

Antimicrobial activity of biochemical substances against pathogens of cultivated mushrooms in Serbia

Ivana Potočnik*, Biljana Todorović, Rada Đurović-Pejčev, Miloš Stepanović, Emil Rekanović and Svetlana Milijašević-Marčić

Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia, Tel./Fax: +381-11-3076 133

**Corresponding author: Ivana.potocnik@pesting.org.rs*

Received: 10 May, 2016

Accepted: 23 May, 2016

SUMMARY

Disease control with few or no chemicals is a major challenge for mushroom growers in the 21st century. An alarming incidence of resistance to antibiotics in bacteria, and to fungicides among mycopathogenic fungi requires effective alternatives. Previous studies have indicated that various plant oils and their components demonstrate strong antimicrobial effects against pathogens on cultivated mushrooms. The strongest and broadest activity to pathogens obtained from mushroom facilities in Serbia was shown by the oils of oregano, thyme and basil. Five oils inhibited the growth of pathogenic bacteria *Pseudomonas tolaasii*: wintergreen, oregano, lemongrass, rosemary and eucalyptus. The essential oils of oregano, geranium and thyme were considerably toxic to the pathogenic fungi *Mycogone perniciosa*, *Lecanicillium fungicola* and *Cladobotryum* spp. The strongest activity against *Trichoderma aggressivum* f. *europaeum* was shown by the oils of basil and mint. Oils of juniper and pine showed neither inhibitory nor lethal effects on mushroom pathogens. Although the fungitoxic activity of oils is not strong, they could be used as a supplement to commercial productus for disease control, which will minimize the quantity of fungicides used.

Keywords: Mushrooms; Pathogens; Essential oils; Biopesticides; Serbia

INTRODUCTION

The most serious diseases of cultivated mushrooms are caused by mushroom virus X, pseudomonads (Grogan, 2008; Milijašević-Marčić et al., 2012) and fungi *Lecanicillium fungicola*, *Mycogone perniciosa*, *Cladobotryum* spp. and *Trichoderma* spp. (Potočnik et al., 2009a, 2009b, 2010a, 2010b; Kosanović et al., 2013). A common method of pathogen control on

farms worldwide is the application of various fungicides. Ongoing pesticide reviews in Europe have resulted in many chemicals being no longer approved for use, mainly from the group of benzimidazoles (Fletcher et al., 1989; Grogan, 2008). A major challenge for mushroom growers in the 21st century is to control diseases with few or no chemicals (Grogan, 2008). Other challenges include antibiotic and fungicide resistance among pathogen populations, managing the existing available pesticides

and the emergence of new pathogens. Few fungicides are officially recommended for mushroom production: prochloraz in the EU countries, and chlorothalonil, thiabendazol and tiophanate-methyl in North America (Beyer & Kremser, 2004; Grogan, 2008). A decreased sensitivity of the pathogenic fungi *Lecanicillium* and *Cladobotryum* to prochloraz has already been reported (Grogan & Gaze, 2000; Gea et al., 2005). Disease control is also provided by biofungicides based on *Bacillus* species (Kosanović et al., 2013; Potočnik et al., 2015). Special attention is now being focused on possible uses of biochemicals, such as extracts or essential oils of different plants, to inhibit pathogen activity.

BIOCHEMICAL SUBSTANCES IN DISEASE CONTROL OF EDIBLE MUSHROOMS

Many compounds have been tested as control agents against edible mushroom diseases. Plant extracts, essential oils (EOs) and their components have demonstrated strong fungistatic and antibacterial effects against pathogenic fungi and bacteria on cultivated mushrooms (Soković & van Griensven., 2006; Glamočlija et al., 2006, 2009; Regnier & Combrinck, 2010). These plants, tested as antimicrobial agents, have been used in herbal medicine, or as flavours (Soković & van Griensven, 2006). Modes of action of EOs in their interaction with bacteria have been revealed, but the mechanism of interaction of EOs with fungi has not been sufficiently explained. It is assumed that carvacrol changes the permeability of cytoplasmic membrane of Gram-positive bacteria, acting as a proton exchanger, decreasing the pH gradient and causing a collapse of the proton motive force and eventually cell death (Ultee et al., 1999; 2002). In general, the EOs with the strongest antibacterial properties contain high percentages of carvacrol and/or thymol, such as oregano or thyme oils (Dorman & Deans, 2000). Voda et al. (2003) found that the action of secondary metabolites is associated with the inhibition of sulfhydryl-containing active sites of fungal enzymes. Essential oils belonging to hydrocarbon monoterpenes have the lowest antifungal activity, while it is supposed that oxygenated terpenes or oils with phenolic structures have a considerable potential (Adam et al., 1998). Essential oils from the group of hydrocarbons tend to be relatively inactive regardless of their structural type, and this inactivity is closely related to their limited hydrogen binding capacity and water solubility. Ketones, aldehydes and alcohols show different levels of activity, which is in connection

with the functional groups present, but also associated with hydrogen-binding parameters in all cases (Soković et al., 2009).

Although fungitoxicity of oils is not strong, they could be used as supplements to commercial preparations for disease control, which will minimize the amounts of fungicides used. Also, some EOs have been confirmed as nontoxic to *A. bisporus* and not causing any negative impact on mushroom yield, e.g. thyme (Regnier & Combrinck, 2010) or tea tree oil (Potočnik et al., 2010a). Essential oils extracted from plants originating from Serbia and those from Germany and Albania were assayed for inhibitory and fungicidal activity against the most important mushroom pathogens.

Oregano (*Origanum vulgare* L.). The highest and broadest EO activity against all investigated pathogens was shown by the oregano oil. Earlier data had shown a broad spectrum of activity of oregano oil in suppressing microbial growth, and indicated its possible uses in integrated management of various diseases (Valero & Salmerón, 2003). Among the 20 components detected in the oregano oil from Germany, eleven with concentrations higher than 0.7% constituted 97.5% of the oil content, while the concentrations of all others ranged from 0.1 to 0.5% (Todorović et al., 2016). Carvacrol was the dominant component, accounting for 75.8% of the oil content, followed by thymol (4.4%), *p*-cymene (3.5%), β -caryophyllene (3.4%) and *g*-terpinene (3.4%). Another sample from Germany, a natural-identical oregano oil consisted of 21 components. Nine of them had concentrations over 0.7%, and constituted 96.2% of total mass. Concentrations of all other components varied from 0.2 to 0.6%. The dominant components in natural-identical oregano from Germany were: carvacrol (64.0%), linalool (17.4%), *p*-cymene (4.4%), thymol (3.6%), *g*-terpinene (2.5%) and β -caryophyllene (1.5%) (Todorović et al., 2016). Carvacrol has been recognized as a major constituent of oregano oil by many authors (Hristova et al., 1999). Tanović et al. (2009) detected a similar composition of an oregano oil from Serbia, having these main components: carvacrol 65%, thymol 14.8 % and β -phellandrene 4.3 %. Conversely, Mockutė et al. (2004) found that the main components of an EO from wild oregano leaves were β -caryophyllene (15.9-21.3%) and germacene D (12.3-16.0%), while carvacrol was detected only in traces. In general, EO yields have been found to peak under hot summer conditions, when species produce oil containing 60-75% phenols, mostly carvacrol (McGimpsey, 1993). Carvacrol was found in the latter study to have the highest antifungal activity of all components tested. Soković & van Griensven

(2006) reported that an oregano EO and its major component carvacrol exhibited a very strong activity against *T. harzianum*, *T. atroviride* and *L. fungicola*. The natural-identical oregano oil from Germany exhibited a remarkable activity against *P. tolaasii* from Serbia, having both the inhibitory and bactericidal effect at 0.32 $\mu\text{g mL}^{-1}$ (Todorović et al., 2016). Another oregano oil from Germany showed a lower effect on bacteria. High antibacterial activity of oregano oil against *P. tolaasii* was also confirmed by Soković and van Griensven (2006). Klouček et al. (2012) reported an ineffectiveness of oregano oil against *P. aeruginosa* even at 500 $\mu\text{g mL}^{-1}$ in the vapour phase, although it contained a huge amount of carvacrol (92%). Wattanasatcha et al. (2012) found thymol to have a stronger antibacterial activity than its isomer carvacrol, applied at equal concentration against *P. aeruginosa*. Tanović et al. (2009) found that oregano oil from Serbia showed the strongest both inhibitory and fungicidal effect on *L. fungicola*, *M. perniciosa* and *Cladobotryum* spp. The World Health Organization (WHO, 2014) confirmed that thymol and carvacrol residues in food are harmless to consumers as long as they do not exceed 50 mg kg^{-1} .

Sage (*Salvia officinalis* L.). Gutierrez et al. (2008) reported significant bacteriostatic and bactericidal activities of sage EO against various *Pseudomonas* species which were attributed to high concentrations of monoterpenes with antibacterial potential (Jalsenjak et al., 1987). A study by Mikiciński et al. (2012) showed a significant antibacterial activity of sage oil against *P. syringae*. These results are consistent with the findings of Pinto et al. (2007) and Delamare et al. (2007), who demonstrated bactericidal effects of sage EO on various Gram-positive and Gram-negative bacteria, thereby contradicting those of Marino et al. (2001), who reported bacteriostatic, but no bactericidal effect of the oil. There is a widespread observation that antimicrobial activity of EOs is more pronounced against Gram-positive than against Gram-negative bacteria (Nostro et al., 2000). This observation was not confirmed by Todorović et al. (2016), as *P. tolaasii* from Serbia was found unaffected by sage oil. Sage was also not active in that study against *L. fungicola* and *Cladobotryum* sp., but showed inhibitory and lethal effects on *M. perniciosa* at the respective concentrations of 0.08 and 0.16 $\mu\text{g mL}^{-1}$ (Tanović et al., 2009).

Thyme (*Thymus vulgaris* L.). Thyme is an evergreen herb with culinary, medicinal, and ornamental uses. The essential oil of common thyme and its major component thymol have shown a very strong activity against *T. harzianum*, *T. atroviride* and *L. fungicola*

(Soković & van Griensven, 2006). The lower antifungal activity of *T. tosevi* oil, in comparison with *T. vulgaris* oil, may be attributed to its lower content of thymol and its precursors (*p*-cymene and γ -terpinene) and to a higher percentage of acetates (*a*-terpinyl acetate and geranyl acetate) which may lead to lower antifungal potential (Soković & van Griensven, 2006; Soković et al., 2009). There is an evident correlation between the high activity of *Thymus* oil and the presence of its phenol components, such as thymol or carvacrol. It seems possible that phenol components may interfere with cell wall enzymes, such as chitin synthase/chitinase, and with α - and β -glucanases of the fungus (Adams et al., 1996). Thyme oil has also been found to suppress highly *P. tolaasii* (Soković & van Griensven, 2006). Soković et al. (2009) found thymol (48.9%) and *p*-cymene (19.0%) to be the main components of a *T. vulgaris* oil, while in *T. tosevi* those were: carvacrol (12.8%), *a*-terpinyl acetate (12.3%), *cis*-myrtenol (11.2%) and thymol (10.4%). Among several commercial essential oils from Serbia tested against *M. perniciosa*, thyme oil revealed the highest antifungal activity with the minimum inhibitory and fungicidal concentration of 0.02 $\mu\text{g mL}^{-1}$ of air (Potočnik et al., 2010b). Thyme oil was found to have an excellent potential for commercial application. Regnier and Combrinck (2010) established a suitable application regime for thyme and lemongrass oils to control the wet bubble disease caused by *M. perniciosa* in commercial production of button mushrooms. They found that a thyme oil concentration of 600 $\mu\text{g mL}^{-1}$ did not significantly reduce total yield. No deduction could be made concerning pathogen control, since no outbreaks were reported on the farm in their survey.

Wintergreen (*Gaultheria procumbens* L.). Wintergreen is a species native to north-eastern parts of North America. Its oil mainly consists of methyl salicylate, and its content was consistent in the studies of Wu et al. (2008) and Jänicke et al. (2003), who reported 96-99% contents, while Hamoud et al. (2012) reported a lower content (87.9%). A remarkable antimicrobial activity of wintergreen oil has been demonstrated in other studies as well (Hammer et al., 1999). Todorović et al. (2016) reported that wintergreen exhibited the strongest activity of all tested oils to *P. tolaasii*, inhibited the growth of bacteria at 0.08 and showed bactericidal effect as well at 0.16 $\mu\text{g mL}^{-1}$ (Todorović et al., 2016). Wintergreen consisted of eight components. Methyl salicylate was the highly dominant component with 96.9% of total oil mass, and limonene was the only other component exceeding 0.7%, having 2.2%. The contents of all other components ranged from 0.1 to 0.2%.

Rosemary (*Rosmarinus officinalis* L.). In traditional medicine, extracts and essential oils from its flowers and leaves are used to treat a variety of disorders. Rosemary contains a number of phytochemicals, including rosmarinic acid, camphor, caffeic acid, etc. Todorović et al. (2016) showed different antibacterial effects of several rosemary oils of different origin: one from Germany, which suppressed the growth of *P. tolaasii* at 0.32 $\mu\text{g mL}^{-1}$, and three from Albania without any activity. Hussein et al. (2011) reported antimicrobial activity of a rosemary oil against *P. aeruginosa*, employing the disc diffusion method. Also, rosemary oil was found to inhibit the growth of *M. perniciosus* at 0.32 $\mu\text{g mL}^{-1}$ (Potočnik et al., 2010b).

Lemongrass (*Cymbopogon flexuosus* Stapf). *Cymbopogon* is one of the most important EO-yielding genera of Poaceae. Its species are widely distributed in semi-temperate to tropical regions of Asia, Africa and America. The species display wide variations in morphological attributes and EO compositions at inter- and intraspecific levels (Rao, 1997). It was confirmed by many authors that EOs of *Cymbopogon* spp., e.g. West-Indian lemongrass (*C. citratus*), East-Indian lemongrass (*C. flexuosus*), citronella (*C. winterianus* and *C. nardus*) or palmarosa (*C. martini*), had strong antifungal and bactericidal properties against bacteria, yeast-like and filamentous fungi in procedures employing disk diffusion (Jänicke et al., 2003), vapor-agar contact (Hamoud et al., 2012), agar well diffusion (Nguefack et al., 2004), microdilution (Simić et al., 2008) and macrodilution methods (Lis-Balchin & Deans, 1997; Regnier & Combrinck, 2010). Citronella oil has been reported inactive against *P. tolaasii* (Simić et al. 2008), but in a study by Todorović et al. (2016), an oil from East-Indian lemongrass suppressed the growth of pathogenic bacteria at 0.32 $\mu\text{g mL}^{-1}$ without exerting a bactericidal effect. Thirty-five components of the lemongrass oil were identified by Todorović et al. (2016). Eighteen of them had concentrations exceeding 0.7% and they accounted for 95.6% of the total oil mass. The most prevalent components were: geraniol (40.7%), neral (26.7%), limonene (8.9%) and geraniol (2.7%), and the oil had only 0.8% of citronellal. In contrast to the East-Indian lemongrass, Nguefack et al. (2004) found that the most active compounds in citronella were citronellal, *trans*-geraniol and citronellol. Regnier and Combrinck (2010) observed that the strong oil activity of West-Indian lemongrass was due to high contents of geraniol 42.5%, neral 31.7%, and limonene 8.9%, which is a very similar content to the East-Indian lemongrass. They reported a lower activity of palmarosa, having 67.1%

geraniol, 17.85% geranylacetate and 2.5% piperitone as its main components. As for some other *Pseudomonas* species, Tyagi and Malik (2010) found that West-Indian lemongrass inhibited *P. fluorescens* at 0.6 $\mu\text{g mL}^{-1}$. Klouček et al. (2012) reported that East-Indian lemongrass oil was not effective against *P. aeruginosa* even at 500 $\mu\text{g mL}^{-1}$ in vapour phase, containing mainly geraniol (42.5%) and neral (32%).

Tea tree oil (*Melaleuca alternifolia* Maiden & Betche). Tea tree, a native herb in Australia, is famous for its oil, which is widely used as an antimicrobial agent for treating an immense variety of medical conditions. Tea tree oil is an active ingredient of two biofungicide preparations: Timorex 66 EC and Timorex Gold EC. Applied at the standard product application rates, it was found to cause a significant reduction in cobweb disease (*C. dendroides*) in an *A. bisporus* experimental growing room (Potočnik et al., 2010a). Two different studies confirmed a considerable *in vivo* inhibition of *T. harzianum* by an addition of tea tree oil to oyster mushroom substrate (Angelini et al., 2008) or button mushroom casing soil (Kosanović et al., 2013). Tea tree oil was also tested against *P. tolaasii* by disc-diffusion and broth microdilution, but it was ineffective (Todorović et al., 2012).

Mint (*Mentha piperita* L.). Peppermint which produces a major essential oil, menthol, has also demonstrated a strong inhibitory activity against the mushroom pathogens *Trichoderma* spp. and *P. tolaasii* (Soković & van Griensven., 2006). Various *Mentha* species (*Lamiaceae*) have been recognized as plants with many useful pharmacological properties. They have been used for their flavours, in herbal medicine and as antimicrobial agents. Soković et al. (2009) found that menthol (37.4%), menthyl acetate (17.4) and menthone (12.7%) were the main components of *M. piperita* oil, whereas carvone (69.5%) and menthone (21.9%) were dominant in *M. spicata*. Their results suggested that carvone and menthol had greater antifungal activities than the other compounds investigated, while limonene showed the lowest antifungal activity. The EOs of peppermint (*M. piperita*), and spearmint (*M. spicata*) from Serbia have great antifungal potentials, inhibiting the growth of *T. viride* at 0.25 $\mu\text{g mL}^{-1}$ (Soković & van Griensven, 2006) and three pathogens of the button mushroom: *L. fungicola*, *T. harzianum* and *P. tolaasii* (Soković et al., 2009). Bouchra et al. (2003) found that *M. pulegium* exhibited a moderate activity against *Botrytis cinerea*, and inhibited 58.5% mycelial growth at 250 $\mu\text{g mL}^{-1}$. Todorović et al. (2016) detected a remarkable activity of peppermint oil against *T. aggressivum* f. *europaeum*, having both an inhibitory and fungicidal effect at 0.64 $\mu\text{g mL}^{-1}$

(Đurović-Pejčev et al., 2014). Chemical composition analyses of the peppermint oil from Serbia showed that its dominant components were menthone (37.02%), menthol (29.57%) and isomenthone (9.06%). Investigation of the antimicrobial activity of peppermint oil to other pseudomonads has shown its strong activity as well. Tyagi and Malik (2010) found that peppermint oil inhibited *P. fluorescens* at 1.12 $\mu\text{g mL}^{-1}$. In spite of the remarkable antimicrobial activity of mint oil, Stanisavljević et al. (2014) found that English horsemint oil (*M. longifolia*) had only a weak effect against *P. aeruginosa* at 25 $\mu\text{g mL}^{-1}$. On the other hand, Todorović et al. (2016) found no antimicrobial activity of mint oil from Serbia against *P. tolaasii* at the tested concentration of 0.32 $\mu\text{g mL}^{-1}$.

Basil (*Ocimum basilicum* L.). Sweet basil is usually used in herbal medicine. A study conducted in Iran has shown a low activity of its EO, i.e. 42.5% inhibition of *B. cinerea* mycelial growth at 0.5 $\mu\text{g mL}^{-1}$ using the macrodilution method (Abdollahi et al., 2010). However, Todorović et al. (2016) found basil oil to be the strongest growth inhibitor among all oils tested against *T. aggressivum* f. *europaeum*. It suppressed pathogen growth at 0.02 $\mu\text{g mL}^{-1}$ but without lethal effect.

St. John's wort (*Hypericum perforatum* L.). Nineteen species of *Hypericum* have been detected in the Serbian flora (Stjepanović-Veseličić, 1972). St. John's wort is used in herbal medicine for its antidepressant, antiviral and antibacterial effects. An oil of St. John's wort from Vranje-Barelić, Serbia, has been found to inhibit *P. tolaasii* at 25 $\mu\text{g mL}^{-1}$ (Saroglou et al., 2007). In a study by Đurović-Pejčev et al. (2014), the EO from plants collected in Ljubovija showed a higher activity against *T. aggressivum* f. *europaeum* at 0.08 $\mu\text{g mL}^{-1}$ than it was reported by Saroglou et al. (2007).

Yarrow (*Achillea millefolium* L.). Yarrow is a native herb in Serbia and a principal constituent of traditional cures and remedies. It has been used in medicine because of its anti-inflammatory, spasmolytic, hemostatic and digestive effects. Interestingly, no antimicrobial activity of yarrow extracts or oils have been observed yet, although the EOs of two other species of this genus, namely *A. clavennae* and *A. alpine*, have been recognized to have some antibacterial properties (Woods-Panzaru et al., 2009). Đurović-Pejčev et al. (2014) found in their study a weak inhibitory effect of yarrow oil on *T. aggressivum* f. *europaeum* at a rate of 0.64 $\mu\text{g mL}^{-1}$.

Walnut (*Juglans regia* L.). Persian or common walnut is an important deciduous tree which is commercially cultivated in Serbia. Green walnuts, shells, kernels and seeds, bark and leaves are used in pharmaceutical and cosmetic industries. Walnut leaves are considered a

source of healthcare compounds and have been intensely used in traditional medicine for treatment of venous insufficiency. Antibacterial activity of a leaf EO of walnut from India was previously reported by Rather et al. (2012). Todorović et al. (2016) reported a mild activity of a walnut oil from Serbia against *T. aggressivum* f. *europaeum*, suppressing its growth of at 0.16 $\mu\text{g mL}^{-1}$.

Juniper (*Juniperus communis* L.). Juniper is widespread in Serbia and grows throughout the temperate regions of Europe, Asia and North America. The EO of juniper female cones has diuretic properties and antiseptic capacities and is a gastrointestinal irritant. A juniper oil from Croatia has shown strong fungicidal activity against yeast, yeast-like fungi and dermatophytes in tests using the diffusion method, and inhibition was below 10% (v/v) (Pepeljnjak et al., 2005). A juniper oil from Serbia has suppressed the growth of *T. aggressivum* f. *europaeum* 83.68% at 0.64 $\mu\text{g mL}^{-1}$ (Đurović-Pejčev et al., 2014). Juniper showed neither total inhibitory nor lethal effects to *T. aggressivum* f. *europaeum*.

Pine (*Pinus* spp.). Four oils of *Pinus* sp. were tested by Todorović et al. (2016) against *T. aggressivum*, i.e. maritime pine (*P. pinaster*), two of Scotch pine (*P. silvestris*) and one of black pine (*P. nigra*). The oils of different *Pinus* species exhibited no antimicrobial effect on *P. tolaasii* from Serbia at any applied concentration. Scotch pine suppressed the growth of *M. perniciosa* at 0.16 $\mu\text{g mL}^{-1}$.

Eucalyptus oil (*Eucalyptus globulus* Labillardie). It has exhibited an activity against *P. tolaasii*, having an inhibitory effect at 0.32 $\mu\text{g mL}^{-1}$ (Todorović et al., 2016).

Lemon (*Citrus lemon* L.). Lemon juice has a low pH and its antibacterial properties are used in traditional medicine. Lemon did not show any activity against *P. tolaasii* (Todorović et al., 2016) but inhibited the growth of *M. perniciosa* at 0.16 $\mu\text{g mL}^{-1}$. (Potočnik et al., 2010b).

Lavender (*Lavandula officinalis* L.). Lavender has anxiolytic effects and a resulting influence on sleep quality. Lavender essential oil was not found active against *P. tolaasii*, (Todorović et al., 2016). However, lavender exhibited a fungicidal effect at 0.32 $\mu\text{g mL}^{-1}$, suppressing the growth of *L. fungicola*, *M. perniciosa* and *Cladobotryum* sp. (Tanović et al., 2009).

Anise (*Pimpinella anisum* L.). Anise did not show an activity against *P. tolaasii* (Todorović et al. 2016), but Tanović et al. (2009) reported its inhibitory and lethal effects on *M. perniciosa* at 0.04 $\mu\text{g mL}^{-1}$. They found anise suppressed the growth of both *L. fungicola* and *Cladobotryum* sp. at 0.16 and 0.08 $\mu\text{g mL}^{-1}$, respectively.

A majority of other tested oils, i.e. cade, two lavender samples, lemon, and both mint oils, showed no activity

against *P. tolaasii* at 0.32 µg mL⁻¹ or lower concentrations. Tanović et al. (2009) revealed a remarkable activity of geranium (*Pelargonium graveolens* L.) against *M. pernicioso*, *L. fungicola* and *Cladobotryum* spp. with respective fungicidal concentrations of 0.02, 0.16 and 0.08 µg mL⁻¹. They found fennel oil (*Foeniculum vulgare* L.) to be active against mushroom pathogens as well, and its lethal oil concentrations were 0.04 to *M. pernicioso* and 0.32 µg mL⁻¹ to both *L. fungicola* and *Cladobotryum* spp. Also, they found that chamomile oil suppressed the growth of *M. pernicioso* at 0.16 µg mL⁻¹, while parsley oil was active against *M. pernicioso* and *Cladobotryum* spp. at 0.04 and 0.32 µg mL⁻¹. The essential oil of *Critmum maritimum*, as well as two of the major components of that oil, limonen and α-pinene, were found to exhibit antifungal activity against *M. pernicioso* (Glamočlija et al., 2009).

CONCLUSION

Oregano, thyme and basil oils have demonstrated the strongest and broadest activity against mushroom pathogens. Five essential oils inhibited the growth of the pathogenic bacteria *Pseudomonas tolaasii* (wintergreen, oregano, lemongrass, rosemary, and eucalyptus). Oregano, geranium and thyme were considerably toxic to the pathogenic fungi *Mycogone pernicioso*, *Lecanicillium fungicola* and *Cladobotryum* spp. The strongest activity against *Trichoderma aggressivum* f. *europaeum* was shown by basil and mint oils. Juniper and pine showed neither inhibitory nor lethal effects on the mushroom pathogens tested. The oils with the strongest antimicrobial activity showed a potential for further *in vivo* experiments against mushroom pathogens.

ACKNOWLEDGEMENT

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, project TR 31043.

REFERENCES

- Abdolahi A., Hassani A., Ghosta Y., Bernousi I., & Meshkatsadat M.H. (2010). Study on the potential use of essential oils for decay control and quality preservation of Tabarzeh table grape. *Journal of Plant Protection Research*, 50(1), 45-52.
- Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., & Arsenakis, M. (1998). Antifungal activity of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. *Journal of Agricultural and Food Chemistry*, 46, 1739-1745. doi 10.1021/jf9708296
- Adams, S., Kunz, B., & Weidenbörner, M. (1996). Mycelial deformations of *Cladosporium herbarum* due to the application of Eugenol and Carvacrol. *Journal of Essential Oils Research*, 8, 535-540.
- Angelini, P., Pagiotti, R., & Granetti, B. (2008). Effect of antimicrobial activity of *Melaleuca alternifolia* oil on antagonistic potential of *Pleurotus* species against *Trichoderma harzianum* in dual culture. *World Journal of Microbiology and Biotechnology*, 24(2), 197-202.
- Beyer, D.M., & Kremser, J.J. (2004). Evaluation of fungicide tolerance and control for three fungal diseases of mushrooms. In Romaine, C.P., Keil, C.B., Rinker, D.L., Royse, D.J. (Eds.), *Science and cultivation of edible and medicinal fungi. Mushroom Science XVI*. (pp 521-529). University Park, PA, USA: Penn State University.
- Bouchra, C., Achouri, M., Hassani Idrissi, L.M., & Hamamouchi, M. (2003). Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers: Fr. *Journal of Ethnopharmacology*, 89, 165-169.
- Delamare, A.P.L., Moschen-Pistorello, I.T., Artico, L., Atti-Serafini, L., & Echeverrigaray, S. (2007). Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. *Food Chemistry*, 100, 603-608.
- Dorman, H.J.D., & Deans, S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88, 308-316.
- Đurović-Pejčev, R., Potočnik, I., Milijašević-Marčić, S., Todorović, B., Rekanović, E., & Stepanović, M. (2014). Antifungal activity of six plant essential oils from Serbia against *Trichoderma aggressivum* f. *europaeum*. *Pesticides and Phytomedicine*, 29(4), 291-297.
- Fletcher J.T., White P.F., & Gaze R.H. (1989). *Mushrooms - Pest and Disease Control* (2nd ed). Newcastle-upon-Tyne, UK: Intercept.
- Gea, F.J., Navarro, M.J., & Tello J.C. (2005). Reduced sensitivity of the mushroom pathogen *Verticillium fungicola* to prochloraz manganese *in vitro*. *Mycological Research*, 109, 741-745.
- Glamočlija, J., Soković, M., Grubišić, D., Vukojević, J., Milenković, I., & Ristić, M. (2009). Antifungal activity of *Critmum maritimum* essential oil and its components against mushroom pathogen *Mycogone pernicioso*. *Chemistry of Natural Compounds*, 45(1), 95-97.

- Glamočlija, J., Soković, M., Vukojević, J., Milenković, I., van Griensven, L.J.L.D. (2006). Chemical composition and antifungal activities of essential oils of *Satureja thymbra* L. and *Salvia pomifera* ssp. *calycna* (Sm.) Hayek. *Journal of Essential Oil Research*, 18, 115-117.
- Grogan, H.M. (2008). Challenges facing mushroom disease control in the 21st century. In Lelley, J.I., Buswell, J.A. (Eds.), *Proceeding of the Sixth International Conference on Mushroom Biology and Mushroom Products*, (pp 120-127). Bonn, Germany: WSM BMP.
- Grogan, H.M., & Gaze, R.H. (2000). Fungicide resistance among *Cladobotryum* spp. – causal agents of cobweb disease of the edible mushroom *Agaricus bisporus*. *Mycological Research*, 104(3), 357-364.
- Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology*, 124, 91-97.
- Hammer, K.A., Carson, C.F., & Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*, 86(6), 985-990.
- Hamoud, R., Sporer, F., Reichling, J., & Wink, M. (2012). Antimicrobial activity of a traditionally used complex essential oil distillate (OlbasÖ Tropfen) in comparison to its individual essential oil ingredients. *Phytomedicine*, 19(11), 969-976.
- Hristova, R., Ristić, M., Brkić, D., Stefkov, G., & Kulevanova, S. (1999). Comparative analysis of essential oil composition of *Origanum vulgare* from Macedonia and commercially available *Origanum herba*. *Acta Pharmaceutica*, 49, 299-305.
- Hussein, A.I., Anwar, F., Nigam, P.S., Sarker, S.D., Moore, J.E., Rao, J.R., & Mazumdar, A. (2011). Antibacterial activity of some Lamiaceae essential oils using resazurin as an indicator of cell growth. *LWT – Food Science and Technology*, 44, 1199-1206.
- Jalsenjak, V., Piljnjak, S., & Kustak, D. (1987). Microcapsules of sage oil, essential oils content and antimicrobial activity. *Pharmazie*, 42, 419-420.
- Jänicke, C., Grünwald, J. & Brender, T. (2003). *Handbuch Phytotherapie*. Stuttgart, Germany: Wissenschaftliche Verlagsgesellschaft GmbH.
- Kloucek, P., Smid, J., Frankova, A., Kokoska, L., Valterova, I., & Pavela, R. (2012). Fast screening method for assessment of antimicrobial activity of essential oils in vapor phase. *Food Research International*, 47(2), 161-165.
- Kosanović, D., Potočnik, I., Duduk, B., Vukojević, J., Stajić, M., Rekanović, E., & Milijašević-Marčić, S. (2013): *Trichoderma* species on *Agaricus bisporus* farms in Serbia and their biocontrol. *Annals of Applied Biology*, 163, 218-230.
- Lis-Balchin, M., & Deans, S.G. (1997). Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *Journal of Applied Microbiology*, 82, 759-762.
- Marino, M., Bersani, C., & Comi, G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *International Journal of Food Microbiology*, 67, 187-195.
- McGimpsey, J. (1993). *Oregano – Origanum vulgare*. *Crop and Food Research, Broadsheet*, 19, 1-4.
- Milijašević-Marčić, S., Todorović, B. Potočnik, I., Stepanović, M., & Rekanović, E. (2012): First report of *Pseudomonas tolaasii* on *Agaricus bisporus* in Serbia. *Phytoparasitica*, 40(3), 299-303.
- Mikiciński, A., Sobiczewski, P., & Berzński, S. (2012). Efficacy of fungicides and essential oils against bacterial diseases of fruit trees. *Journal of Plant Protection Research*, 52(4), 467-471.
- Mockutė, D., Judtentiē, A., & Bernotienė, G. (2004). Volatile constituents of cultivated *Origanum vulgare* L. inflorescences and leaves. *Chemia*, 15, 33-37.
- Nguefack, J., Budde, B.B., & Jakobsen, M. (2004). Five essential oils from aromatic plants of Cameroon: their antibacterial activity and ability to permeabilize the cytoplasmic membrane of *Listeria innocua* examined by flow cytometry. *Letters in Applied Microbiology*, 39(5), 395-400.
- Nostro, A., Germano, M.P., d'Angelo, V., Marino, A., & Cannatelli, M.A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology*, 30, 379-384.
- Pepeljnjak, S., Kosalec, I., Kalodera Z., & Blažević, N. (2005). Antimicrobial activity of juniper berry essential oil (*Juniperus communis* L., *Cupressaceae*). *Acta Pharmaceutica*, 55, 417-422.
- Pinto, E., Salgueiro, L.R., Cavaleiro, C., Palmeira, A., & Gonçalves, M.J. (2007). *In vitro* susceptibility of some species of yeast and filamentous fungi to essential oils of *Salvia officinalis*. *Industrial Crop and Products*, 26, 135-141.
- Potočnik, I., Stepanović, M., Rekanović, E., Todorović, B. & Milijašević-Marčić, S. (2015). Disease control by chemical and biological fungicides in cultivated mushrooms: button mushroom, oyster mushroom and shiitake. *Pesticides and Phytomedicine*, 30, 201-208.
- Potočnik, I., Vukojević, J., Stajić, M., Rekanović, E., Milijašević, S., Todorović, B., & Stepanović, M. (2009a). *In vitro* toxicity of selected fungicides from the groups of benzimidazoles and demethylation inhibitors to *Cladobotryum dendroides* and *Agaricus bisporus*. *Journal of Environmental Science and Health, Part B*, 44(4), 365-370.

- Potočnik I., Vukojević, J., Stajić, M., Rekanović E., Milijašević, S., Stepanović M., & Todorović, B. (2009b). Toxicity of fungicides with different modes of action to *Cladobotryum dendroides* and *Agaricus bisporus*. *Journal of Environmental Science and Health, Part B*, 44(8), 823-827.
- Potočnik, I., Vukojević, J., Stajić, M., Rekanović, E., Stepanović, M., Milijašević, S., & Todorović, B. (2010a). Toxicity of biofungicide Timorex 66 EC to *Cladobotryum dendroides* and *Agaricus bisporus*. *Crop Protection*, 29, 290-294.
- Potočnik, I., Vukojević, J., Stajić, M., Tanović, B., & Rekanović, E. (2010b). Sensitivity of *Mycogone perniciosa*, pathogen of culinary-medicinal button mushroom *Agaricus bisporus* (J. Lge) Imbach (Agaricomycetidae), to selected fungicides and essential oils. *International Journal of Medicinal Mushrooms*, 12(1), 91-98.
- Rao, B.L. (1997). Scope for development of new cultivars of *Cymbopogon*s as a source of terpene chemicals, In Maharaj Krishen Kaul M.K.S. (Ed), *Supplement to cultivation and utilization of aromatic plants*, (pp 71-83). New Delhi, India: National Institute of Science Communication.
- Rather, M.A., Dar, B.A., Dar, M.Y., Wani, B.A., Shah, W.A., Bhat, B.A.... Qurishi, M.A. (2012). Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of *Juglans regia* L. and its constituents. *Phytomedicine*, 19, 1185-1190.
- Regnier, T., & Combrinck, S. (2010). *In vitro* and *in vivo* screening of essential oils for the control of wet bubble disease of *Agaricus bisporus*. *South African Journal of Botany*, 76, 681-685.
- Saroglou, V., Marin, P.D., Rancic, A., Veljic, M., & Skaltsa, H. (2007). Composition and antimicrobial activity of the essential oil of six *Hypericum* species from Serbia. *Biochemical Systematics and Ecology*, 35, 146-152.
- Simić, A. Rančić, A. Soković, M.D. Ristić, M. Grujić-Jovanović, S. Vukojević, J., & Marin, P.D. (2008). Essential oil composition of *Cymbopogon winterianus* and *Carum carvi* and their antimicrobial activities. *Pharmaceutical Biology*, 46(6), 437-441.
- Soković, M., & van Griensven, L.J.L.D. (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. *European Journal of Plant Pathology*, 116, 211-224.
- Soković, M., Vukojević, J., Marin, P., Brkić, D., Vajs, V., & Van Griensven, L.J.L.D., (2009). Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities, *Molecules*, 14, 238-249.
- Stanisavljević, D., Đorđević, S., Milenković, M., Lazić, M., Veličković, D., Randelović, N. & Zlatković, B. (2014). Antimicrobial and antioxidant activity of the essential oils obtained from *Mentha longifolia* L. Hudson, dried by three different techniques. *Records of Natural Products*, 8(1), 61-65.
- Stjepanović-Veseličić, L. (1972). Hypericaceae Lindl. In Josifovic, M. (Ed), *Flora SR Srbije, vol. 3* (pp 102-126). Belgrade, Serbia: Serbian Academy of Sciences and Arts.
- Tanović, B., Potočnik, I., Delibašić, G., Ristić, M., Kostić, M., & Marković, M. (2009). *In vitro* effect of essential oils from aromatic and medicinal plants on mushroom pathogens: *Verticillium fungicola* var. *fungicola*, *Mycogone perniciosa*, and *Cladobotryum* sp. *Archives of biological sciences, Belgrade*, 61(2), 231-238.
- Todorović, B., Milijašević-Marčić, S., Potočnik, I., Stepanović, M., Rekanović, E., Nikolić-Bujanović, Lj., & Čekerevac, M. (2012). *In vitro* activity of antimicrobial agents against *Pseudomonas tolaasii*, pathogen of cultivated button mushroom. *Journal of Environmental Science and Health, Part B, Pesticides, Food Contaminants & Agricultural Wastes*, 47(3), 175-179.
- Todorović, B., Potočnik, I., Rekanović, E., Stepanović, M., Kostić, M., Ristić, M. & Milijašević-Marčić, S. (2016). Toxicity of twenty-two plant essential oils against pathogenic bacteria of vegetables and mushrooms. *Journal of Environmental Science and Health, Part B, Pesticides, Food Contaminants & Agricultural Wastes*. (in press).
- Tyagi, A.K. & Malik, A. (2010). Antimicrobial action of essential oil vapours and negative air ions against *Pseudomonas fluorescens*. *International Journal of Food Microbiology*, 143, 205-210.
- Ultee, A., Bennik, M.H.J. & Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the foodborne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology*, 68, 1561-1568.
- Ultee, A., Kets, E.P.W. & Smid, E.J. (1999). Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology*, 65, 4606-4610.
- Valero, M. & Salmerón, M.C. (2003). Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndalized carrot broth. *International Journal of Food Microbiology*, 85(1-2), 73-81.
- Voda, K., Boh, B., Vrtačnik, M. & Pohleven, F. (2003). Effect of the antifungal activity of oxygenated aromatic essential oil compounds on the white-rot *Trametes versicolor* and the brown-rot *Coniophora puteana*. *International Biodeterioration and Biodegradation*, 51, 51-59.

Wattanasatcha, A. Rengpipat, S. & Wanichwecharungruang, S. (2012). Thymol nanospheres as an effective antibacterial agent. *International Journal of Pharmaceutics*, 434, 360-365.

Woods-Panzaru, S., Nelson, D., McCollum, G., Ballard, L.M., Millar, B.C., Maeda, Y.... Moore, J.E. (2009). An examination of antibacterial and antifungal properties of constituents described in traditional Ulster cures and remedies. *Ulster Medical Journal*, 78(1), 13-15.

World Health Organization Website (WHO). Retrieved on 21/01/2014 from <http://apps.who.int/medicinedocs/en/d/Js2200e/28.html>

Wu, Y.-W., Sun, S.-Q. Zhou, Q., Tao, J.-X. & Noda, I. (2008). Volatility-dependent 2D IR correlation analysis of traditional Chinese medicine "Red Flower Oil" preparation from different manufacturers. *Journal of Molecular Structure*, 882, 107-115.

Antimikrobno delovanje biohemijskih supstanci na patogene šampinjona u Srbiji

REZIME

Početak 21. veka, proizvođači šampinjona su suočeni sa pojavom bolesti koje nanose velike štete. S druge strane, mali je broj raspoloživih fungicida koji imaju odobrenje za primenu u zaštiti gajenih gljiva od bolesti. Usled razvoja rezistentnosti patogenih bakterija i gljiva na antibiotike i fungicide, neophodno je uvođenje alternativnih sredstava zaštite. Istraživanja su pokazala da različita biljna ulja i njihove komponente ispoljavaju jako antimikrobno dejstvo na patogene šampinjona. Etarska ulja origana, majčine dušice i bosiljka su pokazala najjaču i najširu aktivnost za patogene izolovane iz gajilišta šampinjona u Srbiji. Pet etarskih ulja je inhibiralo rast patogene bakterije *Pseudomonas tolaasii*: zimzelena, origana, limunske trave, ruzmarina i eukaliptusa. Ulja origana, geranijuma i majčine dušice su ispoljili najveću toksičnost za patogene gljive *Mycogone pernicioso*, *Lecanicillium fungicola* i *Cladobotryum* spp. Ulje bosiljka je imalo inhibitorno, a nane letalno dejstvo za *Trichoderma aggressivum* f. *europaeum*. Ulja kleke i bora nisu ispoljila ni inhibitorno ni fungicidno dejstvo na patogene šampinjona. Iako fungitoksična aktivnost etarskih ulja nije jaka, ona mogu biti primenjena kao dodatak u zaštiti od bolesti, kako bi se umanjila količina primene hemijskih preparata.

Ključne reči: Pečurke; Patogeni; Etarska ulja; Biopesticidi; Srbija