

INFLUENCE OF ESSENTIAL OILS ON THE GROWTH OF *ASPERGILLUS FLAVUS*

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ABSTRACT

This paper was focused on the determination of the inhibitory effect of selected essential oils on growth of ten isolates of *Aspergillus flavus* and their potential ability to produce mycotoxins *in vitro* by TLC method. The isolates were obtained from moldy bread of domestic origin. We followed the impact of five essential oils at 100% concentration – lemon, eucalyptus, oregano, sage and thyme. The effect of the essential oils we tested the gaseous diffusion method. We isolates grown on CYA (Czapek yeast extract agar), in the dark at 25 ± 1 °C, 14 days. The diameter of colonies grown we continuously measured on the 3rd, 7th, 11th, and 14th day of cultivation. The results of the paper suggest that oregano and thyme essential oil had 100% inhibited the growth of all tested isolates of *Aspergillus flavus*. Lemon, eucalyptus and sage essential oil had not significant inhibitory effects on tested isolates *Aspergillus flavus*, but affected the growth of colonies throughout the cultivation. In addition to the inhibitory effect we witnessed the stimulative effect of lemon, eucalyptus and sage essential oil to some isolates. Together with the antifungal effect of essential oils, we monitored the ability of *Aspergillus flavus* isolates to produce mycotoxins – aflatoxin B₁ (AFB₁) and cyclopiazonic acid (CPA) in the presence of essential oils. Production mycotoxins we have seen in the last (14th) day of cultivation. Lemon and eucalyptus essential oil did not affect the production of mycotoxins. In the case of sage essential oil we were recorded cyclopiazonic acid production in three of the ten isolates from the all three repetitions, while neither isolate did not produced aflatoxin B₁. The production of secondary metabolites was detected in all control samples. From the results we can say that oregano and thyme essential oil could be used as a natural preservative useful in the food industry.

Keywords: essential oils; inhibitory effect; *Aspergillus flavus*; mycotoxins

INTRODUCTION

Microscopic filamentous fungi are significant destroyers of foodstuffs during storage, rendering them unfit for human consumption by retard their nutritive value and sometimes by producing mycotoxins (Kumar et al., 2007). The present and growth of fungi in food may cause spoilage and result in a reduction in quality and quantity (Baratta et al., 2008).

Mold growth on bakery products during storage is a serious economic problem. Mold spoilage of bakery products has been the subject of many studies and a number of species have been implicated. The mold frequently involved are *Penicillium*, *Aspergillus*, *Eurotium* and *Walemia* species (Dantigny et al., 2005).

In addition to the economic losses associated with bakery products, another concern is the possibility of mycotoxin production. Therefore, the presence of toxinogenic fungi and mycotoxins in food and grains stored for long periods of time present a potential hazard to human and animal health. Improper storage conditions offer favorable environment for the growth of *Aspergillus* spp. Some

species such as *Aspergillus flavus* can produce toxic secondary metabolites: like aflatoxins, ochratoxins, cyclopiazonic acid and they affect the food safety (Pardo et al., 2006).

Management of food stuffs contaminations are required to ensure that food commodities remain safe and uncontaminated throughout the supply chain (from 'farm to plate'). Several synthetic preservatives have been effectively used in management of food contamination by *Aspergillus* spp. (Leslie et al., 2008). Effectively, the use of synthetic chemicals to control food deterioration has been restricted because of their carcinogenicity, teratogenicity, high and acute residual toxicity, and other effects on food and humans (Tripathy and Dubey, 2004).

This negative consumer perception of chemical preservatives drives attention towards natural alternatives (Sharma and Tripathi, 2008). 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 attention (Farzaneh et al., 2015). Essential oils are aromatic and

volatile liquids extracted from plants. Essential oils from plants have a broad spectrum of antifungal activity (Yamamoto-Ribeiro et al., 2013). Natural antimicrobial or antifungal substances are promising to replace these synthetic fungicides. The chemicals in essential oils are secondary metabolites, which play an important role in plant defense as they often possess antimicrobial properties (Hyldgaard et al., 2012).

European Union allowed the use of EOs in food and aromatherapy. So, EOs with antimicrobial activity are possible candidates for the preservation of food commodities against *Aspergillus* spp (Razzaghi-Abyaneh et al., 2009). This study was undertaken to investigate the *in vitro* inhibitory effects of selected essential oils on the growth of *Aspergillus flavus* isolates. Together with the antifungal activity of essential oils we determined the ability of *Aspergillus flavus* isolates produce mycotoxins – aflatoxin B₁ and cyclopiazonic acid.

MATERIAL AND METHODOLOGY

Isolation of *Aspergillus flavus*

In this paper, we investigated influence of essential oils on the growth of isolates (10) *Aspergillus flavus* (Table 1) obtained from bread of domestic origin (Slovakian). We followed the impact of the following oils: lemon, eucalyptus, oregano, sage, thyme. All these samples of the essential oils were compared to the control sample. The control sample contained 50 µL of distilled water and it was free of essential oil. These isolates belong to the collection of microorganisms at the Department of Microbiology of the Slovak Agricultural University in Nitra.

Isolates grown on moldy breads we are using microscopic observations included in the genus *Aspergillus*. We restreaked to have their identification media. We used Czapek yeast extract agar (CYA), in malt extract agar (MEA) and the agar with yeast extract and

Table 1 List of isolates.

Number isolate	Label isolates <i>Aspergillus flavus</i>
1	KMi – 48 – MC
2	KMi – 43 – MC
3	KMi – 42 – MC
4	KMi – 41 – MC
5	KMi – 40 – MC
6	KMi – 1 – MC
7	KMi – 6 – MC
8	KMi – 33 – MC
9	KMi – 34 – MC
10	KMi – 35 – MC

Table 2 CYA – Czapek yeast extract agar.

K ₂ HPO ₄	1 g
Czapek concentrate	10 mL
Cu-Zn concentrate	1 mL
Yeast extract	5 g
Sucrose	30 g
Agar	15 g
Distilled water	1 000 mL

Table 3 MEA - In malt extract agar.

Malt extract	20 g
Glucose	20 g
Peptone	1 g
Agar	20 g
Distilled water	1 000 mL

Table 4 YES - Agar yeast extract and sucrose

Yeast exktract	20 g
Sucrose	150 g
MgSO ₄ . 7 H ₂ O	0,5 g
CuSO ₄ . 5 H ₂ O	0,005 g
ZnSO ₄ . 7 H ₂ O	0,01 g
Agar	20 g
Distilled water	1 000 mL

Table 5 Peptone water

Peptone	10 g
NaCl	5 g
Distilled water	1 000 mL

Table 6 The major constituents of essential oils analyzed by Calendula company a.s.

Essential oils	Compound	Amount (%)
Oregano	Carvacrol	44%
	1,8-cineole	minimum 5.0%
Sage	Thujone	minimum 15.0%
	Borneole	minimum 5.0%
	β -pinene	7.0 – 17%
	Sabinene	1.0 – 3.0%
	Limonene	56 – 78%
	γ -terpinene	6.0 – 12%
	β -caryophyllene	maximum 0.5%
Lemon	Neral	0.3 – 1.5%
	α -terpineol	maximum 0.6%
	neryl acetate	0.2 – 0.9%
	Geranial	0.5 – 2.3%
	geranyl acetate	0.1 – 0.8%
	α -pinene	9.0%
	β -pinene	maximum 1.5%
	Sabinene	maximum 0.3%
Eucalypt	Phellandrene	maximum 1.5%
	Limonene	12%
	1,8-cineol	minimum 70%
	camphor	maximum 0.1%
	β -myrcene	1.0 – 3.0%
	γ -terpinene	5.0 – 10%
	p -cymene	15 – 18%
	linalool	4.0 – 6.5%
	terpinene-4-ol	0.2 – 2.5%
	Thyme	thymol
carvacrol		1.0 – 4.0%

sucrose (YES) (Tables 2, 3, 4). As intermediate solution we used peptone water (Table 5). Petri dishes we cultured at 25 °C \pm (CYA and at 37 \pm °C), 7 days in the dark. The agar medium and the peptone water were sterilized at 0.1 MPa for 20 minutes in the autoclave.

Identification of *Aspergillus flavus*

Identification of isolated micromycetes we conducted by mycological keys: **Pitt (1985), Pitt and Hocking (1997), Samson and Frisvad (2004), Klich (2002), Samson et al., (2002), Varga et al., (2007), Tančinová et al., (2012)**. Micromorphological characters we have seen in the microscope. For the preparation of formulations were used Melzer lactic acid solution and the methylene blue. We followed macroscopic features and microscopic features. Identification of the isolates of *Aspergillus flavus* was confirmed by mass spectrometry (MALDI-TOF MS).

Plant essential oils

Essential oils have been bought in Calendula s.a., Nová Lubovňa, Slovakia. The composition of the essential oils specified by the manufacturer – retailer Calendula a.s. (Table 6).

Testing the antifungal activity

The antifungal activity of selected essential oils was investigated by microatmosphere method. The test was performed in sterile Petri dishes (\varnothing 90 mm) containing 15 ml of CYA. Evaluation by filter paper was made by the

method adapted from **Guynot et al. (2003)**. Plates were kept in an inverted position. A sterilized filter paper (square of 1 x 1 cm) was placed in the centre of the lid and 50 μ L of pure essential oil were added to the paper. Blank were made by adding 50 μ L of water to it. Each fungus was inoculated in the centre on Petri dish with needle. Plates were tightly sealed with parafilm and incubated for 14 days at 25 \pm 1 °C (three replicates were used for each treatment). Diameters (\varnothing mm) of the growing colonies were measured at the 3rd, 7th, 11th and 14th day with a ruler. The results were processed using Microsoft Excel 2010.

Investigation of the potential ability of isolates to produce mycotoxins *in vitro*

All isolates of *Aspergillus flavus* were used for toxigenic analyse by thin layer chromatography (TLC) method adapted from **Samson et al. (2002)** modified by **Labuda and Tančinová (2006)**. For the determination of mycotoxins we used Colonies grown on CYA agar, which we have cultivated in the dark at 25 \pm 1 °C for two weeks. Three small pieces (each 1x1 cm) were cut from the colony growing on CYA and placed into 1.5 mL Eppendorf vials. Then 500 μ L of extraction solvent (chloroform/methanol, 2:1) was added to vials containing the agar plugs and shaken on a vortex for at least 2 minutes. Extracts (30 – 50 μ L) were applied afterwards as spots to the TLC plate (Silicagel 60, Merck, Germany) 1 cm apart. Chromatographic plate we put in a developing

system (toluene/ethyl acetate/formic acid, 5:3:1). Visualisation mycotoxins we characterized by Samson et al. (2002). CPA was reflected in the daylight as purple spot with a tail. AFB1 under UV light at 365 nm showed as a blue fluorescent spot (Samson et al., 2002).

Statistical analysis

The size averages of colonies (mm) of ten isolates affected by five essential oils (TREATMENT) on third, seventh, eleventh and fourteenth day of cultivation were analysed by the mixed model as implemented in SAS package (SAS, ver. 8.2, 2001).

The statistical model can be written in the following form:

$$y_{ij} = \mu + \text{TREATMENT}_i / \text{ISOLATES}_j + \text{STRAIN}_j + e_{ij}$$

where:

y_{ij} = the measurements in third, seventh, eleventh and fourteenth day of cultivation independently,

μ = overall mean,

TREATMENT_i = the fixed effects of tested oil (i = control, and five oils),

ISOLATE_j = fixed effect of strain (j = ten isolates),

e_{ij} = random error, assuming $e_{ij} \sim N(0, I \sigma^2)$.

Differences between the levels of the effects were tested by Scheffe multiple range test for studied size of colonies in different days of cultivations. Statistical significances of LSmeans were tested at level <0.05. Results are presented as LSmeans \pm standard error. The size averages of colonies (mm) in figures are presented as arithmetic means (Excel).

RESULTS AND DISCUSSION

The antimicrobial compounds in plant materials are commonly found in the essential oils fractions obtained by steam or supercritical distillation, pressing, or extraction by liquid or volatile solvents. The traditionally most well – known antimicrobial species and herbs are clove, cinnamon, chilli, garlic, thyme, oregano and rosemary. But also bay, basil, sage, anise, coriander, allspice, marjoram, nutmeg, cardamom, mint, parsley, lemongrass, celery, cumin, fennel and many others have been reported to have an inhibitory effect toward microorganisms (Elgayyar et al., 2001).

The aim of our paper was to determine the activity of volatile components of five essential oils – lemon, thyme, eucalyptus, sage and oregano on the growth of *Aspergillus flavus* isolates.

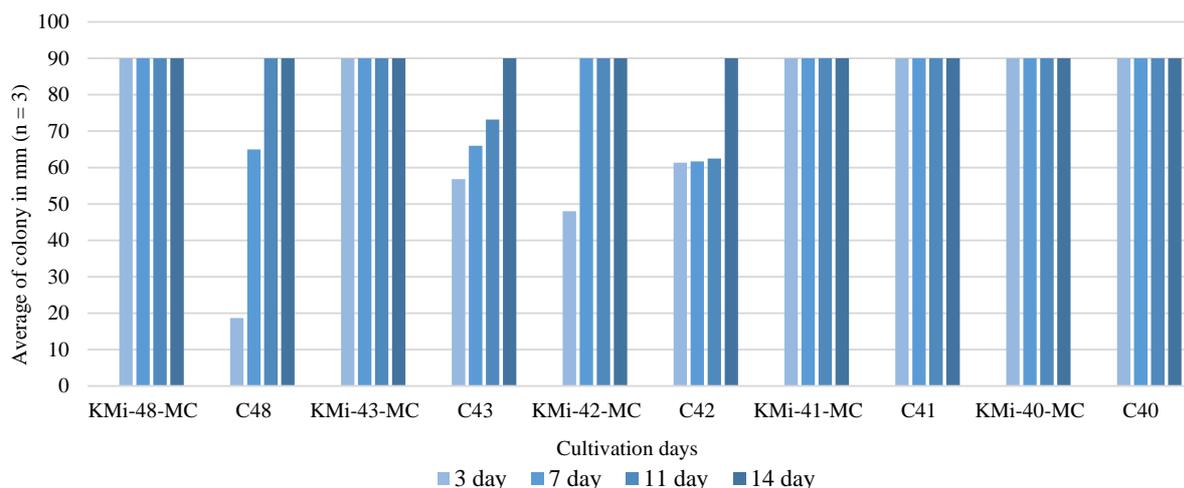


Figure 1 Influence of lemon essential oil on the growth of less sensitive isolates *A. flavus* on CYA at 25 ± 1 ° C. Kmi – x – MC tested isolates, C – control samples, n – the number of repetitions.

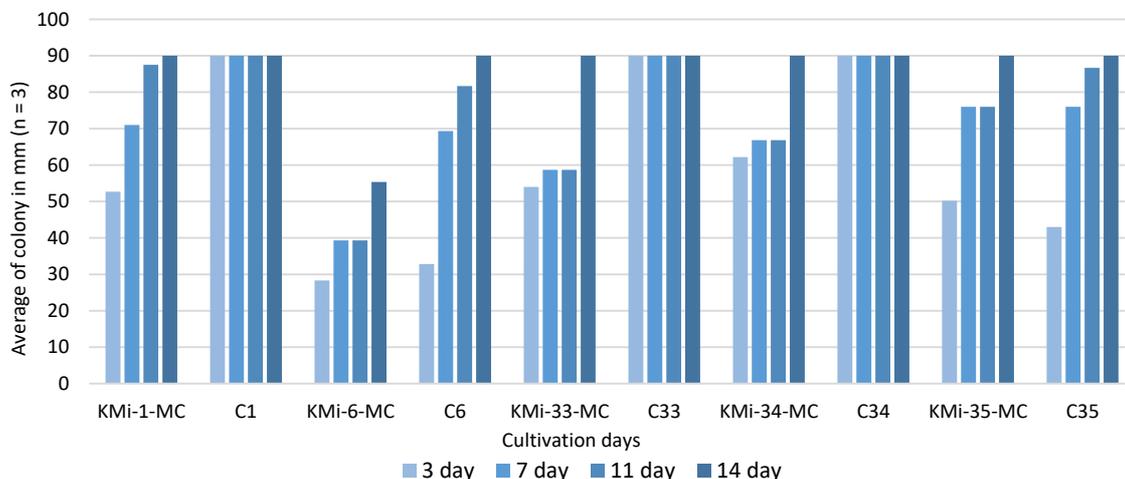


Figure 2 Influence of lemon essential oil on the growth of more sensitive isolates *A. flavus* on CYA at 25 ± 1 ° C.

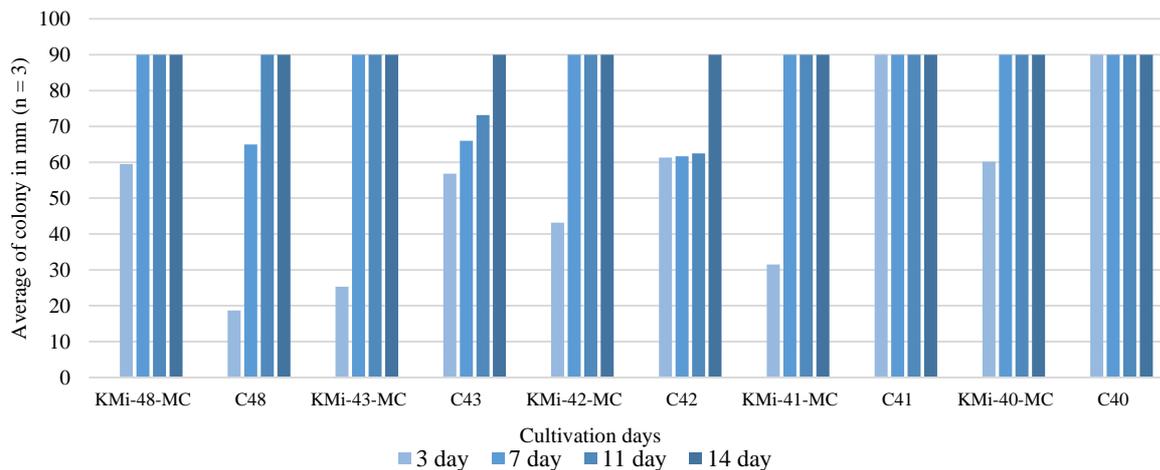


Figure 3 Influence of eucalypt essential oil on the growth of less sensitive isolates *A. flavus* on CYA at 25 ±1 °C. KMi – x – MC – tested isolates, C – control samples, n – the number of repetitions.

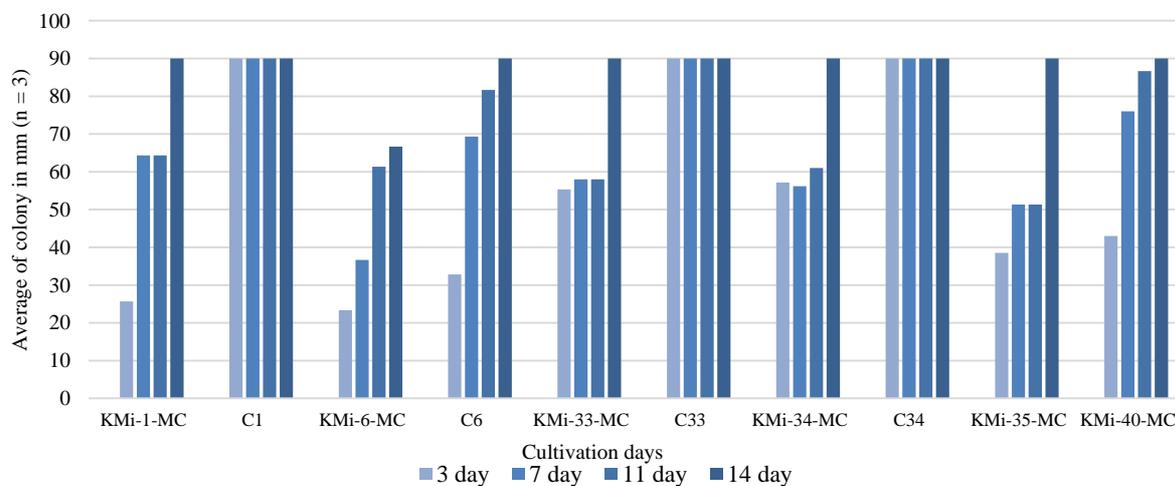


Figure 4 Influence of eucalypt essential oil on the growth of more sensitive isolates *A. flavus* on CYA at 25 ±1 °C. KMi – x – MC – tested isolates, C – control samples, n – the number of repetitions.

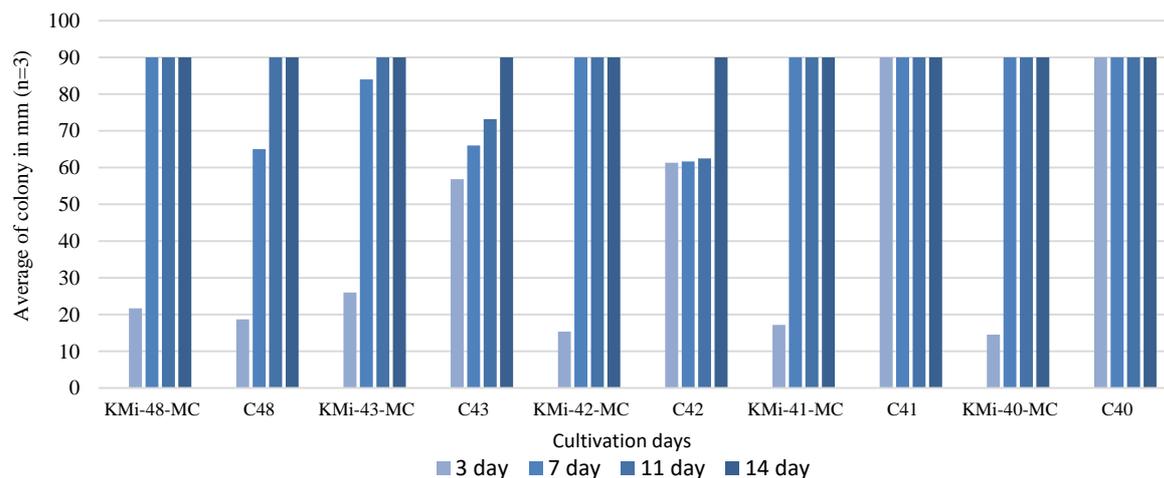


Figure 5 Influence of sage essential oil on the growth of less sensitive isolates *A. flavus* on CYA at 25 ±1 °C. KMi – x – MC – tested isolates, C – control samples, n – the number of repetitions.

A. flavus isolates reacted differently to the presence of the essential oil. For a better overview of the results, we divided them into two groups: the group of isolates which were less sensitive to the effect of essential oils and to the group of isolates which were more affected by essential oil – sensitive isolates. Figures 1 and 2 show the inhibitory

effect of lemon essential oil. The most sensitive isolate was KMi – 6 – MC, where in the oil slowed the growth of their colonies until the last day of cultivation. In isolate KMi – 48 – MC compared to the control sample we may say that the essential oil had a stimulating effect on the growth of the isolate.

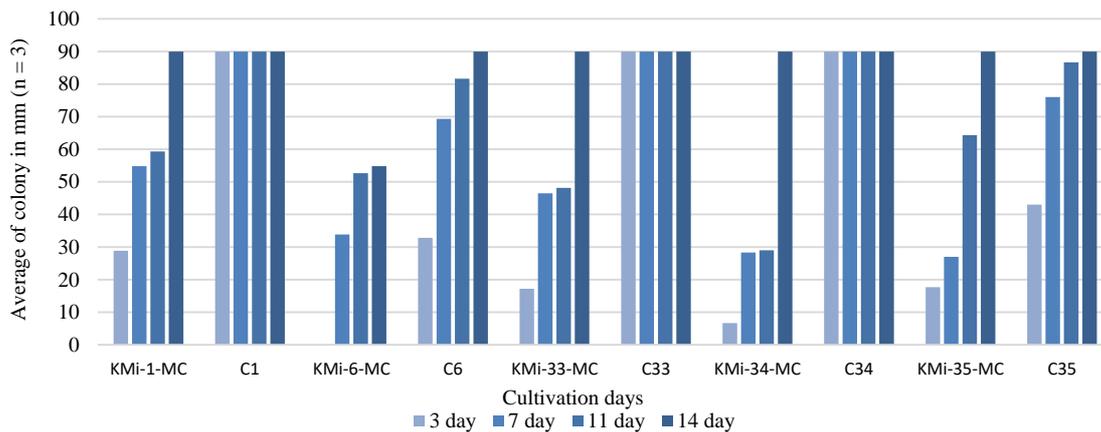


Figure 6 Influence of Sage essential oil on the growth of more sensitive isolates *A. flavus* on CYA at 25 ± 1 °C. KMi – x – MC – tested isolates, C – control samples, n – the number of repetitions.

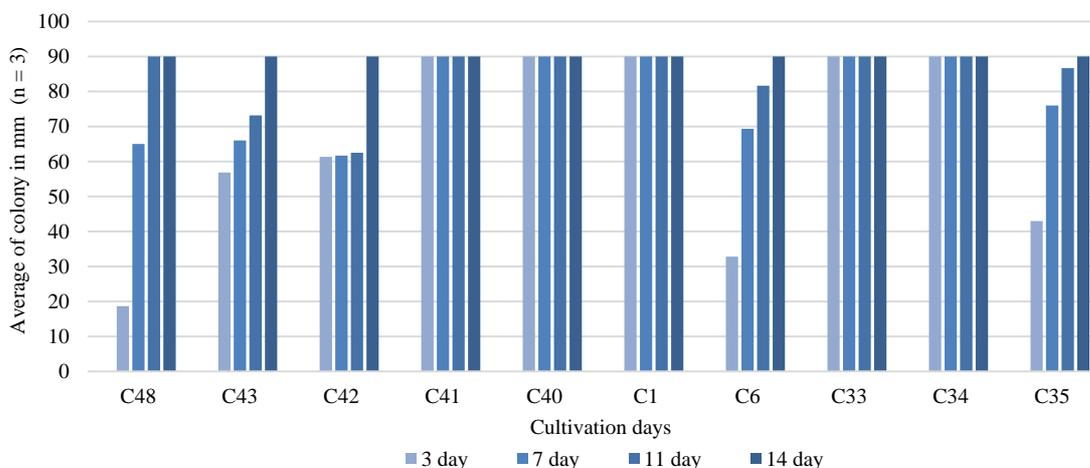


Figure 7 Growth curve of the control samples *A. flavus* isolates without the addition of essential oil on Czapek yeast extract agar at 25 ± 1 °C. C – control samples, n – the number of repetitions.

Eucalyptus essential oil has not expressed full inhibitory effect. Compared with the control sample, we found out that from the seventh day of cultivation had the oil rather than stimulatory inhibitory effects on the growth of colonies. The greatest activity was to isolate KMi – 6 – MC that inhibited growth until the last day of cultivation, as can be seen in the said figures 3 and 4.

Figure 5 and Figure 6 show the inhibitory effect of sage essential oil. This essential oil also has not expressed full inhibitory effect. In the picture 6 we can see isolates, which were most sensitive to the effect of the oil While the greatest effect had on the isolate KMi – 6 – MC, wherein we have not seen the growth of colonies until the third day. Particular isolates differed in the amount of growth in the absence of essential oil (control sample).

Figure 7 shows an overview of the control samples of tested isolates on the 3rd, 7th, 11th and 14th day of cultivation. Control samples of several isolates in comparison to other control samples completely filled Petri dish already on the third day of cultivation.

Oregano and thyme oils have 100% inhibitory effect on growth of all tested isolates disregarding the cultivation day. Lemon, eucalyptus and sage essential oils showed

partial antifungal activity, that means, the tested isolates did not inhibited completely, but affected the growth of colonies in some cases up to the last day of culture. Between individual isolates, we found significant differences in their growth in the presence of essential oils. The inhibitory effect of essential oils on the growth of colonies affects the length of cultivation and specific isolate. That's why *Origanum vulgare* is very well known for significant antifungal character. Table 7 displays the statistical analysis of influence of essential oils on the growth of isolates of *A. flavus* using gaseous diffusion method.

Carmo et al. (2008) dealt with study its inhibitory activity. The aim of their study was to evaluate the antifungal activity of oregano oil towards the microscopic thread-like fungi *Aspergillus*. They focused on species *A. flavus*, *A. parasiticus*, *A. terreus*, *A. ochraceus*, *A. fumigatus* and *A. niger*. The essential oil showed complete inhibitory effect on the growth of colonies throughout the culture to all species tested. Results showed a significant inhibitory character of oregano essential oil.

Table 7 The effect of essential oils on the growth of isolates of *A. flavus* using gaseous diffusion method.

Cultivation days		3 rd day		7 th day		11 th day		14 th day	
Treatment	Isolate	Average	S.e	Average	S.e	Average	S.e	Average	S.e
Control		90 ^a	0.66	90 ^a	0.69	90 ^a	0.66	90.00	0.49
LEO	KMi – 1 – MC	52.67 ^b	0.66	71 ^b	0.69	87.5 ^a	0.66	90.00	0.49
EEO		25.67 ^c	0.66	64.33 ^b	0.69	64.33 ^b	0.66	90	0.49
SEO		28.83 ^c	0.66	54.83 ^c	0.69	59.33 ^b	0.66	90	0.49
Control		32.83 ^a	0.66	69.33 ^a	0.69	81.67 ^a	0.66	90 ^a	0.49
LEO	KMi – 6 – MC	28.32 ^{ac}	0.66	39.33 ^b	0.69	39.33 ^b	0.66	52.33 ^b	0.49
EEO		23.33 ^{bc}	0.66	36.67 ^{bc}	0.69	61.33 ^c	0.66	66.67 ^c	0.49
SEO		0 ^d	0.66	33.83 ^c	0.69	52.67 ^d	0.66	54.83 ^b	0.49
Control		90 ^a	0.66	90 ^a	0.69	90 ^a	0.66	90	0.49
LEO	KMi – 33 – MC	54 ^b	0.66	58.67 ^b	0.69	58.67 ^b	0.66	90	0.49
EEO		55.33 ^b	0.66	58 ^b	0.69	58 ^b	0.66	90	0.49
SEO		17.17 ^c	0.66	46.5 ^c	0.69	48.17 ^c	0.66	90	0.49
Control		90 ^a	0.66	90 ^a	0.69	90 ^a	0.66	90	0.49
LEO	KMi – 34 – MC	62.17 ^b	0.66	66.83 ^b	0.69	66.83 ^b	0.66	90	0.49
EEO		57.17 ^b	0.66	56.17 ^c	0.69	61 ^b	0.66	90	0.49
SEO		6.67 ^c	0.66	28.83 ^d	0.69	29 ^c	0.66	90	0.49
Control		43 ^a	0.66	76 ^a	0.69	86.67 ^a	0.66	90	0.49
LEO	KMi – 35 – MC	50.17 ^b	0.66	76 ^a	0.69	76 ^b	0.66	90	0.49
EEO		38.5 ^a	0.66	51.33 ^b	0.69	51.33 ^c	0.66	90	0.49
SEO		17.67 ^c	0.66	27 ^c	0.69	64.33 ^d	0.66	90	0.49
Control		90 ^a	0.66	90	0.69	90	0.66	90	0.49
LEO	KMi – 40 – MC	90 ^a	0.66	90	0.69	90	0.66	90	0.49
EEO		60.17 ^b	0.66	90	0.69	90	0.66	90	0.49
SEO		14.42 ^c	0.66	90	0.69	90	0.66	90	0.49
Control		90 ^a	0.66	90	0.69	90	0.66	90	0.49
LEO	KMi – 41 – MC	90 ^a	0.66	90	0.69	90	0.66	90	0.49
EEO		31.5 ^b	0.66	90	0.69	90	0.66	90	0.49
SEO		14.42 ^c	0.66	90	0.69	90	0.66	90	0.49
Control		61.33 ^a	0.66	61.67 ^a	0.69	62.5 ^a	0.66	90	0.49
LEO	KMi – 42 – MC	48 ^b	0.66	90 ^b	0.69	90 ^b	0.66	90	0.49
EEO		43.17 ^b	0.66	90 ^b	0.69	90 ^b	0.66	90	0.49
SEO		15.33 ^c	0.66	90 ^b	0.69	90 ^b	0.66	90	0.49
Control		56.83 ^a	0.66	66 ^a	0.69	73.17 ^a	0.66	90	0.49
LEO	KMi – 43 – MC	90 ^b	0.66	90 ^b	0.69	90 ^b	0.66	90	0.49
EEO		25.33 ^c	0.66	90 ^b	0.69	90 ^b	0.66	90	0.49
SEO		26 ^c	0.66	84 ^b	0.69	90 ^b	0.66	90	0.49
Control		18.67 ^a	0.66	65 ^a	0.69	90	0.66	90	0.49
LEO	KMi – 48 – MC	90 ^b	0.66	90 ^b	0.69	90	0.66	90	0.49
EEO		59.5 ^c	0.66	90 ^b	0.69	90	0.66	90	0.49
SEO		21.67 ^a	0.66	90 ^b	0.69	90	0.66	90.00	0.49

* ^{a,b,c} LS Means in the same column at different isolates within the day of cultivation with different letters are different ($p < 0.05$)

** LEO – lemon essential oil, EEO – eucalyptus essential oil, SEO – sage essential oil, S.e – standard error.

Antifungal activity of rosemary and thyme and was observed by Centeno et al. (2010). They are focusing in particular on the species of microscopic filamentous fungi *Aspergillus flavus* and *Aspergillus ochraceus*, which are considered frequent crop and producers of mycotoxins contaminant. They followed the minimum fungicidal concentration (MFC) of selected essential oil. The results confirmed that the analyzed oils have shown inhibitory activity against the testes fungi *Aspergillus flavus* and *Aspergillus ochraceus*.

Abu-Darwish et al. (2013) dealt in its studies with *Salvia officinalis*'s features. The aim was to consider antifungal and anti-inflammatory capabilities of sage essential oil. Antifungal activity of sage essential oil tested on yeast and microscopic filamentous fungi *Aspergillus*. The active ingredients of sage essential oil 1,8-cineole

(50.3%) and camphor (25%) showed only partial antifungal activity against the tested species.

Mekonnen et al. (2016) dealt in their studies with the inhibitory effects of eucalyptus, thyme and rosemary oils towards certain types of microscopic filamentous, which confirmed a partial inhibitory activity of eucalyptus oil on tested species. Baratta et al. (2008) dealt in their studies with the antioxidant and antimicrobial activity of lemon essential oil against *Aspergillus niger* and *Aspergillus flavus*, which confirmed a partial inhibitory effect of lemon oil towards all tested species.

Císarová et al. (2016) in their studies also dealt inhibitory effect of lemon and eucalyptus essential oils, but there were found different results. These essential oils showed very poor inhibitor effects.

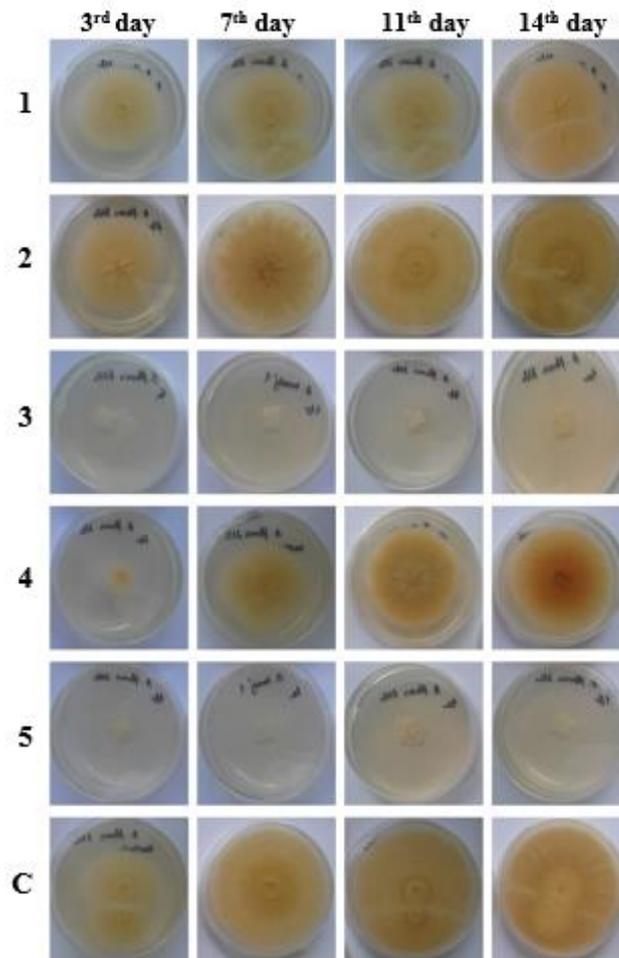


Figure 8 Comparison of the growth of isolate *Aspergillus flavus* KMI – 6 – MC of control sample (C), with the samples of lemon (1), eucalyptus (2), oregano (3), sage (4), thyme (5) essential oil on 3rd, 7th, 11th and 14th day of culture (Foltinová, 2016).

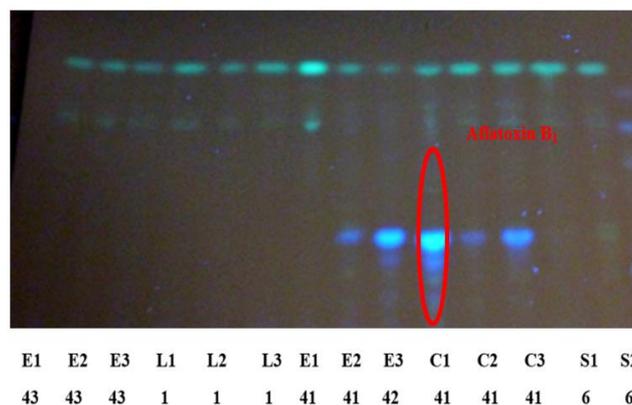


Figure 9 The production of aflatoxin B₁ *Aspergillus flavus* isolates, in vitro TLC method (the 14th day of cultivation, on YES at 25 ± 1 °C) (Foltinová, 2016). E – eucalyptus essential oil, L – lemon essential oil, S – sage essential oil, C – control samples.

In our study, we watched the ability of various isolates of *Aspergillus flavus* to produce mycotoxins – aflatoxin B₁, cyclopiazonic acid (Figure 9 and Figure 10) in conditions *in vitro* by TLC method. All control samples of the tested isolates in the absence essential oil produced mycotoxins in condition *in vitro*. Lemon essential oil had only partial inhibitory effect on the production of aflatoxin B₁ and cyclopiazonic acid, one isolate in the presence of

eucalyptus essential oil produced aflatoxin B₁ and by four isolates was observed cyclopiazonic acid production. None of tested isolates did not produce aflatoxin B₁ in the presence of sage essential oil, but in some isolates was recorded cyclopiazonic acid production. The production of mycotoxins have not been observed in oregano and thyme essential oil, because of their full inhibiting effect on growth of all isolates.

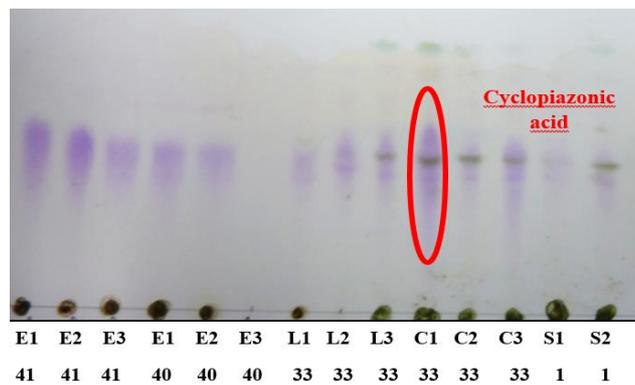


Figure 10 The production of cyclopiazonic acid *Aspergillus flavus* isolates, in conditions *in vitro* by TLC method (the 14th day of cultivation, on CYA at 25 ± 1 °C) (Foltinová, 2016). E – eucalyptus essential oil, L – lemon essential oil, S – sage essential oil, C – control samples.

Aflatoxins are produced by particular species of *A. flavus* and *A. parasiticus*. These mycotoxins are toxic compounds that are carcinogenic, mutagenic and teratogenic effects and present a risk to human and animal health. Antifungal activity of *Thymus vulgaris* against *Aspergillus flavus* and its effects on the production of secondary metabolites (AFB₁, AFB₂) *in vitro* was dealt by Abdel-Wahhab et al. (2010). The results of their work have confirmed the full inhibitory effect of thyme oil on the production of both mycotoxins, which is also consistent with our results.

CONCLUSION

The conclusions indicate that volatile phase of combinations of thyme oil and oregano oil showed good potential to inhibit growth of *Aspergillus flavus*. Even though that essential oils such as lemon, eucalyptus and sage had not antifungal activity like thyme and oregano essential oils, they should find a practical application in the inhibition of the fungal mycelial growth. In our paper we tested the antifungal activity of essential oils against common fungi causing spoilage of bakery products for 3rd, 7th, 11th and 14th days of cultivation. Based on the results of our paper, we can sort essential oils by the strongest inhibitory effect on the growth of *Aspergillus flavus* isolates and production of aflatoxin B₁ and cyclopiazonic acid as follows: oregano and thyme – sage – eucalyptus – lemon. Our paper gives support that essential oils can be used to control plant pathogens such as *Aspergillus flavus*.

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