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ANTI-DECAY POTENTIAL OF FIVE ESSENTIAL OILS AGAINST THE WOOD-DECAYING FUNGI *SERPULA LACRYMANS* AND *TRAMETES VERSICOLOR*

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ABSTRACT

The antifungal efficiency of five selected essential oils – cinnamon, oregano, thyme, basil, and clove against the brown-rot fungus *Serpula lacrymans* and the white-rot fungus *Trametes versicolor* are discussed in the paper. The antifungal screenings were performed in Petri dishes filled with solidified malt agar medium by the poisoned ring-paper method. Two parameters measured on the 4th, 7th, 10th, and 13th days: (1) I_{Soil} – the growth inhibition index of fungal mycelia on the malt agar medium-soil to ϕ12 mm rings of antibiotics test paper impregnated with 0 wt.%, 0.25 wt.%, 1 wt.%, 10 wt.% or 100 wt.% concentration of essential oils; and (2) I_{Paper} – the slowed or totally stopped growth of fungal mycelia on ϕ12 mm rings of antibiotics test paper poisoned with essential oils were evaluated. Antifungal efficiency of basil oil containing mainly linalool is the highest. On the contrary, antifungal efficiency of clove oil containing mainly eugenol is the lowest.

Key words: essential oils, decaying fungi, screening test, fungicidal effect.

INTRODUCTION

Preservation of wood from decay and other biodegradation processes plays an important role during its storage, transportation, and service. Environmental issues from conventional toxic chemical preservatives containing heavy metals, condensed or chlorinated aromatic hydrocarbons, and several other pollutants having a higher toxicity for humans, in connection as well as with their disposal problems, have urged the search for more ecological friendly preservative substances—including natural ones (REINPRECHT 2010, MEDEIROS *et al.* 2016, CAI *et al*. 2019).

This work studies the methods of wood protection related to the use of essential oils from plants. These methods have many advantages: a) plants can be produced in large amounts as agricultural products; b) some essential oils combine antifungal with insecticidal and anti-bacterial effects; c) essential oils are usually health-friendly and many of them are used in medicine, aromatherapy, or cosmetics; and d) essential oils cause only small problems in terms of liquidation of treated products after their service-life. On the contrary, some disadvantages of using essential oils for wood preservation can also occur, e.g., a) high volatility; b) non-stabile concentration of effective compounds (BATISH *et al*. 2008, CAI *et* *al.* 2019); and c) a relatively lower biocidal efficacy in comparison to traditional fungicides (REINPRECHT *et al.* 2013).

Essential oils are natural products of various origin characterized by a strong smell. They have high variability in their chemical composition, both in qualitative and quantitative terms. Their main components belong to various chemical classes such as terpenes, phenols, aliphatic alcohols, ethers, aldehydes, ketones, esters, amines, amides, (DHIFI *et al.* 2016).

A certain fungicidal efficacy of essential oils against decaying fungi, staining fungi, and moulds (microscopic fungi) was demonstrated by XIE *et al. (*2017) as well as by more other researchers. For example, CHENG *et al.* (2008) demonstrated the anti-decay activity of cinnamaldehyde and eugenol congeners; CHITTENDEN and SINGH (2011) found the fungicidal effect of geranium oil and of eugenol and cinnamaldehyde extracts against several decaying and staining fungi; MOHAREB *et al.* (2013) proved that from 18 essential oils, the following six oils isolated from plants had a more significant inhibition effect against the decaying fungi *Hexagonia apiaria* and *Ganoderma lucidum*: *Artemisia monosperma*, *Cupressus sempervirens*, *Citrus limon*, *Thuja occidentalis*, *Schinus molle*, and *Pelargonia graveolens*; SU *et al.* (2013) demonstrated the antifungal activity of essential oils containing α-cadinol and elemol isolated from leaves and fruits of *Juniperus formosana*; REINPRECHT *et al.* (2013) in screening tests with poisoned rings of Whatman paper and PÁNEK *et al.* (2014) in standard tests with preserved wood samples demonstrated that thyme and oregano oils were the most effective against moulds *Aspergillus niger* and *Penicillium brevicompactum* and against the brown-rot fungus *Coniophora puteana*, while clove, savory and birch oils had a lower biocidal efficacy; POP *et al*. (2018), by the CT screening test method in Petri dishes (*i.e*. using two half disks of poisoned Whatman paper with a diameter of 80 mm), demonstrated fungicidal potential of cinnamon and clove oils against the decaying fungi *Postia placenta* and *Trametes versicolor*; BAHMANI and SCHMIDT (2018) tested the bioactivity of 16 essential oils against common moulds (*Aspergillus niger*, *Penicillium commune*) and important decaying fungi (*Coniophora puteana*, *Trametes versicolor*, *Chaetomium globosum*) found the highest efficiency for lavender, lemon grass and thyme oils.

The aim of our experiments is to determine the antifungal potential of selected essential oils against the growth activity of two important wood-decaying fungi *Serpula lacrymans* and *Trametes versicolor*.

MATERIALS AND METHODS

Essential oils

Five essential oils under the label Steaua Divina, Romania—cinnamon, oregano, thyme, basil, and clove (tab. 1)—were used in the experiment as 0.25 wt.%, 1 wt.%, or 10 wt.% ethanol solutions and as the original 100% concentrate.

Antifungal screenings

The antifungal activities of individual essential oils (Table 1) against the brown-rot fungus *Serpula lacrymans* (Schumacher ex Fries) S.F.Gray /*S. lacrymans* (Wulfen) J. Schröt. – by IndexFungorum/, strain BAM 87 (Bundesanstalt für Materialforshung und -prüfung, Berlin), and the white-rot fungus *Trametes versicolor* (Linnaeus ex Fries) Pilat /*T. versicolor* (L.) Lloyd – by IndexFungorum/, strain BAM 116 (Bundesanstalt für Materialforshung und -prüfung, Berlin) were determined by the poisoned ring-paper method (REINPRECHT *et al.* 2003) using paper rings immersed in individual wood-protecting substances.

Tab. 1 Essential oils used in the experiment.

Common name	Scientific name	Major effective components
Cinnamon	*Cynnamomum verum*	Cinnamaldehyde (33%), Linalool (17%), Eugenol (17%), Cinnamyl acetate (12%), Linalyl acetate (2.3%)
Oregano	*Origanum vulgare*	Carvacrol (64%), p-Cymene (12.6%), Terpineol (4.8%), Linalool (3.7%)
Thyme	*Thymus vulgaris*	Thymol (47.6%), γ-Terpinene (30.9%), p-Cymene (8.4%), Carene (3.8%)
Basil	*Ocimum basilicum*	Linalool (47%), β-Elemene (7.8%), Farmesene (6.9%), Guainene (5.3%)
Clove	*Eugenia caryophyllata*	Eugenol (88.6%), Eugenyl acetate (5.6%), β-Caryophyllene (1.4%), 2-Heptanone (0.95%)

The antifungal screenings were performed in glass Petri dishes with a diameter of 100 mm, filled in an autoclave sterilized and in Petri dish solidified 3–4 mm thick layer of 4.5 wt.% malt agar medium (HiMedia, Ltd., India). First, in the central points of Petri dishes the agar malt media were inoculated with ca 5 × 5 mm mycelia of decaying fungi *S. lacrymans* or *T. versicolor*. Then, 4 rings of Antibiotic Test Paper (Fischer Scientific, Czech Republic) with a diameter of 12 mm were placed in each Petri dish, with a distance of 20 mm from the border of the fungal inoculate; into each Petri dish were placed 3 rings of poisoned paper impregnated at atmospheric pressure during 30 seconds with an individual type and concentration of essential oil, and 1 ring of control paper impregnated with 96% ethanol. All paper rings, before placing into Petri dishes, were conditioned 1 hour at a temperature of 22 ± 1 °C. In totally for each series, i.e. for each type of essential oil and its concentration, as well as for each fungus species, six poisoned papers were tested. The antifungal screenings were implemented in sterilized thermostats at a temperature of 22 ± 1 °C for 13 days.

In literature, several other screening methods are recommended for determining the fungicidal performance of potential wood-protecting substances, e.g. POP and VARODI (2017) compared five different screening methods at testing fungicidal efficacy of copper sulphate and biocide Romalit N.

Evaluation of screenings

Evaluation of the antifungal efficiency of individual essential oils was performed based on two fungal growth parameters:

I_{Soil} is the growth inhibition index of fungal mycelium on the solidified malt agar medium, valued from a distance change between the border of growing fungal mycelium and the border of poisoned paper; this index was established to the time when the inhibiting zone of fungal mycelium to the border of control paper (IZc) was already minimal ca 0 mm in a Petri dish; this index could be for lower concentrations of essential oils ascertained only during the first days of screenings; this index also depended on the oil type because bioactive vapours of some essential oils (e.g. basil and cinnamon) in the space of Petri dishes could, in accordance with ZYANI *et al.* (2011), to suppress a growth of fungal mycelium to the control papers, as documented in Figures 1 and 2 by stars in some graphs having 100% growth inhibition indexes and in Figures 4 and 5 by photos for 10 wt.% and 100 wt.% oil concentrations.

$$I_{Soil} = (IZ_{Oil} - IZ_C)/(20 - IZ_C) \times 100 \text{ [\%]} \quad (1)$$

where: IZ_{Oil} is the inhibiting zone to the poisoned paper impregnated with essential oil [mm], IZ_C is the inhibiting zone to the control paper [mm], 20 is the initial distance between the border of fungal mycelium-inoculate and the border of paper [mm].

I_{Paper} is the growth inhibition index of fungal mycelium on the poisoned paper ring impregnated with essential oil; this index is smaller than 100% from the moment when the fungal mycelium starts to contact the oil-poisoned paper or just starts to grow on its surface.

$$I_{Paper} = (\Delta Lc - \Delta L_{Oil})/(\Delta Lc) \times 100 \ [\%] \qquad (2)$$

where: ΔL_C is the mycelium length acquisition on the control paper between two time intervals (e.g. 3 days between 4th and 7th day) [mm], ΔL_{Oil} is the mycelium length acquisition on the poisoned paper impregnated with essential oil between two time intervals of an identical duration, as was determined for the control paper (e.g. 3 days between the 10th and 13th days) [mm].

RESULTS AND DISCUSSION

The results from the antifungal screenings of five essential oils are summarized in Figures 1, 2 and 3 and documented by photographs in Figures 4 and 5. The efficiency of tested essential oils in a concentration of 0.25 wt.% was none or only minimal. All essential oils started to be more effective against the growth of fungal mycelia at using their 10 wt.% concentration, at which their 100 wt. % concentrates totally stopped fungal mycelia growth on the rings of oil-poisoned papers (except for clove oil) and concentrates of basil and cinnamon oils even on the malt agar medium.

Basil oil, containing 47% of linalool, had the best antifungal potential. For example, at its application in 10 wt.% concentration, the growth inhibition indexes of fungal mycelia on the malt agar medium soil were the highest (I_{Soil} = 100% for *S. lacrymans* after 4th day; I_{Soil} = 25% for *S. lacrymans* after 7th day; I_{Soil} = 100% for *T. versicolor* after 4th and 7th day), as shown in Figures 1 and 2, and the growth of mycelia on the oil-poisoned papers occurred only after 10 days (*S. lacrymans*) or 13 days (*T. versicolor*), as shown in Figure 3. However, the efficiency of 10 wt.% concentration of basil oil against fungi stopped after 13 days when mycelia intensively grew on oil-poisoned paper surfaces, as shown in Figures 4 and 5. Generally, achieved results (Figures 1 – 3) indirectly shoved that basil oil will have a more evident antifungal efficiency only at higher amounts in preserved materials.

Clove oil, containing 88.6% of eugenol, proved to be the least effective against decaying fungi. Clove oil had the lowest growth inhibition indexes of fungal mycelia on the malt agar medium, as shown in Figures 1 and 2, at which growth of fungal mycelia on the oil-poisoned papers occurred relatively faster than for other oils, as shown in Figures 3, 4 and 5. For example, at the application of clove oil in 10 wt.% concentration, the results obtained the lowest or no growth inhibition indexes of fungal mycelia on the malt agar medium (I_{Soil} from 31.5% to 0% after 4 and 7 days), as shown in Figures 1 and 2.

Other essential oils had a medium antifungal efficiency in this order “Cinnamon > Thyme > Oregano”, and this order is documented by the growth inhibition indexes of mycelia on the malt agar medium (I_{Soil}), as shown in Figures 1 and 2, and on the oil-poisoned papers (I_{Paper}), as shown in Figure 3.

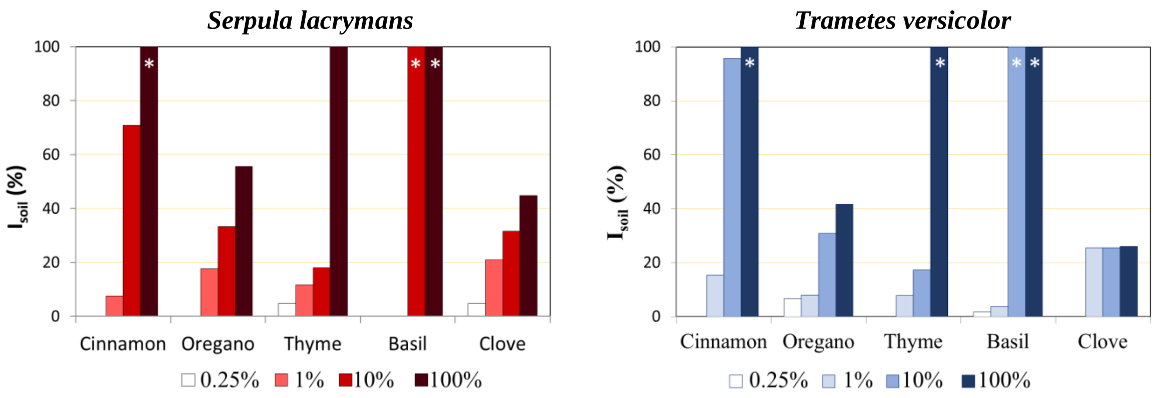

Fig. 1 **I_{Soil} – the growth inhibition index of fungal mycelium on the malt agar medium on its growth to oil-poisoned papers. Evaluated after 4 days (n = 6).**
Note: Stars in some graphs having 100% growth inhibition indexes indicate that growth of fungal mycelium was suppressed also on the malt agar medium to the control papers.

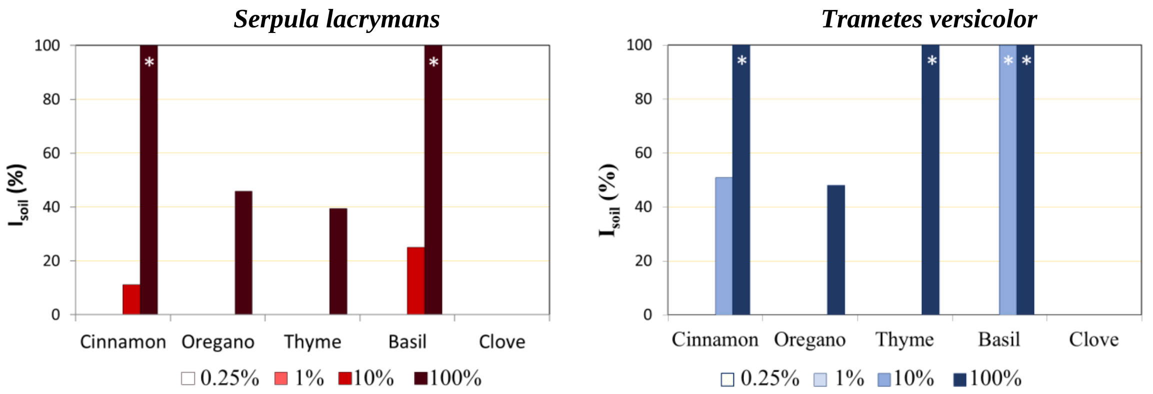

Fig. 2 **I_{Soil} – the growth inhibition index of fungal mycelium on the malt agar medium on its growth to oil-poisoned papers. Evaluated after 7 days (n = 6).**
Note: Stars in some graphs having 100% growth inhibition indexes indicate that growth of fungal mycelium was suppressed also on the malt agar medium to the control papers.

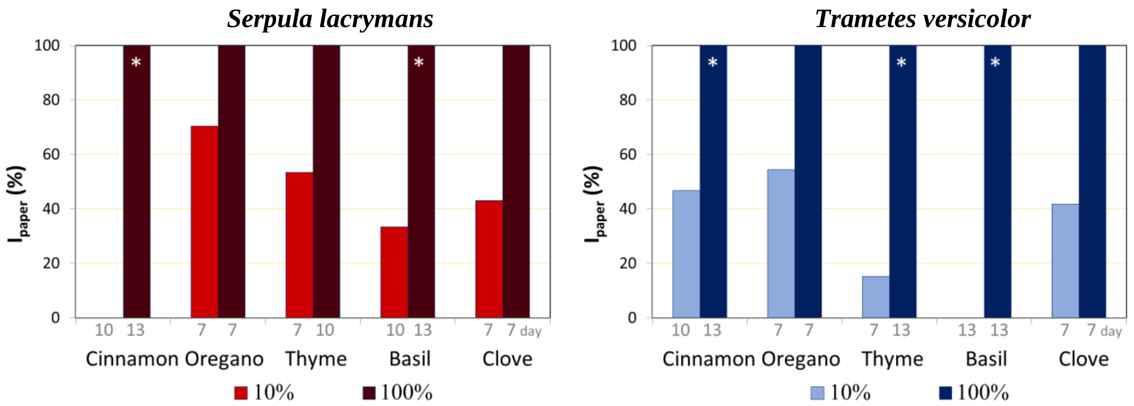

Fig. 3 **I_{Paper} – the growth inhibition index of fungal mycelium on the oil-poisoned paper. Evaluated between 3 days: *7 day* → 3 days between 4th–7th day /quick growth/; *10 day* → 3 days between 7th–10th day; *13 day* → 3 days between 10th–13th day /late or none growth/ (n = 6).**
Note: Stars in some graphs having 100% growth inhibition indexes indicate that growth of fungal mycelium was suppressed also on the control papers due to evaporation of VOC from essential oils.

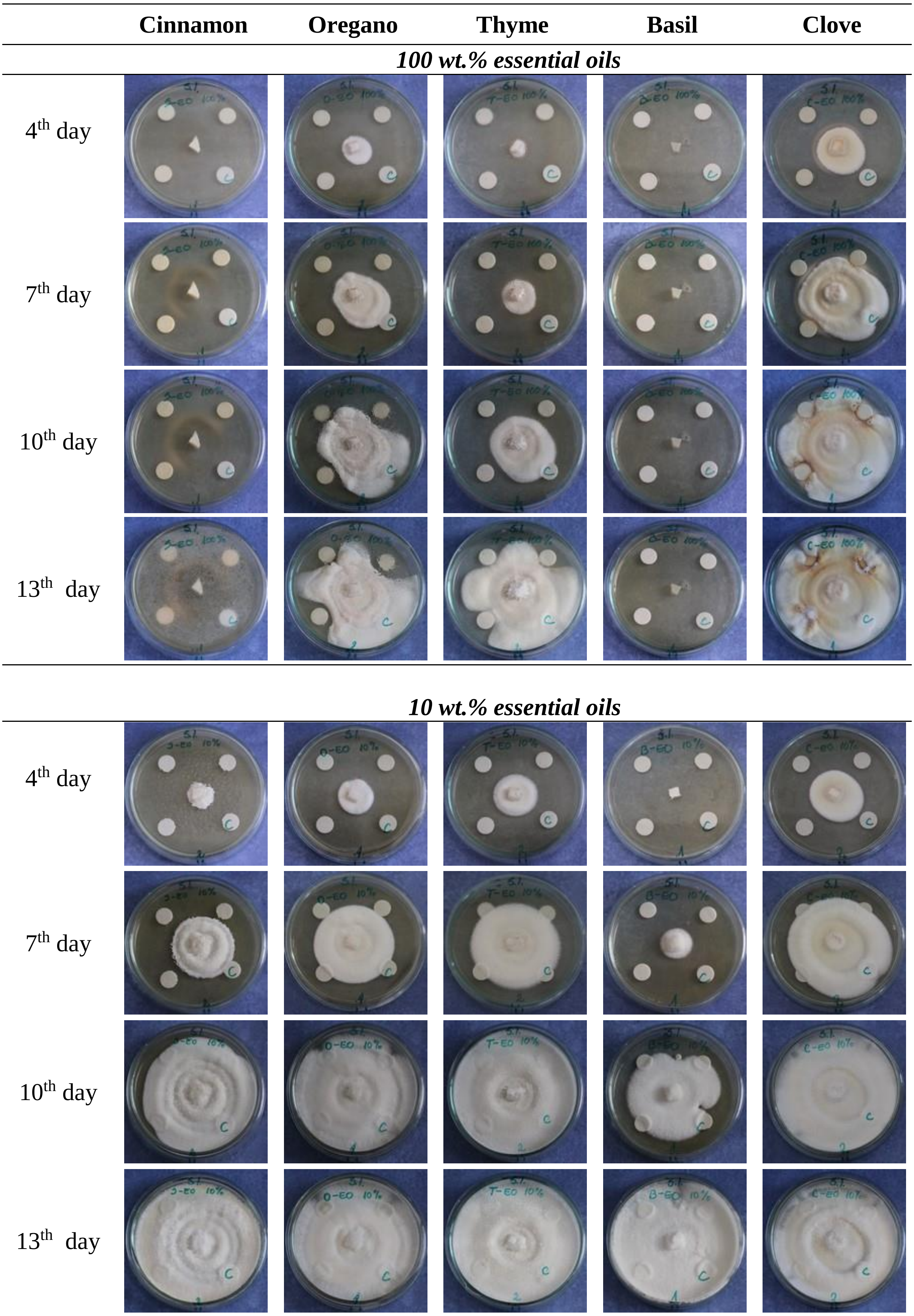

Fig. 4 Photos from the screening of essential oils (100 wt.% and 10 wt.%) against the brown-rot fungus *Serpula lacrymans* by the poisoned ring-paper method (3 poisoned and 1 C-control paper in the dish).

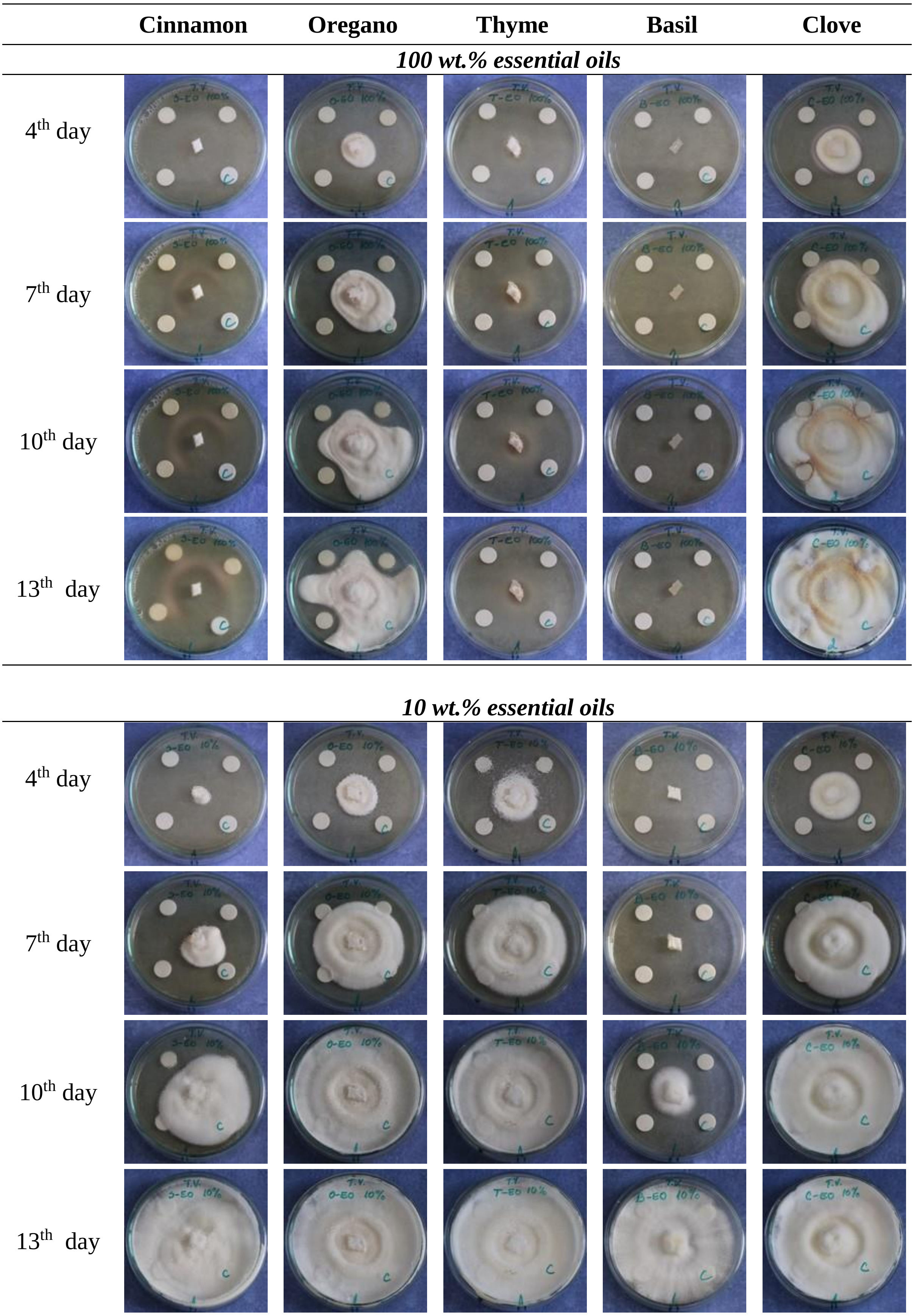

Fig. 5 Photos from the screening of essential oils (100 wt.% and 10 wt.%) against the white-rot fungus *Trametes versicolor* by the poisoned ring-paper method (3 poisoned and 1 C-control paper in the dish).

POP *et al*. (2018), using another screening method for testing two essential oils, for cinnamon oil also established a higher efficiency against decaying fungi *P. placenta* and *T. versicolor* than for clove oil. REINPRECHT *et al.* (2013) in screenings performed similarly with method of this work found that thyme and oregano oils are relatively more effective against *C. puteana* and *T. versicolor* than birch oil, clove oil and savory oil.

Essential oils used in this work had not a higher inhibitory potential against the brown-rot fungus *Serpula lacrymans* than against the white-rot fungus *Trametes versicolor* (Figures 1–3 and Figures 4 and 5). On the contrary, VODA *et al*. (2003) and PÁNEK *et al.* (2014) in experiments with impregnated wood samples found that the efficiency of essential oils is higher against the brown-rot fungus *C. puteana* in comparison to the white-rot fungus *T. versicolor*. This phenomenon related to white-rot fungi was explained by BAYRAMOGLU and ARICA (2009), who have found an apparently higher production of extracellular ligninolytic enzyme laccase by the white-rot fungus *T. versicolor*. Laccase not only disrupts lignin but also inactivates effective phenolic compounds in essential oils, e.g. thymol, carvacrol or eugenol.

Individual types of essential oils obtained from plants contain many different tens to hundreds of constituents (BAHMANI and SCHMIDT 2018). Several constituents of essential oils, e.g. various monoterpenes and phenols, can inhibit growth and enzymatic function of decaying fungi. The individual components of some oil may act synergistically while several compounds may have even a stimulating action on fungi. Various possible action mechanisms by which the activity of wood-damaging fungi may be reduced or inhibited have been proposed by REINPRECHT (2010), i.e. the potential fungicides should have one or more of these effects: inhibition of fungi respiration; inhibition of protein, carbohydrate, nucleic acid and microtubules biosynthesis; disruption of fungi cell membranes due to the inhibition of sterol biosynthesis; inactivation of celluloses, phenoloxidases, and other enzymes produced by fungi.

The fungicidal activities and action mechanisms of an individual essential oil type can be variable, and these are usually more component (Table 1) and usually have an unequal composition in dependence on the place of plant cultivation and the method of oil production (DHIFI *et al.* 2016). In these views, as well as on the basis of several experiments performed in world, including experiments from our laboratories, the general tasks and questions for the future research of essential oils for wood protection are as follows: determine the minimum effective concentration of a defined individual oil type for wood protection; select the most effective oils and their compounds as a function of their efficacy and cost; achieve stability of oils against evaporation, leaching, and UV-radiation in exteriors; produce effective selected oil compounds in factories and test the potential synergistic effect of chemical compounds in oils from naturally produced plants; analyse potential synergistic effects between various oils and additives introduced into different wood species against wood-destroying fungi, moulds, and bacteria.

CONCLUSION

- From the individual essential oils—cinnamon, oregano, thyme, basil, and clove—the relatively highest antifungal efficiency against the brown-rot fungus *Serpula lacrymans* and the white-rot fungus *Trametes versicolor* had basil oil. On the contrary, the antifungal efficiency of clove oil is the lowest.
- Tested essential oils had a comparable efficacy against both wood decaying fungi *S. lacrymans* and *T. versicolor*.

- Essential oils with high antifungal efficiency can potentially be used as health-friendly wood biocides, preferentially in interiors, however unfortunately only in higher concentrations – which may be an issue due to odor and possible skin and other allergies.

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