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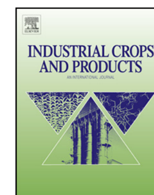

Research paper

Selected essential oil vapours inhibit growth of *Aspergillus* spp. in oats with improved consumer acceptability

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ABSTRACT

Traditionally, chemical pesticides have played a central role in food protection, forcing trends towards reduction of using chemicals in agriculture leads to call for the development of new strategies of protection agricultural product. Antimicrobial volatile substances from plants have become known as a suitable alternative to synthetic pesticides and food preservatives. In this study, we explored the potential of six essential oils retrieved from cinnamon (*Cinnamomum zeylanicum* Nees.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.), clove (*Syzygium aromaticum* L.), lemongrass (*Cymbopogon citratus* [DC] Stapf.) and ginger (*Zingiber officinale* Rosc.) essential oils. Inhibitory activity of essential oils were assessed against three strains of postharvest pathogens of the *Aspergillus parasiticus*, *A. flavus* and *A. clavatus* isolated from oats. In continue, the effect of the essential oils treatment on the sensory profile of the product was evaluated. The results indicated that the essential oils of lemongrass, clove, oregano and thyme have strong antifungal activity; however, only treatment with the oil of lemongrass is acceptable by consumers. The new technology of application of the essential oil vapours was highly effective, suggesting it could be used in the control of postharvest fungal pathogens of grains.
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1. Introduction

Oat (*Avena sativa* L.) is an important cereal in human and animal nutrition (Welch, 1995). Whole-grain oats are a source of many valuable components of a healthy diet, including proteins, fats, fibre, carbohydrates, minerals, and vitamins (Peterson, 2001). However, the presence of microscopic fungi can cause mycotoxin contamination (Kocube et al., 2013), potentially leading to food poisoning and representing a risk to the health of animals and people alike (Rocha et al., 2004). Infection of oat grains by fungi may occur both in the field and during storage (Pitt et al., 2013). Some fungal species belonging to genera of *Fusarium*, *Aspergillus* and *Penicillium*, are known to have the ability to contaminate grain with mycotoxins which are toxic to animals and humans (Medina and Magan, 2011). *A. flavus* produces aflatoxins, potential natural carcinogens, as well as cyclopiazonic acid (Lalitha Rao and Husain, 1985; Sacchi et al., 2009). Usually, cyclopiazonic acid occurs as a co–contaminant with aflatoxins (Pitt et al., 2013). Further important mycotoxins produced by *Aspergillus* include ochratoxins, patulin and fumagillin (Bennett and Klich, 2003; Sheikh-Ali et al., 2014).

One of the traditional ways of managing the risks of contaminated food is through the addition of herbs and spices. Most of them contain essential oils, complex mixtures of natural substances with antimicrobial properties, which have been empirically used as antimicrobial agents in the history and are now extensively studied by scientific methods (Bakkali et al., 2008; Burt, 2004). Hydrodistillation is a common isolation method. Gas chromatography coupled with mass spectrometry (GC–MS) and flame ionization detector (GC-FID) are used for chemical composition analysis of isolated oils (Badawy and Abdelgaleil, 2014; Matos, 2012; Teng et al., 2009). Reviews on antimicrobial activity of essential oils against food related microorganisms (Chutia et al., 2009; Lang and Buchbauer, 2012) were published recently (Otoni et al., 2016; Prakash et al., 2015). Antifungal activity has been reported for many

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of them (Lang and Buchbauer, 2012). Some authors have reported an increased efficiency of essential oils in vapour phase (Bernardos et al., 2015; Gómez-Sánchez et al., 2011; Inouye et al., 2000; López et al., 2007); in this case, they could be used at lower concentrations (Laird and Phillips, 2012). Unfortunately, essential oils have very specific organoleptic properties, meaning that the use of these naturally derived preservatives can alter the taste of food or exceed acceptable flavour thresholds (Hussein et al., 2014). The challenge therefore consists in finding an optimal dose, which protects the product, while still being acceptable to the consumer. A careful selection of essential oils appropriate to the sensory and compositional status of the food system is required. (Gutierrez et al., 2008).

Here, we evaluate the antifungal activity of selected essential oils against three strains of *Aspergillus*, an important postharvest pathogen of cereals, as well as the effect of essential oil treatments on the sensory profile of oats.

2. Material and methods

2.1. Fungal isolates

We studied three strains of *Aspergillus*, namely *Aspergillus clavatus* (KMi28), *Aspergillus flavus* (KMi39) and *Aspergillus parasiticus* (KMi13). These strains were obtained from the collection of microorganisms at the Department of Microbiology of the Slovak University of Agriculture in Nitra. They had initially been isolated from oat seeds in Slovakia and identified based on ITS rDNA and RBP2 sequencing and protein profiling by MALDI-TOF MS analysis (Císarová et al., 2016a). Fungal strains were cultivated on Sabouraud dextrose agar (Oxoid, Czech rep.) at 25 °C for four days prior to *in vitro* examinations.

2.2. Mycotoxin screening

All three *Aspergillus* strains were tested for aflatoxin B1 and G1, cyclopiazonic acid and patulin by thin layer chromatography (TLC) (Labuda and Tancinová, 2006). The mycotoxins were extracted from fresh 5 days old growing colonies on Czapek Yeast Autolysate Agar (Sigma-Aldrich, Germany) was used for cyclopiazonic acid and Yeast extract agar (Sigma-Aldrich, Germany) was used for patulin and aflatoxins B1 and G1, 5 × 5 mm piece was cut from the agar plate and placed into Eppendorf tubes together with 500 μl of extraction solvent (chloroform:methanol, 2:1, v/v). The tubes were then shaken on a vortex for at least 2 min. 30–50 μL of the resulting extract were spotted onto TLC plates (Silicagel 60, Merck, Germany) and left to dry. After developing the plates in toluene:ethylacetate:formic acid (6:3:1, v/v/v), aflatoxin B1 (Rf = 0.65) and aflatoxin G1 (Rf = 0.39) could be visualized directly under UV light (365 nm) as blue and blue-green spots, respectively. Cyclopiazonic acid (Rf = 0.65) was visualized by spraying with Ehrlich reagent (Sigma-Aldrich, Germany), which revealed a violet tailing-spot visible in daylight. Patulin (Rf = 0.32) was visualized by spraying with 0.5% methylbenzothiazolone hydrochloride (Merck, Germany) in methanol, followed by heating to 130 °C for 8 min, revealing a yellow-orange spot. Mycotoxin standards were obtained from Sigma–Aldrich (Germany).

2.3. Essential oil analysis

Cinnamon (*Cinnamomum zeylanicum* Nees.), thyme (*Thymus vulgaris* L.), clove (*Syzygium aromaticum* L.), oregano (*Origanum vulgare* L.), and ginger (*Zingiber officinale* Rosc.) essential oils were obtained from commercial supplier Sigma-Aldrich (Germany). Clove II (*Syzygium aromaticum* L.), oregano II (*Origanum vulgare* L.), lemongrass (*Cymbopogon citratus* [DC] Stapf.) were from Biomedica (Czech rep.). The essential oils were chosen form collection of essential oils at department according to previous results (Kloucek et al., 2012). All essential oils were obtained by hydrodistillation and stored in air-tight sealed glass bottles at 4 °C.

Essential oil constituents were identified and the relative 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 μm film thickness). One microliter of the sample was injected in split mode 1:12, at an injector temperature of 250 °C and an electron ionization energy of 70 eV. Analysis were measured in SCAN mode, mass range was 40–400*m*/*z*. Starting at 60 °C, the oven temperature was increased at a rate of 3 °C/min to a maximum of 231 °C, where it was kept constant for 10 min. The identification of constituents was based on a comparison of their mass spectra and relative retention indices (RI) against the National Institute of Standards and Technology Library (NIST, USA), as well as authentic analytical standards and data from the literature (Adams, 2007). Relative proportions of essential oils constituents were assessed by gas chromatography with flame ionization detector (GC-FID) Agilent 7890A (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS, 30 m × 0.25 mm, 0.25 μm film thickness. The same method as for GC–MS was employed. Carrier gas was nitrogen (constant flow 1 mL/min, 99,999% purity), injector and detector temperatures were 250 °C. Relative proportions were calculated by dividing individual peak area by total area of all peaks, response factor was not taken into account; only compounds over 0.1% were included. Peaks under 0.1% were not counted.

2.4. Antifungal assays in cereals

Vapour-phase antifungal activities of commercially available essential oils – cinnamon leaves (*Cinnamomum zeylanicum* Nees.), thyme (*Thymus vulgaris* L.), clove (*Syzygium aromaticum* L.), oregano (*Origanum vulgare* L.), and ginger (*Zingiber officinale* Rosc.) – were evaluated against strains of *Aspergillus flavus, A. parasiticus* and *A. clavatus* isolated from oats. All experiments were repeated in independent triplicates. Prior to testing, oats were sterilized by washing with 70% ethanol for 30 s, followed by stirring in 3% chloramine solution for two minutes. Excess disinfectant was removed by rinsing with sterile distilled water (Sauer, 1986). Fungal spore suspensions were made by covering fungal colonies with 1 mL of sterile phosphate-buffered saline (Sigma-Aldrich, Germany) with 0.5% Tween 80 (Sigma-Aldrich, Germany) followed by a dilution in 20 mL sterile phosphate-buffered saline with 0.5% Tween 80 to a final concentration 10^6 CFU/ml by adjusting the density to 0.8-1 McFarland units, depending on the strain (Espinel-Ingroff and Kerkering, 1991; Petrikkou et al., 2001). Forty grams of dry sterile oat seeds were transferred to an Erlenmeyer flask, and 20 mL of inoculum was added. This mixture was shaken for 20 min. After inoculation, the seeds were again left to dry under sterile conditions. Essential oils were diluted in ethyl acetate to achieve final concentrations of 125, 250 and 500 μL/L of petri dish volume. Ethyl acetate and temperature treatment alone (see below) were used as controls. One hundred microliters of each concentration were evenly pipetted onto a 4 × 4 cm piece of sterile filter paper. Petri dishes were left opened for one minute to evaporate ethyl acetate (Kloucek et al., 2012).

Five grams of inoculated oats were enclosed into a 90 mm glass petri dish together with the essential oil-infused filter paper. In order to avoid any direct contact between the oat seeds and the essential oils, the filter paper was covered by 85 mm diameter inox metal mesh, onto which the seeds were spread. Petri dishes were closed and left at 50 °C for 2 h followed by 20 min at room temperature. Combinations of all three strains, eight essential oils and

Table 1
Sensory descriptors.

taste	odour
Acceptability	Acceptability
Overall intensity	Overall intensity
Clove	Clove
Thyme	Thyme
Oregano	Oregano
Lemongrass	Lemongrass
Others	Others
Bitter	
Sweet	
Pungent	

three concentrations, in total 72 combinations plus two controls (temperature, ethyl acetate), were tested. All tests were carried out in triplicate.

Treated seeds were then put into 96-well microtitration plates (one seed per well) together with 100 μL of nutrient medium/well. After three days, the wells with visible mycelial growth and wells with visible sporulation were counted.

2.5. Sensory analysis

Sensory testing evaluated the intensity of aroma and taste of each essential oil, as well as the acceptability of the sample. Oats were evaluated by a panel of staff from the Department of Quality of Agricultural Products, Czech University of Life Sciences, Prague, according to international standards and following previously published protocols (Starr et al., 2013). Five samples of raw whole oat grains were subjected to the treatment of 250 μL/L by the procedure described previously, just without inoculation, with a different essential oil each, namely thyme, oregano, clove and lemongrass essential oils, prior to being served to a trained panel of assessors for sensory evaluation. An untreated sample was included as a control. Samples were put to glass bottles submerged with tap water and boiled for 30 min in water bath. All evaluations took place in a sensory evaluation laboratory, which was equipped according to ISO guidelines (ISO 8589:2007). The panel consisted of ten trained experienced assessors according to ISO 8586:2012 (three men and seven women between the ages of 21 and 39 years). In an approach based on descriptive sensory analysis, each panellist evaluated the intensity experienced for each sensory descriptor on a linear graphical oriented unstructured 100 mm scale which was verbally anchored at each end. For each sensory descriptor (Table 1), the left side of the scale corresponded to the lowest intensity and the right side to the highest intensity. Sensory scores of overall acceptability of taste and aroma were based on hedonic scale, where left side is dislike extremely and right side is like extremely.

Samples were served on covered glass petri dishes with randomized four-digit code. A control sample was served first, followed by the remaining samples in random order. Each serving comprised approximately 25 g of seeds. For each essential oil treatment, the intensity and acceptability of taste and odour were evaluated.

2.6. Statistical analysis

The data were homogenous and normal according to Bartlett and Shapiro tests. The results shown in Tables 3 and 4 were tested by one-way ANOVA and Scheffé's method of homogenous subsets. Each of the three parameters – type of EO, concentration, and fungal species, were separately compared by one way ANOVA (Statistica12, StatSoft).

3. Results

3.1. Mycotoxin screening

TLC confirmed toxin production in all tested strains. The presence of aflatoxin B1 and aflatoxins of the G6 group was confirmed in *Aspergillus parasiticus* (KMi13). *Aspergillus clavatus* (KMi28) produces patulin. Cyclopiazonic acid and aflatoxin B1 were confirmed for *Aspergillus flavus* (KMi39).

3.2. Essential oil composition

The major components of the essential oils tested, as identified by GC–MS, are listed in Table 2. Here, eugenol was identified and determined as the major component of cinnamon (72%) and clove oils (78%). The main compounds found in thyme oil were thymol (44%) and *p*-cymene (18%). Carvacrol (70%) was the main compound of oregano essential oil. The major components of lemongrass oil were geranial (40%) and neral (32%). Finally, ginger oil to contain α-zingiberene (32%), camphene (11%) and β-sesquiphellandrene (11%).

3.3. Fungicidal effect of essential oils

The fungicidal effect of the essential oils tested was evaluated three days after treatment, using two parameters: the percentage of seeds with mycelial growth (Table 3) and the percentage of seeds showing mycelial growth with sporulation (Table 4). The essential oils of lemongrass, oregano and thyme showed the strongest inhibitory effect on the mycelial growth of *Aspergillus flavus* and *A. parasiticus*. Among the strains tested, *A. clavatus* was significantly the most resistant, followed by *A. parasiticus* and *A. flavus*. Significant differences of inhibitory effect was observed among all concentrations of essential oils in general, with concentration of 500 μL/L being the most effective. The essential oils at a concentration of 125 μL/L were the least effective. Comparison of the results as separate treatments (Table 3) regardless the variable like type of EO, concentration, or strain resulted in a subdivision of the data into five groups. The most effective were the highest concentrations of lemongrass, oregano and thyme (500 μL/L), and the moderate concentration of lemongrass (250 μL/L). On the other hand the lowest concentration of ginger and clove essential oils were significantly less effective, with effect comparable to the controls.

The effect of the essential oils on the sporulation of the tested strains is shown in Table 4. The essential oils of lemongrass, oregano and thyme showed the strongest inhibitory effect on the sporulation of *Aspergillus flavus* and *A. parasiticus*. *Aspergillus clavatus* was again the most resistant of the tested strains. Lemongrass and oregano at a concentration 500 μL/L were able to inhibit sporulation completely. In same concentration thyme and clove inhibited more than 90% of sporulation. Moderate inhibitory effects were observed for all essential oils at concentration of 250 μL/L, and lemongrass were also effective at a concentration of 125 μL/L. However, most of the essential oils tested at concentration of 125 μL/L were not effective. Comparison of the results as separate treatments (Table 4) regardless the variables like type of EO, concentration, or strain resulted in a subdivision of the data into four groups. The effectiveness against sporulation was comparable among all experiments, with exception of lemongrass (500 μL/L), which was the most effective, and the lowest concentration (125 μL/L) of cinnamon, oregano, ginger and clove essential oils, which were significantly less effective.

Table 2
Essential oils composition, as identified by GC–MS and quantified by GC-FID.

RI[b]	Component	Cinnamon[c]	Clove	Clove II	Ginger	Lemongrass	Oregano	Oregano II	Thyme
938	α-Pinene[a]	1.17		0.12	2.97	0.37	0.41	0.27	1.31
953	Camphene[a]	0.41			11.35	2.13	0.17	0.29	1.52
964	Benzaldehyde[a]	0.18							
980	β-Pinene[a]	0.31					0.80		0.12
982	1-Octen-3-ol					1.86	0.35		0.73
993	β-Myrcene				1.23	0.11	0.19	0.47	1.36
997	3-Octanol						0.52	0.21	
1006	α-Phellandrene	0.36					0.13		0.14
1019	α-Terpinene						0.42	0.91	1.12
1029	p-Cymene[a]	1.79					10.32	7.43	17.88
1031	D-Limonene[a]	0.77				0.42	0.67		0.36
1032	β-Phellandrene				9.11				
1034	Eucalyptol	0.17		0.26	4.80		0.99	1.19	1.47
1062	γ-Terpinen						2.56	3.49	5.27
1074	Linalool oxide					1.23			
1090	Terpinolene						0.16	0.12	0.13
1091	Nonanone					0.11			
1101	Linalool	2.48				1.50	1.45	3.19	4.81
1147	Camphor[a]						0.69	0.89	1.98
1158	(+/−)-citronellal					0.39			
1160	Isoborneol					0.24			
1168	Borneol[a]				1.45	0.81	1.03	0.87	1.85
1179	4-Terpineol[a]	0.11				0.45	0.96	0.77	1.93
1192	α-Terpineol	0.28				0.78		0.41	0.18
1198	γ-Terpineol					0.27			
1202	2-dekanol					0.21			
1234	b-citronellol					0.19			
1238	Thymol methyl ether								0.70
1244	Neral[a]				3.88	31.65			
1247	(−)-carvone[a]		0.12				0.50	0.10	1.01
1259	Geraniol					4.76			
1272	Cinnamaldehyde[a]	1.12							
1273	Geranial[a]				6.31	40.06			
1287	Bornyl acetate					0.17			
1289	Safrole	1.40							
1296	Thymol[a]						4.05	4.06	44.34
1297	Menth-1-en-9-ol					0.12			2.88
1302	Cinnamyl alcohol	0.16							
1306	Carvacrol[a]						67.14	69.78	
1360	Eugenol[a]	72.53	81.74	77.76		0.14		0.15	0.22
1370	m-Eugenol		0.12	0.10		0.21			
1373	Benzenepropyl acetate	0.22				0.15			
1376	Copaene	0.81	0.42			0.14	0.12	0.14	0.16
1387	Geranyl acetate					4.08			
1399	Vanillin	0.18							
1412	Isocaryophillene			0.18					
1420	Caryophyllene[a]	2.69	12.25	7.40		1.99	1.09	1.84	6.94
1445	Cinnamyl acetate	2.01							
1448	Citronellyl propionate					0.37			
1450	Humulen-(v1)			0.17					
1452	α-Caryophyllene	0.52	1.49	2.01		0.24	0.35	0.45	
1478	Germacrene D				0.92				
1481	α-Curcumene				5.36				
1491	Varidiflorene					0.11			
1493	α-Zingiberene				31.71				
1505	α-Bisabolene				8.45		0.11	0.11	
1510	γ-Cadinene					1.95			
1521	β-Sesquiphellandrene				10.44				
1524	δ-Cadinene	0.19		0.46		0.46	0.27	0.44	0.32
1525	Aceteugenol	2.67				0.16			
1531	Eugenol acetate		0.31	7.19					
1570	Caryophyllenyl alcohol		2.54				0.13		
1574	Caryophyllene oxide	1.35		0.94		0.52	1.35	0.44	0.19
1725	Benzyl Benzoate	4.13							
	total	98.01	98.99	96.59	97.98	98.35	96.93	98.02	98.92

[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 proportions were calculated in% by dividing individual peak area by total area of all peaks.

3.4. Sensory properties

Panel members were able to determine all specific tastes and odours of the tested essential oils. In line with their similar chemical composition of thyme and oregano essential oils, samples treated with these oils received similar scores

Odour testing (Table 5) showed that samples treated with lemongrass essential oil were scored higher for acceptability for consumers than all other samples, including the untreated con-

Table 3
Percentage of infected seeds in Scheffé's Homogeneous Subsets.

Essential oil & concentration (μL/L of air)	% infected seeds (average)	
Lemongrass 500	4%	a
Oregano 500	6%	ab
Lemongrass 250	17%	abc
Thyme 500	18%	abc
Oregano M 500	21%	abc
Clove 500	26%	abcd
Oregano 250	36%	abcd
Cinnamon 500	40%	abcde
Lemongrass 125	43%	abcde
Oregano M 250	52%	abcde
Clove 125	52%	abcde
Clove M 500	53%	abcde
Thyme 250	58%	abcde
Clove M 250	61%	abcde
Clove 250	66%	abcde
Cinnamon 250	67%	abcde
Ginger 500	67%	abcde
Oregano 125	74%	bcde
Thyme 125	77%	bcde
Oregano M 125	81%	cde
Cinnamon 125	81%	cde
Ginger 250	82%	cde
Clove M 125	90%	de
Ginger 125	98%	de
Temperature	100%	e
Ethyl acetate	100%	e

The letters in column signify cases with no statistical differences ($p > 0.05$) between samples according the Scheffe's test.

Table 4
Percentage of sporulated samples in Scheffé's Homogeneous Subsets.

Essential oil & concentration (μL/L of air)	% sporulated seeds (average)*	
Lemongrass 500	0%	a
Oregano 500	0%	ab
Lemongrass 250	3%	ab
Thyme 500	6%	ab
Oregano 250	7%	ab
Clove 500	9%	ab
Lemongrass 125	10%	ab
Oregano M 500	11%	ab
Cinnamon 500	16%	ab
Clove M 500	18%	ab
Oregano M 250	21%	ab
Thyme 250	21%	ab
Clove 250	26%	ab
Clove 125	26%	ab
Cinnamon 250	27%	ab
Thyme 125	27%	ab
Clove M 250	31%	ab
Ginger 500	33%	ab
Cinnamon 125	44%	abc
Oregano 125	49%	abcd
Ginger 250	52%	abcd
Oregano M 125	55%	abcd
Clove M 125	59%	bcd
Ginger 125	96%	cd
Temperature	100%	d
Ethyl acetate	100%	d

The letters in column signify cases with no statistical differences ($p > 0.05$) between samples according the Scheffe's test.

trol. Samples treated with thyme and oregano essential oils were perceived to have more additional odours than the other samples. Samples treated with lemongrass and clove essential oils received the highest scores for odour specificity, meaning that these odours were unequivocally identified. Thyme and oregano odours, on the other hand, could not be distinguished from each other. Odour acceptability was ranked as moderate for samples treated with thyme, oregano and clove essential oils.

All samples were evaluated for the intensity and acceptability of their taste, and for the intensity of the specific taste of the essential oil (lemongrass, oregano, thyme, clove) they had been treated with (Table 6). Moreover, bitterness, sweetness and pungency were evaluated for each sample. Samples treated with thyme, oregano and clove essential oils were perceived to have a higher taste intensity than the other samples. Just like their odour, the taste of samples treated with oregano and thyme essential oils was mixed up, while lemongrass and clove oil treated samples were ranked higher for taste specificity. Acceptability of taste scores were very poor for samples treated with thyme, oregano and clove essential oils, while samples treated with lemongrass essential oil achieved a similar score as the untreated control. Thyme, oregano and clove essential oils led to pungency in samples; lemongrass essential oil did so but to a far lesser degree and did not affect the sweetish taste of the oats.

4. Discussion

Fungi from the genus *Aspergillus* play an important role as contaminants of food and animal feed (Sheikh-Ali et al., 2014). Many species of this genus produce mycotoxins (Chulze et al., 2015; Pitt et al., 2013; Sacchi et al., 2009), including these used in our study, which can be dangerous for humans (Reddy et al., 2010; Zain, 2011). An inhibitory effect of essential oils on the growth of different strains of *Aspergillus* spp. has been previously reported (Bernardos et al., 2015; Gumus et al., 2010; Inouye et al., 2000; Li et al., 2013; López-Malo et al., 2005; Silva et al., 2012). Our MICs are generally higher when compared with those obtained by other studies. López et al. (2007) reported MICs between 17.5 and 175 μL/L for the volatile phase of oregano and thyme essential oils against *Aspergillus flavus*. Inouye et al. (2000) found that thyme and cinnamon essential oil applied by gaseous contact exerted a fungistatic effect on *Aspergillus fumigatus* at concentration of 63 μL/L; furthermore, they found these essential oils to be more effective on

Table 5
Odour sensory profile.

	acceptability	overall intensity	clove	thyme	oregano	lemongrass	others
Raw samples							
Thyme	47.1	73.3	3.2	51.3	36.2	10.9	16.9
Oregano	41.8	72.1	4.5	30.1	41.3	6.9	22.1
Clove	47.4	77.5	83.3	1.1	1.2	1.1	4.2
Lemongrass	71.0	81.1	0.9	0.9	7.8	82.3	1.2
Control	57.1	23.2	4.4	4.6	11.6	4.4	3.7
Cooked samples							
Thyme	39.3	70.7	2.9	46.6	38.0	2.4	5.3
Oregano	41.4	82.7	1.4	44.5	46.2	2.2	16.6
Clove	54.0	73.7	82.1	3.1	3.3	1.0	0.9
Lemongrass	80.4	78.0	1.4	1.6	0.7	65.1	1.7
Control	59.0	34.7	0.1	0.1	0.1	0.3	2.4

Table 6
Taste sensory profile.

	acceptability	overall intensity	bitter	sweet	pungent	clove	thyme	oregano	lemongrass	others
Raw samples										
Thyme	25.6	75.7	53.1	12.7	38.8	7.2	45.2	49.3	3.7	14.2
Oregano	25.1	72.4	42.6	14.0	34.7	6.0	33.0	60.1	4.0	17.9
Clove	30.6	73.5	40.2	10.7	33.6	79.9	1.8	2.2	1.8	6.2
Lemongrass	59.6	59.7	12.9	20.2	10.0	1.2	3.0	0.8	54.1	7.2
Control	63.7	39.6	15.0	20.7	2.0	3.3	5.4	8.4	4.5	6.7
Cooked samples										
Thyme	22.1	80.9	58.0	3.3	36.3	3.9	65.3	38.7	3.2	34.9
Oregano	30.0	77.9	41.4	9.0	24.0	6.0	36.5	47.3	2.2	25.7
Clove	45.3	66.3	24.7	5.1	34.4	72.6	5.1	5.1	1.7	11.6
Lemongrass	70.6	67.0	1.9	8.4	13.6	1.3	1.0	1.4	52.3	0.8
Control	63.4	38.4	1.6	8.1	0.6	0.3	0.4	0.3	0.4	4.4

moulds than on bacteria when applied by gaseous contact. Císarová et al. (2016b) reported high efficiency of clove, thyme and oregano essential oils. They found by the micro-atmosphere method minimum inhibitory doses from 31.5 to 62.5 μL/L of air. A variation in reported MICs can be attributed to the different methods used for the evaluation of the antifungal activity in gaseous phase, as well as the type of moulds and essential oils studied. The main difference, however, is that in our study we used real products (oats) and wild strains, rather than *in vitro* testing of laboratory strains. Our results confirmed that oregano, thyme, clove and lemongrass essential oils have strong antifungal properties, and there is great potential for their use in the protection of food against different strains of *Aspergillus* spp. (Císarová et al., 2016a,b; Inouye et al., 2000) as well as in food preservation (Gumus et al., 2010). A recent study suggested that the antifungal activity of cinnamon and thyme oils may be due to their major components, cinnamaldehyde and carvacrol respectively (Ferhout et al., 1999). The essential oils of clove and lemongrass revealed a strong inhibitory effect against the *Aspergillus* strains tested here. Several reports have indicated citral as a fungicidal constituent of lemon grass oil (Allah et al., 1975; Paranagama et al., 2003). Previous work on the antimicrobial activities of several essential oil components has shown cineole, citral, geraniol, linalool and menthol to be active against several yeast-like and filamentous fungi (Džamić et al., 2013; Pattnaik et al., 1997). Thus, the presence of citronellal, linalool and geraniol in lemon grass oil might have played a role in inhibiting sporulation (Pawar and Thaker, 2006).

5. Conclusions

In conclusion, findings suggest that thyme, oregano, clove and lemongrass essential oils are highly effective in vapour phase and could potentially be used in the fight against postharvest fungal pathogens. However, due to their strong aroma and taste, not all of these are acceptable to consumers. In the case of oats, the treatment with lemongrass essential oil inhibited mycelial growth and sporulation of three mycotoxin producing fungi and enhanced acceptability of the product by consumers. In general, it is crucial to find the right combination of product and essential oil. Different concentrations of essential oils can be used to protect some stored food products from heat resistant and saprophytic moulds, giving them a potential as suitable alternatives to chemical additives for use in the food industry.

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References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/mass Spectrometry, 4th ed. Allured Publishing Corporation, Carol Stream, IL, USA.

Allah, M.A.A., Foda, Y.H., Saleh, M., Zaki, M.S.A., Mostafa, M.M., 1975. Identification of the volatile constituents of the Egyptian lemongrass oil. part I. gas chromatographic analysis. Food/Nahrung 19, 195–200, http://dx.doi.org/10.1002/food.19750190302.

Badawy, M.E.I., Abdelgaleil, S.A.M., 2014. Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. Ind. Crops Prod. 52, 776–782, http://dx.doi.org/10.1016/j.indcrop.2013.12.003.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils–a review. Food Chem. Toxicol. 46, 446–475, http://dx.doi.org/10.1016/j.fct.2007.09.106.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497–516, http://dx.doi.org/10.1128/CMR.16.3.497-516.2003.

Bernardos, A., Marina, T., Žáček, P., Pérez-Esteve, É., Martínez-Mañez, R., Lhotka, M., Kouřimskák, L., Pulkrábek, J., Klouček, P., 2015. Antifungal effect of essential oil components against *Aspergillus niger* when loaded into silica mesoporous supports. J. Sci. Food Agric. 95, 2824–2831, http://dx.doi.org/10.1002/jsfa.7022.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods — a review. Int. J. Food Microbiol. 94, 223–253, http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022.

Chulze, S.N., Palazzini, J.M., Torres, A.M., Barros, G., Ponsone, M.L., Geisen, R., Schmidt-Heydt, M., Köhl, J., 2015. Biological control as a strategy to reduce the impact of mycotoxins in peanuts, grapes and cereals in Argentina. Food Addit. Contam. Part A 32, 471–479, http://dx.doi.org/10.1080/19440049.2014.984245.

Chutia, M., Deka Bhuyan, P., Pathak, M.G., Sarma, T.C., Boruah, P., 2009. Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from North East India. LWT – Food Sci. Technol. 42, 777–780, http://dx.doi.org/10.1016/j.lwt.2008.09.015.

Císarová, M., Tančinová, D., Medo, J., 2016a. Antifungal activity of lemon, eucalyptus, thyme, oregano, sage and lavender essential oils against *Aspergillus niger* and *Aspergillus tubingensis* isolated from grapes. Potravinarstvo 10, 83–88, 10.10.5219/554.

Císarová, M., Tančinová, D., Medo, J., Kačániová, M., 2016b. The in vitro effect of selected essential oils on the growth and mycotoxin production of *Aspergillus* species. J. Environ. Sci. Heal., 1–7, http://dx.doi.org/10.1080/03601234.2016.1191887.

Džamić, A.M., Soković, M.D., Ristić, M.S., Grujić, S.M., Mileski, K.S., Marin, P.D., 2013. Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. J. Appl. Pharm. Sci. 4, 1–5, http://dx.doi.org/10.7324/JAPS.2014.40301.

Espinel-Ingroff, A., Kerkering, T.M., 1991. Spectrophotometric method of inoculum preparation for the in vitro susceptibility testing of filamentous fungi. J. Clin. Microbiol. 29, 393–394.

Ferhout, H., Bohatier, J., Guillot, J., Chalchat, J.C., 1999. Antifungal activity of selected essential oils, cinnamaldehyde and carvacrol against *Malassezia furfur* and *Candida albicans*. J. Essent. Oil Res. 11, 119–129, http://dx.doi.org/10.1080/10412905.1999.9701086.

Gómez-Sánchez, A., Palou, E., López-Malo, A., 2011. Antifungal activity evaluation of Mexican oregano (*Lippia berlandieri* Schauer) essential oil on the growth of *Aspergillus flavus* by gaseous contact. J. Food Prot. 74, 2192–2198, http://dx.doi.org/10.4315/0362-028X.JFP-11-308.

Gumus, T., Demirci, A.S., Sagdic, O., Arici, M., 2010. Inhibition of heat resistant molds: *Aspergillus fumigatus* and *Paecilomyces variotii* by some plant essential oils. Food Sci. Biotechnol. 19, 1241–1244, http://dx.doi.org/10.1007/s10068-010-0177-9.

Gutierrez, J., Barry-Ryan, C., Bourke, P., 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int. J. Food Microbiol. 124, 91–97, http://dx.doi.org/10.1016/j.ijfoodmicro.2008.02.028.

Hussein, A.M.S., Shaheen, M.S., Abdel-Kalek, H.H., El-Nor, S.A.H.A., 2014. Production of low calorie bakery product with pleasant flavour, antioxidant and antimicrobial activities. Polish J. Food Nutr. Sci., 64, http://dx.doi.org/10.2478/pjfns-2013-0010.

ISO 8589:2007. Sensory analysis – General guidance for the design of test rooms.

ISO 8586:2012. Sensory analysis – General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors.

Inouye, S., Tsuruoka, T., Watanabe, M., Takeo, K., Akao, M., Nishiyama, Y., Yamaguchi, H., 2000. Inhibitory effect of essential oils on apical growth of *Aspergillus fumigatus* by vapour contact. Mycoses 43, 17–23, http://dx.doi.org/10.1046/j.1439-0507.2000.00538.x.

Kloucek, P., Smid, J., Frankova, A., Kokoska, L., Valterova, I., Pavela, R., 2012. Fast screening method for assessment of antimicrobial activity of essential oils in vapor phase. Food Res. Int. 47, 161–165, http://dx.doi.org/10.1016/j.foodres.2011.04.044.

Kocube, S., Varga, J., Sigeti, D., Baranji, N., Suri, K., Tot, B., Toldi, E., Bartok, T., Mesterhazi, A., 2013. *Aspergillus* species as mycotoxin producers in agricultural products in central Europe. Zb. Matice Srp. za Prir. Nauk., 13–25, http://dx.doi.org/10.2298/ZMSPN1324013K.

Labuda, R., Tancinová, D., 2006. Fungi recovered from Slovakian poultry feed mixtures and their toxinogenity. Ann. Agric. Environ. Med. 13, 193–200.

Laird, K., Phillips, C., 2012. Vapour phase: a potential future use for essential oils as antimicrobials? Lett. Appl. Microbiol. 54, 169–174, http://dx.doi.org/10.1111/j.1472-765X.2011.03190.x.

Lalitha Rao, B., Husain, A., 1985. Presence of cyclopiazonic acid in kodo millet (*Paspalum scrobiculatum*) causing kodua poisoning in man and its production by associated fungi. Mycopathologia 89, 177–180.

Lang, G., Buchbauer, G., 2012. A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. A review. Flavour Fragr. J. 27, 13–39, http://dx.doi.org/10.1002/ffj.2082.

Li, W.R., Shi, Q.S., Ouyang, Y.S., Chen, Y. Ben, Duan, S.S., 2013. Antifungal effects of citronella oil against *Aspergillus niger* ATCC 16404. Appl. Microbiol. Biotechnol. 97, 7483–7492, http://dx.doi.org/10.1007/s00253-012-4460-y.

López-Malo, A., Maris Alzamora, S., Palou, E., 2005. *Aspergillus flavus* growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. Int. J. Food Microbiol. 99, 119–128, http://dx.doi.org/10.1016/j.ijfoodmicro.2004.08.010.

López, P., Sanchez, C., Batlle, R., Nerín, C., 2007. Vapor-phase activities of cinnamon, thyme, and oregano essential oils and key constituents against foodborne microorganisms. J. Agric. Food Chem. 55, 4348–4356, http://dx.doi.org/10.1021/jf063295u.

Matos, O., 2012. Aromatic plants as sources of photoactive biological products useful to crop protection. Acta Hortic., 531–538.

Medina, A., Magan, N., 2011. An Overview of Oat Contamination with Mycotoxins: Strategies for Their Control. Nova Science Publishers, Inc., pp. 97–124.

Otoni, C.G., Espitia, P.J.P., Avena-Bustillos, R.J., McHugh, T.H., 2016. Trends in antimicrobial food packaging systems: emitting sachets and absorbent pads. Food Res. Int., http://dx.doi.org/10.1016/j.foodres.2016.02.018.

Paranagama, P.A., Abeysekera, K.H.T., Abeywickrama, K., Nugaliyadde, L., 2003. Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* Link. isolated from stored rice. Lett. Appl. Microbiol. 37, 86–90.

Pattnaik, S., Subramanyam, V.R., Bapaji, M., Kole, C.R., 1997. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 89, 39–46.

Pawar, V.C., Thaker, V.S., 2006. In vitro efficacy of 75 essential oils against *Aspergillus niger*. Mycoses 49, 316–323, http://dx.doi.org/10.1111/j.1439-0507.2006.01241.x.

Peterson, D.M., 2001. Oat antioxidants. J. Cereal Sci. 33, 115–129, http://dx.doi.org/10.1006/jcrs.2000.0349.

Petrikkou, E., Rodríguez-Tudela, J.L., Cuenca-Estrella, M., Gómez, A., Molleja, A., Mellado, E., 2001. Inoculum standardization for antifungal susceptibility testing of filamentous fungi pathogenic for humans. J. Clin. Microbiol. 39, 1345–1347, http://dx.doi.org/10.1128/JCM.39.4.1345-1347.2001.

Pitt, J.I., Taniwaki, M.H., Cole, M.B., 2013. Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of Food Safety Objectives. Food Control 32, 205–215, http://dx.doi.org/10.1016/j.foodcont.2012.11.023.

Prakash, B., Kedia, A., Mishra, P.K., Dubey, N.K., 2015. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities – potentials and challenges. Food Control 47, 381–391, http://dx.doi.org/10.1016/j.foodcont.2014.07.023.

Reddy, K.R.N., Nurdijati, S.B., Salleh, B., 2010. An overview of plant-derived products on control of mycotoxigenic fungi and mycotoxins. Asian J. Plant Sci. 9, 126–133, http://dx.doi.org/10.3923/ajps.2010.126.133.

Rocha, A.D., de Oliveira, A.B., de Souza Filho, J.D., Lombardi, J.A., Braga, F.C., 2004. Antifungal constituents of *Clytostoma ramentaceum* and *Mansoa hirsuta*. Phytother. Res. 18, 463–467, http://dx.doi.org/10.1002/ptr.1452.

Sacchi, C., González, H.H.L., Broggi, L.E., Pacin, A., Resnik, S.L., Cano, G., Taglieri, D., 2009. Fungal contamination and mycotoxin natural occurrence in oats for race horses feeding in Argentina. Anim. Feed Sci. Technol. 152, 330–335, http://dx.doi.org/10.1016/j.anifeedsci.2009.04.008.

Sauer, D.B., 1986. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology 76, 745–749, http://dx.doi.org/10.1094/Phyto-76-745.

Sheikh-Ali, S.I., Ahmad, A., Mohd-Setapar, S.-H., Zakaria, Z.A., Abdul-Talib, N., Khamis, A.K., Hoque, M.E., 2014. The potential hazards of *Aspergillus* sp. in foods and feeds, and the role of biological treatment: a review. J. Microbiol. 52, 807–818, http://dx.doi.org/10.1007/s12275-014-4294-7.

Silva, F.C., Chalfoun, S.M., De, V.M., Botelho, D.M.S., Lima, N., Luís, R., 2012. Evaluation of antifungal activity of essential oils against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. Rev. Bras. Farmacogn. 22, 1002–1010, http://dx.doi.org/10.1590/S0102-695X2012005000052.

Starr, G., Bredie, W.L.P., Hansen, Å.S., 2013. Sensory profiles of cooked grains from wheat species and varieties. J. Cereal Sci. 57, 295–303, http://dx.doi.org/10.1016/j.jcs.2012.11.014.

Teng, Y., Yang, Q., Yu, Z., Zhou, G., Sun, Q., Jin, H., Hou, T., 2009. In vitro antimicrobial activity of the leaf essential oil of *Spiraea alpina* Pall. World J. Microbiol. Biotechnol. 26, 9–14, http://dx.doi.org/10.1007/s11274-009-0134-z.

Welch, R.W., 1995. Oats in human nutrition and health. In: The Oat Crop. Springer, Netherlands, Dordrecht, pp. 433–479, http://dx.doi.org/10.1007/978-94-011-0015-1_14.

Zain, M.E., 2011. Impact of mycotoxins on humans and animals. J. Saudi Chem. Soc. 15, 129–144, http://dx.doi.org/10.1016/j.jscs.2010.06.006.