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The antifungal activity of essential oils in combination with warm air flow against postharvest phytopathogenic fungi in apples

Adela Frankova [a], Jakub Smid [a], Andrea Bernardos [b], Alena Finkousova [a], Petr Marsik [c, d], David Novotny [e], Veronika Legarová [c], Josef Pulkrabek [a], Pavel Kloucek [c, *]

[a] Czech University of Life Sciences Prague, Department of Crop Production, Kamycka 129, Prague 165 21, Czech Republic
[b] Centro de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Camino de Vera s/n, E-46022 Valencia, Spain
[c] Czech University of Life Sciences Prague, Department of Quality of Agricultural Products, Kamycka 129, Prague 165 21, Czech Republic
[d] Institute of Experimental Botany, Academy of Sciences of the Czech Republic, v.v.i., Rozvojova 263, 165 02 Prague 6, Czech Republic
[e] Crop Research Institute, Division of Crop Protection and Plant Health, Drnovská 507/73, 161 06, Prague 6, Czech Republic

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ABSTRACT

Essential oils (EOs) are strong plant-derived antimicrobials. For their efficient use in the agri-food industry, the problems with technology of their application have to be solved. *In vitro* antifungal activity of cinnamon, oregano, lemongrass and clove essential oils (EOs) was tested by innovative method using EO in combination with warm air flow (WAF). EOs in concentrations from 0.25 to 512 μL/L of air were tested against eleven phytopathogenic fungi. Application of EOs in concentration of 4 and 16 μL/L by WAF method was used for treatment of apples inoculated with *Penicillium expansum*. Detailed sensory analysis of treated apples was performed. The WAF was more effective compare to standard disc volatilization method (DVM), the average minimum inhibitory concentrations *in vitro* were 5.6 μL/L during 5 min WAF treatment, compared to 136 μL/L during the DVM 24 h treatment. EOs applied by WAF delayed the incidence and development of *P. expansum* on apples with minimal adverse effect on their sensory profile. The WAF treatment could be considered for the development of antifungal treatments in the agri-food industry.

1. Introduction

Nowadays, field crops and their products are routinely treated with synthetic protection agents. Although modern fungicides and improved storage technologies has greatly extended the shelf life of agricultural products, postharvest losses still vary from an estimated 5% to more than 20% in industrialized countries. Losses can be as high as 50% in developing countries (Janisiewicz & Korsten, 2002), with molds causing more than 70% of losses in fruits and vegetable storage (Ayala-Zavala, González-Aguilar, & Del-Toro-Sánchez, 2009). Moreover, environmental and human health risks associated with the use of synthetic pesticides, together with the interest of people for healthy living, call for organic food production. However, in organic farming most of the synthetic fungicides have been prohibited due to their negative properties. For all of these reasons, many of the major pesticide manufacturers are focusing on the bio-pesticide industry. This interest in bio-pesticides is partly in response to the demands of major food buyers like Sysco, Wal-Mart and McDonald's, whose suppliers should comply with “sustainable” agricultural practices (Popp, Pető, & Nagy, 2012). These practices have to be highly effective without any adverse effect on human health, the environment, or plants themselves, to allow farmers to limit or eliminate the use of synthetic chemical fungicides (Tripathi & Dubey, 2004; Valero & Giner, 2006).

Natural products in general are an alternative to synthetic pesticides, and among them, EOs are typical antimicrobial agents without harmful residues. Since the 1990s, EOs have been widely studied for their antimicrobial activity. Many of them (e.g. thyme, oregano, clove, cinnamon, horseradish) have been found to be strong antifungals (Prakash, Kedia, Mishra, & Dubey, 2015) not only in direct contact but in the vapour phase as well (Lopez, Sanchez, Batlle, & Nerin, 2005). Antimicrobial activity in the vapour phase allows treatment without soaking or immersing, which could be beneficial in different types of treatment technologies. On the other

* Corresponding author.
E-mail address: kloucek@af.czu.cz (P. Kloucek).

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hand, in higher concentrations EOs could have an impact on the sensory properties of the treated products or even cause damage to plant tissues (Dhima, Vasilakoglou, Gatsis, Panou-Philotheou, & Eleftherohorinos, 2009).

To develop treatment without unfavourable effects, the combination of EOs with another preservation method is requested. As previously reported, the combination of EO with mild heat in a liquid environment allows use shorter treatment time or lower temperature while maintaining the same effect on bacterial pathogens (Espina et al., 2011). Moreover, antifungal activity of volatile compounds increases with vapour pressure, which is positively correlated with temperature, as has been detected for hexanal (Gardini, Lanciotti, Caccioni, & Guerzoni, 1997). An analogous relation is expected with the antifungal activity of EOs due to similar physicochemical properties and antimicrobial modes of action (Lanciotti et al., 2004). Our hypothesis, therefore, is that the increased temperature will increase activity and air flow will facilitate faster evaporation, which allows quick attainment of a homogenous atmosphere in the treated space.

Therefore, the aims of this study were to evaluate the minimum inhibitory concentrations (MICs) of EOs from clove, cinnamon, lemongrass and oregano by a new method combining EO and warm air flow (WAF) against eleven phytopathogenic fungi, to compare the effectiveness of the new method with conventional vapour phase tests. For the evaluation of the WAF treatment on model agriculture products apples inoculated with *Penicillium expansum* were treated and their sensory acceptability was evaluated.

2. Materials and methods

2.1. Essential oils

EOs from *Origanum vulgare* L. (carvacrol 64.56%; p-cymene 5.16%; thymol 2.93%), *Cinnamomum zeylanicum* Blume (Z-cinnamaldehyde 73.06%; limonene 4.98%; linalool 4.97%; cinnamyl acetate 3.70%; eugenol 3.54%), *Caryophyllus aromaticus* L. (eugenol 82.32%; β-caryophyllene 14.44%), *Cymbopogon citratus* Stapf (neral 45.30%; verbenol 33.49%; nerol 3.96%; nerol acetate 3.27%) were purchased from Biomedica s. r.o (Prague, CZ). The composition of used EOs was identified by GC–MS method. The procedure is described by Frankova et al (Frankova, Smid, Kloucek, & Pulkrabek, 2014). In both *in vitro* and *in vivo* assays the same EOs were used.

2.2. In vitro antifungal assay

2.2.1. Microorganisms and preparation of fungal inocula

Eleven phytopathogenic fungal strains were tested. *Botrytis cinerea* Pers. ATCC 12481; *Dendryphion penicillatum* (Corda) Fr. DSM 62543; *Helminthosporium solani* Durieu & Mont. CCM F-511 and *Phoma foveata* Foister CCM F-301 were cultivated on Potato Dextrose Agar (200 g scrubbed and diced potato in 1000 ml distilled water, 15 g agar, 20 g dextrose; pH ± 5.6). *Alternaria alternata* (Fr.) Keissl. CCM 8326; *Aspergillus niger* Tiegh. ATCC 6275; *Cladosporium cucumerinum* Ellis & Arthur; *Claviceps purpurea* (Fr.) Tul. ATCC 20103; *Monilia fructigena* (Pers.) Pers. CCM F-300; *Penicillium digitatum* (Pers.) Sacc. CCM F-382 and *P. expansum* Link ATCC 1117 were cultivated on Sabouraud Dextrose Agar (Oxoid, CZ). The strains were purchased either from the Czech Collection of Microorganisms, Brno, Czech Republic (CCM) or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSM).

The inocula were prepared by dissolving the spores and hyphae from 14-day-old actively growing cultures on agar medium in Mueller-Hinton Broth (MHB) (Oxoid, Brno, CZ) with 0.5% Polysorbate 80 (Sigma–Aldrich). The collected mixture was diluted in MHB and quantified at UV–VIS spectrophotometer Helios ε (Spectronic Unicam, Cambridge, UK) to absorbance 1.5 A at 700 nm (10^5 CFU/mL). The *C. purpurea, M. fructigena* and *P. foveata* do not produce spores, consequently, the inocula contained only mycelium.

2.2.2. Warm air flow treatment (WAF)

To maintain precise temperatures for treatment and EO evaporation, the antifungal tests were performed in a modified gas chromatograph (Hewlett Packard, 5890A). Treatment chamber (glass case with one open side facing the GC fan, inner dimensions 286 × 192 × 260 mm, volume 20L) was inserted into the oven instead of the GC's door and sealed with silicon tape to prevent escape of EO vapours. The heater was dismantled from FID detector and mounted into the treatment chamber. It was used for vaporization of EOs, which were applied to the filter paper wrapped over the heater (Fig. 1). During the 5-min treatment, the treatment chamber temperature was set to 40 °C and the heater temperature to 150 °C. After each run, the treatment chamber was cleaned with ethanol and the oven was heated to 150 °C and then vented. The effectiveness of the cleaning procedure was checked by solid phase micro extraction (SPME) with GC analysis. Petri dishes (PD, 55 mm diameter) filled with 5 ml of agar were inoculated with 50 μL of fungal inocula which was evenly distributed over the whole surface of agar. After that PDs were placed on the bottom of the treatment chamber and treated with WAF. Each treatment run consisted of six PD, five were inoculated with different fungi and one left as contamination control. EOs were tested in concentrations 0.25, 0.5, 1, 2, 4, 8, 16, and 32 μL/L. The effect of WAF itself on the fungal growth was evaluated as well. As a result, there was no inhibition when PD were treated only by WAF. The fungal growth was evaluated after three days of incubation in PD at 25 °C. The lowest concentration completely inhibiting fungal growth has been considered as the MIC. All tests were carried out in triplicate.

2.2.3. Disc volatilization method (DVM)

The disc volatilization method (DVM) described by Kloucek et al. (2012) was used as our comparative method. In brief, tests were performed in 90 mm Petri dishes divided into four sections with 5 ml of agar in each; three compartments were inoculated with

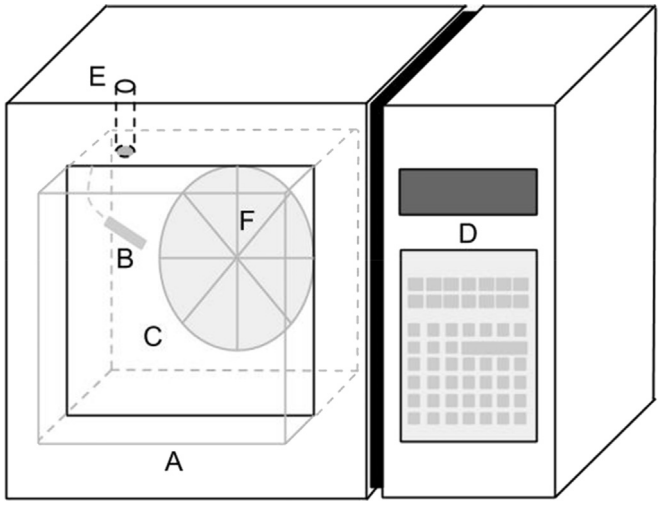

Fig. 1. Gas chromatograph (5890A GAS Chromatograph, Hewlett Packard) modified as the warm air flow treatment chamber. Petri dishes inoculated with fungal pathogens were placed on the bottom of glass case. A – glass case (grey lines); B –heater for evaporation of essential oils; C – oven; D – control panel; E – SPME inlet with septa; F – fan.