ANTIMICROBIAL PROTECTION OF POTATOES USING COMBINATION OF ESSENTIAL OILS AND WARM AIR FLOW

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ABSTRACT

Environmental and human health issues associated with the use of synthetic pesticides, together with the interest of people to healthy eating, call for the development of alternative antimicrobial treatment methods in crop production. Essential oils (EOs) are one of the most antimicrobial active plant secondary metabolites. The aim of this study is to evaluate the antifungal activity of three EOs using new Warm air flow treatment method (WAF). EOs from clove (*Caryophyllus aromaticus*), cinnamon (*Cinnamomum zeylanicum*) and oregano (*Origanum vulgare*) in 4 and 16 $\mu l.l^{-1}$ concentrations was tested in vivo on potatoes against *Phoma foveata.* Potatoes treated by the same manner have been analyzed for their sensory properties. High inhibition was found on potatoes treated with *O. vulgare* EO – the mean of growth were 0.27 mm (16 $\mu l.l^{-1}$) and 2.24 (4 $\mu l.l^{-1}$) and *C. Aromaticus* 1.8 mm (16 $\mu l.l^{-1}$) and 2.87 mm (4 $\mu l.l^{-1}$). Statistically significant differences was found only between two tested samples in organoleptic analyzes. To our knowledge, never have been obtained in such low concentrations inhibiting fungal growth as in our study even in combination with any physical or chemical treatment. Method using evaporation and distribution of EO with warm air flow is suitable for future utilization in antimicrobial protection of any fruit and vegetables. EO vapors with warm air flow could be also applicable e.g. in seed treatment or field applications as well as postharvest treatment of different agricultural products, instead of synthetic fungicides, preferentially by organic farmers.

Key words: hurdle technology; shelf-life; storage; organic farming; *Solanum tuberosum*

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INTRODUCTION

Modern agriculture leads to several major problems in plant production, such as soil degradation, erosion, and problems related to use of synthetic pesticides: loss of biodiversity, disruption of ecosystem and environmental contamination with residues. Moreover, target organisms often become resistant to commonly used pesticides (Shaw 1993). Environmental and human health issues associated with the use of synthetic pesticides, together with the interest of people to healthy eating, call for the development of alternative antimicrobial treatment methods in crop production. Required practices should be highly effective without any adverse effect on human health, environment and plants themselves, allowing to limit or eliminate the use of synthetic chemical fungicides (Tripathi and Dubey 2004).

Essential oils (EOs) are some of the most antimicrobial active plant secondary metabolites, their antimicrobial activity has been widely studied and many of them (e.g. thyme, oregano, clove, cinnamon, horseradish etc.) has been found as strong antimicrobials in last decades. The compounds such as carvacrol, thymol, carvone, eugenol, citran, perillaldehyde, cinnamaldehyd, (Burt 2004; Valverde et al. 2005) citral and geraniol (Inouye et al. 2001) has been found as strongest antimicrobial agents. Arguably, the most interesting area of application for EOs is the inhibition of growth and reduction in numbers of the pathogens (Burt 2004). The EOs can reduce microbial growth in vapor phase similarly as by direct contact (Lopez et al. 2005), so the treatment only by EO vapors is allowed. On the other hand, EOs can cause damage of plant tissues and have impact on organoleptic properties of treated products (Dhima et al. 2009). Currently, development of application method without unfavorable effect on treated products is the main deal for utilization of EOs as antimicrobial agents.

The aim of this study is to evaluate the antifungal activity of three EOs using new Warm air flow treatment method (WAF). EOs from clove (*Caryophyllus aromaticus*), cinnamon (*Cinnamomum zeylanicum*) and oregano (*Origanum vulgare*) in 4 and 16 $\mu l.l^{-1}$ concentrations was tested in vivo on potatoes against *Phoma foveata.* Potatoes treated by the same manner have been analyzed for their sensory properties.

MATERIAL AND METHODS

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). For identification of EOs constituents GC-MS (Varian, Santa Clara, CA, USA) and for relative quantification of EO constituents Agilent 6890 GC-FID (Agilent Technologies, Palo Alto, CA, USA) was used.

Potatoes "Red Anna" were obtained from Department of Crop Production field trials in Uhrivenes. Fungal strain *Phoma foveata* Foister CCM F-301 were purchased from Czech Collection of Microorganisms, Brno, Czech Republic. Inocula were made by dissolving the hyphae from actively growing cultures on agar medium in Mueller-Hinton Broth (Oxoid) with 0.5% Polysorbate 80 (Sigma-Aldrich). Collected mixture was diluted in Mueller-Hinton Broth (Oxoid) and quantified at UV-VIS spektrophotometer Helios ε (Spectronic Unicam, Cambridge, UK) to absorbance 1.5 A at 700 nm.

Gas Chromatograph (Hewlett Packard, 5890A) modified as a warm air flow treatment chamber was used for combined treatment of EO vapors and warm air flow (40°C for 5 min). Glass cube was inserted into the oven instead of door and the detector heater was ejected into the oven and used for vaporization of EOs at accurate temperature (150°C).Whole 6 mm in diameter on the peel was filled by one drop of inocula. After 35 days of storage in 5°C the mycelia growth was measured (the furthermost tissue infected by *P. foveata* from the place of inoculation). Non - treated potatoes and potatoes treated only by warm air flow without EOs was used as controls.

Organoleptic properties of treated potatoes were evaluated by unstructured linear scale. In the Seventeen descriptors on appearance, color, smell, texture, taste and overall score were in the questionnaire.

RESULT AND DISCUSSION

Results of antifungal activity and organoleptic properties are summarized in Fig. 1 and Fig. 2. Inhibition of mycelia growth was found in treating with warm air flow – the mean of growth for control was 10.7 mm and 6.67 for warm air flow treated potatoes without any EO. High inhibition was found on potatoes treated with *O. vulgare* EO – the mean of growth were 0.27 mm (16 µl.l^{-1}) and 2.24 (4 µl.l^{-1}) and *C. Aromaticus* 1.8 mm (16 µl.l^{-1}) and 2.87 mm (4 µl.l^{-1}). *C. zeylanicum* shown only slight inhibition - the mean of growth were 5.91 mm (16 µl.l^{-1}) and 7.4 (4 µl.l^{-1}).

All over the organoleptic analyzes, only two tested samples were statistically significant different. Differences were found between the control and WAF control (descriptor: overall taste) and between *Caryophyllus aromaticus* (conc. 4 µl.l^{-1}) and non – treated control (descriptor: overall score of potatoe sample).

Fig. 1: Phoma foveata growth on treated ¨potatoes – Growth on WAF treated control has been considered as 100 %

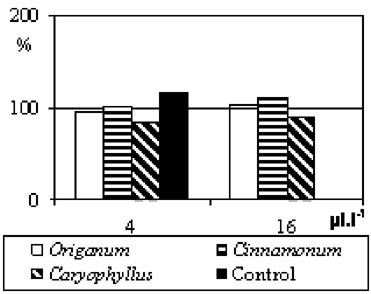
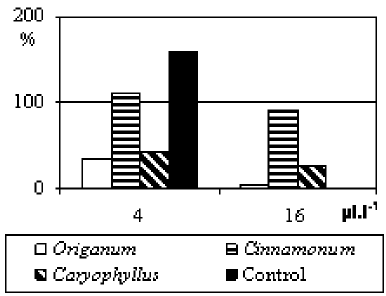


Fig. 2: Overall score of treated potatoes - Growth on WAF treated control has been considered as 100 %



To our knowledge, fungal growth inhibition by EOs have been never obtained in such low concentrations as in our study even in combination with any physical or chemical treatment. *Leptospermum petersonii* oil volatiles at room temperature in vitro did not inhibit *Aspergillus fumigatus*; however, when the oil was heated to 80°C, there was 100% growth inhibition (Hood et al. 2010). Ait-Ouazzou et al. (2011) obtained antibacterial activity of 200 µl.l^{-1} essential oil components in combination with mild heat, pulsed electric fields and different pH. Due to possible adverse effects on different plant products, further investigations e.g. on phytotoxicity, seed germination, fruit and vegetable taste, color and odor changes are being performed. After 72 hours incubation, bean seed treatments with 2, 4, and 8 mg.ml^{-1} of eugenol caused germination reduction of 3%, 7%, and 16%, respectively, which was significantly different from the controls (Lo Cantore et al. 2009). Dipping of fresh-cut kiwifruit in carvacrol solutions at 5–15 mM reduced total viable counts from 6.6 to < 2 log cfu.g^{-1} for 21 days at 4°C; however, undesirable color and odor changes were also observed (Roller and Seedhar 2002). On the other hand, the fungicidal spray containing *Cymbopogon flexuosus* essential oil did not exhibit any phytotoxic effect up to 50 μl.ml^{-1} level on *Malus pumilo* fruit skin when tested in vivo, at 20 μl.ml^{-1} concentration by pre inoculation treatment while in post inoculation treatment 30 μl.ml^{-1} showed complete rotting inhibition (Shahi et al. 2003).

CONCLUSIONS

In this study we have demonstrated that antifungal activity of essential oils from cinnamon, oregano and clove should be used for inhibition of *Phoma foveata* on potatoes. Method using evaporation and distribution of EO with warm air flow is suitable for future utilization in antimicrobial protection of any fruit and vegetables. This method can extend the storage life of treated products. EO vapors with warm air flow could be also applicable e.g. in seed treatment or field applications as well as postharvest treatment of different agricultural products, instead of synthetic fungicides, preferentially by organic farmers.

REFERENCES

AIT-OUAZZOU, A., L. CHERRAT, L. ESPINA, S. LORAN, C. ROTA AND R. PAGAN, 2011. *The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes of food preservation*, Innovative Food Science & Emerging Technologies, 12(3), 320-329.

BURT, S., 2004. *Essential oils: their antibacterial properties and potential applications in foods - a review*, International Journal of Food Microbiology, 94(3), 223-253.

DHIMA, K.V., I.B. VASILAKOGLOU, T.D. GATSIS, E. PANOU-PHILOTHEOU AND I.G. ELEFTHEROHORINOS, 2009. *Effects of aromatic plants incorporated as green manure on weed and maize development*, Field Crops Research, 110(3), 235-241.

HOOD, J.R., D. BURTON, J.M. WILKINSON AND H.M.A. CAVANAGH, 2010. *Antifungal activity of Leptospermum petersonii oil volatiles against Aspergillus spp. in vitro and in vivo*, Journal of Antimicrobial Chemotherapy, 65(2), 285-288.

INOUYE, S., T. TAKIZAWA AND H. YAMAGUCHI, 2001. *Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact*, Journal of Antimicrobial Chemotherapy, 47(5), 565-573.

LO CANTORE, P., V. SHANMUGAIAH AND N.S. IACOBELLIS, 2009. *Antibacterial Activity of Essential Oil Components and Their Potential Use in Seed Disinfection*, Journal of Agricultural and Food Chemistry, 57(20), 9454-9461.

LOPEZ, P., C. SANCHEZ, R. BATLLE AND C. NERIN, 2005. *Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains*, Journal of Agricultural and Food Chemistry, 53(17), 6939-6946.

ROLLER, S. AND P. SEEDHAR, 2002. *Carvacrol and cinnamic acid inhibit microbial growth in fresh-cut melon and kiwifruit at 4° and 8°C*, Letters in Applied Microbiology, 35(5), 390-394.

SHAHI, S.K., M. PATRA, A.C. SHUKLA AND A. DIKSHIT, 2003. *Use of essential oil as botanical-pesticide against post harvest spoilage in Malus pumilo fruits*, Biocontrol, 48(2), 223-232.

SHAW, M.W., 1993. *Theoretical-analysis of the effect of interacting activities on the rate of selection for combined resistance to fungicide mixtures*, Crop Protection, 12(2), 120-126.

TRIPATHI, P. AND N.K. DUBEY, 2004. *Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables,* Postharvest Biology and Technology, 32(3), 235-245.

VALVERDE, J.M., F. GUILLEN, D. MARTINEZ-ROMERO, S. CASTILLO, M. SERRANO AND D. VALERO, 2005. *Improvement of table grapes quality and safety by the combination of modified atmosphere packaging (MAP) and eugenol, menthol, or thymol*, Journal of Agricultural and Food Chemistry, 53(19), 7458-7464.