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INFLUENCE OF EIGHT CHOSEN ESSENTIAL OILS IN THE VAPOR PHASE ON THE GROWTH OF *RHIZOPUS STOLONIFER* AND *RHIZOPUS LYOCOCCUS*

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ABSTRACT
This study aimed to evaluate the fungicidal effect of eight essential oils against five strains of the genus *Rhizopus*. Strains were obtained from various moldy foods, namely *Rhizopus stolonifer* KMi 383 from chestnut, *R. stolonifer* KMi 510 from strawberry, *R. stolonifer* KMi 511 from nectarine, *R. stolonifer* KMi 524 from cherry tomatoes, and *R. lyococcus* KMi 512 from blackberry. The essential oils (EO) used in this study were jasmine EO (extract from *Jasminum officinale* L.), bergamot EO (*Mentha aquatica* L. var. *citrata* (Her.) Fresen), bitter orange EO (from *Citrus aurantium* L.), grapefruit EO (*Citrus paradisi* Macfady), sweet flag EO (East Asian Calamus, from *Acorus calamus* L. var. *angustatus* Bes), star anise EO (from *Illicium verum* J.D.Hook), geranium EO (from *Pelargonium graveolens*), and lemongrass EO (from *Cymbopogon citratus* DC). The semi-quantitative composition of the essential oil samples was determined by gas chromatography coupled with mass spectrometry (GC-MS). The antifungal activity of essential oils against the strains of *R. stolonifer* and *R. lyococcus* was determined during 7 days, using the micro-atmosphere method (0.625 μL.mL^{-1} of air). Two essential oils, geranium and lemongrass, completely inhibited the growth of all isolates. Bitter orange essential oil inhibited the growth of all isolates of *Rhizopus stolonifer*, but isolate of *Rhizopus lyococcus* began to grow after four days of cultivation. In conclusion, certain essential oils are highly effective in the vapor phase. These could be used in further tests of their antifungal activity and could be used in the control of *Rhizopus* spp. or other fungal pathogens.

Keywords: essential oils; *Rhizopus stolonifer*; *Rhizopus lyococcus*; antifungal activity; vapour phase

INTRODUCTION
Rhizopus species including *R. stolonifer* are naturally found in soil, debris, and air. Fungal dispersal mechanisms of *R. stolonifer* are wind, air currents, and some invertebrates such as mites and insects, among others **(Bautista-Baños et al., 2014)**. *Rhizopus stolonifer* is a ubiquitous fungus and can be isolated from many kinds of foods. It grows rampantly at 25 ºC, filling a Petri dish with sparse, dark mycelium in 2 days. It produces barely macroscopic aerial fruiting structures which are at first white, then become black. Given seven undisturbed days, it sheds dry black spores outside the Petri dish, providing an effective inoculum for a continuous chain of future contamination **(Pitt and Hocking, 2009)**. *Rhizopus* rot is common on soft fruits, more abundant in warm humid climates than in cool climates. In several fruits and crops such as strawberries, peaches, avocados, tomatoes, cucumbers, and table grapes, *Rhizopus* rot causes soft rot during transport and storage **(Kassemeyer and Berkelmann-Löhnertz, 2009)**. Infection usually starts from wounding after the cracking of fruits. At first, the lesions (soak with water) are rapidly softened and diseased lesions gradually expanded. The mycelia grow vigorously on the surface of fruits and formed stolons. Colonies are white cottony at first, becoming heavily speckled by the presence of sporangia and then brownish-black. They spread rapidly using stolons fired to various points of the substrate and attach by rhizoids. The color of sporangia is white at first and then turns black with many spores **(Kwon et al., 2001)**. The postharvest handling operations are the main reason *R. stolonifer* succeeds in entering and infecting most horticultural commodities **(Bautista-Baños et al., 2014)**.

Significant postharvest losses occur during the supply chain on fresh produce. Postharvest decay is one of the main factors that determine losses and compromises the quality of fruits and vegetables. Traditionally, postharvest decay control is achieved using chemical fungicides; however, the important concerns relating to environmental and human health require the development of novel methods for the control of postharvest decay **(Mari, Bautista-Baños and Sivakumar, 2016)**. The growing awareness of consumers concerning the relationship between food and health revolutionized the food industry. New techniques such as high pressure, nanotechnology, irradiation, etc., are increasingly used to maximize the

nutritional properties of foods, while new ingredients with functional properties contribute to improving health. The ‘‘elimination” of additives used in a wide variety of foods is demanded, while ‘‘natural” additives are seen as a benefit for both quality and safety **(Viuda-Martos et al., 2008)**. Over the past few years, consumers demand safe, environmentally-friendly, and natural products. It has driven the search for preservation techniques that improve product quality and safety without causing nutritional or sensory losses. Natural antimicrobial essential oils have the potential to provide quality and safety benefits and have fewer impacts on human health **(Ju et al., 2019)**. The development of natural crop protective products as alternatives to synthetic fungicides is currently in the spotlight **(Combrinck, Regnier and Kamatou, 2011; Stević et al., 2014)**.

The present research aimed to determine the inhibitory effect of chosen essential oils on the growth of different *Rhizopus stolonifer* and *Rhizopus lyococcus* strains.

Scientific hypothesis

The chosen essential oils can affect the growth of *Rhizopus* strains.

MATERIAL AND METHODOLOGY

Samples

Essential oils – Hanus Nitra (www.hanus.sk).

Chemicals

Potato dextrose agar (PDA, HIMEDIA India).

Hexane Sigma-Aldrich (HPLC Plus, for HPLC, GC, and residue analysis, ≥95%).

Dimethylsulfoxide (DMSO) Sigma-Aldrich (Hybri-Max™, sterile-filtered, BioReagent, suitable for a hybridoma, ≥99.7%).

Biological Material:

Strains of *Rhizopus* spp. – Collection of microscopic fungi of the Department of Microbiology, SUA in Nitra.

Instruments

Agilent 6890 GC-FID (Agilent Technologies, Palo Alto, CA, USA.

Agilent 7890A GC coupled to an Agilent MSD5975C MS detector (Agilent Technologies, Palo Alto, CA, USA).

EVE™ Automatic cell counter (NanoEnTek, Korea).

Laboratory Methods

Adapted from **Guynot et al. (2003)**.

Fungal culture

We used the strains of *Rhizopus stolonifer* and *Rhizopus lyococcus* obtained from the Collection of Microorganisms of the Department of Microbiology of the Slovak Agricultural University in Nitra. The strains were obtained from moldy plant sources; *Rhizopus stolonifer* KMi 383 (GenBank ID MF461020.1) from moldy chestnut, *R. stolonifer* KMi 510 from moldy strawberry, *R. stolonifer* KMi 511 (GenBank ID KU554577.1) from moldy nectarine *R. stolonifer* KMi 524 (GenBank ID AM933546.1) from moldy cherry tomatoes, and *R. lyococcus* KMi 512 (GenBank ID JN206375.1) from moldy blackberry.

Plant essential oils

The following essential oils were used in the research: jasmine (extract from *Jasminum officinale* L.), bitter orange (from *Citrus aurantium* L.), bergamot EO (*Mentha aquatica* L. var. *citrata* (Her.) Fresen), grapefruit (*Citrus paradisi* Macfady), sweet flag (East Asian Calamus, from *Acorus calamus* L. var. a*ngustatus* Bes), star anise (from *Illicium verum* J. D. Hook), geranium (from *Pelargonium graveolens*), and lemongrass (from *Cymbopogon citratus* DC). Essential oils were stored in air‑tight sealed glass bottles at 4 ±1°C.

Chemical composition of essential oils

The relative composition of essential oils was determined, and the compounds were identified by gas chromatography with mass spectrometry (GC-MS). Essential oils were diluted in hexane to a concentration of l $\mu L.mL^{-1}$. Analyses were carried out using an Agilent 7890A GC coupled to an Agilent MSD5975C MS detector (Agilent Technologies, Palo Alto, CA, USA) with an HP-5MS column (30 m × 0.25 mm, 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 were measured in SCAN mode, the mass range was 40 – 400 m/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. Relatively proportion of EO constituents 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). The same method for GC-MS was used. Relative proportions were calculated by dividing individual peak area by total area of all peaks. The response factor was not taken into account. Only compounds over 0.1% were included. The used standards are listed in Table 1.

Antifungal activity of essential oils

The micro atmosphere method was used to study the effect of essential oils on the growth of strains of *Rhizopus* sp. The test was performed in sterile plastic Petri dishes (Ø 90 mm) containing 15 mL of potato dextrose agar (PDA, HIMEDIA India). Evaluation by filter paper was made by the method adapted from **Guynot et al. (2003)**. Essential oils were tested in concentration 0.625 $\mu L.cm^{-3}$ of air. A sterile filter paper (cca1.5 x 1.5 cm) was placed in the lid of the Petri dish and 50 µL of essential oil was pipetted by micropipette to the paper. Dishes were kept in an 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 on the center of Petri dishes with 5 µL of spore’s suspension (10^5 spores in 1 mL). 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 on the 2nd, 4th, and 7th day 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 (1):

$$\% \text{ of inhibition } = \frac{C-T}{C} x\ 100 \qquad (1)$$

Where:
C is the mean of six replicates of hyphal extension (mm) of controls; 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 of *R. stolonifer* or strain of *R. lyococcus* were used to determine their minimum inhibitory doses (MIDs). EOs dissolved in dimethylsulfoxide (DMSO) were prepared at different concentrations (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 in mL^{-1} was prepared. The EVE™ Automatic cell counter (NanoEnTek, Korea) was used to determine the number of spores. Petri dishes (Ø 90 mm, three-sector, two 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.

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 the control group was evaluated too. The results were mathematically processed using the Microsoft Excel program and statistically evaluated by SAS/9.3 (2010). A used statistical model can be written in the following form:

$$yij = \mu + ISOLATEi /TREATMENTj/ + eij$$

Where:
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).

Probit analyses

The ability of strains to grow in the presence of EO was coded to a 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 the regression equation.

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 the production of essential oils **(Bhattacharya, 2016)**. In this research, we evaluated the antifungal properties of 8 essential oils from families *Oleaceae* (jasmine EO), *Rutaceae* (bergamot EO, bitter orange EO, grapefruit EO), *Acoraceae* (sweet flag EO), *Illiaceae* (geranium EO), *Lamiaceae* bergamot EO (*Mentha aquatica* L. var. *citrata* (Her.) Fresen), *Poaceae* (lemongrass EO). According to authors **(Ben Farhat et al., 2016; Méndez-Tovar et al., 2016; Dušková et al., 2016)**, the effect of the growing seasons, the 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 we used. The identified compounds are listed in Table 1. The major components according to the concrete essential oil were: bergamot EO - (R)-(+)-Limonene (34.7%) and Geraniol (31.1%), bitter orange EO - (R)-(+)-Limonene (31.9%), grapefruit EO - 1,8-Cineole (92.2%), geranium - Citronellol (28.3%), lemongrass EO - β-Citral (28.3%), jasmine EO - (-)-Borneol (37.7%), Star anise EO - Trans-Anethole (87.1%), sweet flag EO - cis-Verbenol (87.6%).

Species of genus *Rhizopus* especially *R. stolonifer* are the most important postharvest pathogens for a great variety of fruits and vegetables. Several articles have been published on the possibilities of influencing the growth of these fungi by plant essential oils. Inhibitory effect of *Thymus vulgaris* EO on the *Rhizopus stolonifer* tested **Bhaskara Reddy et al. (1998)**, **Bosquez-Molina et al. (2010)**. Sage EO (*Salvia officinalis*), savory EO (Satureja horten-sis), and zataria or Shiraz thyme EO (*Zataria multiflora*) were tested by **Alizadeh-Salteh et al. (2010)** and **Alizadeh-Salteh et al. 2013)**. The essential oils from *Mentha piperita, Lavandula angustifolia, Foeniculum vulgare,* and *Cuminum cyminum* were tested by **Hadian et al. (2008)**.

Certain essential oils are highly effective in the vapor phase and could be used in the control of foodborne bacterial pathogens **(López et al., 2007; Nedorostova et al., 2009)**. **Tyagi and Malik (2011)** report significantly higher antimicrobial activity of some essential oils, which we observed in the vapor phase. In our research, we also used the vapor phase to test the effect of selected essential oils on *Rhizopus* growth. The antifungal activity of 7 essential oils against the *Rhizopus stolonifer* (4 strains) and *Rhizopus lyococcus* (1 strain) were determined, using the micro-atmosphere method (625 $\mu L.L^{-1}$ of air). The results are shown in Table 2 and Figure 1.

Two essential oils: lemongrass (*Cymbopogon citratus* DC) and geranium (*Pelargonium graveolens*) completely inhibited the growth of all strains. Lemongrass (*Cymbopogom citratus* DC) essential oil is known due to its broad-spectrum antimicrobial activity **(Leimann et al., 2009)**. According to **Abdulazeez, Abdullahi and James (2016)**, **Božik et al. (2017)**, **Císarová et al. (2020)** lemongrass oil has been also shown to be an effective fumigant for stored food commodities due to its bioactivity in the vapor phase. Lemongrass EO was found to significantly reduce colony development against key postharvest pathogens: *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer*, *A. flavus, A. parasiticus, A. ochraceus, A. westerdijkiae,* and *Aspergillus niger in vitro*. Lemongrass essential oil at concentrations of 0.5% and 1.0% was incorporated into 0.5% and 1.0% chitosan solution and evaluated as means of controlling anthracnose of bell pepper *in vitro* and *in vivo*.

Table 1 Chemical composition of essential oils (in %) determined by by gas chromatography coupled with mass spectrometry (GC-MS).

Compound	Essential oils							
	B	**BO**	**GP**	**GR**	**L**	**J**	**AN**	**S**
Asarone[a]								5.4
Camphenea[a]					0.9	3.5		
(+)-α-Pinene[a]	1.3	1.7	0.6			3.5	0.4	
Estragol							5.3	
β-Phellandrene	0.9	0.3	0.4			1.2		
(-)-β-Pinene[a]	8.9	17.5			1.3			
6-Methylhept-5-en-2-ol					1.2			
β-Myrcene	0.6	1.8	1.4		6.0			
α-Phellandrene			0.2					
p-Cymene[a]	1.8					1.6		
(R)-(+)-Limonene[a]	34.7	31.9			9.6	19.2		
1.8-Cineole[a]			92.2			2.7		1.1
β-Ocimene		0.7						
γ-Terpinene[a]	8.5				0.9			
(-)-Linalool[a]	9.2	3.1		4.3	1.5	4.3	1.0	4.1
(+)-Rose oxide[a]				2.3				
Thujone			0.3					
cis-Limonene oxide			1.4					
(1R)-(+)-Camphor[a]			0.6					
(±)-Citronellal[a]		1.0		2.8				1.3
(-)-Borneol[a]				5.2		37.7		
2-Undecanone[a]						4.6		
Pseudo-limonene						3.6		
5-Methylindole[a]						0.6		
α-Terpineol		1.6						
cis-Verbenol								87.6
4-Carvomenthenol[a]			0.3					
Cinnamaldehyde[a]			0.4					
Citronellol				28.3				
β-Citronellol[a]			0.4					
β-Citral		8.2		0.7	28.3			
(−)-Carvone[a]			0.8					
Geraniol[a]	31.1	6.9		11.8	5.0		1.9	
Trans-Anethole[a]							87.1	
Anisketone							0.4	
α-Citral[a]	0.5	12.2		1.4	35.2			
Neryl acetate		1.8						
Benzyl benzoate						10.9		
Citronellyl formate				13.0				
Carvacrol[a]				3.1				
δ-Elemene				0.6				
α-Copaene				0.7				
Geranyl acetate[a]		6.7		2.2	4.2		0.5	
β-Caryophyllene[a]		1.3		0.8	2.3			
Farnesene[a]					2.0			
Citronellyl propionate[a]				0.8				
Germacrene-D				4.3				
β-Bisabolene				0.7				
γ-Cadinene				0.5				
Myristicin[a]				1.4				
Acetyl eugenol				0.8				
(+)-Carvone acetate				0.6				
Caryophyllene oxide[a]				0.8				
Cedrol[a]				1.2				
γ-Eudesmol				5.7				
Tau-Cadinol				0.6				
β-Eudesmol				2.0				
α-Eudesmol				0.6				
Total	**97.5**	**96.7**	**99**	**97.3**	**98.4**	**98.4**	**96.6**	**99.5**

Note: [a] – identification confirmed by co-injection of authentic standard, GS-MS – gas chromatography coupled with mass spectrometry, B – bergamot, BO – bitter orange, GP – grapefruit, GR – geranium, L – lemongrass, J – jasmine, AN – star anise, S – sweet flag.

Fungal growth was effectively controlled by 0.5% and 1.0% EO *in vitro* **(Ali, Noh and Mustafa, 2015)**. **Moore-Neibel et al. (2011)** described **t**he antimicrobial activity of lemongrass oil against *Salmonella* directly on the leafy greens, romaine, and iceberg lettuces, and both mature and baby spinach described**.**

As mentioned above, the geranium EO completely inhibited the growth of the *Rhizopus* strains used in this study, as well. This essential oil has the potential to be used in the food industry to prolong the shelf life of fresh and processed foods **(Verma, Chandra Padalia and Chauhan, 2016)**.

Bouzenna and Krichen (2013) tested the antifungal activity *Pelargonium graveolens* Eo against *Rhizoctonia solani,* and results showed that the essential oil was highly active at a dose of 12.5 µL.20 mL^{-1} of PDA. **Naeini, Nazeri and Shokri (2011)** reported that *P. graveolens* EO has considerable anti-*Malassezia* activities.

Bitter orange (*Citrus aurantium* L.) essential oil completely inhibited the growth of *Rhizopus stolonifer* strains. But we recorded growth on day seven of cultivation at the *Rhizopus lyococcus* strain, in the presence of this essential oil. Bitter orange oil has been reported to possess various pharmacological properties. Properties of bitter orange oil for food preservation are discussed too **(Anwar et al., 2016)**. In contrast with bitter orange EO, grapefruit essential oil inhibited the growth of *R. lyococcus* completely but only weakly *Rhizopus stolonifer*. **Ng et al. (2016)** show that grapefruit oil (*Citrus paradisi*) exhibits an array of activities encompassing insecticidal and antimicrobial activities.

Table 2 Effect of essential oils (treatment) in the vapor phase on the growth of *Rhizopus stolonifer* and *Rhizopus lyococcus* strains.

Treatment essential oil	Strains of *Rhizopus stolonifer*				Strain of *Rhizopus lyococcus*	std. Error
	383	510	511	524	512	
Second day of cultivation - means						
Star anise	0^{a}	12.42^{b}	7.11^{c}	0.00^{a}	0.00^{a}	0.1548
Bergamot	7.63^{aA}	9.14^{a}	8.87^{a}	7.67^{a}	14.31^{b}	0.1548
Sweet flag	90.00	90.00	90.00	90.00	90.00	0.1548
Geranium	Completely inhibited growth of strains					
Grapefruit	16.16^{aA}	16.19^{a}	28.81^{b}	20.46^{d}	0.00^{c}	0.1548
Jasmine	90.00	90.00	90.00	90.00	90.00	0.1548
Lemongrass	Completely inhibited growth of strains					
Bitter orange	Completely inhibited growth of strains					
Control	90.00^{B}	90.00^{B}	90.00^{B}	90.00^{B}	90.00^{B}	0.1548
Fourth day of cultivation – means						
Star anise	11.61^{aA}	27.92^{bA}	20.615^{cA}	4.64^{dA}	0.00^{eA}	0.2232
Bergamot	9.24^{aA}	14.48^{bA}	13.66^{bcA}	12.35^{bcA}	90.00^{d}	0.2232
Sweet flag	90.00	90.00	90.00	90.00	90.00	0.2232
Geranium	Completely inhibited growth of strains					
Grapefruit	27.75^{aA}	32.63^{bA}	90.00^{c}	39.88^{dA}	0.00^{eA}	0.2232
Jasmine	90.00	90.00	90.00	90.00	90.00	0.2232
Lemongrass	Completely inhibited growth of strains					
Bitter orange	Completely inhibited growth of strains					
Control	90.00^{B}	90.00^{B}	90.00^{B}	90.00^{B}	90.00^{B}	0.2232
Seven day of cultivation – means						
Star anise	22.43^{aA}	90.00^{b}	90.00^{b}	34.47^{cA}	9.77^{dA}	0.2072
Bergamot	16.33^{A}	18.55^{A}	18.41^{A}	21.51^{A}	90,00	0.2072
Sweet flag	90.00	90.00	90.00	90.00	90.00	0.2072
Geranium	Completely inhibited growth of strains					
Grapefruit	36.12^{aA}	90.00^{b}	90.00^{b}	61.61^{c}	0.00^{dA}	0.2072
Jasmine	90.00	90.00	90.00	90.00	90.00	0.2072
Lemongrass	Completely inhibited growth of strains					
Bitter orange	0.00	0.00.	0.00	0.00	14.63^{bA}	0.2072
Control	90.00^{B}	90.00^{B}	90.00^{B}	90.00^{B}	90.00^{B}	0.2072

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

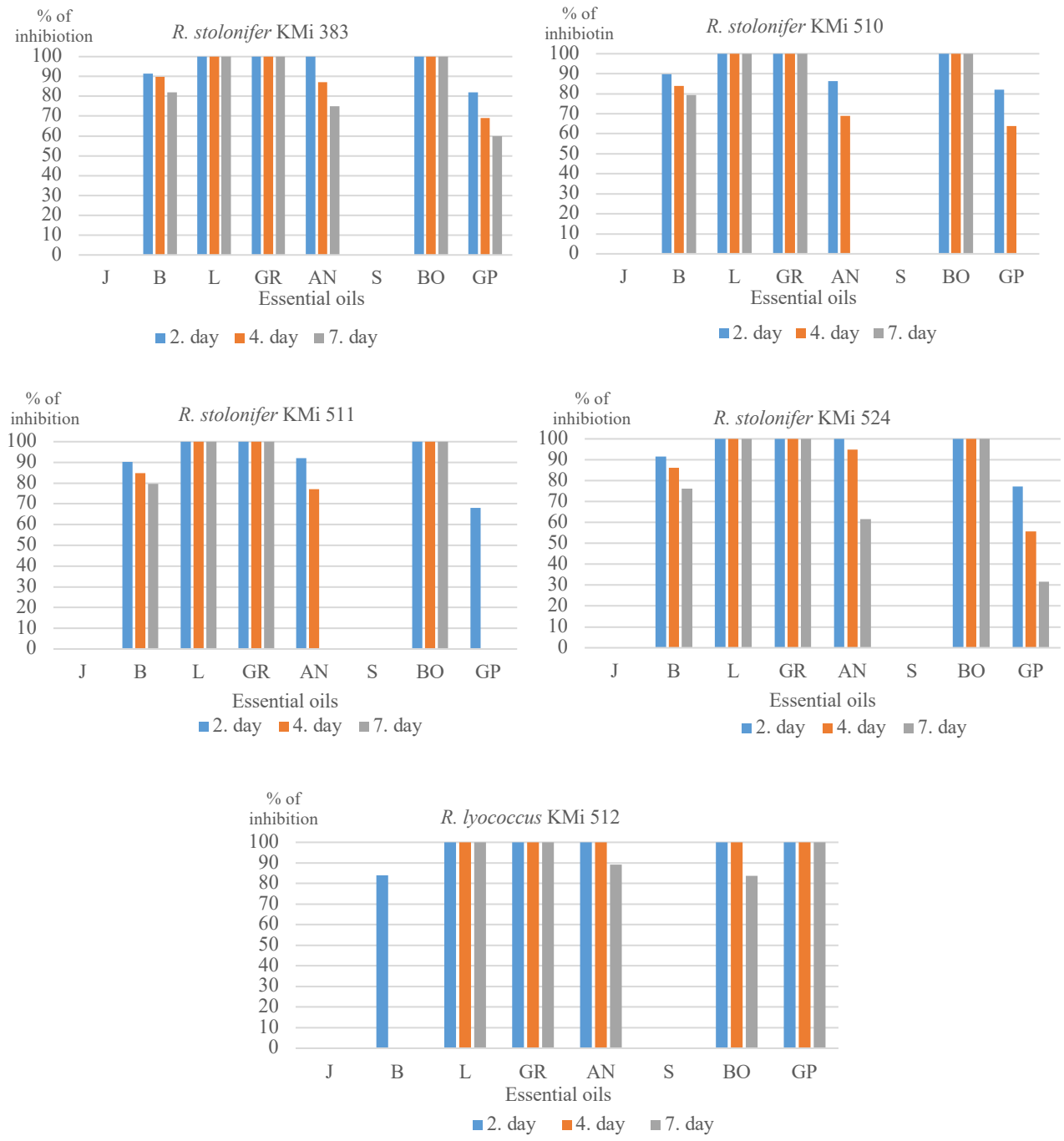

Figure 1 Inhibition of *Rhizopus* spp. growth caused by tested essential oils in the vapor phase. Note: B – bergamot, BO – bitter orange, GP – grapefruit, GR – geranium, L – lemongrass, J – jasmine, AN – star anise, S – sweet flag, R. – *Rhizopus*.

Viuda-Martos et al. (2008) point out the good inhibitory activity of grapefruit EO on the moulds *Penicillium chrysogenum* and *Penicillium verrucosum* too.

The strains of the test species responded differently to the presence of bergamot EO. Significant antifungal activities were observed in all *Rhizopus stolonifer* strains. The strain of *Rhizopus lyococcus* began to grow intensively after two days of cultivation and no growth inhibition was found on the fourth day of cultivation. Some authors e.g. **Avila-Sosa et al. (2016)**, report that bergamot essential oil may be very helpful when applied to food preservation systems.

Jasmine (*Jasminum officinale* L.) and sweet flag (*Acorus calamus* L. var. *angustatus* Bes) essential oils did not inhibit the effect on the growth of tested strains genus *Rhizopus*. Apart from other uses of jasmine essential oil, it is active against various gram-negative, gram-positive bacteria and fungi. This property of jasmine oil allows it to be used in food preservation. It also possesses antioxidant activity **(Ahmed et al., 2016)**. The possibility of using sweet flag EO as a natural preservative in food was indicated by **Miao et al. (2016)** and other authors.

Star anise (*Illicium verum* J. D. Hook) essential oil completely inhibited the growth of two strain *R. stolonifer* (KMi 383 and KMi 524) to the second day of cultivation

and strain *R. lyococcus* to the fourth day of cultivation. The complete inhibition of the germination of spores of *Penicillium expansum* by star anise EO in the vapor phase is reported by **da Rocha Neto et al. (2019)**.

Essential oils that completely inhibited the growth of *R. stolonifer* or *R. lyococcus* isolates were used to determine the minimum inhibitory doses (MIDs). The results are shown in Table 3. High minimum inhibitory doses for *R. stolonifer* and *R. lyococcus* strains were determined. The best results (500 $\mu L.L^{-1}$ of air) for *R. stolonifer* KMi 383, KMi 510, Kmi 524, and *R. lyococcus* Kmi 512 on the 7th day of incubation showed lemongrass EO. MIDs for geranium EO were 625 $\mu L.L^{-1}$ of air for all strains *R. stolonifer* and strain *R. lyococcus* too. The same parameters were determined for geranium Eo for all strains of R. stolonifer and grapefruit Eo for the strain of *R. lyococcus*. On the 14th day of incubation, we found the ame MIDs, respectively higher. It was found that EOs have different effects on individual strains of *R. stolonifer*. **Tripathi and Shukla (2009)** showed absolute fungitoxic activity of geranium EO against Botryodiplodia theobromae (the cause an important postharvest fungal disease of mango), like we. These authors reported MIC 200 ppm for this EO.

Using probit analysis, predicted MIDs90 and MIDs50 were calculated. The results are shown in Table 4. The most effective tested essential oil was lemongrass, less effective bitter orange EO for *R. stolonifer* strains, and grapefruit EO for *R. lyococcus* strain.

The specific compounds isolated from the oils may be non-fungi toxic in nature. Different components of the oils as such may also check the development of races of fungi during their application due to more than one site of action. Fungi can easily develop resistant races against a single component due to its specific mode of action. The exploitation of the essential oils as such would be more economical than a single component as a fungitoxicant **(Tripathi and Shukla, 2009)**. The safe use of EO ingredients is also supported by their self-limiting properties as flavoring substances in food resulting in low levels of use; their rapid absorption, metabolic detoxication, and excretion in humans and other animals; the wide margins of safety between the conservative estimates of intake and the no observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic, developmental and teratology potentials **(Adams et al., 2011)**.

CONCLUSION

In our research, we evaluated the antifungal properties of jasmine EO (extract from *Jasminum officinale* L.), bergamot EO (*Mentha aquatica* L. var. *citrata* (Her.) Fresen), bitter orange EO (from *Citrus aurantium* L.), grapefruit EO (*Citrus paradisi* Macfady), sweet flag EO (East Asian Calamus, from *Acorus calamus* L. var. *angustatus* Bes), star anise EO (from *Illicium verum* J.D.Hook), geranium EO (from *Pelargonium graveolens*), and lemongrass EO (from *Cymbopogon citratus* DC), two essential oils: geranium and lemongrass completely inhibited the growth of all isolates. Bitter orange essential oil inhibited the growth of all isolates of *Rhizopus stolonifer*, but isolate of *Rhizopus lyococcus* began to grow after four days of cultivation. In conclusion, certain essential oils are highly effective in the vapor phase. These could be used in further tests of their antifungal activity and could be used in the control of *Rhizopus* spp. or other fungal pathogens. In further research, we plan to test the effect of selected essential oils in *in vitro* (on selected fruits). In the next part we will test the impact of EOs on sensory quality of selected fruits.

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