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EFFECT OF ESSENTIAL OILS OF *LAMIACEAE* PLANTS ON THE *RHIZOPUS* SPP.

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ABSTRACT

The aim of this study was to evaluate the fungicidal effect of eleven essential oils against six isolates of the genus *Rhizopus*. Isolates were obtained from various moldy foods (chestnut, bread, strawberry, nectarine, blackberry and cherry tomatoes). The essential oils used in this study were extracts of basil (*Oscimum basilicum* L.), hyssop (*Hyssopus officinalis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), mint (*Mentha piperita* L.), oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), summer savory (*Satureja hortensis* L.), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.). Semi-quantitative composition of the essential oil samples was determined by gas chromatography coupled with mass spectrometry (GC-MS). The GC-MS analyses of the essential oils led to identification of 139 compounds, of which 49 were presented in ≥1% amount in at least one essential oil. The antifungal activity of essential oils against the *Rhizopus* spp. was determined, using micro-atmosphere method (0.625 μL.ml^{-1} of air), during 7 days. Seven essential oils: thyme, mint, summer savory, lavender, marjoram, oregano and wild thyme completely inhibited the growth of all isolates. Other essential oils have different effects on the growth of isolates. Basil essential oil stimulated growth of two isolates on the second day of cultivation. The growth of other isolates was, by contrast, inhibited by this essential oil in the same time of cultivation. Hyssop essential oil completely inhibited growth of two isolates, other 4 isolates were inhibited to fourth day of cultivation. In conclusion, certain essential oils are highly effective in vapour phase and can be used in another test of their antifungal activity and could be used in control of *Rhizopus* spp. or other fungal pathogens.

Keywords: essential oils; *Rhizopus* spp.; antifungal activity; vapour phase

INTRODUCTION

Due to an increasing risk of chemical contamination upon the application of synthetic fungicides, to preserve fresh fruits and vegetables, essential oils are gaining increasing attentions **(Farzaneh, 2015)**. Today, it is very important to find out the protection of products of natural origin as an alternative to synthetic fungicides. The promising alternative is the use of the essential oils. Essential oils from plants have great potential as a new source of fungicide to control the pathogenic fungi **(Císarová et al., 2016, Nikkhah et al., 2017)**. In the past decade, due to concerns regarding safety of the chemical control measures, particular attention has been given to the potential applications of essential oils as alternative **(Nikkhah et al., 2017)**.

Essential oils are odiferous, highly volatile substances present in plants. Because of their volatility, these substances can be isolated by means of steam distillation from an aromatic plant of a single botanical species and can be detected by both smell and taste. Individual essential oils are known by the name of the plant from which they are derived and the odor is similar to that of the part of the plant from which they are obtained, although the aroma is generally more intense **(Ríos, 2016)**.

Basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), savory (*Satureja hortensis* L.), sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), wild thyme (*Thymus serpyllum* L.), oregano (*Origanum vulgare* L.) and hyssop (*Hyssopus officinalis* L.) (*Lamiacea* plants) are herbs widely used for culinary purposes. These plants are also used for the production of essential oils.

The use of essential oils is extremely diverse depending on the source, quality, extraction procedure, ect. Essential oils have proven industrial applications in the manufacture of perfumes, cosmetics, soaps, shampoos, or cleaning gels. Another interesting aspect of these oils is their potential as therapeutic agens in aromatherapy or as active principles or excipients of medicine. Another significant application of essential oils is in the agrofood industry, both for producing beverages and for flavoring foods **(Ríos, 2016)**.

Fruit and vegetables are rich in essential vitamins, minerals, fibre, and health promoting compound, and the consumption thereof increased during the past years.

Consumers have a right to good quality produce that is safe for consumption and therefore they are increasingly interested in the nutritional value, good taste and flavour of the fruits and vegetables they consume. According to consumers, the term "quality" can be defined as a fruit with a perfect shape, size, colour, aroma, and an absence of defects such as cuts, bruises or decay **(Sivakumar and Bautista-Baños, 2014)**. Postharvest diseases are one of the major causes for the postharvest loss of horticultural fresh produce during the supply chain **(Mari et al., 2016; Sivakumar and Bautista-Baños, 2014)**.

Rhizopus stolonifer is one of the most common and fastest-growing species in the *Zygomycota* phylum. Disease caused by this fungus is known as soft rot, black mould and *Rhizopus* rot **(Bautista-Baños et al., 2014)**. *Rhizopus* rot is common on soft fruits, more abundant in warm, humid climates than in cool climate. In several fruits and crops such as strawberry, peaches, avocados, tomato, cucumber and table grapes *Rhizopus* rot causes soft rot during transport and storage **(Kassemeyer and Berkelmann-Löhnertz, 2009 Samson et al., 2010)**.

Rhizopus species are considered among the most devastating fungi during storage of various horticultural commodities **(Bautista-Baños et al., 2014**).

Scientific hypothesis

The aim of the present research was to determine the inhibitory effect of eleven essential oils to growth of different *Rhizopus* isolates.

MATERIAL AND METHODOLOGY

Fungal culture

Isolates from moldy foods were transfer on the potato dextrose agar (PDA, HIMEDIA India) and after incubation (25 ±1 °C, 7 days) were identificated to the genus according **Samson et al. (2010)**, **Pitt and Hocking (2009)**. These isolates belong to the collection of microorganisms at the Department of Microbiology of the Slovak Agricultural University in Nitra.

Plant essential oils

The essential oils used in this study were extracts of basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), savory (*Satureja hortensis* L.), sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), wild thyme (*Thymus serpyllum* L.), oregano (*Origanum vulgare* L.) and hyssop (*Hyssopus officinalis* L.).

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 hexan (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}, where 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

Rhizopus spp. were obtainted from moldy foods:

Isolate	Source
KMi 383	chestnut
KMi 392	bread
KMi 510	strawberry
KMi 511	nectarine
KMi 512	blackberry
KMi 524	cherry tomatoes

Figure 1 *Rhizopus* sp. (KMi 524) growing on the cherry tomatoes (Photo: E. Čunderlíková).

retention times of reference standards (nerol, linalool, geraniol, citral, α-pinene and β-pinene). Semi-quantitative content of determined compounds were calculated by dividing individual peak area (excluded by solvent peak area) by total area of all peaks. Peaks under 0.1% were not counted.

Antifungal activity of essential oils

The antifungal activity of selected essential oils was investigated by microatmosphere method. The test was performed in sterile plastic Petri dishes (Ø 90 mm) containing 15 mL of PDA. Evaluation by filter paper was made by the method adapted from **Guynot et al. (2003)**. Essential oils were tested in concentration 0.625 μL.cm^{-3} 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 to confirm no solvent effect of bioactivity. Each isolate was inoculated in the center of Petri dishes with needle. Dishes were tightly sealed with parafilm and incubated for seven days at 25 ±1 °C (three replicates were used for each treatment). Diameters (Ø mm) of the growing colonies were measured at the 2nd, 4th and 7th day with a digital capiler.

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 percentage of growth inhibition using the following equation:

$$\% \text{ of inhibition } = \frac{C - T}{C} \; x \; 100$$

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.

Statistical analysis

The size of colonies of isolates (mm) for each day of cultivation within treatment was evaluated. Also the size of colonies of isolate for each treatment to the same isolate in control group was evaluated too. The results were mathematically processed using the Microsoft Excel program and statistically evaluated by SAS/9.3 (2010). Used statistical model can be written in the following form:

yij = μ + ISOLATEi /TREATMENTj/ + eij,
yij = the measurements for size of colonies,
μ = overall mean,
ISOLATEi = the fixed effects of isolates (i = 1 to 6),
TREATMENTj = the fixed effect of treatment (j = 1 to 5),
eijk = random error, assuming eijkl ~ N(0, I σe2).

RESULTS AND DISCUSSION

According to market data, there are about 400 species, from 67 plant families, which are cultivated on a large commercial scale for production of essential oils. The most important families from this point of view are *Asteraceae* (syn. *Compositae*), *Lamiaceae* (syn. *Labiatae*), and

Table 1 Essential oils tested for the fungicidal effect and their compounds (%)* determined by gas chromatography coupled with mass spectrometry (GC-MS).

	Compound	Essential oils										
		Ros.	Thym.	Mint	Sum. sav.	Lav.	Marj.	Wild thy.	Hys.	Bas.	Sage	Oreg.
1	α-pinene	10.74	1.79	0.73	2.71	1.52	1.85	2.61	0.99	0.30	6,08	
2	β-pinene	7.43	0.17	0.89			0.46	0.18	11.07	0.34	2,34	0,31
3	(+)-4-Carene		1,02		3.76	0.04	9.28	1.27				1.15
4	Camphene	4.66	1.62		0.23	0.06		1.28			5.86	0.31
5	β-Myrcene					0.36		1.25	0.79	0.17	0.73	2.63
6	Sabinene	0.17		0.10	0.70		6.91		1.71	0.10		
7	β-Myrcene	0.92	1.48	0.09	2.49		2.04					
8	D-Limonene	2.82		2.03	0.48	0.32	2.30	1.57	1.10	0.28	1.88	0.48
9	Eucalyptol	43.17	1.64	7.01		1.01		1.16	0.43	4.10	10.84	
10	γ-Terpinene	0.52	5.67		45.09		16.85	10.43			0.94	7.91
11	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-					1,18		0.03				
12	o-Cymene	2.75				0.25	6.30		0.70		1.74	
13	Linalool	0.69	5.80			33.15		5.06	1.14	1.84		0.9
14	endo-Borneol	3.83	2.25		0.15	0.79		2.71			4.45	1.03
15	cis-sabinene hydrate						15.09					
16	cis-α-Bergamotene									2.76		
17	Thujone								0.27		22,37	
18	Thymol		40.41		20.20			12.13				61.45
19	(+)-2-Bornanone	12.80	2.36			0.46		1.47		0.30	19.65	
20	Caryophyllene	3.78	6.77	3.33	0.41	3.32		2.50	2.16	0.15	5.62	2.28

Table 1 Continue.

	Compound	Essential oils										
		Ros.	**Thym.**	**Mint**	**Sum. sav.**	**Lav.**	**Marj.**	**Wild thy.**	**Hys.**	**Bas.**	**Sage**	**Oreg.**
21	Benzene, 4-ethyl -1,2-dimethyl-		19.45					18.07				13.14
22	α-Terpineol	2.31	0.26	0.14		1.17	0.93	1.82	0.27			0.28
23	Humulene	0.47	0.12	0.05		0.08		1.60			6.92	0.35
24	isobornil acetate	1.28										
25	terpinen-4-ol	0.46	2.16							84.89	0.44	
26	Estragole	0.29							0.19			
27	terpinene-4-ol						34.52		1.22			1.1
28	β-thujone								0.25		6.58	
29	3-Hexen-1-ol, (E)-							0.15	0.12			
30	menthofuran			1.62								
31	menthone			22.51								
32	p-menthone			4.22								
33	menthol			6.30								
34	Levomenthol			44.94						0.10		
35	p-Cymene				19.64							
36	Linalyl acetate					38.06						
37	Bornyl acetate			0.16				0.70		0.20	2.29	
38	Pulegone			0.62					2.87			
39	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-				0.41	2.52		2.39				
40	Geranyl acetate					0.98		4.77				
41	(E)-beta-Famesene					2.58						
	(-)-Lavandulol					3.43						
42	Geraniol					0.49		10.74				
43	(-)-beta-Bourbonene								1.66			
44	trans-3-pinanone								25.05			
45	cis-pinane								38.39			
46	Germacrene D									1.08		
47	(-)-Myrtenol									1.63		
48	α-thujene											2.92

Legend: *listed are the components that represented min. 1% in at least one essential oil.
Ros. – rosemary, Thym. – thyme, Sum. sav. – summer savory, Lav. – lavender, Marj. – marjoram, Wild thy. – wild thyme, Hys. – hyssop, Bas. – basil, Oreg. – oregano.

Apiaceae (syn. *Umbelliferae*). Each includes more than 15 species producing essential oils on a large scale **(Bhattacharya, 2016)**. In this study, we evaluated the antifungal properties of 11 essential oils from family *Lamiaceae*. According authors **(Ben Farhat, et al., 2016; Méndez-Tovar et al., 2016; Dušková et al., 2016)**, the growing seasons, different growth stage of plants, climatic conditions of each years 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 139 compounds, 49 from them are presented in ≥1% amount in minimal one essential oil. The identified compounds (49) are listed in Table 1. The major components according concreated essential oil were: basil – estragole (84.98%), hyssop-cis-pinane (38.39%), trans-3-pinanone (25.05%), lavender – linalyl acetate (38.06%), linalool (33.15%), marjorom – terpinene-4-ol (34.52%), γ-Terpinene (16.85%), cis-sabinene hydrate (15.09%), mint – Levomenthol (44.94%), menthone (22.51%), oregano – Thymol (61.45%), Benzene, 4-ethyl-1,2-dimethyl- (13.14%), rosemary – Eucalyptol (43.17%), (+)-2-Bornanone (12.80%), α-pinene (10.74%), sage – Thujone (22.37%), (+)-2-Bornanone (19.65%), Eucalyptol (10.84%), summer savory-γ-Terpinene (45.09%), Thymol (20.20%), p-Cyneme (19.64%), thyme – Thymol (40.41%), Benzene, 4-ethyl-1,2-dimethyl- (19.45%), wild thyme-Benzene, 4-ethyl-1,2-dimethyl- (18.07%), Thymol (12.13%), Geraniol (10.74%), γ-Terpinene (10.45%).

The antifungal activity of 11 essential oils against the *Rhizopus* spp. was determined, using micro-atmosphere method (0.625 μL.cm^{-3} of air). Seven essential oils: thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), summer savory (*Satureja hortensis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.) completely inhibited the growth of all isolates. Other essential oils (Table 2, Figure 2) have different effects on the growth of *Rhizopus* isolates. Basil essential oil stimulated growth of two isolates (KMi 511and KMi 512) on the second day of cultivation. The growth of other isolates was, by contrast, inhibited by this essential oil in the same time of cultivation. Hyssop essential oil completely inhibited growth of two isolates (KMi 510 and KMi 512), other 4 isolates were inhibited to fourth day of cultivation.

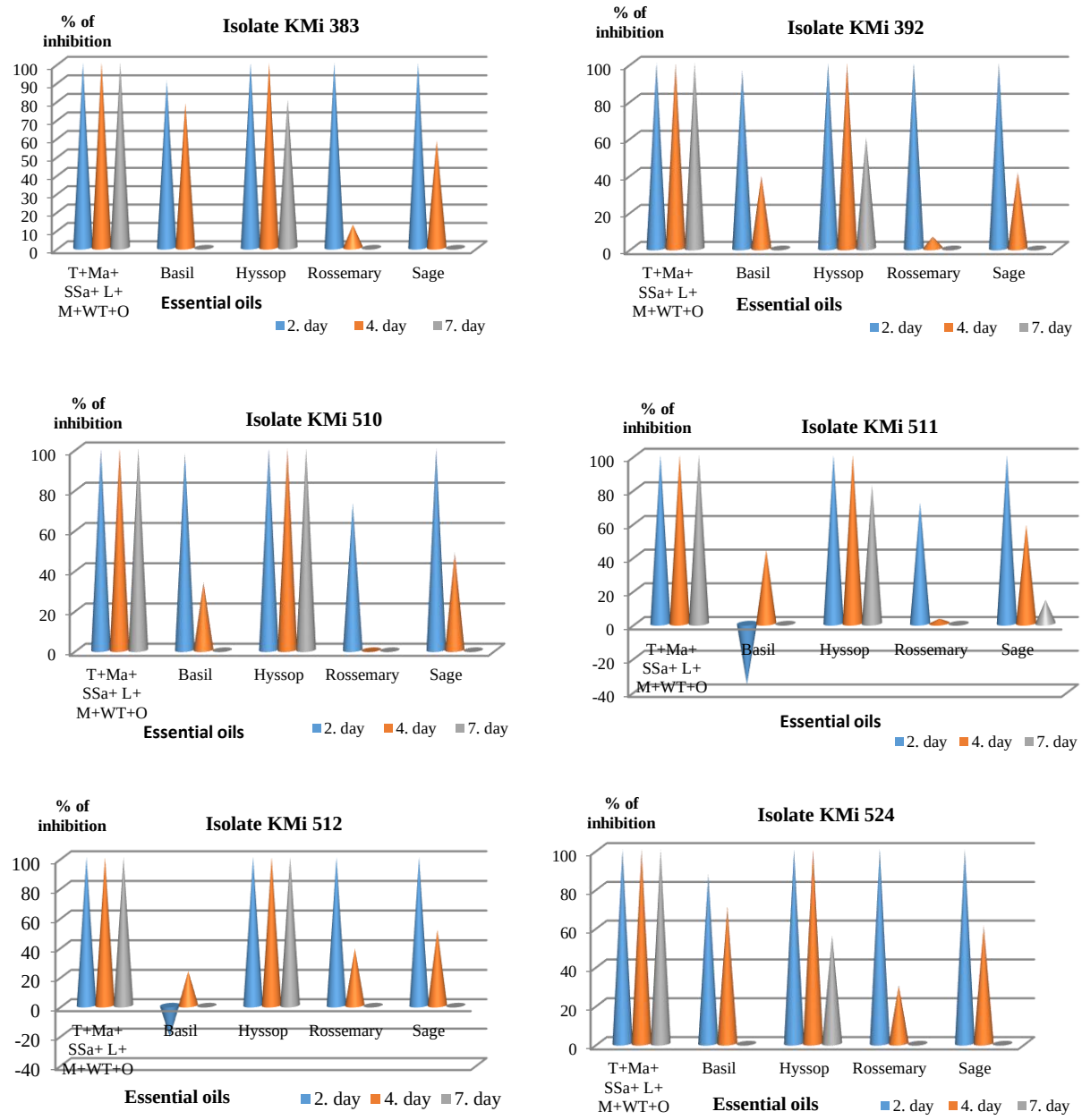

Figure 2 Inhibition of *Rhizopus* spp. growth caused by tested essential oils.
Legend: T– thyme, M – marjoram, SSa – summer savory, L – lavender, M – mint, WT – wild thyme, O – oregano.

The tested antifungal activity (against the *Rhizopus* spp.) of essential oils can be presented as: lavender = marjoram = mint = oregano = savory = thyme = wild thyme >hyssop >sage >rosemary >basil.

Strong inhibition effect (100%) of lavender, thyme and mint determined **Císarová et al. (2016b)**, against *Aspergillus flavus* and *Aspergillus parasiticus*. Inhibition of *Rhizopus stolonifer* growth by thymus essential oil recorded **Bosquez-Molina et al. (2010)**, **Plotto et al. (2003),** too. The strongest antifungal activity of savory essential oil against mycelial growth of *Rhizopus stolonifer* in the vapour phase showed **Alizadeh-Sallteh et al. (2010)**. These authors, like in our research, did not showed a significant influence of sage essential oil on the growth of *Rhizopus stolonifer*. **Farzaneh et al. (2015)** reported that *in vitro* results showed that at the maximum concentration of summer savory essential oil did not possess fungicidal effects on *Aspergillus niger* but they exhibited fungicidal activities against *Penicillium digitatum*, *Botrytis cinerea* and *Rhizopus stolonifer*. **Císarová et al. (2016a)** tested antifungal acitivity of lemon, eucalyptus, thyme, oregano,
sage and lavender essential oils against *Aspergillus niger* and *Aspergillus tubingensis* isolated from grapes. The most effective tested essential oils were oregano and thyme oils, which totally inhibited growth of tested isolates for all days of incubation at 0.625 $\mu L.cm^{-3}$ (in air). Lavender essential oil was less active against tested strains. Antifungal activity of essential oils on *Botrytis cinerea* was tested by **Rattanapitigorn et al. (2006)**. Antifungal activity was examined *in vitro* in plastic Petri dishes containing PDA (like in our research, but essential oils were in different concentrations directly in PDA). Authors reported that lavender, rosemary, peppermint, basil, rose,

Table 2 Effect of essential oils (treatment) on the growth of *Rhizopus* isolates.

Treatment	Isolate	Days of cultivation					
		Second day		Fourth day		Seventh day	
		Lsmean	standarderr	Lsmean	standarderr	Lsmean	standarderr
Basil	**KMi 383**	2.97^{A}	0.72	19.41^{Aa}	4.18	90.00	1.49
Basil	**KMi 392**	7.23^{A}	0.72	54.80	4.18	90.00	1.49
Basil	**KMi 510**	1.97^{A}	0.72	59.82^{bd}	4.18	90.00	1.49
Basil	**KMi 511**	8.25	0.72	50.79^{A}	4.18	90.00	1.49
Basil	**KMi 512**	4.12	0.72	69.11^{bc}	4.18	90.00	1.49
Basil	**KMi 524**	4.62^{A}	0.72	26.40^{Aad}	4.58	90.00	1.49
Rosemary	**KMi 383**	0.00^{Aa}	0.72	78.92	4.18	90.00	1.49
Rosemary	**KMi 392**	0.00^{Aa}	0.72	84.39	4.18	90.00	1.49
Rosemary	**KMi 510**	8.36^{Ab}	0.72	90.00	4.18	90.00	1.49
Rosemary	**KMi 511**	8.72^{b}	0.72	87.55	4.18	90.00	1.49
Rosemary	**KMi 512**	0.00^{a}	0.72	55.30	4.18	90.00	1.49
Rosemary	**KMi 524**	0.00^{Aa}	0.72	63.01	4.18	90.00	1.49
Sage	**KMi 383**	0.00^{A}	0.72	37.66^{A}	4.18	90.00	1.49
Sage	**KMi 392**	0.00^{A}	0.72	53.00	4.18	90.00	1.49
Sage	**KMi 510**	0.00^{A}	0.72	46.46^{A}	4.18	90.00	1.49
Sage	**KMi 511**	0.00	0.72	37.33^{A}	4.18	77.50	1.49
Sage	**KMi 512**	0.00	0.72	43.93^{A}	4.18	90.00	1.49
Sage	**KMi 524**	0.00^{A}	0.72	35.23^{A}	4.18	90.00	1.49
Hyssop	**KMi 383**	0.00^{A}	0.72	0.00^{A}	4.18	17.74^{Aa}	1.49
Hyssop	**KMi 392**	0.00^{A}	0.72	0.00^{A}	4.18	36.12^{Ab}	1.49
Hyssop	**KMi 510**	0.00^{A}	0.72	0.00^{A}	4.18	0.00^{Ac}	1.49
Hyssop	**KMi 511**	0.00	0.72	0.00^{A}	4.18	16.0^{Ad}	1.49
Hyssop	**KMi 512**	0.00	0.72	0.00^{A}	4.18	0.00^{Ae}	1.49
Hyssop	**KMi 524**	0.00^{A}	0.72	0.00^{A}	4.18	39.91^{Ab}	1.49
Mint **Thyme** **Lavender** **Marjoram** **Summer savory** **Oregano** **Wild thyme**	**KMi 383** **KMi 392** **KMi 510** **KMi 512** **KMi 524**	Essential oils completely inhibited growth of isolates					
Control	**KMi 392**	**30.83^{a}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 510**	**51.66^{b}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 511**	**90.00^{c}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 512**	**6.05^{d}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 524**	**3.39^{d}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 524**	**36.67^{a}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**

Legend: a, b, c, d, e – different letters are significant within treatment at the level $p < 0.05$.
A – significant difference ($p < 0.05$) of the same isolate within all treatments to the same isolate in control.

ginger and thyme resulted reduction in colony diameter, but not a complete inhibition of mycelial growth. Antifungal activity of selected 16 essential oils (savory, oregano, thyme, rose, geranium, lavander, coriander, bergamot, lemon, orange, anise, tea tree, violet, basil and calmonile) tested **Stević et al. (2014)** against fungi isolates from medical plant. Among all oils tested, savory, oregano and thyme oils proved to be the best inhibitors of all tested pathogenes. These three essential oils completely inhibited the growth of *Rhizopus* isolates in our experiment as well. As in our experiment, they did not notice a significant effect of basil oil on the testing fungi. **Rattanapitigorn et at. (2006)** reported that although the antifungal activity of plant essential oils is documented, the dose response of the inhibition effect varies widely, depending on the type of essential oils, extraction method, and antifungal test

method. Influence of essential oils addition to the food tested several scientists. **Busatta et al. (2008)** tested addition of different concentrations of marjoram essential oils in sausage on the aerobic heterotrofic bacteria´s growth. A significant reduction in the number of CFU was noted during the first time step (10 days) after a 35-day storage period. At the end of the storage period it was observed that the lowest oil concentration exerted not only antimicrobial activity but also a bactericidal effect, which may as account for the extended shelf-life of certain products. **Michalczyk et al. (2012)** reported that the most signicicant benefit of applying essential oils (coriander and hyssop) to stored ground beef was to inhibit undesirable sensory changes and the growth of *Enterobacteriaceae* bacteria (by 1 – 2 log cycles), especialy at 6 ±1 ºC. Based on our results and the results of other authors, it is important to test not only different species but also different isolates of the same species of moulds to gain important insights into the antifungal properties of essential oils.

CONCLUSION

In this study, we evaluated the antifungal properties of basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), savory (*Satureja hortensis* L.), sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), wild thyme (*Thymus serpyllum* L.), oregano (*Origanum vulgare* L.) and hyssop (*Hyssopus officinalis* L.) essential oils. Seven essential oils: thyme, mint, summer savory, lavender, marjoram, oregano and wild thyme completely inhibited the growth of all isolates. Other essential oils demonstrated different effects on the growth of isolates. Basil essential oil stimulated growth of two isolates on the second day of cultivation. The tested antifungal activity (against the *Rhizopus* spp.) of essential oils can be presented as: lavender = marjoram = mint = oregano = savory = thyme = wild thyme >hyssop >sage >rosemary >basil.In conclusion, certain essential oils are highly effective in vapour phase and can be used in another test of their antifungal activity and could be used in control of *Rhizopus* spp. or another fungal pathogens.

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