	benzaldehyde	1.0						
4	**β–Pinene**[a]		1.1	1.4				
5	β–myrcene							1.0
6	α–phellandrene				9.4			
7	β–phellandrene		1.6					
8	**(R)–(+)–limonene**[a]		14.6	1.7	37.1		15.2	1.1
9	**1,8–cineole**[a]		1.6				1.0	
10	**γ–terpinene**[a]			1.0				
11	**linalool**[a]		1.1			5.1		37.2
12	thujone			1.7				
13	β–terpinen			1.7				
14	**menthol**[a]						1.6	2.8
15	**(+)–fenchone**[a]				4.6			
16	α–terpineol					1.1		1.3
17	**D–dihydrocarvon**[a]				1.3		1.2	
18	**4–carvomenthenol**[a]				1.0			
19	β-citral		31.4					
20	**carvone**[a]			49.5	40.2		74.6	
21	**p–cymene**[a]			32.4	2.9	39.1		
22	**geraniol**							42.1
23	**cinnamaldehyde**[a]	76.0						
24	**α–citrall**[a]		38.0					
25	thymol					43.1		
26	neryl acetate							2.3
27	**geranyl acetate**[a]							5.2
28	**β–caryophyllene**[a]		1.0					1.3
29	cumarine	2.4						
30	**citronelyl propionate**[a]	4.2						
31	o–methoxycinnamaldehyde	11.6						
32	asaron		1.0					
	Total content	**94.8**	**96.0**	**96.9**	**96.5**	**97.7**	**98.6**	**96.3**

Legend: No. – component peak number, [a] – 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**[a]	1.1	5.4			2.1
2	**β–Pinene**[a]		4.0			
3	**α–terpinene**[a]				0.4	
4	β–phellandrene		9.8			
5	**(R)–(+)–limonene**[a]	9.7			27.6	5.1
6	**1,8–cineole**[a]	1.3	52.5		30.2	
7	**γ–terpinene**[a]				19.2	
8	terpinolene					4.5
9	**menthol**[a]		2.6			
10	α–terpineol		1.5			
11	4–allylanisole					4.4
12	estragol			2.7		
13	**p–cymene**[a]		2.2		19.7	
14	**geraniol**			1.3		1.1
15	**trans–anethol**[a]			93.3		79.9
16	α–terpineole acetate		12.2			
17	citronellol acetate	3.7				
18	**β–caryophyllene**[a]		2.6			
19	(+)–ledene	1.7				
20	myristicin	1.1				
21	(–)–spathulenol	1.5				
22	asaron	1.1				
23	dihydrocoumarin	53.2				
	Total content	**97.3**	**96.9**	**98.8**	**99.2**	**99.9**

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).**

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 (citrata), 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 MID_{50} were obtained in treatment with thyme EO for strain KMi524 MID_{50} 99.81 µL/L of air after 7. days and MID_{50} 99.93 µL/L of air after 14. days of cultivation, respectively. The lowest MID_{90} value was determined for strain KMi524 (153.63 µL/L) in treatment with thyme EO after 14. days of cultivation. The highest MID_{90} was determined for strain KMi510 (581.95 µL/L) in treatment with cumin EO after 14. days of cultivation. This strain was the least sensitive with the highest MID_{90} also for EOs of thyme, cinnamon and litsea. As conclusion can be conclude that EOs show promising antifungal activities and may be provide environmentally safer and more acceptable antifungal agents for fruit and vegetables conservation, especially in vapor phase. However, further *in vivo* studies are needed to improve the knowledges about the influence of EOs on sensory properties and quality of fruit and vegetables.

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