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Antifungal effect of five essential oils against important pathogenic fungi of cereals

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ABSTRACT

Essential oils from five species of medicinal and food plants were tested as mycelial growth inhibitors against five important pathogenic fungal species that cause stem, leaf and ear diseases of cereals. An agar dilution method was used to determine the inhibitory effect and effective dose of essential oils extracted from *Pimpinella anisum*, *Thymus vulgaris*, *Pelargonium odoratissimum*, *Rosmarinus officinalis* and *Foeniculum vulgare* on the fungi *Oculimacula yallundae*, *Microdochium nivale*, *Zymoseptoria tritici*, *Pyrenophora teres* and *Fusarium culmorum*. All essential oils used in our experiment affected the growth of these fungi. Ultimately, the best antifungal activity (on the basis of inhibitory effect) was demonstrated by *Thymus vulgaris*. The chemical compositions of the essential oils were determined by gas chromatography/mass spectrometry analysis.

1. Introduction

Cereal grains constitute the most important human food, serving as the primary source of calories for the majority of the world's population (Lorenz and Lee, 1977). Cereals can be infected by a number of fungal pathogens, such as *Oculimacula yallundae*, *Microdochium nivale*, *Mycosphaerella graminicola*, *Pyrenophora teres*, *Fusarium* spp. and others. Cereal eyespot is a fungal disease that is widespread in cool, wet growing areas of the world. The disease is caused by the pathogen species *O. yallundae* and *O. acuformis* (Crous et al., 2003). High incidence of eyespot on stem bases can lead to significant economic losses (Hardwick et al., 2001). The fungal pathogen *M. nivale* (teleomorph *Monographella nivalis*) is among the most important seed- and soil-borne pathogens of winter cereals. The main hosts of *M. nivale* are wheat, rye, triticale, barley and oats. Infection by *M. nivale* can result in severe yield losses due to reduced emergence and seedling blight. *M. nivale* is also involved in the cereal stem–base disease complex. A range of different *Fusarium* species have been associated with Fusarium head blight of cereals, but *Fusarium graminearum* (teleomorph *Gibberella zeae*), *Fusarium culmorum* and *Fusarium avenaceum* (teleomorph *Gibberella avenacea*) appear to predominate, depending on climatic conditions (Parry et al., 1995). Apart from losses in grain yield and reductions in baking and seed quality, the major risk due to *Fusarium* head blight is contamination of the crop with toxic fungal secondary metabolites known as mycotoxins, which have negative consequences in human food and animal feed (Placinta et al., 1999). *M. graminicola* is the teleomorph of *Zymoseptoria tritici*. This pathogen is the causal agent of Septoria tritici blotch of wheat. Septoria tritici blotch is currently the most important foliar disease of wheat in Europe and many other temperate parts of the world. Net blotch is a foliar disease of barley that has a worldwide distribution and can cause substantial yield losses (Steffenson et al., 1991). The causal agent is the heterothallic ascomycete *P. teres* (anamorph: *Drechslera teres*).

The intensification of agricultural production to satisfy food needs is increasing the importance and destructiveness of fungal diseases attacking cultivated crops. Synthetic pesticides play a major role today in crop protection programmes, and the need for

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pesticides is growing with increasing production intensification. The widespread use of pesticides has resulted in the development of pest resistance, pest resurgences, outbreaks of new pests, toxicity to non-target organisms and hazardous effects on the environment, thus endangering ecosystem sustainability (Jeyasankar and Jesudasan, 2005; Ntalli and Menkissoglu-Spiroudi, 2011). It has been occasionally suggested that diseases such as cancer (Klotz et al., 1997; Tessier and Matsumura, 2001; Parron et al., 2010), allergies, neurological disorders, endocrine disruption (McKinlay et al., 2008; Mnif et al., 2011) and reproductive disorders (Roeleveld and Bretveld, 2008; Wilson et al., 2009) may be connected to pesticide exposure. For these reasons, it is necessary to seek new plant protection alternatives that are environmentally and medically acceptable. The use of botanical pesticides appears to be an acceptable alternative. Inasmuch as there are not sufficient plant-based extracts for such purposes, it is necessary to find promising, new plant extracts or substances that could lead to new botanical pesticides (Pavela, 2010; Pavela et al., 2013).

Essential oils, also known as volatile oils, are complex mixtures of volatile compounds biosynthesised by plants. These mainly include terpenes, terpenoids, as well as aromatic and aliphatic compounds, all of which are characterized by low molecular weight (Bassole and Juliani, 2012). Their insecticidal (Regnault-Roger, 1997; Pavela, 2011), antimicrobial (Deba et al., 2008), antiviral (Rao et al., 1986; Bishop, 1995) and antifungal (Bouchra et al., 2003; Daferera et al., 2003; Sokmen et al., 2004; Vilela et al., 2009; Zabka et al., 2009; Zabka and Pavela, 2013) effects and activities against nematodes (Douda et al., 2010) have been previously examined. The most attractive aspect of using essential oils as fungicides (or pesticides generally) is their low toxicity for mammals (Isman, 2000).

In the present study, we describe *in vitro* antifungal activity of some essential oils on significant fungal plant pathogens. The pathogenic species selected for testing in this study are among the most serious cereal pathogens. They are causative agents of diseases causing serious losses to agricultural production. Some of them have not been tested for the effectivity of essential oils. The objective of the study was to select the most effective essential oil that would be promising for the development of new botanical fungicides against a spectrum of fungal pathogens causing the most serious cereal diseases. When selecting the essential oils, their prices, the availability of the plant species used for producing those oils, and the potential for growing these in Central European climatic conditions all were taken into account.

2. Materials and methods

2.1. Origin and isolation of essential oils

The essential oils used in this study were purchased from Essential Oil University, 16224 Charlestown-Bethlehem Road, Charlestown, IN 47111, USA. The standards used to identify the essential oil components were purchased from Sigma–Aldrich (Prague, Czech Republic), and hexane (Merck, Prague, Czech Republic) was used as a solvent for chemical analysis.

2.2. Fungal strains

Two strains were used from each of the 5 species of the tested fungi. All isolates were acquired in the territory of the Czech Republic. *O. yallundae* strains Oy13 and Oy14 and *M. nivale* strains Mn177 and Mn30 were isolated from the stem bases of wheat collected in April 2013. *Pyrenophora teres* Ptt52 and Ptt17 strains were collected from leaves of spring barley collected in May 2013. *F. culmorum* strains Fc107 (year of collection 2010) and Fc289 (year of collection

Table 1
Inhibition effect of essential oils on fungal pathogens at concentration 1 μL mL^{-1}.

Essential oil	% Inhibition of target fungi (mean ± SE)									
	M. nivale		*P. teres*		*F. culmorum*		*Z. tritici*		*O. yallundae*	
	Mn177	Mn30	Ptt52	Ptt17	Fc107	Fc289	Zt88	Zt96	Oy13	Oy14
P. anisum	33.59 ± 9.21	34.38 ± 3.83	80.79 ± 5.90	57.3 ± 9.27	10.53 ± 1.89	4.94 ± 3.12	42.67 ± 8.78	63.71 ± 8.15	11.82 ± 2.79	41.51 ± 3.9
T. vulgaris	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	99.71 ± 0.73	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
P. odoratissimum	37.5 ± 3.83	67.19 ± 1.56	100 ± 0.00	100 ± 0.00	66.76 ± 14.10	65.45 ± 18.75	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
F. vulgare	33.59 ± 4.06	41.41 ± 5.58	59.3 ± 9.23	57.3 ± 5.02	21.6 ± 3.66	18.97 ± 6.32	66.67 ± 10.21	72.5 ± 6.86	55.45 ± 4.72	60.38 ± 4.8
R. officinalis	5.47 ± 3.41	1.56 ± 0.53	23.81 ± 4.61	20.76 ± 11.4	14.4 ± 3.12	18.81 ± 3.88	17.67 ± 8.07	24.17 ± 15.85	9.09 ± 4.45	2.86 ± 1.71