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COMPARATIVE ASSESSMENT OF ANTIFUNGAL POTENTIAL OF CLOVE (*EUGENIA CARYOPHYLLATA)* AND CINNAMON (*CINNAMOMUM VERUM)* ESSENTIAL OILS

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Abstract:
The paper refers to the comparative evaluation of the potential antifungal effect of two essential oils: clove (Eugenia caryophyllata) essential oil and cinnamon (Cinnamomum verum) essential oil, against a brown rot fungus (Postia placenta) and a white rot fungus (Trametes versicolor). The screening test employed in this research demonstrated the biocidal antifungal potential of the two essential oils against both fungi tested. Cinnamon essential S-EO showed biocidal effect against white rot Trametes versicolor from 1% concentration and against brown rot Postia placenta from 10% concentration. Clove essential oil C-EO showed biocidal effect against both types of fungi, brown and white rot, from 1% concentration. Both esssential oils tested were more active against the brown rot fungus Postia placenta than the white rot fungus Trametes versicolor.

Key words: *antifungal potential; essential oils; screening, white rot; brown rot.*

INTRODUCTION

Essential oils (EOs) are complex and volatile natural compounds formed by aromatic plants as secondary metabolites (Bakkali et al. 2008). They are characterized by a strong smell.

According to the European Pharmacopoeia 7th edition, EOs are defined as: "*Odorant product, generally of a complex composition, obtained from a botanically defined plant raw material, either by driving by steam of water, either by dry distillation or by a suitable mechanical method without heating. An essential oil is usually separated from the aqueous phase by a physical method that does not lead to significant change in its chemical composition*" (Asbahani et al. 2015).

Essential oils, as natural products of various origin, have a very high variability in their chemical composition, both in qualitative and quantitative terms, their main components belonging to various chemical classes: from terpenes and phenols to alcohols, ethers, oxides, aldehydes, ketones, esters, amines, amides (Dhifi et al. 2016).

This diversity and richness of their chemical composition endures different EOs with antibacterial, antifungal, or insecticide properties, opening diverse areas of utilisation or research interest for novel applications.

EOs have been found suitable for use in many fields, such as: nutrition (M. Viuda-Martos et al. 2008, Nedorostova et al. 2009, Gatto et al. 2011, Janatova et al. 2015, Munhuweyi et al. 2018) pharmaceutics (Bouaziz et al. 2009, Zabka et al. 2014) and agriculture (Roman Pavela 2005, Righi et al. 2010, Bomfim et al. 2015) or conservation of cultural heritage, such as old documents (Borrego et al. 2012).

Also, the antifungal properties of certain essential oils can be exployted to protect wood against mold and rot attack (Macías et al. 2005, Wang et.al 2005, Yang and Clausen 2007, Cheng et al. 2008, Singh and Chittenden 2008, Zyani et al. 2011, Mohareb et al. 2013, Panek et al. 2014, C.M. de Medeiros et al. 2016, Zhang et al. 2016, Xie et al. 2017). Several EOs, such as: cinnamon EO, rosemary EO, thyme EO, tea tree EO, clove EO, oregano EO, lavander EO, sage EO, basil EO and various testing methods were employed, so that the results are difficult to compare.

The intererest in utilisation of essential oils in wood preservation and other fields is very much related to the fact that they are natural products, which are expected to bring the beneffit of biocidal properties with low toxicity to humans and low environmental impact (Yang and Clausen 2007, Nedorostova et al. 2009, Chittenden and Singh 2010, Turek and Stintzing 2013, Panek et al. 2014).

The present paper is part of a PhD research project looking at the bioprotection of wood in the syntagm of efficiency vs. eco-impact, aiming to bring a contribution to the testing of selected essential oils as potential eco-products for wood preservation.

The paper refers to the comparative evaluation of the potential antifungal effect of two essential oils: clove (*Eugenia caryophyllata)* and cinnamon (*Cinnamomum verum)*, against a brown rot fungus (*Postia placenta*) and a white rot fungus (*Trametes versicolor*), frequenly involved in wood bidegradation. An original screening test method, selected from a set of five, presented in a previous publication (Pop and Varodi 2017), was employed.

These EOs were selected on the basis of the literature study. Clove essential oil was proven to have biocidal properties against both white rot fungi and brown rot fungi. (Chittenden and Singh 2010, Zyani et al. 2011, Panek et al. 2014). Cinnamon essential oil also demonstrated biocidal properties against both white rot fungi and brown rot fungi. (Wang et al. 2005, Chittenden and Singh 2010).

OBJECTIVES:

1.Assessing the biocidal (antifungal) potential of the two EOs employing a previously developed screening test.

2. Determination of the concentrations range in which the antifungal effect of the two EOs is manifested.

3. Comparative assessment of their biocidal effect against two types of fungi (brown rot and white rot).

METHODOLOGY (MATERIALS / METHODS)

Two essential oils were selected and tested: Cloves (*Eugenia caryophyllata)* C-EO and Cinnamon *(Cinnamomum verum)* S-EO, purchased commercially, under the label Steaua Divina, in 10ml bottles. The two essential oils were employed in four variants: undiluted and diluted with ethyl alcohol (reagent for analysis, 96%) at tree different ratios (1:10; 1:100, 1:400), resulting 4 different volumetric concentrations, referred to in this paper as 100%, 10%, 1% and 0.25%, respectively.

Two fungi were used: one brown rot (*Postia placenta*) and one white rot (*Trametes versicolor*).

The screening test employed was the CT test (Pop and Varodi 2017). Culture medium (40g malt extract, 20 agar, 940ml distilled water) was prepared, sterilised and poured in sterile Petri dishes. Half-moon shaped Whatman papers (see Fig.1) were employed as support for impregnation with the solutions of EOs. They were previously sterilised with steam at 121ºC for 20 minutes. Impregnation was achieved by full immersion for 15 seconds at 20ºC. Treatment and further conditioning (15 minutes) were conducted in sterile conditions in the bacteriological cupboard. Control samples were prepared by impregnation with ethyl alcohol, employing a similar procedure.

The gellified culture medium was innoculated centrally with the test fungus and then two half-moon shaped Whatman papers, one treated (EO) and one control (ethyl alcohol) were placed as shown in Fig.1.

After sealing with parafin paper, the dishes were placed in the culture chamber (CLIMACEL 404 Comfort BMT Czech Republic) at a temperature of 23±2°C and a humidity of 75±5%.

Three replicates were employed for each EO and each concentration for each fungus. Moreover, two Petri dishes, each containing two control papers, as well as an innoculated dish as control of fungus virrulence, were included in the test for both fungi.

Evolution of fungal development was monitorised during the test at 3, 5, 7, 9 and 11 days after innoculation. A qualitative (photographic) and a quantitative assessment was performed.

The qualitative assessment looked at the fungal growth (development) and the preferential orientation (ethyl alcohol vs. essential oil) (Fig.1).

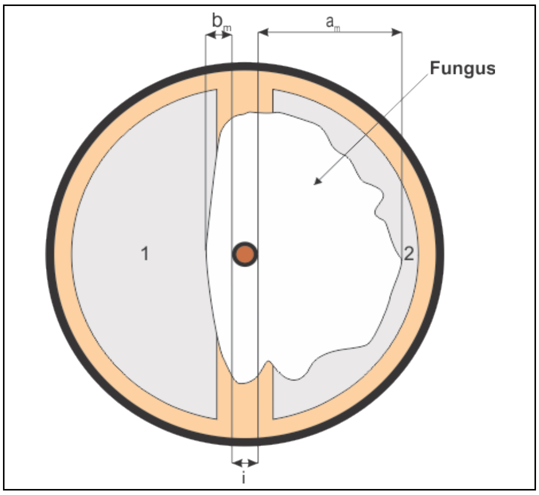

Fig. 1.
Principle of the CT screening test and parameters measurement for calculation of the relative development index (ID) and growth reduction index (IRD).

The quantitative assessment was subsequently made on the photographs, as explained in the scheme in Fig.1. Two indexes were calculated: the relative fungal development index (ID), defined to highlight preferential growth towards control, and a growth reduction index (IRD), according to equations 1 and 2, respectively.

$$ID=b_m/a_m*100 \quad \text{(eq. 1)}$$
$$IRD=(a_m-b_m)/a_m*100 \quad \text{(eg. 2)}$$

where:
ID - relative fungal development index, [%];
IRD - growth reduction index, [%];
a_m - the measured maximum growth (measured in mm) of the fungus towards the control solution zone, [mm];
b_m - the measured maximum growth of the fungus towards the area of the test solution, [mm].

RESULTS AND DISCUSSIONS

Both qualitative and quantitative results were obtained according to the previously presented methodology.

The qualitative results referring to the assessment of the potential biocidal, antifungal effect of the tested essential oils are presented in Fig. 2 and Fig. 3 for clove essential oil (C-EO) and Fig.4 and Fig.5 for cinnamon essential oil (S-EO). The pictures in these figures illustrate comparatively the evolution of fungal development during the test (for a maximum period of 11 days) for the respective oils at the four tested concentrations, for *Trametes versicolor* (Fig. 2, Fig. 4) and *Postia placenta* (Fig. 3, Fig. 5).

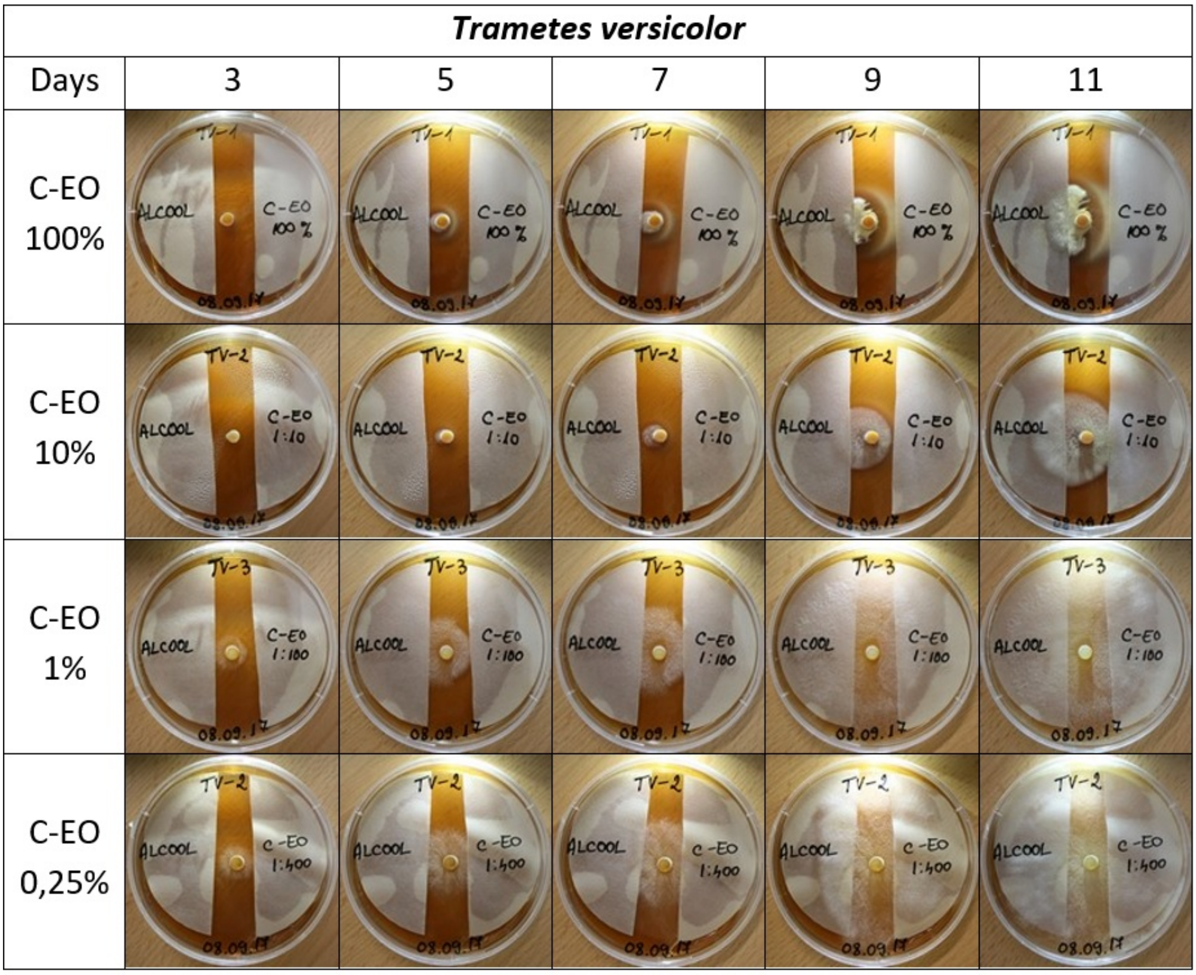

Fig. 2.
Assessment of biocidal effect of clove essential oil C-EO at different concentrations agaist the white rot fungus Trametes versicolor - evolution of fungal development throughout the test (max. 11 days).

Considering the Fig. 2 (C-EO/*Trametes versicolor*) it can be observed that at a concentration of 0.25%, the fungus did not show a preferential growth, but it developed relatively uniformously, covering the whole surface of the the Petri dish after 11 days. At 1% concentration, the fungus showed a preferential growth towards the control area in the first part of the test (up to 7days), ultimately covering the whole surface of the Petri dish. At 10% and 100% concentrations, the fungus presented a clear delay and much slower growth, with clear preference for the control area.

At the end of the test (11 days) the fungus did not covered the whole surface of the plate, but only about 23% and 10% respectively. These demonstrate the antifungal potential of clove oil against the white rot *Trametes versicolor* at concentrations from 10% above.

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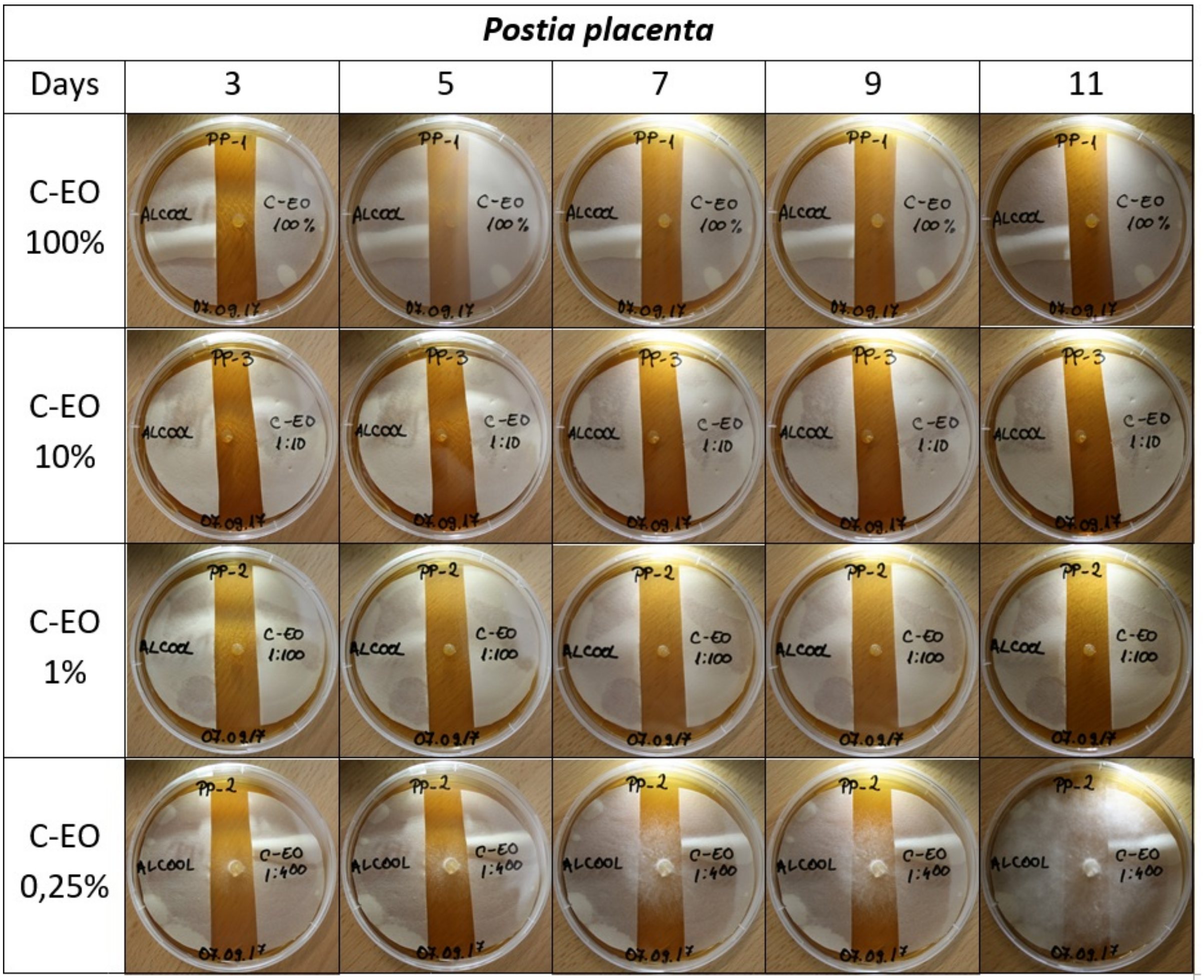

Fig. 3.
Assessment of biocidal effect of clove essential oil C-EO at different concentrations agaist the brown rot fungus Postia placenta - evolution of fungal development throughout the test (max. 11 days).

Considering the pictures in Fig. 3 (C-EO/*Postia placenta*) it can be observed that at a concentration of 0.25%, the fungus did not show a preferential growth, but it developed relatively uniformously, covering the whole surface of the the Petri dish after 11 days.

At concentrations of 1%, 10% and 100%, the fungus did not developed at all, the essential oil sterilizing the entire Petri dish, case described as absolute inhibition, cooresponding to growth reduction index of 100% (IRD= abs (100%)).

These demonstrate the potential antifungal effect of C-EO against the brown rot fungus *Postia placenta*, at concentrations starting from 1%. Comparing these results with the ones presented earlier for the same C-EO against the white rot fungus *Trametes versicolor*, it could be concluded that C-EO is more efficient against brown rot than white rot fungi. This is in accord with findings of Panek et al. 2014.

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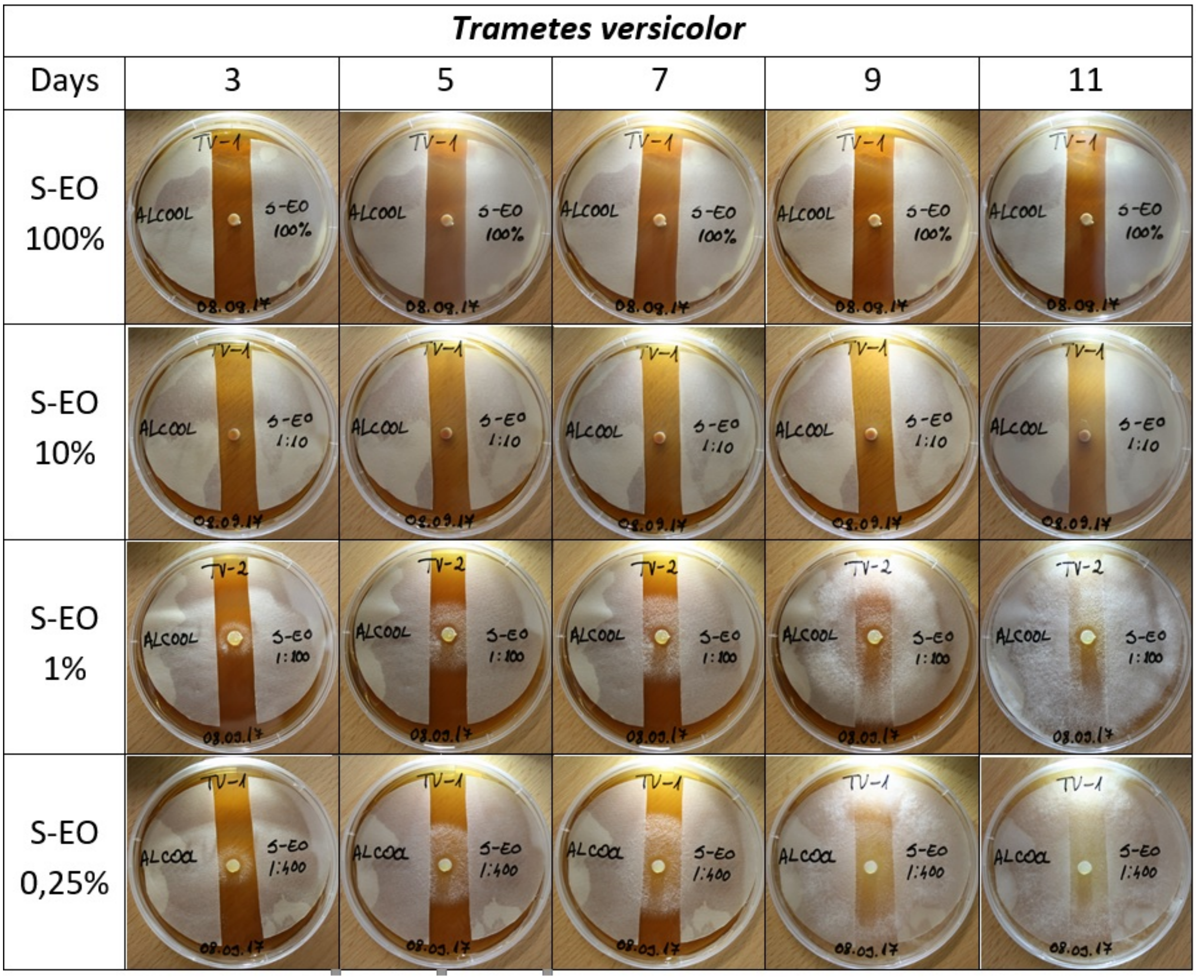

Fig. 4.
Assessment of biocidal effect of cinnamon essential oil S-EO at different concentrations agaist the white rot fungus Trametes versicolor - evolution of fungal development throughout the test (max. 11 days).

Considering the pictures in Fig. 4 (S-EO/ *Trametes versicolor*) it can be observed that at a concentration of 0.25% and 1% the fungus did not show a preferential growth, but it developed relatively uniformously, covering the whole surface of the the Petri dish after 11 days.

At concentrations of 10% and 100% S-EO, it was registered absolute inhibition of fungus, which did not developed at all, the essential oil sterilizing the entire Petri dish (IRD= abs (100%)).

This would suggest that S-EO can act as biocide against the white rot fungus *Trametes versicolor*, but the minimum concentration, which should be somehow lower than 10% (absolute inhibition), could not be determined by these tests and more research would be necessary for clarification.

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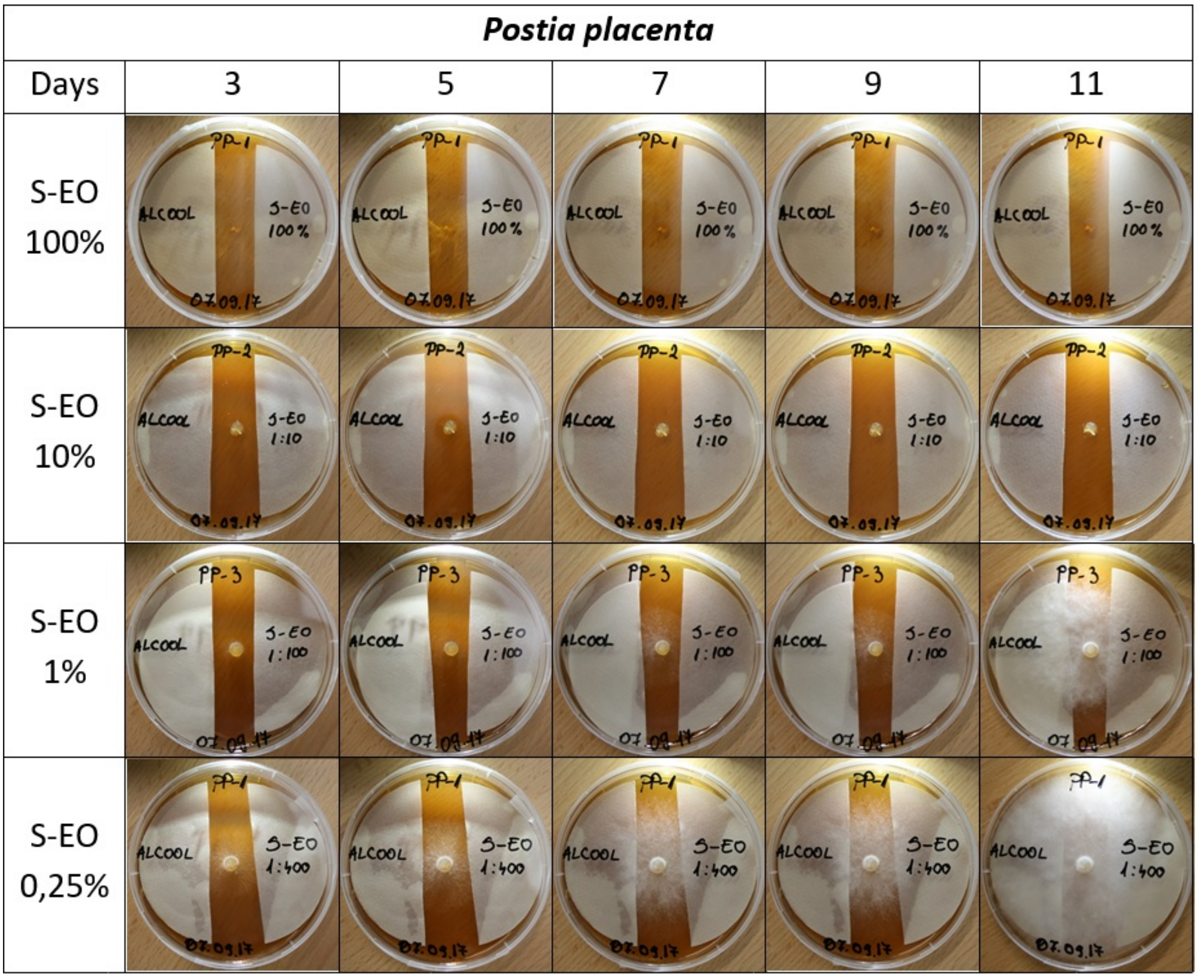

Fig. 5.
Assessment of biocidal effect of cinnamon essential oil S-EO at different concentrations agaist the brown rot fungus Postia placenta - evolution of fungal development throughout the test (max. 11 days).

Considering the pictures in Fig.5 (S-EO/*Postia placenta*) it can be observed that at a concentration of 0.25%, the fungus did not show a preferential growth, but it developed relatively uniformously, covering the whole surface of the the Petri dish after 11 days.

At the concentration of 1% S-EO, the fungus development was delayed and showed a slight preferential growth towards the control area. At concentrations of 10% and 100%, the fungal development was totally inhibited (IRD= abs (100%)).

These results indicate the antifungal effect of S-EO against the brown rot *Postia placenta,* at concentrations from 1% above.

Comparing the results obtained for S-EO in the tests with the two types of fungi, a higher efficiency against brown rot fungi compared to white rot is suggested. This is similar with the case of C-EO and consistent with literature information (Panek et al. 2014). A possible explanation is related to the composition of these EO, namely the chemical components with recognised antifungal activity (e.g phenols) and the biochemical processes associated to the biodegradation of wood by white rot and brown rot fungi (Voda et al. 2003). The brown rot fungi are capable to degrade only polysaccharides (cellulose, hemicelluloses), while the white rot fungi can degrade also lignin due to a more complex enzymatic system. As lignin is of poly-phenolic structure it is reasonable to believe that these fungi will not be stopped in their evolution by phenols (Bayramoglu and Arica 2009).

The quantitative results referring to the biocidal-antifungal effect of the two tested essential oils, expressed by the growth reduction index (IRD) values, are presented comparatively in Fig. 6 for the white rot fungus *Trametes versicolor* and Fig. 7 for the brown rot *Postia placenta.* The graphs are referring to the IRD values calculated after 7 days of incubation. This period of time was considered as relelevant, in relation to the development stage of the fungus (where growth occurred), stage which allowed differentiation of preferential growth (if the case) and possibility of measuring the maximum radial growth values with the view of calculating the growth reduction index values (IRD, %).

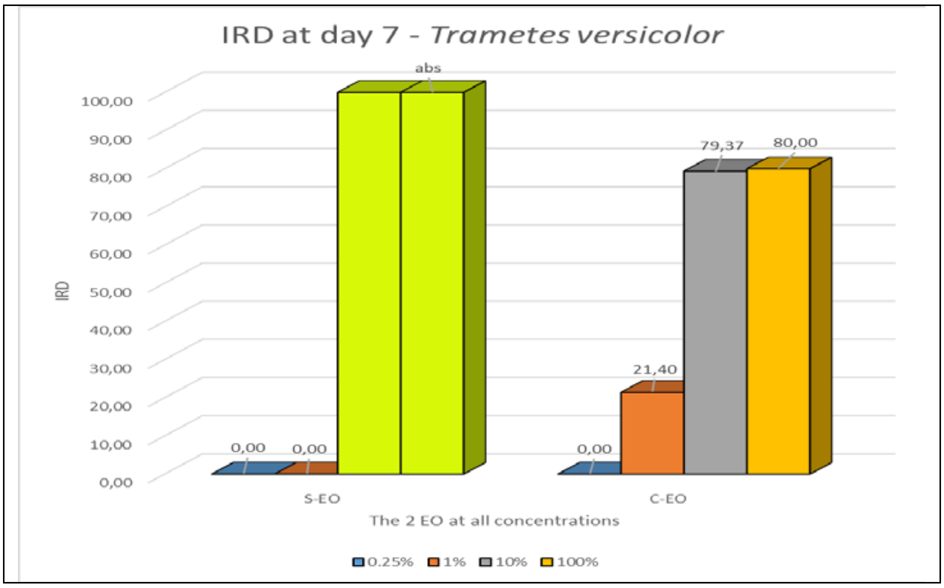

Fig. 6.
Growth reduction index (IRD) of white rot fungus Trametes versicolor after 7 days incubation for and clove (C-EO) and cinnamon (S-EO) essential oils at all concentrations.

As shown in Fig. 6, the development of white rot fungus (*Trametes versicolor*) was totally inhibited (IRD= abs (100%)) by cinnamon essential oil S-EO at concentrations of 100% and 10%, while no antifungal potential was registered at the lower concentrations of 1% and 0.25% (IRD=0%).

In contrast, clove essential oil (C-EO) demonstrated biocidal antifungal potential against the white rot fungus *Trametes versicolor*, starting from 1% concentration (IRD=21.4%). For the higher concentrations of 10% and 100%, IRD values of about 80% were obtained, demonstrating significant antifungal activity, though total inhibition of C-EO *Trametes versicolor* development was not registered even at 100% concentration.

Comparing the antifungal potential of C-EO and S-EO against the white rot fungus *Trametes versicolor,* based on the results obtained in this research, it seems reasonable to say that C-EO starts to be active from a lower concentrations (1%), but S-EO seems more active than C-EO at higher concentrations of 10% and 100%, totally inhibiting the fungal development.

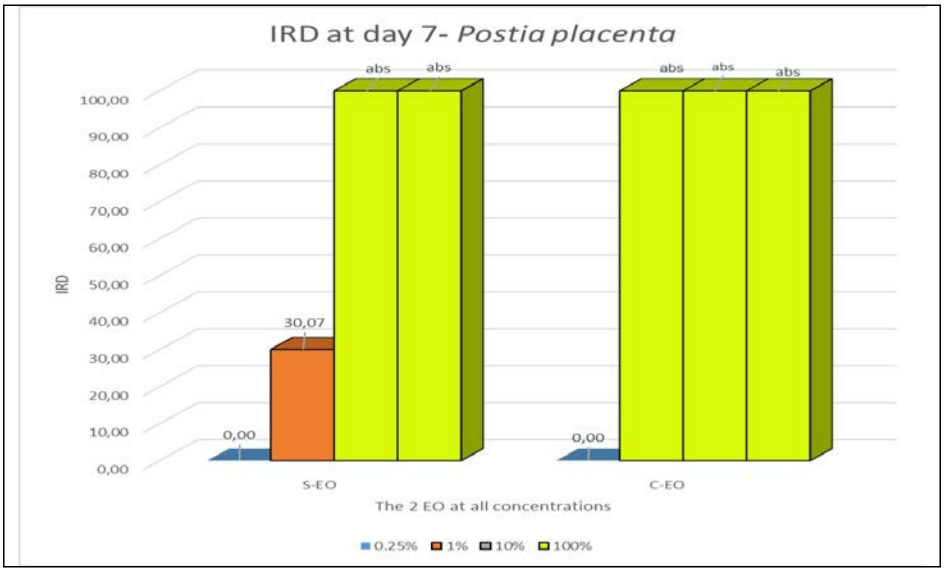

Fig. 7.
Growth reduction index (IRD) of brown rot fungus Postia placenta after 7 days incubation for and clove (C-EO) and cinnamon (S-EO) essential oils at all concentrations.

As shown in Fig. 7, the development of brown rot fungus (*Postia placenta*) was totally inhibited (IRD= abs (100%)) by cinnamon essential oil S-EO at concentrations of 100% and 10%, while no antifungal potential was registered at the lowest concentration of 0.25%. A reduction of fungal growth, coreesponding to a IRD value of about 30% was obtained at a concentration of 1%.

Clove essential oil C-EO totally inhibited the development of the brown rot fungus *Postia placenta* (IRD= abs (100%)) when employed at concentarations of 1%, 10% and 100%, while no antifungal potential was registered at the lowest concentration of 0.25%.

Comparing the antifungal potential of C-EO and S-EO against the brown rot fungus *Postia placenta,* based on the results obtained in this research, it seems reasonable to say that both are active at concentrations from 1% above, but C-EO is more active than S-EO at all the three active concentrations tested (1%, 10% and 100%), totally inhibiting the fungal development. For S-EO total inhibition was registered only at concentrations of 10% and 100%.

CONCLUSIONS

The following conclusions resulted from this research:

1. The screening test employed in this research demonstrated the biocidal antifungal potential of clove (*Eugenia caryophyllata)* essential oil (C-EO) and cinnamon *(Cinnamomum verum)* essential oil (S-EO) against the white rot fungus *Trametes versicolor* and the brown rot fungus *Postia placenta*.
2. The antifungal potential of both EOs tested depends on the concentration and type of rot fungus. Cinnamon essential oil (*Cinnamomum verum*) S-EO shows biocidal effect against white rot *Trametes versicolor* from 1% concentration and against brown rot *Postia placenta* from 10% concentration. Clove essential oil (*Eugenia caryophyllata)* C-EO shows biocidal effect against both types of fungi, brown and white rot, from 1% concentration.
3. Both EOs seem more active against the brown rot fungus *c* which is in accordance to literature and related to the biochemical processes associated to the biodegradation of wood by white rot and brown rot fungi.

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