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SELECTED PLANT ESSENTIAL OILS OF THE LAMIACEEAE AND APIACEAE FAMILY AS THE ANTIFUNGAL AGENTS IN THE VAPOUR PHASE AGAINST *RHIZOPUS STOLONIFER* AND *RHIZOPUS ORYZAE* ISOLATED FROM MOULDING BREADS

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ABSTRACT

Species of the *Rhizopus* genus are often involved in the moulding of bread and bakery products. Essential oils (EOs) are a suitable alternative to extend the shelf life of food. This research was focused on testing the effect of selected EOs on the growth of *Rhizopus* species. Tested strains were isolated from mouldy breads. The antifungal activity of EOs against *R. stolonifer* and *R. oryzae* strains was determined by micro-atmospheric method (625 µL EO/L air) during 7 days of cultivation. Eight EOs, thyme, red thyme, savory, caraway, marjoram, wild thyme, and oregano 100% inhibited the growth of all strains. Basil, sage, and anise EOs only partially inhibited the growth of strains. Subsequently, the minimum inhibitory doses were determined using micro-atmospheric method. Minimal inhibition doses were 250 µL/L for thyme and red thyme EO, 250 and 500 µL/L for savory EO, 250 and 500 µL/L for oregano EO, 500 and >500 µL/L for wild thyme EO, >500 µL/L for caraway and marjoram EOs. Strains of the genus *Rhizopus* reacted differently to the presence of EOs. Thyme and red thyme were evaluated as the most effective EOs in this study.

Keywords: essential oils, *Rhizopus*, antifungal activity, vapour phase, Lamiacea, Apiaceae

INTRODUCTION

Bakery products, as an important part of a healthy diet, have a limited shelf life. The microbiological deterioration of these products not only affects the quality properties and results in economic loss, but also endangers the health of the consumer **(Gavahian *et al.*, 2020).** According to **Saranjar *et* Sivasakthivelan (2016),** the deteriorations of bakery products due to the growth of moulds range between 1 and 5% of the production. Species of the genera *Aspergillus* (including species previously classified in the genus *Eurotium*), *Monilia, Mucor, Cladosporium, Fusarium, Penicillium* and *Rhizopus* are common bakery spoilers **(Coda *et al.*, 2008; Tančinová *et al.*, 2012; Saranjar *et* Sivasakthivelan, 2016).** Moulds are the main cause of bread waste production **(Bandalan *et* Lauzon, 2018).** *Rhizopus stolonifer* or so called "black bread mould" is relatively common on the bread surface and causes moulds of the products **(Vazhacharickal *et al.*, 2015).** Plant-based antimicrobials, including essential oils (EOs), have gained interest as a potential alternative to synthetic preservatives because they are environmentally friendly and generally recognized as safe **(Maurya *et al.*, 2021).** EOs are important plant extracts that have attracted great interest for their various biological activities and their potential to replace chemical preservatives in the field of food preservation. Over the past few years, consumer demand for safe, ecological, and natural products has driven the search for preservation techniques that improve product quality and safety without causing nutritional or sensory losses. Natural antimicrobial EOs have the potential to provide quality and safety benefits and have fewer impacts on human health **(Ju *et al.*, 2019).** As they are recognized as GRAS, they can be applied in small or large quantities depending on the effects of the bioactive compounds **(Herman *et al.*, 2019).** They are a good source of bioactive compounds that have antimicrobial properties **(Tongnuanchan *et* Benjakul, 2014).** EOs extend the shelf life of food by inhibiting the growth of spoilage and pathogenic microorganisms **(Ribeiro-Santos *et al.*, 2017).** The antimicrobial activity of the essential oils cannot be connected to a single mechanism but to diverse mechanisms **(Sakkas *et* Papadopoulou, 2017).**
The aim of the present research was to determine the antifungal activities of basil, thyme, red thyme, savory, sage, marjoram, wild thyme, oregano, aniseed, and caraway EOs in the vapour phase against *Rhizopus stolonifer* and *Rhizopus oryzae* strains isolated from mouldy breads.

MATERIAL AND METHODS

Fungal cultures

The strains of *Rhizopus stolonifer* KMi-368 (GenBank ID MF461020.1), *Rhizopus stolonifer* KMi-411 (GenBank ID AM933546.1) and *Rhizopus oryzae* KMi-392 (GenBank ID FN421345.1) used in the research were obtained from the Collection of Microorganisms of the Department of Microbiology of the Slovak University of Agriculture in Nitra. The strains were originally isolated from mouldy breads.

Essential oils

EOs from the Lamiaceae and Apiaceae families were used in the research. From Lamiaceae family were used: basil (from *Oscimum basilicum* L.), thyme and red thyme (from *Thymus vulgaris* L.), savory (from *Satureja hortensis* L.), sage (*Salvia officinalis* L.), marjoram (from *Origanum majorana* L.), wild thyme (from *Thymus serpyllum* L.), and oregano (from *Origanum vulgare* L.) EOs. From Apiaceae family were used: aniseed (from *Pimpinella anisum* L.) and caraway (from *Carum carvi* L.) EOs. 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). The determination methodology is described in detail in the article **Tančinová *et al.* (2021).**

Antifungal activity of essential oils

The microatmosphere method was used to study the effect of EOs on the growth of *Rhizopus stolonifer* and *R. oryzae* strains. Sterile plastic Petri dishes with a diameter of 90 mm were used in the experiment. 5 µL of *Rhizopus* spores' suspensions (10^6 spores in 1 mL) were inoculated on 15 mL potato dextrose agar medium (PDA, HIMEDIA India). The spore concentration was determined using an EVE™ Automatic cell counter (NanoEnTek, Korea). The EOs were applied to sterile filter papers Whatman No. 1 (cca 1.5 x 1.5 cm) and placed in the Petri dish lids at a concentration of 625 µL/L of air. Filter paper discs impregnated with

sterilized distilled water were used as a control to confirm no solvent effect of bioactivity. 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 calliper. Inhibition of mycelial growth was calculated by 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 EO. Inhibition of fungal growth in each day was analysed by two-way analysis of variance (ANOVA) and samples were pairwise compared by Tukey HSD difference. Statistical analyses were done in R (R core team, 2020).

Minimum inhibitory doses

EOs that completely inhibited the growth of all *R. stolonifer* or *R. oryzae* strains were used to determine their minimum inhibitory doses (MIDs). EOs dissolved in dimethylsulfoxid (DMSO) were prepared at different concentrations (500, 250, 125, 63, 31.25, and 15.63 µL EO/L of air). Spore suspensions were prepared as in the previous experiment. 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 the 25±1°C and measured after 7 days. The MIDs (expressed as microliters of EOs per volume unit of atmosphere above the strain growing on the agar surface) was defined as the lowest concentration of the oil which did not permit any visible growth after 7 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.

RESULTS AND DISCUSSION

Essential oils

EOs are a complex mixture of fragrant, volatile organic compounds obtained from some aromatic plant materials (**Tariq *et al.*, 2019**; **Jahani *et al.*, 2020**). According to the authors (**Bączek et al., 2019**; **Casiglia *et al.*, 2015**; **Casiglia *et al.*, 2019; Dušková *et al.*, 2016; Farhat *et al.*, 2016; Jarić *et al.*, 2015; Kirillov *et al.*, 2016; Lemos *et al.*, 2017; Méndez-Tovar *et al.*, 2016; Nazzaro *et al.*, 2017**), seasonal variability, different stage of plant growth, climatic conditions, existence of chemo- and ecotypes, and other factors affect the composition of EOs. In view of the above, the composition of oils used in the research was determined. The main components of the EOs are listed in the table 1.

Antifungal activity of essential oils

The antifungal activity of ten EOs against the *Rhizopus stolonifer* (2 strains) and *Rhizopus oryzae* (1 strain) were determined, using the micro-atmosphere method (625 µL/L of air). The results are shown in table 2. Fungal growth was affected by EOs in all strains (ANOVA $p>0.001$). However, strains reacted differently to applied EOs ($p>0.001$). Interactions between the analysed factors were also found (isolate*essential oil; $p>0.001$), thus some EOs inhibited the growth of certain isolates in a different extent. In this first part of the study, seven out of ten EOs completely inhibited the growth of all *Rhizopus* strains. For the other three EOs, we observed a partial inhibitory effect on the tested strains.

The weakest inhibitory effect was determined for *Oscimum basilicum* L. **(**basil) EO. In the first measurement (2nd day of cultivation), inhibition of radial growth was 92-67%, depending on the strain. In another measurement (4th day of cultivation), the EO already inhibited the growth of only one strain of *R. stolonifer* KMi-392 (43%). Strains *R. stolonifer* KMi-411 and *R. oryzae* KMi-368 grew as controls. At the last measurement (7th day), all strains grew all over the Petri dish. Differences were noted in the sporulation of strains. Strains without EO treatment sporulated as early as the first measurement. In *Rhizopus stolonifer* KMi-392 strain, we observed slight sporulation (one of three replicates) at the last measurement. In the *Rhizopus oryzae* KMi-368 and *Rhizopus stolonifer* KMi-411strains, the sporulation was detected at the second, respectively third measurement. Contrary to our results, **Al-Maskri *et al.* (2011)** reported strong activity of basil EO (measured by determining zone of inhibition) against *Aspergillus fumigatus, Aspergillus niger, Penicillium italicum* and *Rhizopus stolonifer*. **Stanojevic *et al.* (2017)** state antimicrobial activity of basil EO on the *Escherichia coli, Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Providencia stuartii,* coagulase-positive *Staphylococcus, Streptococcus* group D, *Salmonella* spp., and *Candida albicans*. **Ngamakeue *et* Chitprasert (2016)** pointed to the antimicrobial properties of encapsulated basil EO, too. But **Tančinová *et al.* (2018)** observed the stimulating effect of basil EO on the growth of *Rhizopus* strains (2 from 6 tested) on the second day of cultivation.

Salvia officinalis L**.** (sage) EO partially inhibited the growth of tested strains. Radial growths were recorded on the second measurement. The effectiveness of inhibition in the second measurements was 0-77%, depending on the strain. Sporulation was detected in all strains at the third measurement (7th day). The inhibitory effect of sage EO (in the vapor phase) on the fungi (*Penicillium roqueforti, P. corylophilum, Aspergillus flavus*, and *A. amstelodami*), isolated from mouldy bakery products is reported by **Suhr *et* Nielsen (2003).** Partial inhibitory effect of sage EO on *Aspergillus niger* and *Aspergillus tubingensis* we demonstrated in the previous study (**Císarová *et al*.,2016). Yilar *et al*. (2018)** described antifungal ability against plant pathogens, specifically: *Alternaria solani, Rhizoctonia solani, Aspergillus niger, Penicillium italicum, Sclerotinia sclerotiorum, Monilia laxa, Botrytis cinerea*, and *Ascochyta rabiei*.

Pimpinella anisum L. (aniseed) EO is a plant used in foodstuffs as an ingredient in famous liqueurs, confectionery, and bakery products. Its essential oil is one of the best-selling on the market and used on an industrial level **(Iannarelli *et al.*, 2018).** Aniseed EO partially inhibited the growth of tested strains. The radial growth of *Rhizopus stolonifer* KMi-392 strain was partially inhibited throughout the experiment (100%-61%). The inhibition effect on the *Rhizopus oryzae* KMi-368 strain was 93 and 71%, on the second, respectively forth day. But on the seventh day the radial growth was like in control set and the sporulation was also observed in this time. The *Rhizopus stolonifer* KMi-411 strain was completely inhibited on the second day, only. In further measurements, the strain has grown, and on the seventh day growing through the entire Petri dishes. Sporulation was observed on the seventh day of cultivation. Weak inhibitory activity on the growth of some *Candida albicans* isolates is reported by **Bona *et al.* (2016).** In contrast to us, **Aminifard *et* Bayat (2018)** showed strong fungicidal effect on the growth of *Penicillium digitatum* in an *in vitro* experiment. **Hu *et al.* (2019)** examined the antifungal activity of anise EO by agar diffusion assay against *Aspergillus niger, A. oryzae*, and *A. ochraceus* (isolated from mouldy breads). They reported MIC 1 mg/mL (*A. niger* and *A. oryzae*) and 0.5 mg/mL (*A. ochraceus*). The authors reported that aniseed EO had a lower impact on the tested species than clove and cinnamon EOs.

Table 1 Main components of essential oils identified by gas chromatography with mass spectrometry

Essential oils	Main components (in %)
Anise	trans anetol (93.3)
Caraway	(-)-Carvone (49.5) p-Cymene (22.7) p-Cymene-2,5-dione (9.7), (+)-α-Tujén (6.8)
Basil	Estragol (88,6) 1,8-Cineole (3.5)
Thyme	(+)-Menthofuran (43.1) p-Cymene (39.1) (-)-Linalool (5.1)
Red thyme	(+)-Menthofuran (51.1) p-Cymene (16.5) γ-Terpinene (4.6) (-)-Linalool (4.2)
Savory	γ-Terpinene (38.1) Carvacrol (33.5) p-Cymene (17.5)
Sage	α-Thujone (23.0) (-)-Isopulegol (20.1) 1,8-Cineole (11.0) Camphene (6.0) (+)-α-pinene (5.1)
Marjoram	(+/-) Menthol (25.7) γ-Terpinene (13.1) (-)-Linalool (12.8) α-Terpineol (8.6) α-Terpinene (8.0) β-Phellandrene (5.4)
Wild thyme	Carvacrol (19.7) Thymol (17.3) p-Cymene (16.7) γ-Terpinene (12.0) Geraniol (7.1)
Oregano	Carvacrol (71.3) p-Cymene (9.8) γ-Terpinene (5.5)

Table 2 Average growth of fungal strains (diameter in mm; n = 6) and percentage of growth inhibition of strains treated by essential oils (625 µL/L of air) on PDA at 25±1°C

Essential oils	Day of cultivation																	
	2nd						4th						7th					
	Tested strains of genus *Rhizopus*																	
	R. oryzae		*R. stolonifer*				*R. oryzae*		*R. stolonifer*				*R. oryzae*		*R. stolonifer*			
	KMi-368		KMi-392		KMi-411		KMi-368		KMi-392		KMi-411		KMi-368		KMi-392		KMi-411	
	Average ± sd	% of inh.	Average ± sd	% of inh.	Average ± sd	% of inh.	Average ± sd	% of inh.	Average ± sd	% of inh.	Average ± sd	% of inh.	Average ± sd	% of inh.	Average± sd	% of inh.	Average ± sd	% of inh.
Basil	17.04±0.21c	81	7.23±1.76b	92	29.45±4.60b	67	90.00±0.00d	0	90.00±0.00b	0	51.06±4.11c	43	90.00±0.00b	0	90.00±0.00b	0	90.00±0.00c	0
Thyme	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100
Red thyme	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100
Savory	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100
Sage	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	21.04±0.50b	77	90.00±0.00b	0	50.13±6.02c	44	90.00±0.00b	0	90.00±0.00b	0	90.00±0.00c	0
Anise	5.99±0.08b	93	0.00±0.00a	100	0.00±0.00a	100	25.81±0.37c	71	90.00±0.00b	0	17.44±0.58b	81	90.00±0.00b	0	90.00±0.00b	0	34.97±0.49b	61
Caraway	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100
Marjoram	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100
Wild thyme	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100
Oregano	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100	0.00±0.00a	100
Control	90.00±0.00d		90.00±0.00c		90.00±0.00c		90.00±0.00d		90.00±0.00b		90.00±0.00d		90.00±0.00b		90.00±0.00b		90.00±0,00c	

Averages accompanied by the same letter (in columns) are not significantly different (ANOVA, Tukey test; $p<0.05$).
Legend: n - number of measurements, PDA – potato dextrose agar, inh. – inhibition, sd - standard deviation.

The basil EO affected tested strains differently on the second and fourth day, when significantly the slowest growth was measured on *R. stolonifer* KMi-392 strain. On the second day, the average of *R. stolonifer* KMi-392 colony was halved compared to *R. oryzae* KMi-368 strain and only a quarter compared to *R. stolonifer* KMi-411 strain. Despite there was not visible growth of any strain after sage EO treatment on the second day, on the fourth day *R. stolonifer* KMi-392 strain overgrowths whole plate, while *R. stolonifer* KMi-411 strain produced 50 mm and *R. oryzae* KMi-368 strain only 21 mm colony with significant differences (Tukey test $P<0.05$). Only *R. oryzae* KMi-368 strain showed visible growth on the second day after anise EO application. On the fourth day, *R. stolonifer* KMi-392 strain overgrew Petri plate while colonies of *R. oryzae* KMi-368 and *R. stolonifer* KMi-411 strains were significantly (Tukey test $P<0.05$) smaller with diameters 25.8 mm and 17.4 mm respectively. Even on the seventh day colonies of *R. stolonifer* KMi-411 strain reached only 35 mm while *R. stolonifer* KMi-392 strain filled whole 90 mm plate. Imbibition activity of EOs is not constant for all tested strains and there is a need to consider the probability of more resistant strains occurrence in site of EOs application.

Thymus vulgaris L. (thyme, and red thyme), *Satureja hortensis* L (savory), *Origanum majorana* L. (marjoram), *Thymus serpyllum* L. (wild thyme), *Origanum vulgare* L. (oregano), and *Carum carvi* L. (caraway) EOs completely inhibited the growth of all strains. These seven EOs were used in another experiment.

Minimal inhibitory doses and probit analyses

The results of minimal inhibitory doses (MIDs) are listed in table 3 and $MIDs_{90}$, respectively $MIDs_{50}$ are in table 4. In this study, two different *Thymus vulgaris* EOs were used: thyme and red thyme. *Thymus vulgaris* has been used as a food flavouring and in gastronomy for a very long time. This plant is widely used in traditional medicine and folk medicine, too **(Hosseinzadeh, 2015; Komaki *et al*., 2016; Satyal *et al.*, 2016; Kuete, 2017; Kubatka *et al*., 2019).** MIDs were 250 µL/L of air for all strains, in case of thyme and red thyme EOs. $MIDs_{90}$ on the seventh day for thyme EO were 203.40 µL/L (*R. stolonifer* KMi-392), 151.11 µL/L (*R. stolonifer* KMi-411), and 144.73 µL/L (*R. oryzae* KMi-368). **Reyes-Jurado *et al.* (2019)** also tested antifungal activity of thyme EO in vapour phase (against *Aspergillus nomius, Eupenicillium hirayamae, Penicillium cinnamopurpureum*, and *P. viridicatum*, which were isolated from stored wheat grains). They determined MICs of thyme EO between 0.1–0.5 µg/mL of air. **Al-Shahrani *et al.* (2017)** tested antifungal activity of thyme EO by micro-dilution method. They show minimal inhibitory concentrations and minimal fungicidal concentrations ranged between 2.5 and 10 mg/mL. MIC_{50} against *Aspergillus niger* was 5 mg/mL and against *Aspergillus flavus* 2.5 mg/mL. **Pinto *et al.* (2021)** indicated that vapours of red thyme EO significantly reduced *Penicillium* decay of oranges during cold storage.

Kim *et al.* (2019) pointed to the strong antifungal activity of *Thymus vulgaris* and *Satureja hortensis* (savory) EOs against the phytopathogenic fungi (*Raffaelea quercus*-mongolicae and *Rhizoctonia solani*). In our research, MIDs of savory EO were 250 µL/L of air (*R. stolonifer* KMi-411, *R. oryzae* KMi-368) and 500 µL/L of air (*R. stolonifer* KMi-392). MID_{90} were between 167.60 – 311.26 µL/L of air. Tested strains differed in their resistance to savory EO. **Ferdes *et al.* (2017)** tested the antifungal effects of savory and other essential oils (sage, rosemary, anise, and quinoa) against mycelium growth of *Aspergillus niger, Aspergillus oryzae, Mucor pusillus* and *Fusarium oxysporum* in *in vitro* conditions. All the tested EOs showed inhibitory effect. The savory essential oil showed inhibitory effect in the concentration 10 µg/mL. The essential oils of savory and sage showed a great inhibitory effect against *M. pusillus* when compared with other EOs. Compared to these authors, savory EO was more effective than sage EO in our research.

The highest MID values were determined for essential oils from *Origanum majorama* L. and *Carum carvi* L. MID of marjoram EO was >500 µL/L of air for all strains. $MIDs_{90}$ on the seventh day for marjoram EO were 549.57 µL/L (*R. stolonifer* KMi-392), 511.64 µL/L, (*R. stolonifer* KMi-411), and 541.85 µL/L (*R. oryzae* KMi-368). **Nikkhah *et* Hashemi (2020)** stated MIC of marjoram EO on the *Alternaria alternata* 5 000 g/L, and on the *Penicillium expansum* 10 000 g/L. MIC of marjoram EO on the *Botrytis cinerea* 2500 µg/mL and on the *Penicillium expansum* 5000 µg/mL determinated by a broth microdilution method was declared by **Nikkhah *et al.* (2017)**. MID of caraway EO was >500 µL/L of air for all strains, like for marjoram EO. $MIDs_{90}$ on the seventh day for caraway EO were 541.85 µL/L (*R. stolonifer* KMi-392), 541.85 µL/L, (*R. stolonifer* KMi-411), and 549.53 µL/L (*R. oryzae* KMi-368). Caraway (*Carum carvi* L.) is a plant with spicy tasting seeds. Caraway has been used for a long time in traditional medicine systems, folk medicine and as a food supplement, also **(Lasram, 2019; Rasooli *et* Allameh, 2016; Sachan *et al*., 2016).**

Table 3 The inhibitory effect (in %) of essential oils on the growth of *Rhizopus stolonifer* and *Rhizopus oryzae* colonies (n = 6) on PDA at 25±1°C after 7 days of cultivation

Essential oil	µL/L	Strains of *Rhizopus* genus		
		R. oryzae KMi-368	*R. stolonifer* KMi-392	*R. stolonifer* KMi-411
Thyme	500	100	100	100
	250	100	100	100
	125	50	33,33	66.66
	62.5	0	16.66	33.33
Red thyme	500	100	100	100
	250	100	100	100
	125	83.33	66.66	33.33
	62.5	50	50	0
	31.25	33.33	16.66	0
	15.625	16.66	0	0
Savory	500	100	100	100
	250	100	66.66	100
	125	16.66	50	16.66
	62.5	0	0	0
Caraway	500	16.66	33.33	33.33
Marjoram	500	33.33	16.66	83.33
Wild thyme	500	66.66	100	100
	250	66.66	83.33	83.33
	125	33.33	33.33	50
	62.5	16,66	0	16.66
Oregano	500	100	100	100
	250	66.66	100	100
	125	50	66.66	66.66
	62.5	33.33	50	66.66
	31.25	16.66	0	33.33

Legend: PDA – potato dextrose agar, n = number of repetitions

Table 4 Minimal inhibition doses (µL/L) estimated by probit analyses at 7th day of cultivation

MID µL/L	Essential oils						
	Thyme	**Red thyme**	**Savory**	**Caraway**	**Marjoram**	**Wild thyme**	**Oregano**
	***Rhizopus stolonifer* KMi-411**						
MID_{50}	99.93	132.03	142.86	510.18	469.82	153.39	74.15
MID_{90}	156.11	153.92	167.60	541.85	511.64	256.76	160.63
	***Rhizopus stolonifer* KMi-392**						
MID_{50}	135.22	87.94	178.07	510.18	520.78	176.26	93.04
MID_{90}	203.40	161.64	311.26	541.85	549.57	261.89	153.51
	***Rhizopus oryzae* KMi-368**						
MID_{50}	125.00	64.21	142.86	520.78	510.18	284.11	160.86
MID_{90}	144.73	143.79	167.60	549.53	541.85	554.52	334.36

MIDs of oregano EO were 500 µL/L of air for *R. oryzae* and 250 µL/L for *R. stolonifer* strains. $MIDs_{90}$ on the seventh day for oregano EO were 153.51 µL/L (*R. stolonifer* KMi-392), 160.63 µL/L, (*R. stolonifer* KMi-411), and 334.36 µ/L (*R. oryzae* KMi-368). **Hossain *et al*. (2016)** tested antifungal activities of basil, cinnamon, eucalyptus, mandarin, oregano, peppermint, tea tree and thyme EOs on the growth of *Aspergillus niger, A. flavus, A. parasiticus* and *Penicillium chrysogenum*. These authors reported that thyme and oregano proved to be the most effective against all fungal species tested. **Boudine *et al*. (2016)** pointed out the antifungal activity of oregano EO, too. These scientists tested antifungal activity of oregano EO and thymol on the growth of *A. niger, A. flavus, Penicillium*

sp., *Fusarium* sp., and *Mucor* sp., isolated from corn. They state that, oregano essential oil and thymol demonstrate an antifungal effect against all tested isolates. MIDs of wild thyme EO were 500 µL/L of air for *R. stolonifer* and >500 µL/L for *R. oryzae*. $MIDs_{90}$ on the seventh day for wild thyme EO were 261.89 µL/L (*R. stolonifer* KMi-392), 256.76 µL/L (*R. stolonifer* KMi-411), and 554.52 µL/L (*R. oryzae* KMi-368). **Wesołowska *et al.* (2015)** draw attention to antimicrobial activity of wild thyme EO to the strain of *E. coli* (MIC=0.025 µL/mL) and to the yeast *Candida albicans* (MIC=0.05 µL/mL). **Nardoni *et al.* (2015)** reported about strong antifungal activity of EO from *Thymus serpyllum* L. on the selected dermatophyte species.

CONCLUSION

In our research, the influence of ten selected EOs obtained from plants of the Lamiaceae and Apiaceae families on the growth of three strains of the *Rhizopus* genus was tested. At the highest concentration used (625 µL EO/L of the air) thymus, red thymus, savory, caraway, marjoram, wild thyme, and oregano completely inhibited the growth of *Rhizopus oryzae* and *R. stolonifer* strains. The minimum inhibitory doses were: 250 µL/L for thyme and red thyme EOs, 250 and 500 µL/L for sage and oregano EOs, 500 and >500 µL/L for wild thyme and >500 µL/L for caraway and marjoram EOs. The tested strains reacted differently to the presence of EOs, not only at the species level but also within the same species. The most effective essential oils can be used to suppress the growth of *R. stolonifer* and *R. oryzae*.

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