Chemosphere 112 (2014) 443–448

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Antifungal activity and chemical composition of twenty essential oils against significant indoor and outdoor toxigenic and aeroallergenic fungi

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HIGHLIGHTS

- Antifungal activity of 20 Essential oils (EOs) was investigated and MIC evaluated.
- Significant allergenic, toxigenic and pathogenic fungi were used as a target species.
- GC–MS analysis was used and the abundance of active compounds (AC) was revealed.
- Molecular structure of the main active compounds correlated with activity.
- Superior activity of some EOs was discussed with respect to AC abundance.

ARTICLE INFO

Article history:
Received 16 January 2014
Received in revised form 2 May 2014
Accepted 4 May 2014
Available online 2 June 2014

Handling Editor: A. Gies

Keywords:
Essential oil
Phenolic compounds
Allergen
Indoor fungi
Antifungal efficacy
Botanical fungicides

ABSTRACT

Health affecting, loss-inducing or otherwise harmful fungal pathogens (molds) pose a serious challenge in many areas of human activities. On the contrary, frequent use of synthetic fungicides is undesirable in some cases and may be equally problematic. Moreover, the ever more increasing fungal resistance against commercial synthetic fungicides justifies development of rising efforts to seek new effective, while environmentally friendly alternatives. Botanical fungicides based on Essential oils (EOs) undoubtedly provide such an alternative. The study explores the efficacy of 20 EOs against *Alternaria alternata, Stachybotrys chartarum, Cladosporium cladosporioides* and *Aspergillus niger*, related to abundance of majority active substances. Minimum inhibitory concentration (MIC_{100} and MIC_{50}) was evaluated. GC–MS analysis revealed high abundance of highly effective phenolic compounds whose different molecular structures correlates with differences in EOs efficacy. The efficacy of some EOs, observed in our study, can be similar to the levels of some synthetic fungicides used in medicine and agriculture e.g. sometimes problematic azole-based formulations. Thanks to the EOs environmental safety and natural origin, they offer the potential to become an alternative where the use of synthetic fungicides is impossible for various reasons.

1. Introduction

Contamination by toxigenic, allergenic and pathogenic fungi (molds) poses a great challenge in all areas of human activities. Occurrence of these hazardous fungi and the potential of their suppression play a crucial role in terms of economy, hygiene and health. The immediate effect of hazardous fungi on human health is clearly the most serious threat. Production of hazardous secondary metabolites in the contaminated substrate, for example, in foods and other agricultural commodities stored at inadequate conditions, has been a well-known cause both of chronic and acute harm to human health worldwide (Tournas, 2005; Gottschalk et al., 2009). Similarly, contamination of the air by allergenic or toxic spores poses an immediate threat to human health. A huge rise in the concentration of hazardous spores in the air has been seen particularly in unventilated humid areas, such as poorly ventilated storage areas or human homes in areas struck by floods (Rao et al., 2007). Important species of this group, sometimes called indoor fungi, include *Alternaria alternata, Stachybotrys chartarum, Cladosporium cladosporioides and Aspergillus niger* (Johanning et al., 1996; Lee, 2003; Vermani et al., 2004; Wilson et al., 2004; Prester, 2011). These problematic species can cause numerous health problems due to their ability to produce extremely toxic (Amuzie et al., 2010) and/or allergenic secondary metabolites on the surface of their spores. Their inhalation is a common cause of

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http://dx.doi.org/10.1016/j.chemosphere.2014.05.014
0045-6535/

numerous allergies, asthma and other bronchopulmonary and other health problems, often associated also with the so called sick building syndrome (Johanning et al., 1996; Sander et al., 1998; Bush and Prochnau, 2004; Shah et al., 2004; Wilson et al., 2004; Saenz-de-Santamaria et al., 2006; Chou et al., 2008; Poll et al., 2009; Rid et al., 2009; Straus, 2009; Amuzie et al., 2010). Some of these species have been also mentioned as opportunistic pathogens that are able to directly infect internal animal or human tissues under certain conditions (Kim et al., 2003a; Shah et al., 2004; Xavier et al., 2008). Elimination of fungal occurrence in the cases above, such as contaminated stored foods, contaminated residential areas and serious human mycoses using synthetic fungicides tends to be highly problematic for many reasons. In this respect, the problem is posed particularly by acute and chronic toxicity of synthetic fungicidal components with harmful side effects on human health, the environment, and long-term persistence of their residues (Zarn et al., 2003; Nakanishi, 2007; Costa et al., 2008; Howard et al., 2008; Scordino et al., 2008; Gubbins and Heldenbrand, 2010).

In the light of these problems, there is a growing need to explore and develop new, environmentally safe substances which will undergo quick and natural degradation in the environment. Alternative methods of suppressing pathogenic and toxigenic fungi, based on the use of natural plant substances, often result in research focused on the development of highly effective essential oils. Plant essential oils have been suggested as alternative sources for antifungal treatment (Singh et al., 2009; Zabka et al., 2009; Kumar et al., 2010). The antifungal effect of individual essential oils is determined by representation and content of individual biologically active substances. Phenolic substances undoubtedly belong to components offering the highest antifungal efficacy. Natural phenolic substances are among the most antifungal active substances present in plant essential oils. In spite of their high antifungal, antibacterial and insecticidal efficacy, they show a very low toxic effect on homeothermic animals (Xu et al., 2008; Ahmad et al., 2011). For the purpose of our study, we focused on exploring the efficacy of 20 essential oils, widely used for medicinal purposes, that can be considered as components of environmentally safe botanical fungicides. Their biological efficacy was targeted against the complex of 4 important allergenic, toxigenic and pathogenic fungi *A. alternata, S. chartarum, C. cladosporioides and A. niger*. Individual efficacies were compared and explored up to the level of minimum inhibitory concentrations (MIC) together with the content of individual active substances.

2. Materials and methods

2.1. Origin and isolation of the essential oils

All plants within our experiment are widely used in medicine. Plant material from 20 plant species (Table 1) was purchased from Byliny Mikes Company, Czech Republic. Individual parts of the plants were dried at 40 °C. The dried samples were subjected to hydrodistillation for 2 h using a Clevenger-type apparatus. The oil obtained was separated from water and dried over anhydrous Na_2SO_4. All essential oils were stored at 4 °C until further assay.

2.2. Fungal strains

All target pathogenic and toxigenic fungal strains were obtained from collection of phytopathogenic fungi maintained in the Crop Research Institute, v.v.i., Czech Republic, Prague.

A. alternata, S. chartarum and *C. cladosporioides* strains were isolated originally from an infected stored plant material. *A. niger* was isolated from a contaminated stored corn. Strains were preserved on the slant agar (Potato Carrot Agar) at 4 °C. Subcultivations on Petri dishes and other manipulations with these strains were carried out in the Bio Security Level two (BSL 2) laboratory with respect to the spore toxicity health risk of some species used in our experiments.

2.3. Experimental design used to determination of inhibitory effect

Inhibitory effect of essential oils on mycelial radial growth of fungi was tested by the agar dilution method. Each essential oil was properly diluted in Potato Dextrose Agar (PDA) at concentration 1 μl mL^{-1}. The prepared Petri dishes (9.0 cm diameter) were aseptically inoculated with assay disc (0.4 cm) cuts from the periphery of 7 day-old culture of the target fungi. The control sets were prepared subsequently using sterile distilled water instead of oil. All experiments were performed in quadruplicates. The incubation was carried out at 21 °C for seven days. The percent inhibition of the radial growth of the target fungi was calculated according to the following formula. Percent inhibition = (DC–DT)/DC × 100, where DC is the colony diameter of the control sets and DT is the colony diameter of the treated sets. The minimum inhibitory concentration (MIC) of the essential oils with the most significant activity was determined by method of graded concentration of the oils (0.02–7 μl mL^{-1}) in the PDA. Cultivation was carried out the same way as before (at 21 °C, for seven days). The MIC_{100} was regarded as the lowest concentration of oil that did not permit any visible growth when compared with control sets. The MIC_{50} was regarded as the concentration of plant extract that results in a 50% inhibition of visible growth when compared to control sets (Zabka et al., 2009, 2011).

2.4. Statistical analysis

The Probit analysis was applied to assess the MIC_{50} and MIC_{100} values for each effective extract (Finney, 1971). The EPA Probit Analysis Program (Version 1.5) was used for statistical evaluation. The MIC values were statistically calculated and associated with Chi-square values significant at $P < 0.05$ level. MIC_{50} and MIC_{100} were assessed for each extract showing basic fungal growth inhibition higher than 50%.

2.5. Used method of chemical composition analysis

The identification of the major chemical components of the oil samples was done in a complete HP 6890 gas chromatograph using a mass selective detector HP 5973, equipped with Chemstation software and Wiley 275 spectra data. A HP-Innowax fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The chromatographic conditions were: column temperature 60 °C (8 min), 60–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min), interface 180 °C, split ratio 1:100, carrier gas, He (55.4 kPa), flow rate 1.0 ml/min, ionization energy 70 eV, mass range 40–350, volume injected 0.5 μl, solvent cut, 3.5 min. GC analysis was performed on a HP 5973 gas chromatograph with FID detector using a HP-Innowax fused silica capillary column (30 m × 0.25 mm, 0.50 μm film thickness). The chromatographic conditions were: column temperature 40 °C (8 min), 40–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min), injector temperature 250 °C, split ratio 1:50, detector temperature 250 °C, carrier gas hydrogen (34 kPa), flow rate 1.0 ml/min, volume injected 0.2 μL. The qualitative and quantitative chemical compositions of essential oils in terms of majority substances are reported in Table 4.

Table 1
Investigated plant material, source of essential oils.

Plant source of essential oil	Family	Origin	Plant part used	Yield (% w/w on dry weight basis)
Carum carvi L.	*Apiaceae*	Czech Republic	Seeds	2.2
Citrus aurantium L.	*Rutaceae*	Tunisia	Peel	1.5
Citrus bergamia Risso & Poit.	*Rutaceae*	USA	Peel	1.6
Coriandrum sativum L.	*Apiaceae*	USA	Seeds	2.2
Juniperus communis L.	*Cupressaceae*	Croatia	Aerial parts	1.1
Lavandula angustifolia Mill.	*Lamiaceae*	USA	Flower	3.6
Mentha arvensis L.	*Lamiaceae*	India	Aerial parts	1.0
Mentha pulegium L.	*Lamiaceae*	USA	Aerial parts	1.2
Ocimum basilicum L.	*Lamiaceae*	USA	Aerial parts	1.3
Ocimum citriodorum Vis.	*Lamiaceae*	USA	Aerial parts	1.2
Origanum majorana L.	*Lamiaceae*	Egypt	Aerial parts	1.2
Origanum vulgare L.	*Lamiaceae*	Turkey	Aerial parts	1.1
Pimenta racemosa (Mill.) J.W. Moore	*Myrtaceae*	Jamaican	Leaf	1.9
Rosmarinus officinalis L.	*Lamiaceae*	Corsica	Aerial parts	1.9
Salvia officinalis L.	*Lamiaceae*	Spain	Aerial parts	2.4
Salvia sterea L.	*Lamiaceae*	USA	Aerial parts	1.8
Tanacetum vulgare L.	*Asteraceae*	USA	Aerial parts	0.9
Thymus satureoides Coss. & Balansa.	*Lamiaceae*	Morocco	Aerial parts	1.6
Thymus vulgaris L.	*Lamiaceae*	Spain	Aerial parts	0.8
Zingiber cassumunar Roxb.	*Zingiberaceae*	Thailand	Root	0.9

3. Results

All the tested essential oils used in our study showed antifungal efficacy to varying extents. Significant differences in terms of efficacy were seen among individual essential oils. The values of essential screening inhibition are presented in Table 2. The tested essential oils could be divided in 3 groups based on achieved inhibition and based on the overall spectrum of inhibited pathogens. Essential oils from *Citrus aurantium*, *Citrus bergamia*, *Juniperus communis*, *Ocimum basalicum*, *Salvia officinalis*, *Tanacetum vulgare* and *Zingiber cassumunar* can be mentioned as those providing the least efficacy. This least effective group failed to reach 50% antifungal inhibitive effect in any one target pathogen. The second group comprising *C. aurantium*, *Levandula angustifolia*, *Mentha arvensis*, *Mentha pulegium* and *Salvia sclerea* exceeded the 50% inhibitory effect boundary level in all target pathogenic fungi except the most resistant fungal species *A. niger*. An exception can be found in this group of effective essential oils for *Origanum majorana* where the determined 50% efficacy threshold failed to be found not only for *A. niger*, but also for *C. cladosporioides*. The group of most effective essential oils including *Coriandrum sativum*, *Ocimum citriodorum*, *Origanum vulgare*, *Carum carvi*, *Pimenta racemosa*, *Thymus satureoides* and *Thymus vulgaris* achieved high inhibition levels up to 100% across the entire spectrum of target pathogenic fungi. In particular, this applies to *O. vulgare*, *P. racemosa*, *T. satureoides* and *T. vulgaris* where 100% inhibition levels were achieved for all target pathogens. MIC_{50} and MIC_{100} values were found in subsequent experiments and statistically determined for all essential oils whose inhibitory effects exceeded 50%. Based on the lowest determined MIC values, the highest antifungal efficacy of the aforementioned species *O. vulgare*, *P. racemosa* and *T. vulgaris* against target fungal species was clearly confirmed. The absolutely highest efficacy was observed for *O. vulgare* essential oil where the MIC_{50} values ranged from 0.007 μl mL^{-1} to 0.043 μl mL^{-1} and MIC_{100} values from 0.011 μl mL^{-1} to 0.092 μl mL^{-1} across the entire target fungal spectrum (Table 3). Compared to the values of other effective essential oils such as *C. carvi* or *C. sativum*, the MIC values clearly indicate that the efficacy determined for *O. vulgare* is ten times up to hundred times higher. Chemical compositions in terms of majority substances of individual essential oils are provided in

Table 2
Inhibitory effect of essential oils on pathogenic and toxinogenic fungi at concentration 1 μl mL^{-1}.

Esential oils	% Inhibition of target fungi (mean ± SE)			
	Alternaria alternata	*Stachybotrys chartarum*	*Cladosporium cladosporioides*	*Aspergillus niger*
Carum carvi	99.29 ± 0.05	100	100	100
Citrus aurantium	27.63 ± 0.05	20.31 ± 0.00	46.55 ± 0.05	14.86 ± 0.25
Citrus bergamia	18.42 ± 0.19	28.12 ± 0.00	18.96 ± 0.05	12.61 ± 0.19
Coriandrum sativum	100	98.73 ± 0.12	63.64 ± 0.08	65.32 ± 0.17
Juniperus communis	11.84 ± 0.09	31.03 ± 0.00	31.03 ± 0.00	1.80 ± 0.17
Mentha arvensis	100	99.33 ± 0.04	94.55 ± 0.06	48.15 ± 0.00
Mentha pulegium	88.57 ± 0.08	95.97 ± 0.11	91.82 ± 0.10	44.44 ± 0.10
Lavandula angustifolia	50.71 ± 0.31	77.85 ± 0.34	52.73 ± 0.00	22.84 ± 0.13
Ocimum basilicum	11.84 ± 0.15	25.78 ± 0.21	27.59 ± 0.06	4.05 ± 0.05
Ocimum citriodorum	100	98.66 ± 0.05	100	100
Origanum majorana	65.71 ± 0.08	93.29 ± 0.11	29.09 ± 0.17	25.93 ± 0.00
Origanum vulgare	100	100	100	100
Pimenta racemosa	100	100	100	100
Rosmarinus officinalis	14.47 ± 0.18	43.75 ± 0.00	27.59 ± 0.06	0.45 ± 0.05
Salvia officinalis	18.42 ± 0.05	13.58 ± 0.07	29.31 ± 0.05	0.90 ± 0.08
Salvia sclerea	65.00 ± 0.10	62.42 ± 0.54	52.81 ± 0.11	17.28 ± 0.17
Tanacetum vulgare	7.89 ± 0.17	4.68 ± 0.70	15.52 ± 0.05	6.31 ± 0.12
Thymus satureoides	100	100	100	100
Thymus vulgaris	100	100	100	100
Zingiber cassumunar	1.31 ± 0.05	33.59 ± 0.21	48.28 ± 0.10	21.17 ± 0.15

Table 3
Antifungal eficacy of essential oils against target fungal pathogens, values of MIC_{50} and MIC_{100}.

Esential oils	Target fungal species (μl mL^{-1})											
	Alternaria alternata			*Stachybotrys chartarum*			*Cladosporium cladosporioides*			*Aspergillus niger*		
	MIC_{50}	MIC_{100}	Chi[a]	MIC_{50}	MIC_{100}	Chi[a]	MIC_{50}	MIC_{100}	Chi[a]	MIC_{50}	MIC_{100}	Chi[a]
Carum carvi	0.305	0.589	0.685	0.154	0.260	0.254	0.317	0.368	2.904	0.518	0.701	3.033
Coriandrum sativum	0.478	0.946	2.123	0.478	0.902	2.321	0.833	1.732	0.066	0.786	1.527	0.006
Lavandula angustifolia	0.873	5.352	0.799	0.593	1.540	2.22	0.961	2.499	5.278	>1	–	–
Mentha arvensis	0.413	0.823	1.351	0.323	0.522	0.006	0.497	0.942	3.12	>1	–	–
Mentha pulegium	0.390	0.999	0.355	0.446	0.882	2.912	0.342	0.865	0.746	>1	–	–
Ocimum citriodorum	0.247	0.508	1.133	0.233	0.439	0.853	0.323	0.399	0.187	0.211	0.418	4.846
Origanum majorana	0.625	4.196	0.597	0.486	0.923	0.258	>1	–	–	>1	–	–
Origanum vulgare	0.034	0.092	0.125	0.007	0.011	0.021	0.028	0.066	2.811	0.043	0.072	1.211
Pimenta racemosa	0.128	0.393	0.829	0.054	0.075	0.221	0.045	0.055	2.526	0.042	0.059	2.221
Salvia sclerea	0.653	1.990	0.895	0.674	1.456	5.929	0.882	3.064	3.321	>1	–	–
Thymus satureoides	0.196	0.302	1.258	0.085	0.241	2.303	0.117	0.289	0.851	0.245	0.461	1.233
Thymus vulgaris	0.077	0.146	2.822	0.038	0.062	1.545	0.022	0.040	5.468	0.047	0.068	2.132

[a] Chi-square value, significant at $P < 0.05$ level.

Table 4. According to the GC–MS analysis performed, the most effective essential oils contained high amount of carvacrol 86.9% in the case *O. vulgare*, thymol 60.2% and eugenol 64% for *T. vulgaris* and *P. racemosa*, respectively. Relatively constant interspecies differences in terms of resistance against essential oils could be observed among target pathogens. The highest resistance against the used essential oils was exhibited by *A. niger* for which the highest MIC values were determined. Moreover, *A. niger* exhibited the highest resistance also in the sense of the lowest number of effective essential oils (only 7 of the 20 tested in total), with inhibitory effect over 50% at the initial concentration 1 μl mL^{-1}. On the contrary, *S. chartarum* was the least resistant species in most cases, showing the lowest MIC values.

4. Discussion

Our study compared antifungal efficacy of 20 different essential oils against 4 important toxigenic, allergenic and pathogenic fungi that pose medical, health-related as well as economic risks. As indicated by experimental results, it is now clear that the predominant majority of our tested essential oils provided effective growth inhibition among the fungal species above. Nevertheless, several extraordinarily effective candidates can be selected even in this group of effective essential oils, which in some cases exhibit an effect ten times up to hundred times higher. One of the main objectives of the study was to compare differences in efficacies and subsequently to select the most effective essential oils that could be considered as those actually most appropriate for the development and use of alternative, environmentally safe pesticides. Thanks to the high inhibitory efficacy and extremely low MIC values, essential oils from *O. vulgare*, *T. vulgaris* and *P. racemosa* can be indicated as essential oils providing extraordinary efficacy against the target fungal species: *A. alternata, S. chartarum, C. cladosporioides and A. niger*. The marked antifungal effect of *O. vulgare*, *T. vulgaris* and *P. racemosa* against the aforementioned toxigenic and allergenic fungal pathogens is most likely based on the high contents of these simple, natural phenolic compounds. As shown by GC–MS analysis for the most effective essential oils, majority presence especially of carvacrol and thymol was found for *O. vulgare* and *T. vulgaris*, and also of eugenol for *P. racemosa*. These substances can clearly be selected as the most effective phenolic compounds, especially carvacrol and thymol (Zabka and Pavela, 2013). The compounds are positional isomers with their phenolic hydroxyl group at a different location. Their molecular structure and relative position of functional groups are responsible for their strong ability to dissolve and accumulate in the cellular membrane, resulting in cellular membrane destabilization, which can be attributed to a more effective proton transfer disruption (Rao et al., 2010; Ahmad et al., 2011). Similar results were observed in a previous study of insecticidal properties in a complex of essential oils (Pavela, 2011). Thymol and carvacrol showed the highest efficacy against insects; this fact could be explained by membrane proton transfer disruption and consequently 4-aminobutyrate (GABA) blockade in the insect's nervous system. Although being of interest that GABA was previously found in the fungal cell metabolic pathways (Kumar and Punekar, 1997), antifungal properties of these compounds are connected simply to their ability to block ATP and ergosterol synthesis (Ultee et al., 2002; Ahmad et al., 2011). The high content of eugenol as the majority component of *P. racemosa* essential oil based on GC–MS analysis seems to be responsible for the third highest antifungal effect in our study. The slightly lower antifungal efficacy of eugenol than in the case of thymol and carvacrol could be explained by lower hydrophobicity and a different molecular structure of eugenol, especially due to presence of the methoxy group, which results in a lower ability to release a proton from the hydroxyl group (Ben Arfa et al., 2006). On the other hand, essential oils with a high content of eugenol provided a very good antifungal effect in a previous study (Zabka et al., 2009). This effect of high eugenol content was observed also in this study for the above mentioned *P. racemosa* essential oil. In addition, except for significant phenolic compounds, GC-MS analysis revealed a great number of various monoterpenes which are also described as antifungal (Garcia et al., 2008). However, their practical utilization is limited considering their rapid fumigation (Isman, 2000). Therefore, in our opinion, the aforementioned less volatile phenols play the main role in the antifungal effect of essential oils used in practical applications. In practical use, chemical composition of essential oils may generally provide an important factor with an impact on preserving their efficacy. Essential oils are exposed both to chemical and physical factors that enhance the loss of their biological activity. Oxidation of mono- and sesquiterpene compounds could lead to the loss of their biological activity. A higher content of more hydrogenated compounds leads to the highest susceptibility to oxidation (Kim et al., 2003b). Each essential oil could therefore be affected based on its chemical composition. In addition, temperature and light are also factors that enhance oxidation processes (Isman, 2000).

The efficacy of essential oils from *O. vulgare*, *T. vulgaris* and *P. racemosa*, observed in our study, achieves even the levels of some synthetic fungicides used in medicine and agriculture; for example, the widely used and often problematic azole-based formulations

Table 4
GC–MS analysis of effective essential oils.

Essential oil	Major compounds, FID%
Carum carvi	Carvon 68.7; limonen 21.5; alfa-pinen 6.2
Citrus aurantium	Linalool 45.5; limonen 15.5; linalool acetat 12.9; beta-pinen 11.5; alfa-terpineol 4.7; geranyl acetat 3.1; beta-E-ocimen 1.1
Coriandrum sativum	Linalool 66.7; limonen 5.7; alfa-pinen 4.1; geraniol 4.5; geranyl acetat 3.8; camphor 3.3; para-cymen 2.6; beta-pinen 2.4; alfa-terpineol 2.2
Lavandula angustifolia	Linalool acetat 31.8; linalool 30.8; lavandolol acetat 7.4; beta-Z-ocimen 4.3; alfa-humulen 1.5; beta-caryophyllen 4.2; borneol 2.8; terpin-4-ol 2.7; limonene 1.8; alfa-terpineol 1.7; beta-E-ocimen 1.6; 1,8-cineol 1.1
Mentha arvensis	Menthol 42.1; menthon 21.7; isomenthon 13.6; limonen 4.2; menthyl acetat 3.8; pulegon 2.4; isopulegol 1.6; beta-pinen 1.5; neo-menthol 1.4; piperiton 1.3;
Mentha pulegium	Pulegon 83.5; menthon 4.1; isomenthon 2.9; isopulegol 1.8; neo-iso-isopulegol 1.7; alfa-humulen 1.1
Ocimum citriodorum	Geranial 31.1; neral 23.6; linalool 16.4; limonene 8.9; 1,8-cineol 3.3; eugenol 2;; beta-caryophyllen 1.2; citronellal 1.2; myrcen 1.2; alfa-pinen 1.1
Origanum majorana	Terpin-4-ol 26.5; linalool 16.2; gamma-terpinen 12.5; trans-sabinen hydrat 8.2; alfa-terpinen 6.8; sabinen 3.8; alfa-terpineol 3.2; alfa-terpinolen 2.8; para-cymen 2.3; cis-sabinen hydrat 2; linalool acetat 2; beta-caryophyllen 1.9; beta-phellandren 1.6; eremophilen 1.4; menth-2-en-1-ol 1.3; limonene 1.1; myrcen 1.1
Origanum vulgare	Carvacrol 86.9; linalool 4.6; para-cymen 2.7; thymol 2; gamma-terpinen 1.4
Pimenta racemosa	Eugenol 64; myrcen 14.6; chavicol 7.7; beta-caryophyllen 4.9; limonene 2; linalool 1.6
Salvia sclerea	Linalool acetat 59.8; linalool 20.9; thunbergol 5.2alfa-terpineol 4.4; geranyl acetat 2.2; germacren D 1.7; beta-caryophyllen 1.4; neryl acetat 1.1; myrcen 1
Thymus satureoides	Borneol 29.8; thymol 12; carvacrol 10; alfa-terpineol 7.6; camphen 7.6; alfa-pinen 4.6; beta-caryophyllen 4.8; para-cymen 3.8; linalool 3.2; methyl ether thymol 2.9; bornyl acetat 1.8; terpin-4-ol 1.6; camphor 1.2; gamma-terpinen 1.1; cis-pinan 1
Thymus vulgaris	Thymol 60.2; para-cymen 19.9; carvacrol 5.9; linalool 5.8; geraniol 1.6; gamma-terpinen 1.4; beta-caryophyllen 1.1; terpinyl acetat 1.1

(Pujol et al., 2000; Zarn et al., 2003; Therese et al., 2006; Costa et al., 2008). On the contrary, natural essential oils from *O. vulgare*, *T. vulgaris* and *P. racemosa* may provide a similar antifungal effect, moreover free of any risk of hazardous residues forming in the environment. Thymol and carvacrol are significantly less cytotoxic than conventional antifungals (Ahmad et al., 2011). They are quickly metabolized and excreted. The main metabolic route is esterification. A minor pathway also consists in oxidation of the terminal methyl groups to primary alcohols (Austgulen et al., 1987). Thanks to this fact, they are subject to quick biodegradation in the organism and/or environment (Guenette et al., 2007; Hu and Coats, 2008). In addition, natural phenols such as thymol, carvacrol and eugenol, which are majority antifungal compounds of these essential oils, tend to be contrarily described in literature as, for example, anti-inflammatory, antibacterial, antifungal, anticarcinogenic, antiplatelet compounds and as compounds providing other diverse activities (Ruberto and Baratta, 2000; Baser, 2008; Landa et al., 2009; Kokoskova et al., 2011).

In conclusion, we can note that essential oils from *O. vulgare*, *T. vulgaris* and *P. racemosa*, particularly thanks to their naturally high content of phenols such as thymol, carvacrol or eugenol, evidently with the highest antifungal effects, provide a very promising and effective alternative in the field of antifungal applications. Thanks to their environmental safety and natural origin, they offer the potential to become an alternative particularly where the use of synthetic fungicides is impossible for various reasons. The practical importance of botanical pesticides based on essential oils shows a rising tendency every year, in accordance with the development of environmental trends and integrated management, gaining ever more ground in the control of harmful fungi.

Acknowledgement

This study was supported by Grants of the Czech Republic Ministry of Education, Youth and Sports (LH11133).

References

Ahmad, A., Khan, A., Akhtar, F., Yousuf, S., Xess, I., Khan, L.A., Manzoor, N., 2011. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur. J. Clin. Microbiol. Infect. Dis. 30 (1), 41–50.

Amuzie, C.J., Islam, Z., Kim, J.K., Seo, J.H., Pestka, J.J., 2010. Kinetics of Satratoxin G tissue distribution and excretion following intranasal exposure in the mouse. Toxicol. Sci. 116 (2), 433–440.

Austgulen, L.T., Solheim, E., Scheline, R.R., 1987. Metabolism in rats of para-cymene derivatives – carvacrol and thymol. Pharmacol. Toxicol. 61 (2), 98–102.

Baser, K.H.C., 2008. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr. Pharm. Des. 14 (29), 3106–3119.

Ben Arfa, A., Combes, S., Preziosi-Belloy, L., Gontard, N., Chalier, P., 2006. Antimicrobial activity of carvacrol related to its chemical structure. Lett. Appl. Microbiol. 43 (2), 149–154.

Bush, R.K., Prochnau, J.J., 2004. Alternaria-induced asthma. J. Allergy Clin. Immunol. 113 (2), 227–234.

Costa, L.G., Giordano, G., Guizzetti, M., Vitalone, A., 2008. Neurotoxicity of pesticides: a brief review. Front. Biosci. 13, 1240–1249.

Finney, D.J., 1971. Probit analysis. Cambridge University Press, London.

Garcia, R., Alves, E.S.S., Santos, M.P., Aquije, G., Fernandes, A.A.R., dos Santos, R.B., Ventura, J.A., Fernandes, P.M.B., 2008. Antimicrobial activity and potential use of monoterpenes as tropical fruits preservatives. Braz. J. Microbiol. 39 (1), 163–168.

Gottschalk, C., Barthel, J., Engelhardt, G., Bauer, J., Meyer, K., 2009. Simultaneous determination of type A, B and D trichothecenes and their occurrence in cereals and cereal products. Food Addit. Contam. Part A-Chem. 26 (9), 1273–1289.

Gubbins, P.O., Heldenbrand, S., 2010. Clinically relevant drug interactions of current antifungal agents. Mycoses 53 (2), 95–113.

Guenette, S.A., Ross, A., Marier, J.F., Beaudry, F., Vachon, P., 2007. Pharmacokinetics of eugenol and its effects on thermal hypersensitivity in rats. Eur. J. Pharmacol. 562 (1–2), 60–67.

Howard, A., Hoffman, J., Sheth, A., 2008. Clinical application of voriconazole concentrations in the treatment of invasive aspergillosis. Ann. Pharmacother. 42 (12), 1859–1864.

Hu, D., Coats, J., 2008. Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory. Pest Manag. Sci. 64 (7), 775–779.

Chou, H., Tam, M.F., Lee, L.H., Chiang, C.H., Tai, H.Y., Panzani, R.C., Shen, H.D., 2008. Vacuolar serine protease is a major allergen of Cladosporium cladosporioides. Int. Arch. Allergy Immunol. 146 (4), 277–286.

Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop Prot. 19 (8–10), 603–608.

Johanning, E., Biagini, R., Hull, D., Morey, P., Jarvis, B., Landsbergis, P., 1996. Health and immunology study following exposure to toxigenic fungi (Stachybotrys chartarum) in a water-damaged office environment. Int. Arch. Occup. Environ. Health 68 (4), 207–218.

Kim, S.H., Yong, H.C., Yoon, J.H., Youn, H.Y., Yoshioka, N., Kano, R., Hasegawa, A., 2003a. Aspergillus niger pulmonary infection in a dog. J. Vet. Med. Sci. 65 (10), 1139–1140.

Kim, S.I., Roh, J.Y., Kim, D.H., Lee, H.S., Ahn, Y.J., 2003b. Insecticidal activities of aromatic plant extracts and essential oils against Sitophilus oryzae and Callosobruchus chinensis. J. Stored Prod. Res. 39 (3), 293–303.

Kokoskova, B., Pouvova, D., Pavela, R., 2011. Effectiveness of plant essential oils against *Erwinia Amylovora*, *Pseudomonas Syringae* Pv. Syringae and associated saprophytic bacteria on/in host plants. J. Plant Pathol. 93 (1), 133–139.

Kumar, A., Shukla, R., Singh, P., Anuradha, Dubey, N.K., 2010. Efficacy of extract and essential oil of Lantana indica Roxb. against food contaminating moulds and aflatoxin B-1 production. Int. J. Food Sci. Technol. 45 (1), 179–185.

Kumar, S., Punekar, N.S., 1997. The metabolism of 4-aminobutyrate (GABA) in fungi. Mycol. Res. 101, 403–409.

Landa, P., Kokoska, L., Pribylova, M., Vanek, T., Marsik, P., 2009. In vitro anti-inflammatory activity of carvacrol: Inhibitory effect on COX-2 catalyzed prostaglandin E-2 biosynthesisb. Arch. Pharm. Res. 32 (1), 75–78.

Lee, T.G., 2003. Health symptoms caused by molds in a courthouse. Arch. Environ. Health 58 (7), 442–446.

Nakanishi, T., 2007. Potential toxicity of organotin compounds via nuclear receptor signaling in mammals. J. Health Sci. 53 (1), 1–9.
Pavela, R., 2011. Insecticidal and repellent activity of selected essential oils against of the pollen beetle, Meligethes aeneus (Fabricius) adults. Ind. Crop. Prod. 34 (1), 888–892.
Poll, V., Denk, U., Shen, H.D., Panzani, R.C., Dissertori, O., Lackner, P., Hemmer, W., Mari, A., Crameri, R., Lottspeich, F., Rid, R., Richter, K., Breitenbach, M., Simon-Nobbe, B., 2009. The vacuolar serine protease, a cross-reactive allergen from Cladosporium herbarum. Mol. Immunol. 46 (7), 1360–1373.
Prester, L., 2011. Indoor exposure to mould allergens. Arh. Hig. Rada. Toksikol. 62 (4), 371–380.
Pujol, I., Aguilar, C., Gene, J., Guarro, J., 2000. In vitro antifungal susceptibility of Alternaria spp. and Ulocladium spp. J. Antimicrob. Chemother. 46 (2), 337–338.
Rao, A., Zhang, Y.Q., Muend, S., Rao, R., 2010. Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. Antimicrob. Agents Chemother. 54 (12), 5062–5069.
Rao, C.Y., Riggs, M.A., Chew, G.L., Muilenberg, M.L., Thorne, P.S., Van Sickle, D., Dunn, K.H., Brown, C., 2007. Characterization of airborne molds, endotoxins, and glucans in homes in New Orleans after Hurricanes Katrina and Rita. Appl. Environ. Microbiol. 73 (5), 1630–1634.
Rid, R., Onder, K., MacDonald, S., Lang, R., Hawranek, T., Ebner, C., Hemmer, W., Richter, K., Simon-Nobbe, B., Breitenbach, M., 2009. Alternaria alternata TCTP, a novel cross-reactive ascomycete allergen. Mol. Immunol. 46 (16), 3476–3487.
Ruberto, G., Baratta, M.T., 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem. 69 (2), 167–174.
Saenz-de-Santamaria, M., Postigo, I., Gutierrez-Rodriguez, A., Cardona, G., Guisantes, J.A., Asturias, J., Martinez, J., 2006. The major allergen of Alternaria alternata (Alt a 1) is expressed in other members of the Pleosporaceae family. Mycoses 49 (2), 91–95.
Sander, I., Raulf-Heimsoth, M., Siethoff, C., Lohaus, C., Meyer, H.E., Baur, X., 1998. Allergy to Aspergillus-derived enzymes in the baking industry: identification of beta-xylosidase from Aspergillus niger as a new allergen (Asp n 14). J. Allergy Clin. Immunol. 102 (2), 256–264.
Scordino, M., Sabatino, L., Traulo, P., Gagliano, G., Gargano, M., Panto, V., Gambino, G.L., 2008. LC/MS/MS detection of fungicide guazatine residues for quality assessment of commercial citrus fruit. Eur. Food Res. Technol. 227 (5), 1339–1347.
Shah, A., Maurya, V., Panjabi, C., Khanna, P., 2004. Allergic bronchopulmonary aspergillosis without clinical asthma caused by Aspergillus niger. Allergy 59 (2), 236–237.
Singh, P., Kumar, A., Dubey, N.K., Gupta, R., 2009. Essential oil of aegle marmelos as a safe plant-based antimicrobial against postharvest microbial infestations and aflatoxin contamination of food commodities. J. Food Sci. 74 (6), M302–M307.
Straus, D.C., 2009. Molds, mycotoxins, and sick building syndrome. Toxicol. Ind. Health 25 (9–10), 617–635.
Therese, K.L., Bagyalakshmi, R., Madhavan, H.N., Deepa, P., 2006. In-vitro susceptibility testing by agar dilution method to determine the minimum inhibitory concentrations of amphotericin B, fluconazole and ketoconazole against ocular fungal isolates. Indian J. Med. Microbiol. 24 (4), 273–279.
Tournas, V.H., 2005. Spoilage of vegetable crops by bacteria and fungi and related health hazards. Crit. Rev. Microbiol. 31 (1), 33–44.
Ultee, A., Bennik, M.H.J., Moezelaar, R., 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68 (4), 1561–1568.
Vermani, M., Vijayan, V.K., Kausar, M.A., Agarwal, M.K., 2004. Quantification of airborne Aspergillus allergens: redefining the approach. J. Asthma 47 (7), 754–761.
Wilson, S.C., Brasel, T.L., Carriker, C.G., Fortenberry, G.D., Fogle, M.R., Martin, J.M., Wu, C., Andriychuk, L.A., Karunasena, E., Straus, D.C., 2004. An investigation into techniques for cleaning mold-contaminated home contents. J. Occup. Environ. Hyg. 1 (7), 442–447.
Xavier, M.O., Sales, M.D.U., Camargo, J.D.P., Pasqualotto, A.C., Severo, L.C., 2008. Aspergillus niger causing tracheobronchitis and invasive pulmonary aspergillosis in a lung transplant recipient: case report. Rev. Soc. Bras. Med. Trop. 41 (2), 200–201.
Xu, J., Zhou, F., Ji, B.P., Pei, R.S., Xu, N., 2008. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Lett. Appl. Microbiol. 47 (3), 174–179.
Zabka, M., Pavela, R., 2013. Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi. Chemosphere 93 (6), 1051–1056.
Zabka, M., Pavela, R., Gabrielova-Slezakova, L., 2011. Promising antifungal effect of some Euro-Asiatic plants against dangerous pathogenic and toxinogenic fungi. J. Sci. Food. Agric. 91 (3), 492–497.
Zabka, M., Pavela, R., Slezakova, L., 2009. Antifungal effect of Pimenta dioica essential oil against dangerous pathogenic and toxinogenic fungi. Ind. Crop. Prod. 30 (2), 250–253.
Zarn, J.A., Bruschweiler, B.J., Schlatter, J.R., 2003. Azole fungicides affect mammalian steroidogenesis by inhibiting sterol 14 alpha-demethylase and aromatase. Environ. Health Perspect. 111 (3), 255–261.