JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

INHIBITORY EFFECT OF ESSENTIAL OILS FROM SOME *LAMIACEAE* SPECIES ON GROWTH OF *EUROTIUM* SPP. ISOLATED FROM BREAD

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doi: 10.15414/jmbfs.2018.8.2.857-862

ARTICLE INFO

Received 6. 7. 2018
Revised 22. 9. 2018
Accepted 24. 9. 2018
Published 1. 10. 2018

Regular article

OPEN ACCESS

ABSTRACT

Bread is considered an intermediate - moisture food product that is prone to mould spoilage. Growth of spoilage fungi is currently controlled with the addition of chemical preservatives. Consumers demand more natural products and there is a need to reduce the amount of chemical preservatives added to foods. Essential oils (EOs) and their constituents emerged as promising and effective compounds to protect foods from lipid peroxidation and provide microbiological food safety. So the aim of our study was to determinate microscopic fungi involved in contamination of bread target the genus *Eurotium* and to evaluate the antifungal activity of 5 EOs by vapor contact against the fungal species of this genus. In all samples after three days of cultivation fungi created colonies on the surface of the crust and the pieces of the bread samples stored on DRBC were after three days of cultivation covered with fungi colonies. Altogether 195 isolates were recovered from 8 bread samples and assigned to 10 fungal genera. The most frequently genus of fungi was *Penicillium*, *Aspergillus*, *Cladosporium*, *Epicoccum* and *Eurotium*. Based on phylogenetic and morphological studies, five different *Eurotium* species were identified: *E. amstelodami, E. chevalieri, E. herbariorum, E. rubrum* and *E. repens.* In this study, we explored the potential of five essential oils retrieved from some *Lamiaceae* species, concretely: basil (*Ocinum basilicum* L.), lavender (*Lavandula angustifolia* Mill.), oregano (*Origanum vulgare* L.), mint (*Metha piperita* L.) and sage (*Salvia officinalis* L.). Gas chromatography–mass spectrometry (GC-MS) analysis allowed for the identification of 28 compounds as main constituents. Inhibitory activity of EOs was assessed against 5 species of genus *Eurotium* (2 isolates for each specie). In all studied strains, the essential oils caused significant differences ($P<0.05$) on the mycelium growth. Our result showed that the best antifungal effect was shown by lavender and oregano (100%) to all species of tested fungi followed by mint (with the best MGI 79.89% to *E. rubrum*) >basil (with the best Mycelial growth Inhibition (MGI) 93.65% to *E. herbariorum*) and the low effect had sage essential oil (with the best MGI 68.62% to *E. repens*). The present study demonstrated the potential food preservative ability of essential oils from some *Lamiaceae* species.

Keywords: antifungal activity, bread, *Eurotium*, essential oils, vapor

INTRODUCTION

Baked goods are considered to be one of the most important products of the food industry. Bread is a staple food in many countries and consumed daily all over the world. Within the European Union (EU) the production of bread is relatively stable showing low growth in most western countries (**Elsanhoty *et al.*, 2013**). However, like many processed foods, bakery products, are subject for physical, chemical and microbiological spoilage (**Saranraj and Sivasakthivelan, 2016**). The ingredients contained in bread are supportive to growth of microorganisms and multiplication at different stages of bread production, slicing and wrapping. The main microbial spoilage types of bread are moldiness and ropiness which are troublesome for bakers. Mold growth often begins within a loaf of sliced bread, where is a moisture more available than on the surface, especially in the crease (**Salim-ur-Rehman *et al.*, 2007**). Mould spoilage is a serious and costly problem for bakeries. Filamentous fungi involved in bread spoilage include *Rhizopus* sp., and *Mucor* sp., *Penicillium* sp., *Eurotium* sp., *Aspergillus* sp. and *Monilia sitophilia*. Storage of bread under low humidity conditions retards mould growth. However, fungi such as *Eurotium* spp. are the most common causes of spoilage of dried cereals capable of growth at 0.75 a_w (**Dao and Dantigny, 2011**). *Eurotium* species are usually the first fungi to colonize improperly water allowing other species (**Saranraj and Sivasakthivelan, 2016**) and losses of bakery products due to mould spoilage vary between 1-5 % depending on seasons, type of products and methods of processing. Nowadays, storage fungi are commonly controlled by synthetic chemicals; however, these agents usually impact the human health and the environment adversely (**Nakahara *et al.*, 2013**). In bakery products, preservatives (salts of propionic and sorbic acids) are added to prevent growth of spoilage fungi. However, in recent years, there has been consumer pressure to reduce using of preservatives (**Saladino *et al.*, 2017**). Furthermore, the use of fungicides is more harmful in the post-harvest period because of the short time between treatment and consumption. Moreover some fungi have shown resistance against broad spectrum fungicides such as benzimidazoles, imazalil and prochloraz due to repeated usage (**Lima *et al.*, 2015; Farzaneh *et al.*, 2015**). Consequently, interest in more natural, non-synthesized, antimicrobials as potential alternatives to conventional antimicrobials for extending shelf life and combat foodborne pathogens has increased. Aromatic plants and their components have been examined as potential inhibitors of bacterial and fungal growth and most of their properties have been linked to essential oils and other secondary plant metabolites. Historically, essential oils from different sources have been widely promoted for their potential antimicrobial capabilities (**Calo *et al.*, 2015**). The essential oils from *Lamiaceae* species are accredited with antioxidant, antiseptic, anti-inflamatory, antimicrobial and fungicidal properties (**Burt, 2004**; **Kumar *et al.*, 2014**). For all these reasons, research has focused on finding natural alternatives to traditional solutions (**Cherrat *et al.*, 2014**). The good natural antimicrobial has to fulfil some requirements such as: (a) to be active in low concentrations in its natural form, (b) to be inexpensive, (c) not to generate sensorial changes in the

product, (d) to inhibit a wide range of spoilage and pathogenic microorganisms and (e) not to be toxic. EOs have been identified as natural food additives which can find useful application in food preservation (**Davidson *et al.*, 2013**). The object of our study was to determinate microscopic fungi involved in contamination of bread with the target the genus *Eurotium* and to evaluate the antifungal activity of 5 EOs by vapor contact against the fungal species of this genus.

MATERIAL AND METHODS

Fungal isolation

Eight samples of bread (collected from a local markets) were mycological tested. Three samples contained rye/wheat flour (no.1, 2 and 8), two samples (no. 3, 7) contained wheat/potato flour and three breads samples were made from wheat flour only (no. 4, 5 and 6). The aqueous activity (a_w) was measured for each sample (Table 1). After analysis the bread samples were three days stored at room temperature in a plastic bag, in the bread box at temperature 25±1°C. After that we detected contamination of samples by microscopic filamentous fungi with a goal to *Eurotium* spp. occurring in used types of bread in these ways of storage, which are the most used at our homes. Fungi that created visible colonies on the surface of the bread were inserted directly on MEA (Malt Extract agar; Merck, Germany, **Klich, 2002**). All the bread samples (the middle part) were cut to cubes of sides 1.5 x 1.5 x 1.5 cm and were given in number of five pieces directly on plates with DRBC (Dichloran Rose Bengal Chloramphenicol agar; Merck, Germany) and DG18 medium (Dichloran-Glycerol medium base; Merck, Germany). Cultivation was proceeded for three days in the dark 25±1°C. All experiments were performed in triplicate. Obtained results were evaluated and expressed in relative density (RD) and isolation frequency (Fr) at the genus. The isolation frequency (%) is defined as the percentage of samples within which the species or genus occurred at least once and relative density (%) is defined as the percentage of isolates of the species or genus, occurring in analysed samples (**Gautam *et al*., 2009**). These values were calculated according following formula:

$$Fr\ (\%) = (ns/N)*100 \qquad RD\ (\%) = (ni/Ni)*100$$

Where ns = number of samples with species or genus; N = total number of samples; ni = number of isolates of a species or genus; Ni = total number of isolated fungi (**Gonzáles *et al*. 1996**).

Table 1 The flour content (reported by the producer) and a_w value of used bread samples

No.	Samples	a_w Bread composition reported by the producer (%)
1.	Whole grain bread	0.877
		47% WF, 2% PF
2.	Flax bread	0.901
		33% WF, 16% RF
3.	Potato bread	0.909
		47% WF, 2% PF
4.	Wheat bread	0.922
		47% WF
5.	Wheat bread	0.921
		41% WF
6.	Wheat bread	0.922
		43% WF
7.	Potato bread	0.906
		47% WF, 2% PF
8.	Ray bread	0.895
		34% WF, 17% RF

Legend: No.-number of sample, WF-Wheat flour, PF-Potato flour, RF-Ray flour, a_w- Water activity

Fungal identification

Isolates of the genus *Eurotium* were identified to the species level according to morphological characteristics based on microscopy. To determine particular species, diagnostic literature was used as follows: **Pitt and Hocking, 1985**; **Klich, 2002**; **Samson *et al.*, 2002**. Strains were inoculated in three points on the following identification media: CYA (Czapek yeast autolysate agar; Merck, Germany) with 20% and 40% (w/v) sucrose (CY20S, CY40S, respectively) and DG-18 medium. Inoculated media were incubated at 25, 30 and 37 °C in the dark for 7 days.

Essential oils samples

The essential oils from some *Lamiaceae* species were used in this study, concretely: basil (*Ocinum basilicum* L.), lavender (*Lavandula angustifolia* Mill.), oregano (*Origanum vulgare* L.), mint (*Metha piperita* L.) and sage (*Salvia officinalis* L.). The EOs were supplied by Calendula company a.s. (Nová Ľubovňa, 238 A, Slovakia). All essential oils were extracted by hydrodistillation and stored in air-tight sealed glass bottles at 4°C.

GC-MS analysis of essential oils

Essential oils constituent were identified and the relatively composition of the oil was determined by gas chromatography followed by mass spectrometry (GC-MS). Prior to the analysis, essential oils were diluted in hexane to a concentration of l µL/mL. Analyses were carried out using an Agilent 7890A GC coupled to an Agilent MSD5975C MS detector (Agilent Technologies, Palo Alto, CA, USA) with a 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 at electron ionization energy of 70 eV. Analysis were measured in SCAN mode, mass range was 40-400m/z. Starting at 60°C, the oven temperature was increased at a rate of 3°C/min to a maximum of 231°C, where it was kept constant for 10 min. The identification of constituents was based on a comparison of their mass spectra and relative retention indices (RI) against the National Institute of Standards and Technology Library (NIST, USA), as well as authentic analytical standards and data from the literature (**Adams, 2007**). 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 1% were included. Peaks under 1% were not counted.

Antifungal activity of EOs

The antifungal activity of selected EOs was investigated by the method adapted from **Guynot *et al.* (2003)**. The test was performed in sterile Petri dishes (Ø 90 mm) containing 15 mL of CYA. Essential oils were diluted in ethyl acetate to give final volume 100 µL. Plates were kept in an inverted position. A sterilized filter paper (square of 1 x1 cm) was placed in the centre of the lid and 100 µl of EOs were added to the paper at concentration 500 µL/L of air. Blank was made by adding 100 µL of ethyl acetate to it. Each fungus was inoculated in the centre on Petri plates with needle-inoculated. Plates were tightly sealed with parafilm and incubated for 7, 11 and 21 days at 25±1 °C (three replicates were used for each treatment). Diameters of the growing colonies were measured. The antifungal activity was expressed in terms of percentage of mycelial growth inhibition and calculated according to the following formula:

$$\text{Mycelial growth Inhibition: MGI \%} = [(d_c-d_t)/d_c] *100$$

Where d_c=average (mm) increase in mycelial growth in control, d_t=average (mm) increase in mycelial growth in treatment (**Marandi *et al.*, 2011**).

Statistical analysis

The basic variation-statistical values including means, standard deviation, growth inhibition percentage (%) were calculated from the obtained data using the SAS statistical program (one- factorial variance analysis ANOVA) at $P<0.05$ level of significance.

RESULTS AND DISCUSSION

Mycological evaluation of bread samples

Bakery products, especially bread, are intermediate moisture content products which are highly perishable. The most common forms of bread deterioration are moisture loss and microbiological spoilage. Spoilage of wheat bread and the other bakery products by colonisation and growth of fungi represents more than 90% of the total microbial contamination (**Magan *et al*., 2003**; **Arroyo *et al*., 2008**). Moreover, during bread preparation, such as baking, only the vegetative forms of microorganisms are removed, but bacteria and fungi spores are able to survive (**Nowicki *et al.*, 1988**). In all samples after three days of cultivation fungi created colonies on the surface of the crust and also the pieces of the bread samples stored on DRBC were after three days of cultivation covered with fungi colonies. Our results showed, that 195 isolates were recovered from 8 bread samples and assigned to 10 fungal genera, including *Mycelia sterilia* (isolates without sporulation). One of the objectives of this study was to detected contamination of samples by microscopic filamentous fungi with goal to *Eurotium* spp.. So the isolates of the genera *Eurotium* have been identified to the species level.

The filamentous fungi isolated from bread samples and their isolation frequency (Fr %) are indicated in Figure 1 and relative density (RD %) in Figure 2. Isolates of the genus *Penicillium* were found in all tested samples with the highest isolation frequency 100% and higher relative density 50.26% (98 isolates).

According to **Hocking and Pitt** (**1980**) species of genus *Penicillium*, *Aspergillus*, *Eurotium*, *Chrysosporium* and *Wallemia* are the most frequently occurring xerophilic fungi in bread samples. Also **Viljoen and Von Holy** (**1997**) recovered 97 isolates from genus *Penicillium* (37.1%), *Aspergillus* (18.6%) and *Cladosporium* (13.4%) as the most frequented in the bread samples. The second highest isolation frequency was detected for the genus *Aspergillus* (87.5%) with relative density of 16.41% (32 isolates), followed by genus *Cladosporium* (Fr 75%) (RD 5.64%), *Eurotium* (RD 5.13%) and *Epicoccum* (RD 4.62%) with isolation frequency of 62.5%, for both. Similar results were obtained in previous study, but no isolates of the genus *Eurotium* were occurred in bread samples (**Tančinová *et al.*, 2012**). Isolation frequency of another four genera [*Mycelia sterilia* and *Trichoderma* (RD 1.03%), *Rhizopus* (RD 5.64%), *Alternaria* (RD 2.05%)] was less than 40%. Isolates of the genus *Mucor* was detected in samples with low isolation frequency (Fr 37.5%) but with a higher relative density (RD 8.21%), in comparison with another genus with isolation frequency less than 40%. **Vytřasová *et al.*, (2002**) in their study isolated *W. sebi*, *E. amstelodami*, *E. chevalieri*, *E. herbariorum* and *E. rubrum* from 10 different bread samples. Their also found that during the identification of xerophilic fungi, *Eurotium* species were often accompanied by *Penicillium* and *Emericella* species. These findings correlated with our results (Figure 1).

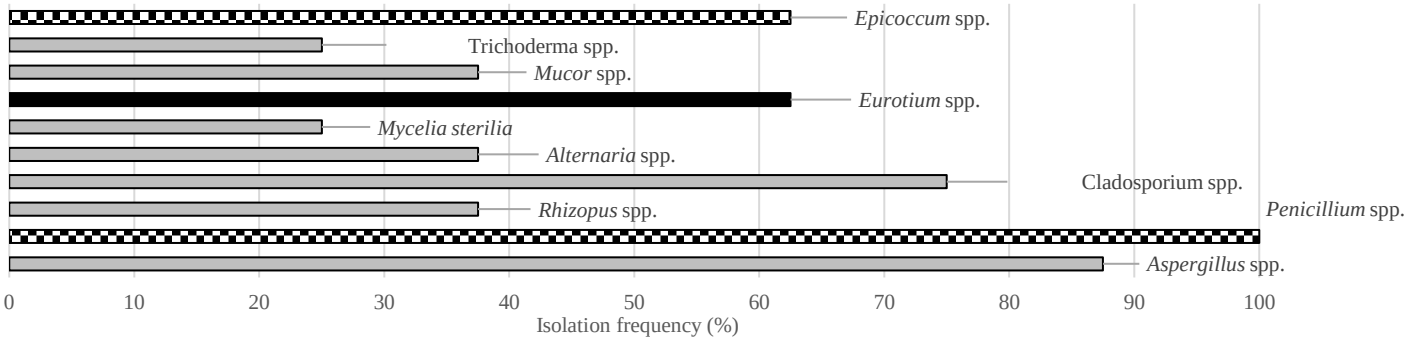

Figure 1 Isolation frequency (Fr) (%) isolated genus from bread samples (n = 8)

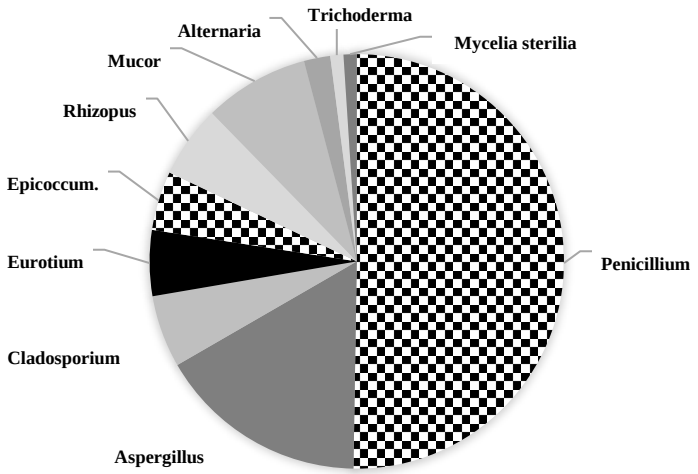

Figure 2 Relative density (RD) (%) isolated genera from bread samples (n = 8)

In our study were isolated the genus *Eurotium* from five bread samples (no. 1, 2, 4, 6 and 7). Based on phylogenetic and morphological studies 10 isolates of the genus *Eurotium* were identified to the species. In total, five different *Eurotium* species were identified: *E. amstelodami*, *E. chevalieri*, *E. herbariorum*, *E. rubrum* and *E. repens*. For each species two isolates were detected. The characteristics of the strains did not differ significantly from known species described in the literature (Figure 3). The colonies of all isolates had relatively slow growth on CY20S and MEA with a diameters between 20-32 mm after 7 days of incubation; were usually plane, low and voluminous with different colours (from pale to brown yellow, greyish greeny); reverse were pale, olive, orange or brown. All strains were able to developing cleisthothecia after 10 days of cultivation. Conidiophores borne from aerial hyphae; ascospores yellow, ellipsoidal, with rough walls and with two conspicuous longitudinal ridges (*E. amstelodami*) (Figure 3 X,Y); smooth walled, with two prominent, parallel flanges (*E. chevalieri*) (Figure 3 C,D,E,F) and with a shallow longitudinal furrow and roughened ridges (*E. herbariorum*) (Figure 3 CH,I,J); conidia on CY20S and MEA from ellipsoidal to spherical.

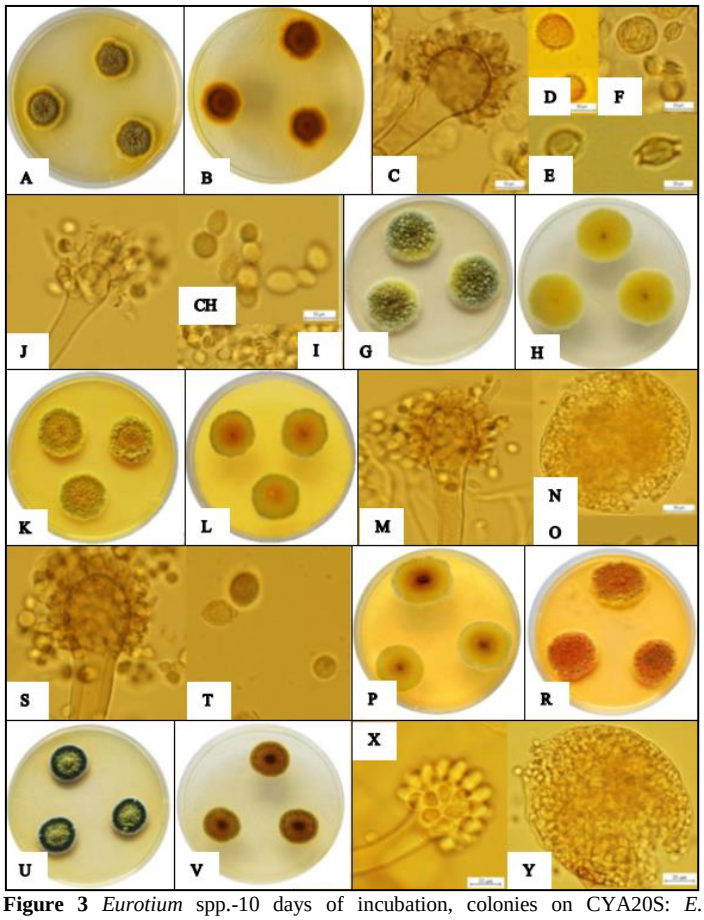

Figure 3 *Eurotium* spp.-10 days of incubation, colonies on CYA20S: *E. chevalieri* (A-top, B-reverse), *E. herbariorum* (G-top, H-reverse), *E. repens* (K-top, L-reverse), *E. rubrum* (P-top, R-reverse) and *E. amstelodami* (U-top, V-reverse); microstructures (scale bar = 10, 20 µm): *E. chevalieri* (C- heads, conidiophore, D-conidia, E,F-ascospore), *E. herbariorum* (CH, I-conidia, J-head, conidiophore), *E. repens* (M-head, conidiophore, N-cleisthothecium, O-conidia), *E. rubrum* (S-head, conidiophore, T-conidia) and *E. amstelodami* (X-head, conidiophore, Y-cleisthothecium)

Essential oils analyses by using GC-MS

Qualitative and quantitative analysis of the essential oils is listed in Table 2.

Table 2 Qualitative and quantitative analysis (%) of the used essential oils by GC-MS

RI[b]	Component	Basil[c]	Lavender	Oregano	Mint	Sage
938	**α-pinene[a]**			1.20		5.10
981	**β-Pinene[a]**		1.00			2.30
993	β-Myrcene			1.30		
1029	**p-Cymene[a]**			9.80		1.80
1031	**D-Limonene[a]**				1.90	1.90
1034	1.8-Cineole	3.50	1.10		7.20	11.00
1043	β-trans-ocimene		1.60			
1053	β-Ocimene		1.40			
1062	γ-Terpinene			5.50		1.1
1101	(-)-Linalool	1.20	35.50			
1107	α-Thujone					23.00
1119	Fenchyl alcohol					6.00
1148	(−)-Isopulegol					20.10
1158	(±)-Citronellal				23.10	
1168	**Borneol[a]**				8.50	4.20
1177	(−)-Menthol				40.80	
1185	Menth-1-en-4-ol		2.50		1.20	
1192	α-Terpineol		1.20			
1200	Estragol	88.6				
1259	**Geranial[a]**		35.10			
1287	(−)-Bornyl acetate					2.30
1296	**Thymol[a]**			2.2		
1297	2-Undecanone		3.10		5.8	
1306	**Carvacrol[a]**			71.10		
1419	β-Caryophyllene		3.90	1.4	3.40	5.60
1437	α-Bergamotene	2.00				
1455	α-Caryophyllene		2.40			7.00
1574	Caryophyllene oxide					
	total	**95.30**	**88.80**	**92.50**	**91.90**	**91.40**

Legend: [a] - Identification confirmed by co-injection of authentic standard; [b] - RI: identification based on Kovat's retention indices (HP-5MS capillary column) and mass spectra; [c] - relative proportion were calculated in % by dividing individual peaks area by total area of all peaks.

The main components of basil essential oil (*Ocinum basilicum* L.) were estragol (88.06%). Carvacrol was identified and determined as the major component of oregano (*Origanum vulgare* L.) (71.10%) oil. Lavender (*Lavandula angustifolia* Mill.) oil was comprised mainly of geranial (35.10%) and (-)-Linalool (35.50%) while sage (*Salvia officinalis* L.) constituted mainly α-Thujone (23.00%), (-)-Isopulegol (20.10%) and 1.8-Cineole (10.00%). In mint (*Mentha piperita* L. L.) essential oil, the main components were (-)-Menthol (40.80%) and (±)-Citronellal (23.10%).

Antifungal activity of EOs on the growth of *Eurotium* spp.

In this study was evaluated the antifungal activity of 5 essential oils to inhibit bakery spoilage fungi - species of genus *Eurotium* by vapour contact. The effect of EOs basil, lavender, oregano, mint and sage on fungal growth of *Eurotium* spp. is showed in Table 3. In all studied strains, the essential oils caused significant differences ($P<0.05$) on the mycelium growth. Among the EOs, the ones that totally inhibited growth of all isolates were lavender and oregano with mycelial growth inhibition of 100% after 21st days of cultivation (Table 4). Basil, sage and mint were also able to inhibit the growth of mould, but depending of the isolates and time of cultivation. The most sensitive isolate was *Eurotium amstelodami* (2 isolates). Oregano and lavender EOs had antifungal activity against this strain at all days (7th, 11th and 21st) of the cultivation. The essential oil of mint inhibited its growth until 7 days completely with mycelial growth inhibition of 60.37% and basil EO was able to inhibit its growth until 11days of cultivation completely with mycelial growth inhibition of 93.65% after 21 days of cultivation.

Table 3 Antifungal activity of selected essential oils to *Eurotium* spp.

Species	Days	**Essential oils** (mean colony diameter in mm ± SD) (500µL/L of air)					Control
		Basil	Lavender	Oregano	Mint	Sage	
Eurotium chevalieri (n=2)	**7th**	$10.50^{b} \pm 3.32$	$0^{a} \pm 0$	$0^{a} \pm 0$	$5.77^{b} \pm 6.31$	$7.42^{b} \pm 1.80$	$25.42^{c} \pm 1.51$
	11th	$15.67^{c} \pm 1.8$	$0^{a} \pm 0$	$0^{a} \pm 0$	$10.83^{b} \pm 1.47$	$20.33^{d} \pm 3.95$	$32.33^{e} \pm 3.20$
	21st	$22.00^{c} \pm 1.67$	$0^{a} \pm 0$	$0^{a} \pm 0$	$16.25^{b} \pm 2.27$	$27.40^{d} \pm 3.04$	$41.17^{e} \pm 2.32$
Eurotium herbariorum (n=2)	**7th**	$5.75^{a} \pm 1.60$	$0^{a} \pm 0$	$0^{a} \pm 0$	$3.17^{a} \pm 3.48$	$6.25^{a} \pm 7.02$	$27.08^{b} \pm 6.81$
	11th	$10.42^{c} \pm 2.11$	$0^{a} \pm 0$	$0^{a} \pm 0$	$6.25^{b} \pm 0.75$	$11.25^{c} \pm 2.82$	$39.67^{d} \pm 3.44$
	21st	$17.42^{c} \pm 3.00$	$0^{a} \pm 0$	$0^{a} \pm 0$	$10.83^{b} \pm 1.47$	$15.00^{c} \pm 1.55$	$45.33^{d} \pm 0.82$
Eurotium repens (n=2)	**7th**	$5.00^{b} \pm 1.09$	$0^{a} \pm 0$	$0^{a} \pm 0$	$2.67^{ab} \pm 3.33$	$0^{a} \pm 0$	$27.00^{c} \pm 5.08$
	11th	$10.67^{b} \pm 3.70$	$0^{a} \pm 0$	$0^{a} \pm 0$	$7.50b^{a} \pm 3.44$	$4.67^{ab} \pm 5.28$	$34.83^{c} \pm 7.80$
	21st	$17.50^{b} \pm 6.20$	$0^{a} \pm 0$	$0^{a} \pm 0$	$19.08^{b} \pm 9.75$	$13.10^{b} \pm 7.17$	$41.75^{c} \pm 5.13$
Eurotium rubrum (n=2)	**7th**	$9.25^{b} \pm 7.75$	$0^{a} \pm 0$	$0^{a} \pm 0$	$1.00^{a} \pm 1.67$	$7.00^{ab} \pm 8.07$	$26.92^{c} \pm 2.05$
	11th	$16.92^{b} \pm 9.84$	$0^{a} \pm 0$	$0^{a} \pm 0$	$5.00^{a} \pm 3.90$	$20.17^{b} \pm 4.20$	$35.25^{c} \pm 4.37$
	21st	$26.67^{b} \pm 9.70$	$0^{a} \pm 0$	$0^{a} \pm 0$	$8.58^{a} \pm 5.10$	$34.30^{bc} \pm 4.69$	$42.67^{c} \pm 1.97$
Eurotium amstelodami (n=2)	**7th**	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$5.17^{a} \pm 3.06$	$26.17^{b} \pm 7.52$
	11th	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{ab} \pm 0$	$8.83^{b} \pm 2.54$	$13.17^{b} \pm 4.87$	$38.50^{c} \pm 5.92$
	21st	$2.75^{a} \pm 2.22$	$0^{a} \pm 0$	$0^{a} \pm 0$	$17.17^{b} \pm 7.25$	$20.20^{b} \pm 3.54$	$43.33^{c} \pm 4.92$

Legend: Data in the column followed by different letters are significantly different in 95.0% Tukey HSD test, n= number of isolated tested, SD-standard deviation

Species *Eurotium repens* (2 isolates) was sensitive to sage until 7 days of cultivation. Moreover sage EO had higher mycelial growth inhibition (68.62%) after 21days of cultivation in a comparison with basil (58.08%) and mint (54.30%) essential oils. The best antifungal effects were shown after 21days of cultivation by mint with mycelial growth inhibition for *Eurotium chevalieri* (2 isolates) 60.53%, *E. herbariorum* (2 isolates) 76.11% and *E. rubrum* (2 isolates) 79.89%, respectively. These species were sensitive to oregano and lavender at all days of the cultivation, as well as other tested species of genus *Eurotium*. Our result showed that the best antifungal effect was shown by lavender and oregano (100%) to all species of tested fungi followed by mint (with the best MGI 79.89% to *E. rubrum*) >basil (with the best MGI 93.65% to *E. herbariorum*) and the low effect had sage essential oil (with the best MGI 68.62% to *E. repens*). Also authors **Kocić-Tanackov *et al.* (2012)** demonstrated that antifungal activity of oregano extract completely inhibited the growth of *Aspergillus wentii* and growth of *A. carbonarius* and *A. niger* was reduced.

Table 4 Mycelial growth inhibition (%) of *Eurotium* spp. by tested essential oils after 21st days of cultivation

Essential oils	Species				
	Mycelial growth inhibition (%)				
	E. chevalieri	*E. herbariorum*	*E. repens*	*E. rubrum*	*E. amstelodami*
Basil	40.56%	61.57%	58.08%	37.50%	**93.65%**
Lavender	**100%**	**100%**	**100%**	**100%**	**100%**
Oregano	**100%**	**100%**	**100%**	**100%**	**100%**
Mint	60.53%	76.11%	54.30%	**79.89%**	60.37%
Sage	33.45%	66.91%	**68.62%**	19.62%	53.38%

In case of mint and basil EOs, similar results were obtained in previous studies. The oregano, thyme, clove, lavender, mint sage and eucalyptus essential oils were tested against three strains of *A. flavus*. The best antifungal activity was shown by oregano, thyme, clove and lavender, but mint essential oil was able to inhibit growth of *Aspergillus* sp. until 14 days of cultivation. Sage and eucalyptus EOs showed very low or no effect on the growth of tested fungi (**Císarová and Tančinová, 2015**). Moreover, sage essential oil showed very low inhibitory effect on the growth of tested aspergilli in another study, but was able to inhibit production of mycotoxins by tested fungi. Basil essential oil was able to inhibit growth of all tested strains *Eurotium* spp. on all days of cultivation or after 14th days (**Císarová *et al.*, 2014**). The results of authors **Alves-Silva *et al.* (2013)** showed that the basil EO showed higher antifungal activity against *Mucor racemosus* and *Penicillium chrysogenum*. The present study confirmed the antifungal activity of tested essential oils. Carvacrol and Geranial were the major components of oregano and lavender EOs in this study. These terpenoids are active against a broad spectrum of microorganisms, with Carvacrol (a monoterpenoid phenol) as one of the most active components. Carvacrol and p-Cymene (its monoterpene precursor) are present in oregano and thyme respectively, and they have a promising potential to be used as natural preservatives when both are used together (**Gutiérrez-del-Río *et al.*, 2018**). The Menthol was the major component of mint EOs. Authors **Abbaszadeh *et al.* (2014)** demonstrated that Menthol was found with the most potent inhibitory activity for *Cladosporium* spp. and *Aspergillus* spp. (with the MIC of 125 mg/mL), followed by *Fusarium oxysporum*, *Penicillium* spp., *Rhizopus oryzae*, *Botrytis cinerea* and *Alternaria alternata*. Estragol (Methyl chavicol) is usually the major component of the basil EOs and these EOs with following chemical composition: 1.8-Cineole, Linalool, Camphor, Methyl chavicol, and Eugenol are often related with greater antifungal activity (**Pirbalouti *et al.*, 2013**). So the antifungal activity of the essential oils or extract is directly related to chemical composition of the EO, climatic, soil or seasonal variation during growing of plants, extraction used and last but not least to microbial species tested.

CONCLUSION

Nowadays, mould growth is still a cause of high losses to the bread-producing industry. Bread is known as a high moisture product with a_w values between 0.96 and 0.98 and is included in commodities with short durability. After three days of storage was found the visible destruction of bread by growing fungi in both: bread samples stored in plastic bag on the surface of the crust and on the pieces of the bread samples stored on DRBC. Our results showed that 195 isolates were recovered from 8 bread samples and assigned to 10 fungal genera. The most frequently genus of fungi was *Penicillium*, *Aspergillus*, *Cladosporium*, *Epicoccum* and *Eurotium*. One of the objectives of this study was to detect contamination of samples by microscopic filamentous fungi with goal to *Eurotium* spp. In total, five different *Eurotium* species were identified: *E. amstelodami*, *E. chevalieri*, *E. herbariorum*, *E. rubrum* and *E. repens*. These species were used in next - essential oils studies. The present study demonstrated the potential food preservatives ability of oregano, lavender, mint, basil and sage EOs. The best antifungal activity was shown by oregano and lavender EOs, but other essential oils also showed inhibition effect on the growth of fungi *Eurotium* spp. In bakery products, preservatives (salts of propionic and sorbic acids) are added to prevent growth of spoilage fungi. However, in recent years, consumers demand to reduce the use of chemical preservatives. So the essential oils could become an effective modern alternative without health hazards for customers especially for bakery products.

Acknowledgments: This work was supported by project no. FPPV-09-2019 and by Slovak Research and Development Agency under Contract no. APVV-16-0289.

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