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EFFECT OF ESSENTIAL OILS OF *LAMIACEAE* PLANTS ON THE *PENICILLIUM COMMUNE*

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ABSTRACT

The aim of this research was to determine the inhibitory effect of vapor phase of eight essential oils (EOs) on the growth of seven strains of *Penicillium commune* isolated from moldy milk products. Another objective was to determine the minimum inhibitory doses (*in vitro* and probit analyses) of EOs, which at concentration 625 $\mu L.L^{-1}$ of air completely inhibited the growth of all strains. The antifungal activity was evaluated by the micro-atmosphere method. Thyme, red thyme, peppermint, mint, and savory completely inhibited the growth of strains during cultivation at 25 °C and 5 °C. Basil, rosemary, and sage EOs have different effects on the growth of *P. commune* strains. EOs that completely inhibit the growth of all strains were used to determine their minimum inhibitory doses (MIDs). The best results 62.5 $\mu L.L^{-1}$ of air and 125 $\mu L.L^{-1}$ of air 7th day at 25 °C of incubation showed red thyme EO. Similar results were also found in thyme EO, but the MID inhibiting the growth of the one strain was 250 $\mu L.L^{-1}$ of air. MIDs of savory and peppermint were from 125 to 500 $\mu L.L^{-1}$ of air. Mint EO had the highest MID (from 250 to 625 $\mu L.L^{-1}$ of air). On the 14th day of incubation we found the same MIDs, respectively higher. It was found that EOs have different effects on individual strains of *P. commune*. According to probit analyses, the most effective tested EOs were red thyme and thyme, less effective peppermint and savory, and the least effective was mint EO.

Keywords: *P. commune*, essential oils, antifungal activity, vapor phase

INTRODUCTION

Molds are the most common cheese spoilage organisms which can lead to economic loss as well as raising public health concerns due to the production of mycotoxins **(Cheong *et al.*, 2014)**. According to **Pitt and Hocking (2009),** cheese is very susceptible to mold growth. Spoilage is generally confined to molds which are psychrotolerant. **Lund *et al.* (1995)** reported that 91 % of isolates from cheeses from Denmark, France, Greece, UK and other countries were *Penicillium* species. *Penicillium commune* was the most widespread and most frequent occurring (42 %) species. *P. commune* is one of the most important cheese contaminant **(Kure and Skaar, 2000; Kure *et al.*, 2001, Garnier *et al.*, 2017).** The growth of *P. commune* on cheese results in discoloring of the surface and production of off flavors. In spite of the fact that the dairy staff daily clean and disinfect the production plants and environments, periodical growth of *P. commune* on cheese is a major problem **(Lund *et al.*, 2003)**.
Today's consumers demand food that is minimally technologically processed and without synthetic preservatives or additives, because of the possible adverse health effects. Therefore, the food industry is now focused on finding solutions that fully satisfy the criteria of consumers while retaining the food safety. Application of natural antimicrobial agents such as extracts, essential oils and components of spices, and other aromatic plants could be significant in resolving these problems. These agents may be useful as additives in limiting or preventing the development of harmful fungi in food, as food surface protectants, or in modified atmosphere packaging of food **(Kocic-Tanackov *et al.*, 2014)**. Essential oils have deserved much attention in the past decades for their antimicrobial activity, since many of them have demonstrated efficacy against food-borne pathogenic and spoilage microorganisms **(Bassanetti *et al.*, 2017)**.
The aim of the present research was to determine the inhibitory effect of vapor phase of eight essential oils on the growth of seven different strains of *P. commune* isolated from moldy milk products. Another objective was to determine the minimum inhibitory doses of essential oils, which at a concentration 625 $\mu L.L^{-1}$ of air completely inhibited the growth of all the strains.

MATERIAL AND METHODS

Plant essential oils

The essential oils used in this study were extract of basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme and red thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita* L.), mint (*Mentha crispate* L.) savory (*Satureja hortensis* L.), and sage (*Salvia officinalis* L.). Essential oils were commercially produced.

Chemical composition of essential oils

Semi-quantitative composition of the essential oil samples was determined by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 7890B oven coupled with Agilent 5977A mass detector (Agilent Technologies Inc., Palo Alto, CA, USA) and CombiPal autosampler 120 (CTC Analytics AG, Zwingen, Switzerland). Prior to the analysis, essential oil samples were diluted in hexane (HPLC ≥ 97 %, Sigma Aldrich GmbH, Germany) to a concentration of 10 $\mu L\ mL^{-1}$. One microliter of diluted sample was injected in inlet operated in split mode (1:10; 250 °C). Separation was achieved using a ZB-WAXplusTM capillary column (10 m × 0.1 mm × 0.10 µm) (Phenomenex Inc., Torrance, CA, USA) and the following oven temperature programme: 50 °C for the first 5 minutes, increased to 240 °C at the rate of 3 °C min^{-1}, and it was kept constant for 2 minutes. Helium was used as carrier gas at the constant flow (1.2 mL min-1). The mass detector parameters were as follows: ionization energy of filament: 70 eV, transfer line temperature: 250 °C, MS source temperature: 230 °C, quadrupole temperature: 150 °C. The mass spectrometer was programmed under electron impact (EI) in a full scan mode at m/z 40-400. The identification of compounds was carried out by comparing of mass spectra (over 80 % match) with a commercial database NIST® 2014 and the retention times of reference standards (nerol, linalool, geraniol, citral, α-pinene and β-pinene). Semi-quantitative content of determined compounds was calculated by dividing individual peak area (excluded by solvent peak area) by total area of all peaks. Peaks under 0.1 % were not counted.

Fungal culture

Seven strains (Table 1) from different moldy milk products were used. These strains belong to the Collection of Fungi of Department of Microbiology; Faculty of Biotechnology and Food Sciences SUA in Nitra, Slovakia. 5 day old cultures cultivated on Czapek yeast extract agar (CYA) at 25 ± 1 °C were used for each experiment (CYA, **Pitt et Hocking, 2009**).

Table 1 Origin of the strains *Penicillium commune*

Strain	Origin
P. commune KMi 177	cheese flavored with pepper
P. commune KMi 270	smoked cheese (block)
P. commune KMi 276	smoked cheese (slices)
P. commune KMi 277	smoked cheese (slices)
P. commune KMi 370	sour cream
P. commune KMi 402	sour cream
P. commune KMi 403	parenica (pasta filata)

Antifungal activity of essential oils

The antifungal activity of selected essential oils was evaluated by the micro-atmosphere method. The test was performed in sterile plastic Petri dishes (Ø 90 mm) containing 15 mL of CYA. The evaluation by filter paper was made by the adapted method from **Guynot *et al.* (2003).** Essential oils were tested in concentration 625 $\mu L.L^{-1}$ of air. A round sterile filter paper (Ø 9 cm) was placed in the lid of Petri dish and 50 µL of essential oil was pipetted by micropipette to the paper. Dishes were kept in inverted position. Filter paper discs impregnated with sterilized distilled water were used as a control. Each strain was inoculated in the center of Petri dishes with sterilized needle. Dishes were tightly sealed with parafilm and incubated for 14 days at 25 ±1 °C and for 35 days at 5 ±1 °C (four replicates were used for each treatment). The diameters (Ø mm) of the growing colonies (from the reverse side) were measured at the 3rd, 7th, 11th, and 14th day - strains cultivated at 25 ±1 °C and 3rd, 7th, 11th,14th, 21st, 28th and 35th day – cultivated at 5 ±1 °C with a digital caliper.

Inhibition of mycelial growth

According to **Cakir *et al.* (2005)** and **Kordali *et al.* (2008),** growth inhibition of treated samples (T) against control (C) was calculated by the percentage of growth inhibition using the following equation:

$$\% \text{ of inhibition} = (C-T)/C \times 100$$

where, C is the mean of six replicates of hyphal extension (mm) of controls and T is the mean of six replicates of hyphal extension (mm) of plates treated with either essential oil.

Minimum inhibitory doses (MIDs)

Essential oils that completely inhibit the growth of all strains were used to determine their minimum inhibitory doses (MIDs). EOs dissolved in DMSO were prepared at different concentrations (625, 500, 250, 125, 63, 31.25, and 15.63 $\mu L.L^{-1}$ of air). For each fungal strain, a conidial spore suspension of 10^6 spore's ml^{-1} was prepared. Petri dishes (Ø 90 mm, two-sector, three replicates) containing 15 mL of CYA were inoculated by 5µl spores suspension. Cultivation was carried out at the 25 ± 1 °C and measured after 7 and 14 days. The MID (expressed as microliters of EOs per volume unit of 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.

Probit analyses

The ability of strains to grow in the presence of EO was coded to binomial scale (1 – growth observed, 0 – without growth). Such data were processed by probit analysis in Statgraphics Centurion XV (Statgraphics) software. Doses that inhibit the growth in 50 % respectively 90 % of cases (MID50 and MID90) were reversely predicted from regression equation.

Statistical analysis

Average diameters of developed colonies were compared using 2-factor ANOVA with interaction. Average values for strains as well as essential oils were compared using Tukey post hoc test. Analysis was performed in Statgraphics.

RESULTS AND DISCUSSION

In this study, evaluating the antifungal properties of eight essential oils from family *Lamiaceae*. Essential oils are complex mixtures of low molecular weight compounds extracted from plants by steam distillation and various solvents. Terpenoids and phenylpropanoids are the major constituents, which provide characteristic aroma and biological properties to essential oils (**Raut *et* Karuppayil, 2014).** According to authors **(Ben Farhat, *et al*., 2016; Méndez-Tovar *et al*., 2016; Dušková *et al*., 2016)** the effect of the growing seasons, different growth stage of plants, and climatic conditions of each year in terms of the essential oil content and composition were proven. Based on the above, we also focused on the composition of the essential oils used. The GC-MS analyses of the essential oils led to identification of 98 compounds, 34 from them are presented in ≥1 percentage amount in minimal one essential oil. The identified compounds (34) are listed in Table 2. The major components according to the concrete essential oil were: basil - Estragole (84.98 %); red thyme - Thymol (33.65 %) and o-xylene (43.85 %); rosemary – Eucalyptol (43.17 %), (+)-2-Bornanone (12.80 %) and α-pinene (10.74 %); thyme – Thymol (40.41 %) and Benzene, 4-ethyl-1,2-dimethyl- (19.45 %); peppermint – Levomenthol (44.94 %) and menthone (22.51 %); savory - γ-Terpinene (45.09%), Thymol (20.20 %) and p-Cymene (19.64 %); sage –Thujone (22.37 %), (+)-2-Bornanone (19.65 %) and Eucalyptol (10.84 %); mint - (-)-Carvone (72.62 %) and D-Limonene (15.23 %).

Table 2 Essential oils tested for the fungicidal effect and their compounds [%]* determined by gas chromatography coupled with mass spectrometry (GC-MS)

	Compound	Essential oils							
		Basil	Red thyme	Rosemary	Thyme	Peppermint	Savory	Sage	Mint
1	α-pinene	0.30	3.49	10.74	1.79	0.73	2.71	6.08	0.61
2	β-pinene	0.34	0.66	7.43	0.17	0.89	3	2.34	0.87
3	(+)-4-Carene				1.02		3.76		
4	Camphene		0.96	4.66	1.62		0.23	5.86	
5	β-Myrcene	0.17	1.2					0.73	0.52
6	β-Myrcene			0.92	1.48	0.09	2.49		
7	D-Limonene	0.28	1.03	2.82		2.03	0.48	1.88	15.23
8	Eucalyptol	4.10	0.9	43.17	1.64	7.01		10.84	0.91
9	γ-Terpinene			0.52	5.67		45.09	0.94	0.08
10	o-Cymene			2.75				1.74	0.32
11	Linalool	1.84	7.12	0.69	5.80				
12	endo-Borneol		1.02	3.83	2.25		0.15	4.45	
13	cis-α-Bergamotene	2.76							
14	Thujone							22.37	
15	Thymol		33.65		40.41		20.20		
16	(+)-2-Bornanone	0.30	0.41	12.80	2.36			19.65	
17	Caryophyllene	0.15	1.43	3.78	6.77	3.33	0.41	5.62	0.63
18	Benzene, 4-ethyl-1,2-dimethyl-				19.45				
19	α-Terpineol		1.59	2.31	0.26	0.14			0.11
20	Humulene			0.47	0.12	0.05		6.92	
21	Longifolene			0.16					1.62
22	isobornil acetate			1.28					

23	terpinen-4-ol		0.13	0.46	2.16			0.44	0.23
24	Estragole	84.89		0.29					
25	β-thujone							6.58	
26	menthofuran					1.62			
27	menthone					22.51			
28	p-menthone					4.22			0.65
29	menthol					6.30			
30	Levomenthol	0.10				44.94			
31	p-Cymene						19.64		
32	Bornyl acetate	0.20				0.16		2.29	
33	(-)-Carvone								72.62
34	o-xylene		43.85						

Legend: *listed are the components that represented min. 1 % in at least one essential oil

Antifungal activity of essential oils

The antifungal activity of eight essential oils against seven strains of *P. commune* was determined, using micro-atmosphere method (625 µL.L^{-1} of air). Five essential oils: thyme, red thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita* L.), mint (*Mentha crispate* L.) savory (*Satureja hortensis* L.) mint (*Mentha piperita* L.), completely inhibited the growth of all strains during cultivation at 25 °C and 5 °C. Other essential oils: basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L), and sage (*Salvia officinalis* L.) (Tab 3) have different effects on the growth of *P. commune* strains. Inhibitory effect (P <0.001) on the growth of the all strains of *P. commune* was recorded in all essential oils. Significant differences were observed between the individual strains (Table 3), which are documented in Figure 1, too.

Table 3 Effect of essential oils (treatment) and strains on the growth of *Penicillium commune*

	Temperature of incubation										
	25 °C				5 °C						
	Average diameter (in mm)										
	3rd day	7th day	11th day	14th day	3rd day	7th day	11th day	14th day	21st day	28th day	35th day
Essential oil											
Basil	2.7 d	16.0 d	28.7 d	36.6 d	0.0 a	0.0 a	0.0 a	0.0 a	1.0 c	3.2 c	7.1 c
Thyme	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Rosemary	2.1 c	11.7 c	22.2 c	30.0 c	0.0 a	0.0 a	0.2 a	1.6 b	4.4 d	7.3 d	11.1 d
Red thyme	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Peppermint	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Savory	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Sage	1.0 b	7.0 b	19.4 b	26.5 b	0.0 a	0.0 a	0.0 a	0.0 a	0.7 b	2.4 b	4.8 b
Mint	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
control	15.3 e	30.2 e	40.0 e	45.7 e	0.4 b	4.8 b	10.2 b	14.9 c	24.8 e	31.2 e	37.8 e
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Strain											
KMi 177	2.2 BC	6.0 B	10.5 C	12.6 B	0.0 A	0.7 CD	1.5 D	1.9 C	3.6 C	4.8 C	7.0 B
KMi 270	3.8 D	6.9 C	10.7 C	14.0 C	0.1 B	0.3 A	0.8 A	1.3 A	3.4 C	5.0 CD	6.9 B
KMi 276	2.4 C	4.6 A	9.0 A	13.0 B	0.2 C	0.7 D	1.2 BC	2.1 C	2.9 B	5.1 D	6.9 B
KMi 277	2.1 B	8.8 D	11.5 D	14.3 C	0.0 A	0.8 D	1.3 C	2.4 D	5.0 E	6.9 F	10.0 C
KMi 370	1.2 A	5.8 B	10.0 B	12.7 B	0.0 A	0.5 BC	1.0 B	1.6 B	2.2 A	3.2 A	4.4 A
KMi 402	1.1 A	4.1 A	8.6 A	11.3 A	0.0 A	0.4 AB	1.0 B	1.5 AB	2.7 B	3.5 B	4.8 A
KMi 403	3.7 D	14.4 E	25.6 E	29.9 D	0.0 A	0.4 B	1.2 BC	2.0 C	4.3 D	5.7 E	7.4 B
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Legend: Averages followed by the same letter (in columns) is not statistical different at α = 0.05 (ANOVA, Tukey test) small letters – difference within treatment, capital letters – different within strains.

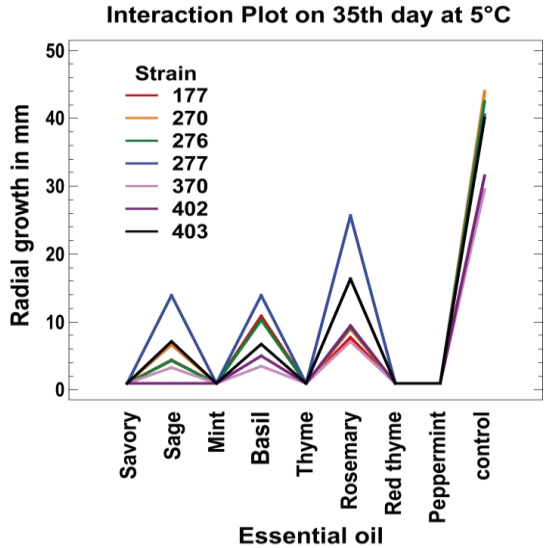
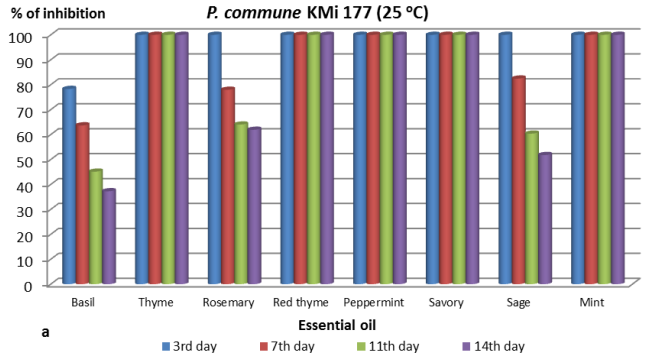
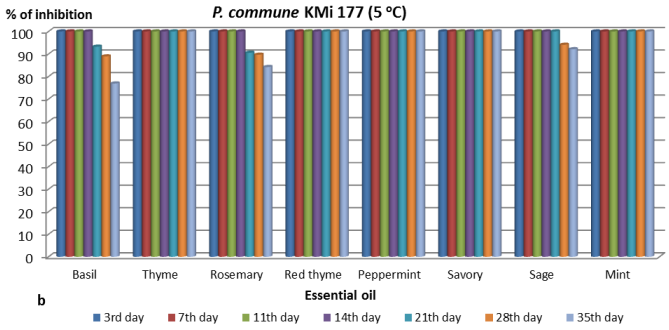


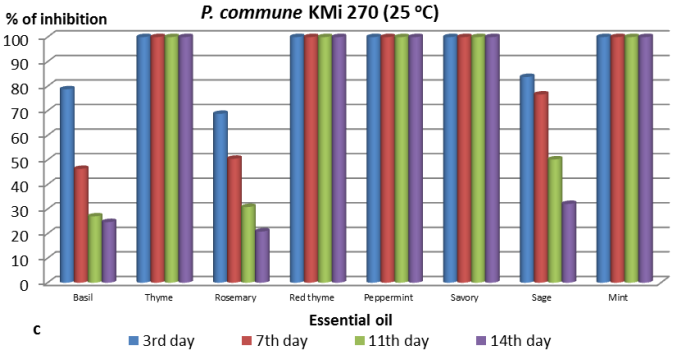
Figure 1 Growth of strains of *P. commune* on the 35th day of incubation at 5 °C in the presence/absence of essential oil

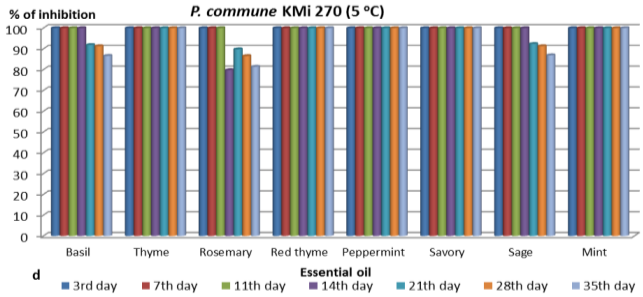
All strains of *P. commune*, without essential oil at the atmosphere - controls, were grown on the first measurement (3rd day of incubation) at 25 °C. Dairy products are stored at low temperatures, so an additional cultivation temperature of 5 °C was used. At 5 °C (controls), the growth of two strains (KMi 270 and KMi 276) was recorded on the 3rd day and other five strains on the 7th day. Inhibition of mycelial growth (percentage of inhibition) is shown in Figure 2. Thyme, red thyme, savory, peppermint, and mint essential oils completely inhibited the growth of the strains of *P. commune* at 5 °C, respectively 25 °C, throughout the experiment. Basil, rosemary, and sage essential oils inhibited the growth of the strains of *P. commune*, but their inhibition effects were depending on the individual strains. Sage essential oil 100 % inhibited the growth of the strain *P. commune* KMi 402 at 5 °C, for example. The stimulation effect was observed in one case. Basil essential oil stimulated growth of *P. commune* KMi 277 (Fig 2g) at the 14th day of incubation at 25 °C. Strong inhibition effect (100 %) of thyme and mint determined **Císarová *et al.* (2016b)** on *Aspergillus flavus* and *Aspergillus parasiticus*. Thyme oil (625 µL.L^{-1} of air) totally inhibited growth of *Aspergillus niger* and *Aspergillus tubingensis* **(Císarová *et al.* 2016a)**. The 100 % inhibition effect of thyme, red thyme, mint, and savory essential oils (625 µL.L^{-1} of air) against *Rhizopus* spp. has shown **Tančinová *et al.* (2018).** According **Elshafie *et al.* (2015)**, thyme essential oils can be utilized against *Monilinia laxa*, *Monilinia fructigena*, and *Monilinia fructicola*. **Alizadeh-Saltech *et al.* (2010)** tested the influence of vapor phase of sage, savory, and zataria essential oils on the growth of *Rhizopus stolonifer*. Sterile filter paper discs soaked with 3, 6, 12, 24 or 48 µl pure essential oils were placed on the inner

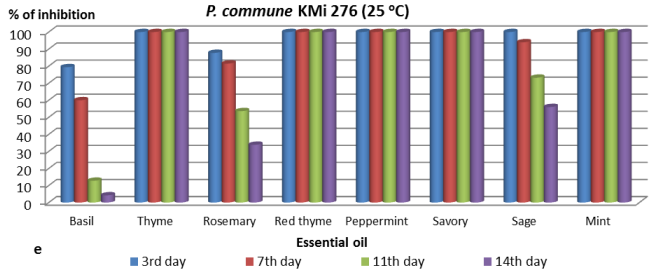
surface of the Petri dish lid. Sage oil did not have an acceptable inhibitory effect on the *R. stolonifer*. Savory, and zataria, but showed strong antifungal activity against *R. stolonifer*. The 100 % inhibitory effect of the savory essential oil and the partial inhibitory effect of sage essential oil have been observed in our experiment, too. **Servili *et al.* (2017)** recommended exposure to volatiles of the rosemary and peppermint essential oils an innovative method to control the postharvest gray mold of table grapes. **Sarkhosh *et al.* (2017)** tested the inhibitory properties of five essential oils: mint, savory, thyme, cinnamon, and lavender against anthracnose (*Colletotrichum gloesporioides* Penz) of avocado fruit. Authors report the potential of using savory and thyme essential oils as biological fungicides for increasing the storage time of avocado fruit. Thyme oil highly reduced 64 % of *Botrytis cinerea* colonization on pretreated detached leaves of tomato compared to untreated control **(Ben-Jabeur, *et* al., 2015)**. **Combrinck *et al.* (2011)** tested antifungal properties of eighteen essential oils (including peppermint and thyme oils) on the fungal growth of five plant pathogens (*Lasiodiplodia theobromae, Colletotrichum gloesporioides, Alternaria citri, Botrytis cinerea,* and *Penicillium digitatum*). Thyme oil proved to be the most effective inhibitor, totally inhibiting all of the pathogens tested at concentrations of 1000 $\mu l.l^{-1}$ (medium) and lower, with the exception of resistant *Penicillium* strains. The concrete essential oil can have different influence on the growth of species of molds. It is very individual. In our research, we noticed differences between the tested strains within *P. commune*. Therefore, it is necessary to use more strains of the same species in the trials so that the results are not influenced by the individual attributes of the concrete strain.

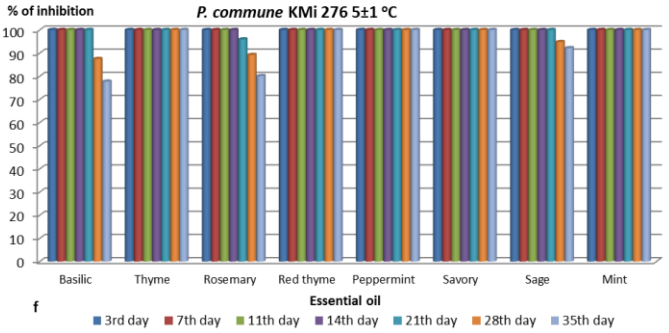


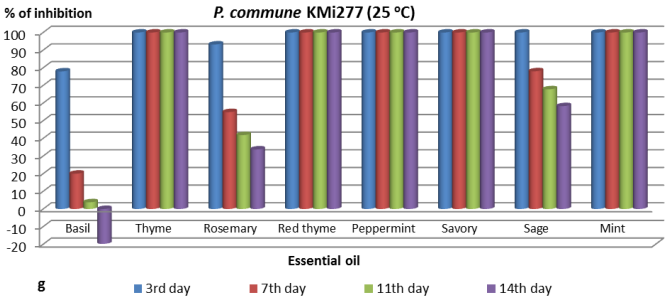


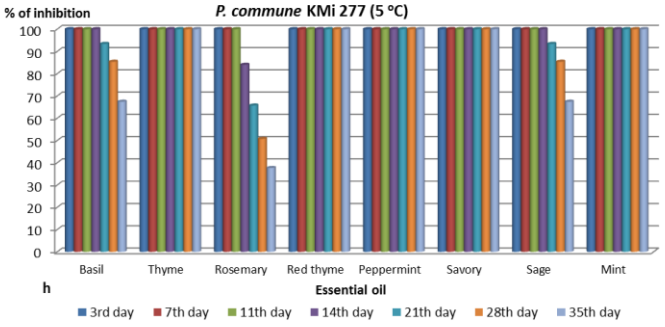












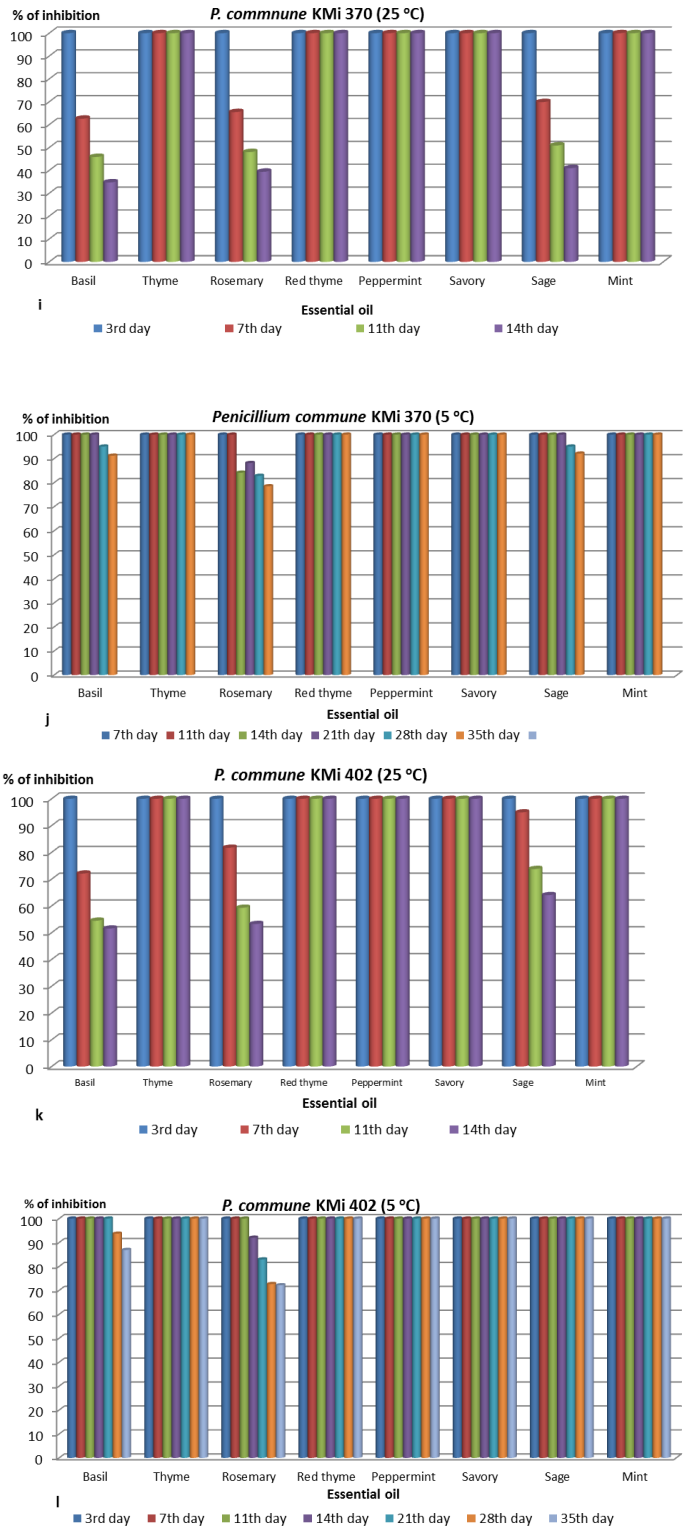


Figure 2 Inhibition of *Penicillium commune* strains growth causing by tested essential oils

In this study essential oils were able to inhibit growth of all strains at all days of cultivation at the highest concentration (625 µL.L^{-1} of air) and they were used for determination of MIDs. The results are shown in Table 4. The best results 62.5 µL.L^{-1} of air for strain KMi 177, KMi 276, KMi 277, KMi 370 and 125 µL.L^{-1} of air for KMi 270, KMi 402 and KMi 403 at 7th day of incubation showed red thyme essential oil. Similar results were also found in thyme essential oil, but the MID inhibiting the growth of the strain KMi 403 was 250 µL.L^{-1} of air. MIDs of savory and peppermint were from 125 to 500 µL.L^{-1} of air depending on the strains. Mint essential oil had the highest MID (from 250 to 625 µL.L^{-1} of air). On the 14th day of incubation, we found the same MIDs, respectively higher. It was found that EOs have different effects on individual strains of *P. commune*.

Table 4 The growth (in %) of colonies (n = 6) of *Penicillium commune* on CYA at 25 ° C after 7 and, respectively, 14 days of cultivation after application of essential oils

Essential oil	µL.L^{-1} of air	Strain of *Penicillium commune*													
		KMi 177		KMi 270		KMi 276		KMi 277		KMi 370		KMi 402		KMi 403	
		Day of cultivation													
		7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th	7th	14th
Thyme	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	125	0	0	0	0	0	0	0	0	0	0	0	0	100	100
	62.5	50	50	100	100	66.66	100	100	100	0	0	66.66	83.33	100	100
	31.3	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	15.6	100	100	100	100	100	100	100	100	100	100	100	100	100	100

	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Red thyme	125	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	62.5	0	50	100	100	0	33.33	0	0	0	0	50	66.66	16.66	16.66
	31.3	100	100	100	100	66.66	83.33	100	100	83.33	83.33	100	100	100	100
	15.6	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	33.33	0	0	0	0
	250	0	0	0	66.66	0	33.33	0	0	33.33	100	0	83.33	66.66	83.33
Peppermint	125	0	50	100	100	66.66	83.33	0	66.66	100	100	100	100	100	100
	62.5	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	31.3	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	15.6	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	250	0	0	0	0	0	0	0	0	0	0	0	0	33.33	33.33
Savory	125	0	0	33.33	66.66	0	33.33	66.66	66.66	100	100	66.66	100	50	100
	62.5	100	100	100	100	66.66	66.66	100	100	100	100	100	100	100	100
	31.3	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	15.6	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0	0	0	50	66.66
	250	0	0	0	50	16.66	16.66	0	100	0	16.66	0	33.33	100	100
Mint	125	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	62.5	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	31.3	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	15.6	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Legend: CYA - Czapek yeast extract agar

Using probit analysis, predicted MIDs90 and MIDs50 were calculated. The results are shown in Table 5. The most effective tested essential oils were red thyme and thyme, less effective peppermint and savory. MIDs of mint essential oil were the highest. The most resistant strain was KMi 403 with the highest MID90 for thyme, peppermint, savory, and mint essential oils. The highest MID90 of red thyme was determinate for KMi 270. **Radaelli *et al.* (2016)** tested antimicrobial activities of six essential oils (basil, rosemary, peppermint, marjoram, and thyme) against *Clostridium perfringens*. The essential oil from *Thymus vulgaris* showed the lowest MIC (minimum inhibitory concentration) against *Clostridium perfringens*. The authors, as well as our research showed a significant antimicrobial activity of thyme essential oil.

Table 5 Minimal inhibition doses estimated by probit analyses

Strain of *P. commune*	MID	Essential oil									
		Thyme		Red thyme		Peppermint		Savory		Mint	
		Day of cultivation									
		7	14	7	14	7	14	7	14	7	14
KMi 177	MID90	74.24	74.94	53.46	74.94	104.85	143.92	104.85	105.03	211.10	211.11
	MID50	62.51	63.02	46.93	63.02	94.06	125.00	94.06	94.31	193.74	193.75
KMi 270	MID90	104.85	105.03	104.85	105.03	211.10	294.02	136.48	153.07	211.10	280.37
	MID50	94.06	94.31	94.07	94.31	193.74	261.07	119.19	132.06	193.74	250.00
KMi 276	MID90	80.29	105.03	42.57	78.36	153.23	305.97	80.29	153.48	256.22	256.22
	MID50	66.99	94.31	34.16	53.70	132.10	213.83	66.99	100.02	230.86	230.87
KMi 277	MID90	104.85	105.03	53.47	53.71	104.85	153.07	153.23	153.07	211.10	393.29
	MID50	94.07	94.31	46.93	47.06	94.07	132.06	132.10	132.06	193.74	365.34
KMi 370	MID90	53.47	53.71	46.92	46.76	268.70	524.25	211.10	211.11	211.10	256.22
	MID50	46.93	47.06	38.00	37.80	240.54	487.72	193.74	193.75	193.74	230.87
KMi 402	MID90	80.29	89.43	74.24	81.06	211.10	314.06	153.23	211.11	211.10	268.70
	MID50	66.99	74.37	62.52	67.56	193.74	277.56	132.10	193.75	193.74	240.54
KMi 403	MID90	211.11	211.11	65.32	65.32	294.02	314.06	295.74	300.70	533.37	540.97
	MID50	193.75	193.75	55.95	55.95	261.08	277.56	186.87	240.54	500.00	510.31

Legend: *P.* – *Penicillium*, MID – minimum inhibitory doses

In our research, we have confirmed the ability of the tested oils family *Lamiaceae* to inhibit (partially or completely) the growth of *P. commune* strains. The strains used in our experiment were isolated directly from the moldy dairy products produced in Slovakia. However, the individual strains responded differently on the same oil, and therefore it necessary to use more than one strain in research. Testing should be supplemented by testing the influence of oils on the sensory properties of foods. According to **Servilli *et al.* (2017),** the interaction of food matrix components with the essential oils need to be investigated before their application is proposed for commercial practice. A challenge for the application of essential oils is their strong aroma even at low concentrations, which might adversely affect the organoleptic properties of the food being treated. Concentration of these substances applied in cheeses should be considered carefully because of their possible negative impacts on organoleptic properties **(Khorshidian et al., 2018).**

CONCLUSION

In this study, we evaluated the antifungal properties of basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme, red thyme (*Thymus vulgaris* L.), mint (*Mentha crispate* L.), peppermint (*Mentha piperita* L.), savory (*Satureja*

hortensis L.), and sage (*Salvia officinalis* L.) essential oils. Five essential oils: thyme, red thyme, peppermint, mint, and savory completely inhibited the growth of all strains during cultivation at 25 °C and 5 °C also. Other essential oils: basil, rosemary, and sage have different effects on the growth of *P. commune* strains. Essential oils that completely inhibit the growth of all strains were used to determine their minimum inhibitory doses (MIDs). The best results 62.5 $\mu L.L^{-1}$ of air and 125 $\mu L.L^{-1}$ of air 7th day at 25 °C of incubation showed red thyme essential oil. Similar results were also found in thyme essential oil, but the MID inhibiting the growth of the one strain was 250 $\mu L.L^{-1}$ of air. MIDs of savory and peppermint were from 125 to 500 $\mu L.L^{-1}$ of air depending on the strains. Mint essential oil had the highest MID (from 250 to 625 $\mu L.L^{-1}$ of air). On the 14th day of incubation we found the same MIDs, respectively higher. It was found that essential oils have different effects on individual strains of *P. commune*. According to probit analyses, the most effective tested essential oils were red thyme and thyme, less effective peppermint and savory. MIDs of mint essential oil were the highest. The most resistant strain was KMi 403 with the highest MID90 for thyme, peppermint, savory, and mint essential oils. The highest MID90 of red thyme was determinate for KMi 270.

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