

# Toxicity of plant essential oils to *Cryphonectria parasitica* (Murr.) Barr, the causal agent of chestnut blight

Jelena Luković<sup>1</sup>, Ivana Potočnik<sup>1\*</sup>, Emil Rekanović<sup>1</sup>, Svetlana Milijašević-Marčić<sup>1</sup>, Biljana Todorović<sup>1</sup>, Miroslav Kostić<sup>2</sup> and Zlatan Radulović<sup>3</sup>

<sup>1</sup>*Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia*

<sup>2</sup>*Institute for Medicinal Plant Research „Dr Josif Pančić“, Tadeuša Koščuška 1, 11000 Belgrade, Serbia*

<sup>3</sup>*Institute of Forestry, Kneza Višeslava 3, 11030 Belgrade, Serbia*

\*Corresponding author: [ivana.potocnik@pesting.org.rs](mailto:ivana.potocnik@pesting.org.rs)

Received: 9 September 2019

Accepted: 4 October 2019

## SUMMARY

Twenty-two essential oil samples from Germany and Albania were assayed to test their inhibitory and fungicidal activity against *Cryphonectria parasitica* (Murr.) Barr, the major causal agent of chestnut blight on chestnut trees, using the fumigant macrodilution method. Test concentrations of the oils in air phase were: 0.02, 0.04, 0.08, 0.16 and 0.32  $\mu\text{l ml}^{-1}$ , and minimum inhibitory and fungicidal concentrations were determined. The strongest activity was shown by two samples of mint oil from Germany and Albania, and black pine at the concentration of 0.02  $\mu\text{l ml}^{-1}$  of air for both test isolates (M1 and 4S). The tested plant essential oils also included: eucalyptus, sage, silver fir and cade. Only three samples of EOs (mint (2) and black pine) were lethal to both isolates. Sage and silver fir oils were more toxic to the M1 isolate, while cade and eucalyptus oils were more toxic to the 4S isolate. Fifteen oils: mint (2), black pine, cade, eucalyptus, silver fir, sage, pine (4), oregano, lavender, and rosemary (2), inhibited the growth of *Cryphonectria parasitica*, and seven oils (two samples of mint, eucalyptus, black pine, sage, silver fir and cade) showed fungicidal effects on the pathogen.

**Keywords:** essential oils; chestnut blight; antifungal activity; biofungicides

## INTRODUCTION

The major causal agent of chestnut blight on chestnut trees is *Cryphonectria parasitica* (Murr.) Barr. The pathogen belongs to Sordariomycete (ascomycete) fungi in the family Cryphonectriaceae. Its major hosts are species in the genus *Castanea* (Fagaceae), particularly the

American chestnut (*Castanea dentate* (Marsh.) Borkh.) and European chestnut (*Castanea sativa* Mill.), as well as Chinese and Japanese chestnuts (*Castanea mollissima* Blume and *Castanea crenata* Siebold and Zucc.) (Roane et al., 1986). Other hosts include mainly oaks (*Quercus* spp.), maples (*Acer* spp.), European hornbeam (*Carpinus betulus* L.) and American chinquapin (*Castanea pumila* L.)

(Diller, 1965; Radocz & Tarcali, 2009; Tziros et al., 2015; Rigling & Prospero, 2018). Chestnut blight was introduced in Europe (first detected point was near Genova, Italy) from North America in 1938, thus endangering the survival of European sweet chestnut (Dutech et al., 2012). In Serbia, the pathogen has spread to sweet chestnut trees in the environs of Kruševac, Vranje, Vršački Breg and Kosovo (Marinković & Karadžić, 1985; Karadžić & Milenković, 2013; Karadžić et al., 2019). In 2012, *C. parasitica* was first identified on sessile oak (*Quercus petraea* (Matt.) Lebl.) trees in Serbia (location Vršački Breg) (Karadžić & Milenković, 2013; Karadžić et al., 2019).

The fungus *C. parasitica* is a bark pathogen, which causes perennial necrotic lesions (so-called cankers) and infects stems, branches and eventually twigs. Manifestation of symptoms induced by the pathogen on susceptible hosts varies depending on the virulence of a particular *C. parasitica* strain and the age of infected tree parts (Prospero & Rigling, 2013; Rigling & Prospero, 2018). Bark cankers on smooth-barked young stems/branches are orange to reddish-brown on the surface. The pathogen is hard to discern on older stems/branches with thick bark until longitudinal splits appear in the bark (Diller, 1965). Chestnut blight cankers are characterized by mycelial fans and fruiting bodies of the pathogen. The tree may react to canker by producing epicormic shoots below it. Non-lethal, superficial or callusing cankers on susceptible host trees are usually associated with mycovirus-induced hypovirulence (Bryner et al., 2013).

Disease management strategies have focused either on hypovirulence (biological control) or resistance breeding. To prevent the introduction and spreading of *C. parasitica*, quarantine regulations have been adopted worldwide, affecting the movement and trade of chestnut wood and bark, seeds and living plants. In Europe, the European and Mediterranean Plant Protection Organization (EPPO) still recommends the regulation of *C. parasitica* as an A2 quarantine organism locally present in the EPPO region (EPPO, 2018). After *C. parasitica* introduction into a new area, eradication efforts by cutting and burning infected trees have mostly failed (Prospero & Rigling, 2013). Treatment with chemicals does not seem to be a practical option for *C. parasitica* control because the use of chemicals in forests, in most countries, is restricted or prohibited, and fungicides may be phytotoxic or induce resistance in the pathogen. However, Trapiello et al. (2015) showed that the application of triazole (epoxiconazole) fungicides may be helpful under certain management conditions, e.g. in nurseries. In Europe, the

mycovirus *Cryohonectria parasitica* 1 (CHV-1) acts as a successful biological control agent of chestnut blight by causing the so-called hypovirulence. CHV-1 infects *C. parasitica* and reduces its parasitic growth and sporulation capacity. Hypovirulence is present in many chestnut-growing regions of Europe, while disease management in North America is mainly focused on resistance breeding (Rigling & Prospero, 2018).

The antifungal properties of essential oils (EOs) and their constituents have been reported in several studies, most of which focused on the inhibition of fungal mycelial growth *in vitro*. Essential oils, due to their very strong activity, could be promising control agents in future extensive research and *in vivo* experiments. They could be used as supplements to commercial products for disease control, which would minimize the amount of fungicides used. *In vitro* experiments have shown that the volatile phase of certain essential oils, such as those of Scottish pine, eucalyptus, juniper, orange, rosemary and thyme, applied at a concentration of 0.65  $\mu\text{l ml}^{-1}$  of air, inhibit the growth of various soil-borne pathogens: *Fusarium* spp., *Rhizoctonia* sp. and *Pythium* sp. (Tanović et al., 2007). The essential oils of oregano (*Origanum vulgare* L.) and thyme (*Tymus vulgaris* L.) were found in another study to be effective against several phytopathogenic fungi: *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Phytophthora infestans*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Botrytis cinerea*, *Monilinia fructicola*, *Rhizopus stolonifer*, *Macrophomina phaseolina* and *Pythium* sp. (El-Mohamedy et al., 2013). The oils of oregano, common thyme and peppermint (*Mentha piperita* L.) have demonstrated high *in vitro* activity against *Trichoderma* green mould, while adding tea tree oil [*Melaleuca alternifolia* (Maiden and Betche) Cheel] to oyster mushroom substrate or button mushroom casing layer has resulted in considerable *in vivo* inhibition of *Trichoderma harzianum* Rifai (Soković et al., 2009; Kosanović et al., 2013). In an analysis of 22 essential oils, those of wintergreen (*Gaultheria procumbens* L.), lemongrass (*Cymbopogon flexuosus* Stapf.) and oregano, demonstrated the strongest antimicrobial activity against three bacteria that are pathogenic to common bean, tomato and cultivated mushroom (Todorović et al., 2016), while the oils of mint (*Mentha piperita* L.), eucalyptus (*Eucalyptus globulus* Labillardie), black pine (*Pinus nigra* L.) and cade (*Juniperus oxycedrus* L.) showed the strongest antifungal activity against *Verticillium dahliae* Klebahn, a pathogen of pepper (Luković et al., 2019).

The purpose of this study was to assess the vapour antifungal activities of 22 essential oil samples against *C. parasitica* by evaluating their minimal inhibitory and

fungicidal concentrations *in vitro*, using the fumigant macrodilution method in an attempt to promote their use as alternative products for biological control.

## MATERIAL AND METHODS

### Essential oil samples

Twenty-two samples of plant essential oils (EOs) were provided by the Institute for Medicinal Plant Research “Dr. Josif Pančić”, Belgrade (Serbia) (Table 1). They included 11 EOs produced by Frey & Lau, Ulzburg (Germany). The characteristics of samples in this group fully conformed with the quality control requirements given by the Fifth European Pharmacopoeia (Ph. Eur.

5.0). Of the two listed oregano oil samples, the latter was declared nature-identical. The other samples consisted of oils acquired from the Albanian companies Xherdo Co. sh.p.k., Tirana, and Agroherbal sh.p.k, Mamurras.

### Test organism and inoculum preparation

The isolates 4S and M1 of *Cryphonectria parasitica* from the collection of the Institute of Forestry were isolated from infected chestnut trees at two locations near Vranje (Sobina and Muhovac, Serbia) in 2010 (Karadžić et al., 2019). Conidia were harvested by flooding the plates with 10 ml of sterile distilled water and Tween 20 (v/v 0.01%), and then filtration through a double layer of cheesecloth. Conidial suspension was prepared in sterile saline and adjusted to a concentration of approximately  $10^6$  conidia ml<sup>-1</sup>.

**Table 1.** List of tested essential oils

Essential oils	Source*	Designation	Remark
Anise ( <i>Illicium verum</i> Hooker)	Frey & Lau	S0100154	Ph. Eur. 5.0 <sup>+</sup>
Cade ( <i>Juniperus oxycedrus</i> L.)	Xherdo Co.	-	-
Eucalyptus ( <i>Eucalyptus globulus</i> Labillardie)	Frey & Lau	S0100321	Ph. Eur. 5.0 <sup>+</sup>
Lavender ( <i>Lavandula angustifolia</i> Mill.)	Frey & Lau	P0123527	Ph. Eur. 5.0 <sup>+</sup>
Lavender ( <i>Lavandula angustifolia</i> Mill.)	Agroherbal	-	-
Lemon ( <i>Citrus limon</i> L.)	Frey & Lau	P0119551	Ph. Eur. 5.0 <sup>+</sup>
Lemongrass ( <i>Cymbopogon flexuosus</i> Stapf.)	Frey & Lau	P0120241	Ph. Eur. 5.0 <sup>+</sup>
Mint ( <i>Mentha piperita</i> L.)	Agroherbal	No. 41/A	-
Mint ( <i>Mentha piperita</i> L.)	Frey & Lau	P0123884	Ph. Eur. 5.0 <sup>+</sup>
Oregano ( <i>Origanum vulgare</i> L.)	Frey & Lau	P0125062	Ph. Eur. 5.0 <sup>+</sup>
Oregano ( <i>Origanum vulgare</i> L.)	Frey & Lau	P0125412	Ph. Eur. 5.0 <sup>+</sup>
Pine (Black) ( <i>Pinus nigra</i> L.)	Agroherbal	-	-
Pine (Maritime) ( <i>Pinus pinaster</i> Aiton)	Frey & Lau	P0125332	Ph. Eur. 5.0 <sup>+</sup>
Pine (Maritime) ( <i>Pinus pinaster</i> Aiton)	Agroherbal	No. 2	-
Pine (Scotch) ( <i>Pinus silvestris</i> L.)	Frey & Lau	P0124319	Ph. Eur. 5.0 <sup>+</sup>
Rosemary ( <i>Rosmarinus officinalis</i> L.)	Frey & Lau	P0124476	Ph. Eur. 5.0 <sup>+</sup>
Rosemary ( <i>Rosmarinus officinalis</i> L.)	Agroherbal	No. 1	-
Rosemary ( <i>Rosmarinus officinalis</i> L.)	Agroherbal	No. 41	-
Rosemary ( <i>Rosmarinus officinalis</i> L.)	Xherdo Co.	-	-
Sage ( <i>Salvia officinalis</i> L.)	Xherdo Co.	-	-
Silver fir ( <i>Abies alba</i> Mill.)	Xherdo Co.	-	-
Wintergreen ( <i>Gaultheria procumbens</i> L.)	Frey & Lau	P0117394	Ph. Eur. 5.0 <sup>+</sup>

\*Frey & Lau, Ulzburg (Germany)

Agroherbal sh.p.k., Mamurras (Albania)

Xherdo Co. sh.p.k., Tirana (Albania)

<sup>+</sup> Conforms with requirements of the 5<sup>th</sup> European Pharmacopoeia (Ph. Eur. 5.0)

## Antifungal activity of essential oils *in vitro*

Antifungal activity was tested on PDA medium in glass Petri plates (R=100 mm). The medium was inoculated with the test fungi by pipetting 20  $\mu\text{l}$  of conidial suspension into each well cut into plate center (R=10 mm). The inoculum was then exposed to the volatile phase of EOs for three days at 20°C. The oils were applied as single drops onto the inner side of each plate cover at concentrations of 0.02, 0.04, 0.08, 0.16 and 0.32  $\mu\text{l ml}^{-1}$  of air inside Petri plates using the micropipette. The bottom of each plate was immediately placed upon the cover. The plates were sealed with parafilm to prevent gas exchange with the outside environment. Oil concentrations that completely inhibited fungal growth after three-day exposure at 20°C were considered to be fungistatic and the lowest of these concentrations was determined as the minimum inhibitory concentration (MIC). The plates were then opened and ventilated in a laminar flow hood for 30 min in order to remove volatiles and check fungicidal effects. Oil concentrations were considered fungicidal if no fungal growth was observed three days after ventilation. The lowest concentration that caused fungicidal effect was defined as the minimum fungicidal concentration (MFC) (Tanović et al., 2006). Four replicates per treatment were used and the experiment was repeated twice.

## Essential oil analysis

Analyses of the essential oils were performed by gas chromatography (GC) using two detector types. Analytical gas chromatography (GC/FID) analysis of the oils was carried out on a Hewlett-Packard HP-5890 Series II GC device equipped with a split-splitless injector and automatic liquid sampler (ALS), attached to an HP-5 column (25 m x 0.32 mm, 0.52  $\mu\text{m}$  film thickness) and fitted to a flame ionization detector (FID). Carrier gas flow rate ( $\text{H}_2$ ) was 1  $\text{mL min}^{-1}$ , split ratio 1:60, injector temperature was 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40-260°C (at a rate of 4°C  $\text{min}^{-1}$ ). Solutions of essential oil samples in ethanol (~1%) were consecutively injected by ALS (1  $\mu\text{l}$ , split ratio=1:20). Area percent reports, obtained as a result of standard processing of chromatograms, were used as a base for quantification purposes. The same analytical conditions as those mentioned for the GC/FID were employed for gas chromatography/mass spectrometry (GC/MS) analysis, along with column HP-5MS (30 m x 0.25 mm, 0.25  $\mu\text{m}$  film thickness), using a Hewlett-Packard HP G 1800C Series II GCD system. Instead of hydrogen, helium

was used as the carrier gas. Transfer line was heated to 260°C. Mass spectra were acquired in EI mode (70 eV), in  $m/z$  range 40-450. Sample solutions in ethanol (~1 %) were injected by ALS (200 nL, split ratio=1:20). Oil components were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental data on retention indices were determined by using calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1.), compared to those from available literature (Adams, 2007) and used as an additional tool to approve MS findings.

## RESULTS

The growth of *C. parasitica* isolate M1 was inhibited by 11 essential oils (EOs), while isolate 4S was inhibited by 14 out of 22 tested oil samples, which were applied in a range of concentrations from 0.02 to 0.32  $\mu\text{l ml}^{-1}$  of air (Table 2). Eleven oil samples showed neither inhibitory nor lethal effects on isolate M1, while eight oil samples had neither inhibitory nor lethal effects on isolate 4S. Growth inhibition of the test pathogen after three days was achieved by both mint oil samples, eucalyptus, black pine, cade, sage, silver fir, three samples of pine and one sample of oregano against isolate M1 and two samples of mint, eucalyptus, black pine, cade, sage, silver fir, three samples of pine, one oregano sample, a sample of lavender and two samples of rosemary against isolate 4S. Three oil samples (two mint samples and one of black pine) exhibited lethal effects on both pathogen isolates. In addition, two more EOs (sage and silver fir) had lethal effects on isolate M1 and two EOs (cade and eucalyptus) on isolate 4S. The strongest growth inhibitors of *C. parasitica* were two samples of mint (from Germany and Albania) and the black pine EO, having MIC and MFC values of 0.02  $\mu\text{l ml}^{-1}$  of air for both isolates. Sage and silver fir were the next most effective EOs against isolate M1. Their MIC and MFC were 0.02  $\mu\text{l ml}^{-1}$  and 0.04  $\mu\text{l ml}^{-1}$ , respectively. Cade and eucalyptus EOs showed antifungal activity against isolate 4S, having MIC values of 0.08 and 0.16  $\mu\text{l ml}^{-1}$ , respectively, and MFC of 0.32  $\mu\text{l ml}^{-1}$  of air. The following oils also demonstrated good inhibitory effects against both isolates: oregano, three pine samples, lavender and rosemary, having different MICs, and MFCs exceeding 0.32  $\mu\text{l ml}^{-1}$  of air (Table 2). Fungicidal effects were shown by seven samples of oils (two samples of mint, black pine, sage, silver fir, cade and eucalyptus), having different MFCs (0.02, 0.04 and 0.32  $\mu\text{l ml}^{-1}$  of air) after four-day exposure.

**Table 2.** Effective concentrations of essential oils ( $\mu\text{g ml}^{-1}$  of air) against two isolates (M1 and 4S) of *Cryphonectria parasitica* (Murr.) Barr.

Essential oils	Isolate M1		Isolate 4S	
	MIC <sup>a</sup>	MFC <sup>b</sup>	MIC <sup>a</sup>	MFC <sup>b</sup>
Anise S0100154	>0.32	>0.32	>0.32	>0.32
Cade Xherdo Co.	0.16	>0.32	0.08	0.32
Eucalyptus S0100321	0.16	>0.32	0.08	0.32
Lavender P0123527	>0.32	>0.32	>0.32	>0.32
Lavender Agroherbal	>0.32	>0.32	0.32	>0.32
Lemon P0119551	>0.32	>0.32	>0.32	>0.32
Lemongrass P0120241	>0.32	>0.32	>0.32	>0.32
Mint No. 41/A, Agroherbal	0.02	0.02	0.02	0.02
Mint P0123884	0.02	0.02	0.02	0.02
Oregano P0125062	>0.32	>0.32	>0.32	>0.32
Oregano P0125412	0.16	>0.32	0.16	>0.32
Pine (Black) Agroherbal	0.02	0.02	0.02	0.02
Pine (Maritime) P0125332	0.32	>0.32	0.32	>0.32
Pine (Maritime) No. 2, Agroherbal	0.16	>0.32	0.32	>0.32
Pine (Scotch) P0124319 Ph.	0.32	>0.32	0.32	>0.32
Rosemary PO124476	>0.32	>0.32	0.32	0.32
Rosemary No. 1, Agroherbal	>0.32	>0.32	>0.32	>0.32
Rosemary No. 41, Agroherbal	>0.32	>0.32	0.32	>0.32
Rosemary Xherdo Co.	>0.32	>0.32	>0.32	>0.32
Sage Xherdo Co.	0.04	0.04	0.04	>0.32
Silver fir Xherdo Co.	0.02	0.04	0.02	>0.32
Wintergreen P0117394	>0.32	>0.32	>0.32	>0.32

<sup>a</sup>Minimal concentration of essential oil causing complete inhibition of pathogen growth after tree-day exposure (minimum inhibitory concentration)

<sup>b</sup>Minimal concentration of essential oil showing lethal effect on the pathogen (minimum fungicidal concentration)

Only three EO samples (two of mint, and black pine) were lethal to both isolates. Sage and silver fir oils were more toxic to isolate M1, while cade and eucalyptus oils were more toxic to isolate 4S. Eight oils (anise, lemon, lemongrass, two samples of rosemary, a sample of lavender and wintergreen) did not inhibit pathogen growth at all. Three more samples (one of lavender and two of rosemary) did not inhibit the growth of isolate M1.

## DISCUSSION

The results of this study indicate that some of the tested essential oils have the ability to suppress the growth of *C. parasitica* *in vitro*. Of the 22 essential oil samples analyzed, 14 (two mint samples, cade, eucalyptus, black pine, silver fir, sage, lavender, oregano, four samples of pine and rosemary) inhibited the growth of *C. parasitica*, while seven oils (two samples of mint, eucalyptus, black

pine, silver fir, sage and cade) had fungicidal effects on the pathogen.

Analyzing the same 22 essential oils from Germany and Albania, Todorović et al. (2016) found those of wintergreen, lemongrass and oregano to demonstrate the strongest antimicrobial activity against three pathogenic bacteria: *Xanthomonas campestris* pv. *phaseoli*, *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas tolaasii*, the pathogens of common bean, tomato and cultivated mushroom. On the other hand, mint oil samples showed the strongest activity at 0.02  $\mu\text{l ml}^{-1}$  of air, followed by eucalyptus, black pine and cade in a study against *V. dahliae* (Luković et al., 2019), all oils being lethal to the tested fungus. Similar results were obtained in the current study, where the strongest activity towards two isolates of *C. parasitica* was shown by two samples of mint oil and black pine at 0.02  $\mu\text{l ml}^{-1}$  of air, followed by eucalyptus, black pine, cade, sage and silver fir, which were also lethal. Lee et al. (2008) tested

11 Myrtaceae plant essential oils, using the fumigation method, and found that *Leptospermum petersonii* EOs had the most potent antifungal activity against three phytopathogenic fungi (*Phytophthora ramorum*, *C. parasitica* and *Fusarium circinatum*). Inhibition rate of *C. parasitica* by *Eucalyptus citridora* EO was 29.4%, while *Leptospermum petersonii* EO achieved 98.5% inhibition. Lee et al. (2008) also tested the antifungal activity of several identified compounds of Myrtaceae plant essential oils. In a test with *C. parasitica*, the inhibition rates of the compounds nerol and geraniol were 61.7% and 68.9%, respectively, at concentration  $28 \times 10^{-3} \text{ mg ml}^{-1}$  of air. An analysis of essential oils obtained from 40 plant species against three phytopathogenic fungi in another study showed that the inhibition rate of patchouli (*Pogostemon patchouli*) essential oil was 51%, while the other oils had weak activity against *C. parasitica* (Lee et al., 2009).

*In vitro* experiments have also shown that the volatile phase of certain essential oils, such as those of Scottish pine, eucalyptus, juniper, orange, rosemary and thyme, applied at the concentration of  $0.65 \mu\text{l ml}^{-1}$  of air, inhibited the growth of some soil-borne pathogens: *Fusarium* spp., *Rhizoctonia* sp. and *Pythium* sp. (Tanović et al., 2007). In another study, eucalyptus and clove oils showed fungistatic effects on *Fusarium culmorum* and *Alternaria alternata* (Byron & Hall, 2002). Moreover, the essential oils of oregano and thyme were effective against several phytopathogenic fungi: *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Phytophthora infestans*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Botrytis cinerea*, *Monilinia fructicola*, *Rhizopus stolonifer*, *Macrophomina phaseolina* and *Pythium* sp. (El-Mohamedy et al., 2013). Essential oils of mint, thyme and oregano have demonstrated high *in vitro* activity against *Trichoderma* green mould, while tea tree oil added to oyster mushroom substrate or button mushroom casing layer has resulted in considerable *in vivo* inhibition of *Trichoderma harzianum* (Soković & Van Griensven, 2006; Kosanović et al., 2013; Đurović-Pejčev et al., 2014). Đurović-Pejčev et al. (2014) analyzed six essential oils, and found only the oil of peppermint to exhibit lethal effect on *Trichoderma aggressivum* f. *europaeum*.

The EOs of mint, black pine and eucalyptus were some of the most successful oils tested in the current study in terms of their highest inhibition and lethal activity. A previous study had shown that the main components of mint essential oil were: menthone (37.02%), menthol (29.57%) and isomenthone (9.06%) (Đurović-Pejčev et al., 2014). The composition of black pine EO was analyzed by Koutsaviti et al. (2015). *P. nigra* EO was dominated by  $\alpha$ -pinene (24.9 – 28.9%) and germacrene

D (20.3 – 31.9%). In the study by Luković et al. (2019), 1.8-cineole was identified as the dominant oil component of eucalyptus essential oil (82.8%).

The results of this study show that mint, eucalyptus, black pine, silver fir, sage and cade essential oils qualify, based on their potentials, for further *in vivo* experiments against *C. parasitica*. The strongest inhibitory and fungicidal effects were displayed by the oils of two samples of mint, black pine, eucalyptus, silver fir and cade. It will be of great importance in further research to test these oils *in vivo* as potential biofungicides for the control of chestnut blight.

## ACKNOWLEDGEMENT

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

## REFERENCES

- Adams, R.P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry* (4<sup>th</sup> ed.). Carol Stream, IL, USA: Allured Publishing Corporation.
- Bryner, S.F., Sotirovski, K., Akilli, S., Risteski, M., Perlerou, C., & Rigling, D. (2013). Informative value of canker morphology on the presence or absence of virus infection in chestnut blight cankers. *Forest Pathology*, 43(6), 496-504. doi: <https://doi.org/10.1111/efp.12063>
- Byron, E.M., & Hall, A.M. (2002). Inhibition of common cereal pathogenic fungi by clove oil and eucalyptus oil. In T. Margini (Ed.), *Proceedings of Brighton Crop Protection Conference – Pest & Diseases 2* (pp 765-768). Brighton, UK: University of Brighton Press.
- Diller, J.D. (1965). Chestnut blight. *USDA Forest Pest Leaflet*, 94. Retrieved from [https://www.fs.usda.gov/Internet/FSE\\_DOCUMENTS/fsbdev2\\_043617.pdf](https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fsbdev2_043617.pdf)
- 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. doi:10.2298/pif1404291d
- Dutech, C., Barres, B., Bridier, J., Robin, C., Milgroom, M.G., & Ravigne, V. (2012) The chestnut blight fungus world tour: Successive introduction events from diverse origins in an invasive plant fungal pathogen. *Molecular Ecology*, 21(16), 3931-3946. doi: 10.1111/j.1365-294X.2012.05575.x

- El-Mohamedy, R.S.R., Abdel-Kader, M.M., Abd-El-Kareem, F., & El-Mougy, N.S. (2013). Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato rot pathogens *in vitro*. *Journal of Agricultural Technology*, 9(6), 1507-1520.
- EPPO (2018). EPPO A2 list of pests recommended for regulation as quarantine pests - version 2019-09. Retrieved from [https://www.eppo.int/ACTIVITIES/plant\\_quarantine/A2\\_list](https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list)
- Karadžić, D., & Milenković, I. (2013). *Cryphonectria parasitica* (Murill.) Barr occurrence of parasitic fungus on sessile oak in Serbia. *Šumarstvo*, 1-2, 1–8 (in Serbian with English summary)
- Karadžić, D., Radulović, Z., Sikora, K., Stanivuković, Z., Golubović Ćurguz, V., Oszako, T., & Milenković, I. (2019). Characterisation and pathogenicity of *Cryphonectria parasitica* on sweet chestnut and sessile oak trees in Serbia. *Plant Protection Science*, 55(3), 191-201. doi:10.17221/38/2018-pps
- 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(2), 218-230. doi: 10.1111/aab.12048
- Koutsaviti, K., Giatropoulos, A., Pitarokili, D., Papachristos, D., Michaelakis, A., & Tzakou, O. (2015). Greek *Pinus* essential oils: Larvicidal activity and repellency against *Aedes albopictus* (Diptera: Culicidae). *Parasitology Research*, 114(2), 583-592. doi:10.1007/s00436-014-4220-2
- Lee, Y.S., Kim, J., Lee, S.G., Oh, E., Shin, S.C., & Park, I.K. (2009). Effects of plant essential oils and components from oriental sweetgum (*Liquidambar orientalis*) on growth and morphogenesis of three phytopathogenic fungi. *Pesticide Biochemistry and Physiology*, 93(3), 138-143.
- Lee, Y.S., Kim, J., Shin, S.C., Lee, S.G., & Park, I.K. (2008). Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi. *Flavour and Fragrance Journal*, 23, 23-28. doi:10.1002/ffj.1850
- Luković, J., Todorović, B., Milijašević-Marčić, S., Rekanović, E., Kostić, M., Đurović-Pejčev, R., & Potočnik, I. (2019). Antifungal activity of plant essential oils against *Verticillium dahliae* Klebahn, the causal agent of Verticillium wilt of pepper. *Pesticides & Phytomedicine*, 34(1), 39-46. doi:<https://doi.org/10.2298/PIF1901039L>
- Marinković P., & Karadžić D. (1985). The most important diseases in nurseries, plantations and forests of Kosovo. *Šumarstvo*, 2-3, 29-38 (in Serbian with English summary).
- Prospero, S., & Rigling, D. (2013). Chestnut blight. In: P. Gonthier and G. Nicolotti, (eds), *Infectious forest diseases* (pp 318-338). Wallingford, UK: CAB International.
- Radocz, L., & Tarcali, G. (2009). "Chestnut blight" infection on oaks in the Carpathian basin. *Cereal Research Communications*, 37, 265-268.
- Rigling, D., & Prospero, S. (2018). *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. *Molecular Plant Pathology*, 19(1), 7-20.
- Roane, M.K., Griffin, G.J., & Elkins, J.R. (1986). Chestnut blight, other *Endothia* diseases, and the genus *Endothia* (APS Monograph Series 53). St. Paul, MN: APS Press.
- Soković, M.D., Vukojević, J., Marin, P.D., Brkić, D.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(1), 238-249. doi: 10.3390/molecules14010238
- 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. doi: 10.1007/s10658-006-9053-0
- Tanović, B., Milijašević, S., & Obradović, A. (2007). *In vitro* effect of plant essential oils on of some soil-borne pathogens. *Acta Horticulturae*, 729, 483-487. doi: 10.17660/ActaHortic.2007.729.79
- Tanović B., Potočnik I., Stanisavljević B., Đorđević M., & Rekanović E. (2006). Response of *Verticillium fungicola* var. *fungicola*, *Mycogone perniciosa* and *Cladobotyrum* sp. mushroom pathogens to some essential oils. *Pesticides & Phytomedicine*, 21, 231-237.
- 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*, 51(12), 832-839. doi: 10.1080/03601234.2016.1208462.
- Trapiello, E., Gonzalez-Varela, G., & Gonzalez, A.J. (2015). Chestnut blight control by agrochemicals in *Castanea sativa* under managed conditions. *Journal of Plant Diseases and Protection*, 122(3), 120-124.
- Tziros, G.T, Nakopoulou, Z.G., & Diamandis, S. (2015). *Cryphonectria parasitica*, the chestnut blight fungus, causes cankers on *Quercus frainetto* in Greece. *Australian Plant Disease Notes*, 10, 19.

# Toksičnost različitih etarskih ulja za *Cryphonectria parasitica* (Murr.) Barr, glavnog prouzrokovača raka kore kestena

## REZIME

Primenom makrodilucione fumigantne metode ispitana je inhibitorna i fungicidna aktivnost 22 uzorka etarskih ulja iz Nemačke i Albanije za *Cryphonectria parasitica* (Murr.) Barr, prouzrokovača raka kore kestena. Efikasnost etarskih ulja testirana je na različitim koncentracijama (0,02, 0,04, 0,08, 0,16 i 0,32  $\mu\text{l ml}^{-1}$  vazdušne faze) i na osnovu njih su određene minimalne inhibitorne i fungicidne koncentracije. Najjače dejstvo ispoljila su dva uzorka etarskog ulja nane i etarsko ulje crnog bora, sa minimalnim inhibitornim i fungicidnim koncentracijama od 0,02  $\mu\text{l ml}^{-1}$  vazdušne faze za oba testirana izolata (M1 i 4S), zatim etarska ulja eukaliptusa, jele, žalfije i crvene kleke. Samo tri etarska ulja (dva uzorka nane i crni bor) bila su letalna za oba izolata. Etarska ulja žalfije i jele bila su toksičnija za izolat M1, dok su etarska ulja eukaliptusa i crvene kleke bila toksičnija za izolat 4S. Petnaest od 22 ispitivana uzorka etarskih ulja ispoljila su inhibitorni efekat: dva uzorka nane, crvena kleka, eukaliptus, crni bor, lavanda, žalfija, jela, tri uzorka bora, origano i dva uzorka ruzmarina, dok je sedam etarskih ulja ispoljilo i fungicidni efekat (dva uzorka nane, eukaliptus, crni bor, žalfija, jela i crvena kleka).

**Ključne reči:** etarska ulja; rak kore kestena; antifungalno delovanje; biofungicidi