Volume 57(2):113-116, 2013
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Evaluation of five essential oils for the control of food-spoilage and mycotoxin producing fungi

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ABSTRACT The inhibition of growth and aflatoxin production by essential oils (EOs) of cinnamon, clary sage, juniper, lemon and marjoram were investigated on food-spoilage fungi *Aspergillus parasiticus var.globosus, Fusarium graminearum* and *Fusarium culmorum.* The antifungal effect of the EOs was observed by determination of growth-rate (mm/day) and antifungal index (%) using reversed Petri-dish method. Aflatoxin production of *A. parasiticus* was monitored by thin layer chromatography (TLC). The growth of *A. parasiticus* was significantly decreased (P<0,001) by marjoram and clary sage EOs. TLC results revealed only a slight effect on aflatoxin production: cinnamon and clary sage EOs found to decrease the amount of aflatoxin B1 and G2. *F. graminearum* and *F. culmorum* showed almost similar response to the EOs. In both cases cinnamon EO caused a total inhibition of growth, while lemon EO was ineffective. Juniper significantly (P<0,003) inhibited the growth of *F. culmorum* but had no effect on *F. graminearum*. The EOs tested in this study may be potential antimicrobial compounds for use as food preservatives and anti-aflatoxin agents. **Acta Biol Szeged 57(2):113-116 (2013)**

KEY WORDS

essential oils
growth- inhibition
aflatoxin
Aspergillus
Fusarium

Many fungal species produce mycotoxins contaminating various foods and feed (Naeini et al. 2010). Among them, aflatoxin synthetised by several fungal species from which *Aspergillus flavus* and *Aspergillus parasiticus* are the most important ones. This toxin is produced during plant growth, harvest, storage, and food processing. Aflatoxin is extremely hazardous: it has a hepatotoxic, carcinogenic and immunosuppressive effect. It may also affect the epidemiology of many diseases and it poses serious health risks in those countries where the toxin content of food and feed commodities are not properly monitored and controlled (Williams et al. 2004). *Fusarium culmorum*, and more recently, *F. graminearum* are the most common causes of Fusarium ear blight and both of them produce trichothecenes and *F. graminearum* also produces zearalenone. Many of these mycotoxins are possible immunosuppressants (Hope et al. 2005).

Global warming may strongly influence the occurrence and distribution of mycotoxin producing fungi. Climate change can lead to the increasing abundance of thermotolerant species (especially in extremely hot years) in regions with temperate climate, including Central Europe. This is accompanied with the appearance of their mycotoxins in agricultural products (Tóth et al. 2013). From all these reasons, mycotoxins are in the focus of food safety concerns today. There is an increasing need to find efficient and easy-to-use mycotoxin-reducing strategies (Naeini et al. 2010).

Several authors demonstrated that, most of the essential oils extracted from aromatic plants, have antimicrobial, antifungal effect and/or antioxidant properties. That means they have also a potential to act against mycotoxin-producing fungi. There are some results which suggest that in an experimental system the extent of inhibition of fungal growth and aflatoxin production depends on the concentration of essential oils used (Atanda et al. 2006; Sindhu et al. 2011). A great advantage of EOs is their bioactivity in the vapour-phase, a characteristic that makes them attractive as feasible fumigants for stored product protection (Tripati and Dubey 2004). It is also worth to mention that EOs proved their potential as antimicrobial compounds in food preservation (Nguefack et al. 2004). The recent study has the aim to investigate the antifungal and anti-aflatoxin production activity of five essential oils on three mycotoxin-producing fungi, *A. parasiticus, F. culmorum* and *F. graminearum.*

Materials and Methods

Microorganisms

Aspergillus parasiticus CBS 260.67 was obtained from the Centralbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). *Fusarium graminearum* NRRL 28436 and *Fusarium culmorum* NRRL 29379 were provided by the

Accepted March 17, 2014
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Table 1. Chemical composition (%) of the essential oils tested.

Essential oil	Compound	Chemical composition (%)
Lemon	limonene	83.20
	β-pinene	9.54
	γ-terpinene	5.60
Juniper	α-pinene	40.70
	β-pinene	36.00
	o-cymene	18.90
Marjoram	terpinen-4-ol	33.58
	γ-terpinene	19.53
	β-phellandrene	8.00
Clary sage	linalyl acetate	84.00
	linalool	13.60
Cinnamon	cinnamic aldehyde	93.10
	cinnamyl- acetate	2.50

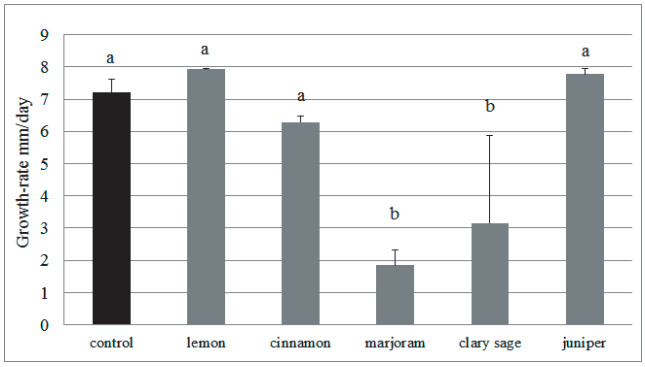

Figure 1. Average growth rate ± SD of *A. parasiticus* after treatment with different EOs (20 mg). Different letters on the top of columns represent significantly different results (P<0,05).

Table 2. Antifungal index of the investigated essential oils (20 mg/paper disc).

	Antifungal index (%)				
	Cinna-mon	Lemon	Marjo-ram	Clary sage	Juniper
A. parasiticus	14,93	-3,17	76,47	28,05	-0,45
F. graminearum	96,67	0	43,33	9,26	0
F. culmorum	96,67	0	68,7	73,7	0

Figure 2. Average growth rates ± SD of *F. culmorum* after treatment with different EOs (20 mg). Different letters on the top of columns represent significantly different results (P<0,05).

National Center for Agricultural Utilization Research (NRRL, Peoria, Illinois). Potato dextrose agar (PDA, Difco) was used for the cultivation of *Fusarium* species and malt extract agar (0,5% malt extract, 0,5% yeast, 0,5% glucose, 2% agar) for *A. parasiticus.*

Essential oils

Five essential oils – cinnamon *(Cinnamomum zeylanicum),* clary sage *(Salvia sclarea)*, juniper *(Juniperus communis)*, lemon *(Citrus lemon)* and marjoram *(Origanum majorana)* - were used in the experiments. They were purchased from Aromax Natural Products Zrt. (Budapest, Hungary). The composition of the oils was determined by GC-MS (Agilent; GC: 6850 Series II; MS: 5975C VL MSD) using an Agilent 19091S-433E colonna at the laboratory of Aromax. EOs were prepared by steam distillation of leaves or bark of the plants, except lemon EO; that was cold-pressed from lemon peel. Table 1 presents the chemical compositions of these essential oils.

Antifungal activity of EOs

To investigate the antifungal effect of the EOs reversed Petri-dish method was used. Petri-dishes (9 cm diameter) contained malt extract agar (for *A. parasiticus*) or potato dextrose agar (for *Fusarium* species) were inoculated at the centre with a mycelial disc (3 mm diameter) taken from the periphery of a fungus colony grown for 72 h.

Paper discs (10 mm in diameter) were fixed to the inner top of the Petri-dish with a drop of agar and impregnated with different amounts of EOs (5 mg, 20 mg or 40 mg). Control plates contained discs with distilled water. Plates were closed with parafilm and were incubated in reversed position at 25°C for 10 days. The colony diameter was recorded each day.

The growth-rate was calculated by linear regression of the linear phase of colony growth curves. The growth of fungal cultures containing different concentrations of the investigated EOs was compared with the control culture growing without any EOs (Passone et al. 2012).

Antifungal index was calculated by the following formula:

Antifungal index (%) = $(1- (D_t/D_c))x\ 100$

Dt diameter of EO treated mould colonies (mm)

Dc diameter of control mould colonies (mm) (Shukla et al. 2012).

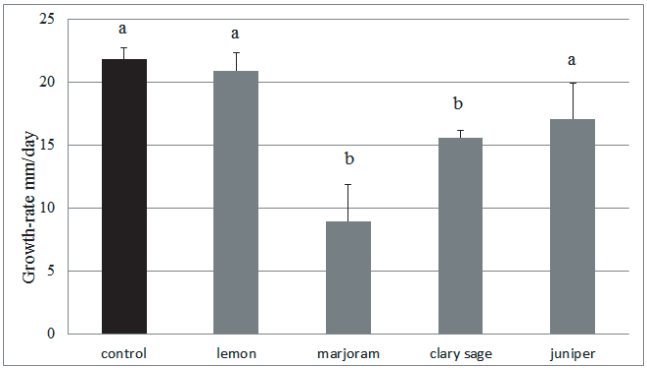

Figure 3. Average growth rates ± SD of *F. graminearum* after treatment with different EOs (20 mg). Different letters on the top of columns represent significantly different results (P<0,05).

Determination of aflatoxin production

Effect of the EOs on aflatoxin production was investigated at 5 and 40 mg/paper disk concentration of the EOs. At the 10th day of growth 10 round discs (12 mm diameter) were cut from the colony of *A. parasiticus* and were extracted with 10 mL chloroform. 1 mL extract was evaporated to dryness and redisolved in 100 µL chloroform.

Aflatoxin standard (B1, B2, G1, G2) produced by Trilogy Analytical Laboratory (Washington MO, Missouri, USA ((TS-108 (P86) was used in 5 µg/ml concentration.

One µl of each sample and 20 µl of the standard were used to the thin-layer chromatography on silica gel F60 aluminium sheets. The chromatogram was developed in a mixture of toluene-ethyl acetate-formic acid in 6:3:1 ratio. The bands were visualized and photographed under UV light. Colour intensity of bands representing aflatoxin types were compared visually.

All experiments were made in three replicates per treatment, except for the identification of aflatoxins which was carried out once.

Statistical analysis

Results were analysed using the Windows R-2.8 programme. One-way ANOVA was used to determine the differences between the control and treated samples.

Results and Discussion

For the proper comparability of the results, fungal growth-rates and antifungal indexes are presented using 20 mg/paper disk EO concentration. In most cases there were no inhibitory effect at lower concentrations and no growth at higher concentrations.

The growth rate of *A. parasiticus* compared to the untreated sample showed a minimal enhancement by juniper and lemon oil but these changes were not significant (P>0,05). Marjoram and clary sage decreased the growth rate significantly (P<0,05) (Fig. 1). Marjoram EO showed the best growth inhibition with the antifungal index of 76.4% (Table 2). The inhibitory effect of clary sage also differed significantly (P<0,001) from the control sample.

F. graminearum and *F. culmorum* showed almost similar response after treatment with the EOs (Figs. 2-3). Both of the controls grew very quick: the colony diameter of the control sample of *F. graminearum* has reached 90 mm (diameter of the Petri dish) on the 5th day and the control *of F. culmorum* on the 4th day. Lemon EO was ineffective in both cases. Juniper significantly (P<0,003) inhibited the growth of *F. culmorum* but had no effect on *F. graminearum* (Table 2).

In cases of *Fusarium* strains cinnamon EO was the best inhibitor, a total inhibition of growth was obtained with this EO. Marjoram significantly inhibited the growth of *F. culmorum* (p<0.001, antifungal index: 68.7%) and *F. graminearum* (p<0.001, antifungal index: 43.33%). Clary sage inhibited the growth of both *Fusarium* species, having an antifungal index of 73.7% for *F. culmorum* and an antifungal index of 9.26% for *F. graminearum (*Table 2).

Results regarding aflatoxin production can be seen on Figure 4. Results of TLC show that mycotoxin production of *A. parasiticus* was slightly influenced by the EOs. Compared to the control samples there seems to be a difference in B1, G1 production in the case of treatment with 5 mg cinnamon, and 5 mg and 40 mg of clary sage but further quantitative studies are needed to confirm this examination.

This study has reported the antifungal properties of five essential oils tested against three food spoilage and mycotoxin producing fungi. Higher antifungal activity was found in the EOs from cinnamon, marjoram and clary sage. Cinnamon EO caused complete inhibition of the growth of the tested fungi.

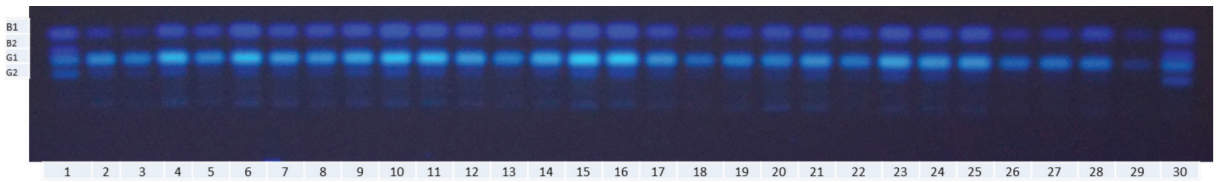

Figure 4. Thin layer chromatography of aflatoxin production by *A. parasiticus.* Standard (1, 30), control samples (2, 3, 4), juniper 5 mg (5,6,7), juniper 40 mg (8, 9, 10), lemon 5 mg (11, 12, 13), lemon 40 mg (14, 15, 16), cinnamon 5 mg (17, 18, 19), cinnamon 40 mg (20, 21), marjoram 5 mg (22, 23, 24), clary sage 5 mg (25, 26, 27), clary sage 40 mg (28, 29).

This result is similar to the findings of Soliman and Badeaa (2002) where the effect of cinnamon EO was tested on *A. flavus, A. parasiticus, A. ochraceus* and *F. moniliforme*. Many previous studies had verified cinnamon oil as a fungicide agent against a lot of fungi and showed its high fungicidal activity (Sinha et al. 1993; Mukherjee and Nandi 1994). This activity of cinnamon EO is mainly due to its major component, cinnamaldehyde which is a powerful fungistatic agent (Burt 2004; Bakkali et al. 2008).

Further studies in connection with the mycotoxin-reducing effect of these EOs will be necessary to reveal all the circumstances of this process.

Acknowledgement

This research was supported by the grants of the Hungarian-Romanian Intergovernmental S&T Cooperation Programme TÉT_10-1-2013-0019.

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