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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.

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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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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.