

COMPARATIVE ASSESSMENT OF THE BIOCIDAL POTENTIAL OF 3 ESSENTIAL OILS

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Abstract: *Essential oils can be used as wood preservatives with antifungal properties. These kinds of products can be considered potential ecological wood preservatives for special applications, such as furniture conservation. The present paper refers to a laboratory screening test designed to allow a quick and reliable evaluation of the potential biocidal effect of 3 types of essential oils. The tested products were: basil (*Ocimum Basilicum*), thyme (*Thymus vulgaris*), and oregano (*Origanum vulgare*) essential oils at 4 different concentrations. Two types of fungi were employed as biological material: a white rot (*Trametes versicolor*) and a brown rot (*Postia placenta*). Oregano and thyme essential oils demonstrated biocidal effect against both tested fungi, being more active against brown rot than white rot. This effect depends on the concentration of the essential oils. The biocidal potential of basil essential oil is very low.*

Key words: *essential oils; oregano essential oil; thyme essential oil; basil essential oil; white rot; brown rot.*

1. Introduction

Essential oils (EOs) are complex and volatile natural compounds, which are characterized by a strong smell and are formed by aromatic plants as secondary metabolites [1].

As natural products of various origin, essential oils have a very high variability in their chemical composition, both in qualitative and quantitative terms, and their main components belong to various chemical classes: from terpenes, heterocycles, and phenols to alcohols,

ethers or oxides, aldehydes, ketones, esters, amines, amides [4].

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

The antifungal properties of certain essential oils can be exploited to protect wood against mould and rot attack [5-7, 10, 14, 18, 19, 21, 22]. Several EOs, such as: cinnamon EO, rosemary EO, thyme EO, tea tree EO, clove EO, oregano EO,

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lavender EO, sage EO, basil EO, and various testing methods were employed, so the results are difficult to compare.

The interest in the utilisation of essential oils in wood preservation and in other fields is very much related to the fact that they are natural products which are expected to bring the benefit of biocidal properties with low toxicity to humans, and low environmental impact [9], [14], [16], [19].

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

Screening tests are most used in preliminary biocidal potential assessment because they are aimed at rapidly identifying potentially biocidal substances, as revealed in the test by inhibiting the development or the lethal effect on inoculated fungi on a sterile culture medium [12]. In previous research, authors compared various tests which were adapted or taken from the literature. Their comparative evaluations aimed at selecting a useful test to continue the research. For this purpose, the tests were done with classic protection substances, with recognized biocidal potential.

This paper refers to the comparative evaluation of the potential antifungal effect of three essential oils: basil (*Ocimum Basilicum*), thyme (*Thymus vulgaris*), and oregano (*Origanum vulgare*), against a brown rot fungus (*Postia placenta*) and a white rot fungus (*Trametes versicolor*) which are frequently involved in wood biodegradation.

Oregano essential oil has proven to have fungicidal properties [3, 10, 18, 20],

antimicrobial properties [8, 9], and insecticidal properties [11].

Thyme essential oil has proven to have fungicidal properties [10, 15, 18-20], antioxidant properties [13], antimicrobial properties [9], and insecticidal properties [11]. Basil essential oil has proven to have fungicidal properties [22], antimicrobial properties [9], and insecticidal properties [11].

2. Objectives

The actual objectives in this research phase were as follows:

1. Assessment of the biocidal (antifungal) potential of the EOs employing a previously developed screening test;
2. Determination of the concentration range in which the biocidal effect is manifested.
3. Comparative assessment of the biocidal effect against two types of fungi (brown rot and white rot).

3. Methodology (Materials and Methods)

Three essential oils were selected and tested: basil (*Ocimum Basilicum*) B-EO, thyme (*Thymus vulgaris*) T-EO, and oregano (*Origanum vulgare*) O-EO, all commercial products under the label Steaua Divina (10 ml bottles). The three essential oils were employed in four variants: undiluted and diluted with ethyl alcohol (reagent for analysis, 96%) at three 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, as presented in a previous publication [12]. The jellified culture medium was inoculated centrally with the test fungus, and then two half-moon

shaped Whatman papers, one treated with essential oil (EO) and one control (ethyl alcohol), were placed as shown in Figure 1.

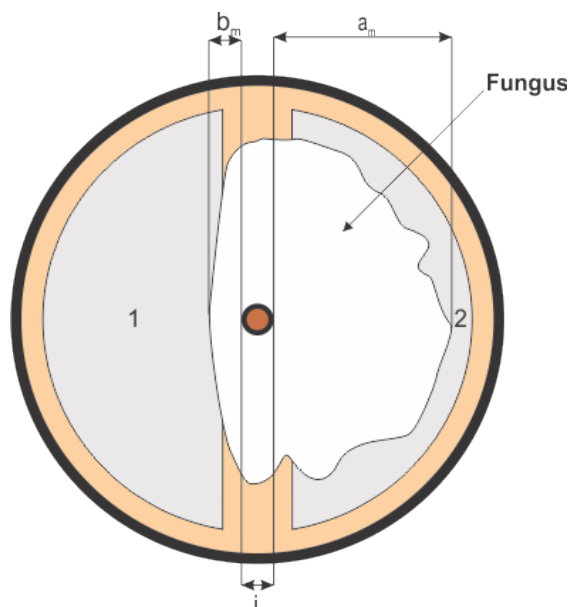


Fig. 1. Parameter measurement made for Fungus Development Index (ID) and Growth Reduction Index (IRD) calculation

After being sealed with paraffin paper, the dishes were placed in the culture chamber (CLIMACEL 404 Comfort BMT Czech Republic) at a temperature of $23 \pm 2^\circ\text{C}$ and a humidity of $75 \pm 5\%$.

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 inoculated dish as control of fungus virulence, were included in the test for both fungi.

The evolution of fungal development was monitored during the test at 3, 5, 7, 9, and 11 days after inoculation. A qualitative (photographic) and a quantitative assessment were performed. The qualitative assessment looked at the

fungal growth (development) and the preferential orientation (ethyl alcohol vs. essential oil) (Figure 1). The quantitative assessment was subsequently made on the photographs, as explained in the scheme in Figure 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:

$$ID = b_m / a_m * 100 \quad (1)$$

$$IRD = (a_m - b_m) / a_m * 100 \quad (2)$$

where:

ID is 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].

4. Results and discussions

Both the qualitative and the 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 Figure 2 and Figure 3 for thyme essential oil (T-EO), Figure 4 and Figure 5 for oregano essential oil (O-EO), and Figure 6 and Figure 7 for basil essential oil (B-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* (Figures 2, 4 and 6) and *Postia placenta* (Figures 3, 5 and 7).

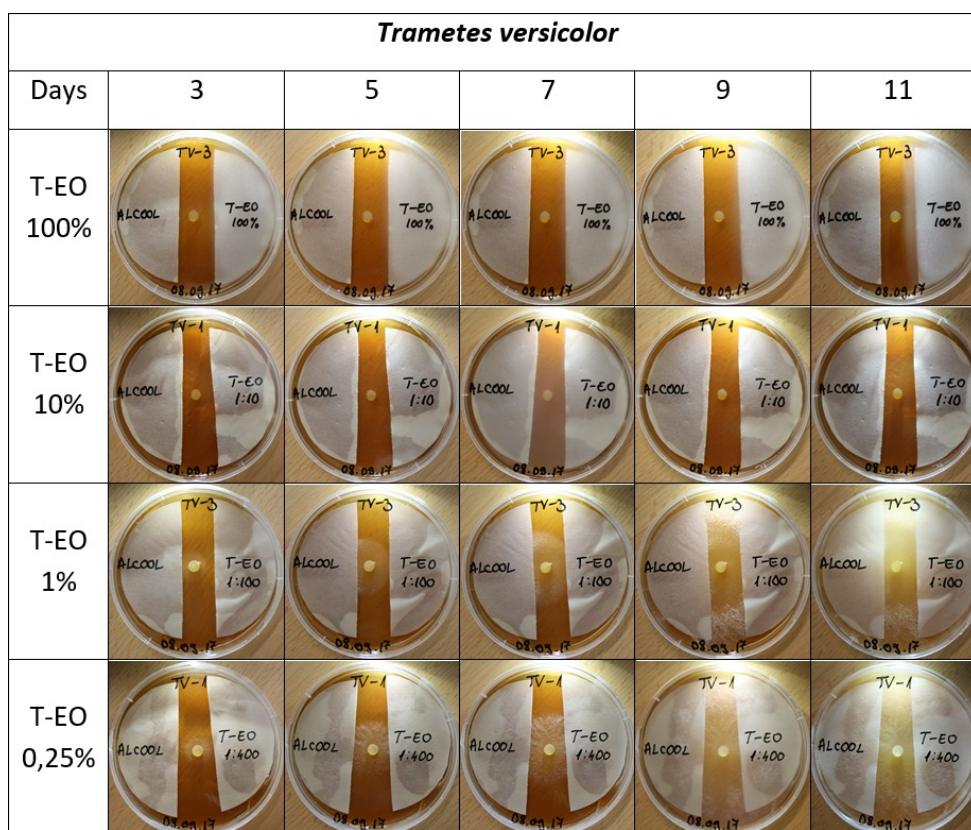


Fig. 2. Assessment of biocidal effect of thyme essential oil T-EO at different concentrations against the white rot fungus *Trametes versicolor* - evolution of fungal development throughout the test (max. 11 days)

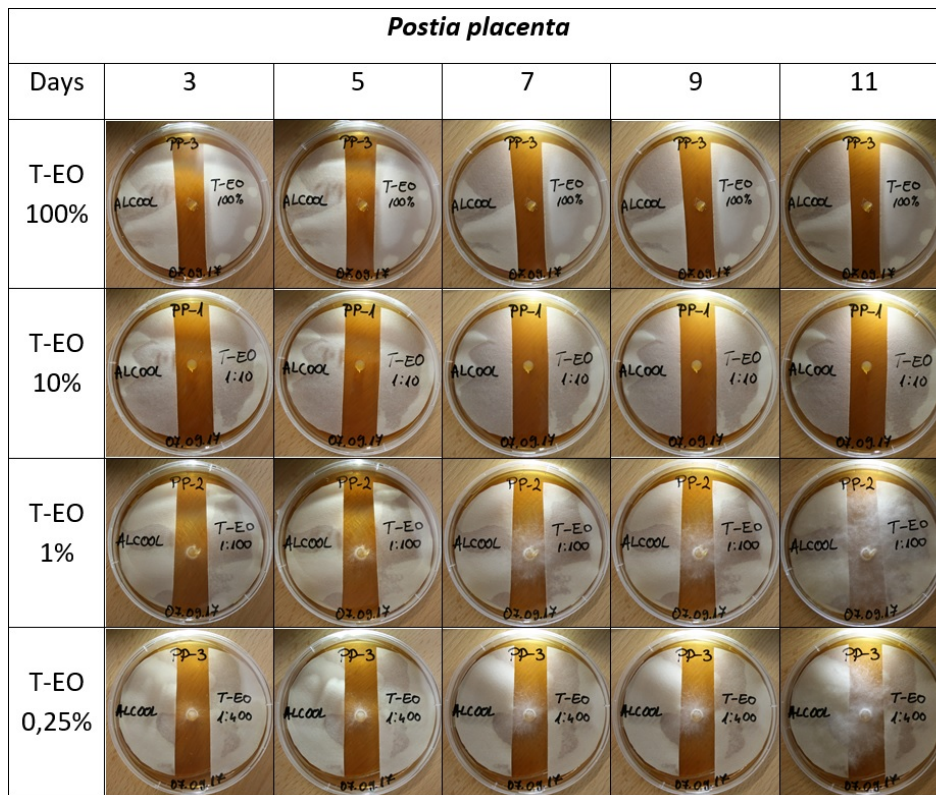


Fig. 3. Assessment of biocidal effect of thyme essential oil T-EO at different concentrations against the brown rot fungus *Postia placenta* - evolution of fungal development throughout the test (max. 11 days)

Considering Figure 2 (T-EO/*Trametes versicolor*), it can be observed that at 0.25% concentration, the fungus did not show a preferential growth, but it developed relatively uniformly, covering the whole surface of 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 7 days), ultimately covering the whole surface of the Petri dish. At 10% and 100% concentrations, the fungus did not develop at all and the essential oil sterilized the entire Petri dish, a case described as absolute inhibition, corresponding to a growth reduction

index of 100% (IRD = abs (100%)). These demonstrate the potential antifungal effect of T-EO against the white rot fungus *Trametes versicolor*, at concentrations starting from above 1%.

Considering the pictures in Figure 3 (T-EO/*Postia placenta*), it can be observed that at 0.25% concentration the fungus has a preferential growth to the control area. At 1% concentration the fungus did not show a preferential growth, but it developed relatively uniformly, covering the whole surface of the Petri dish after 11 days.

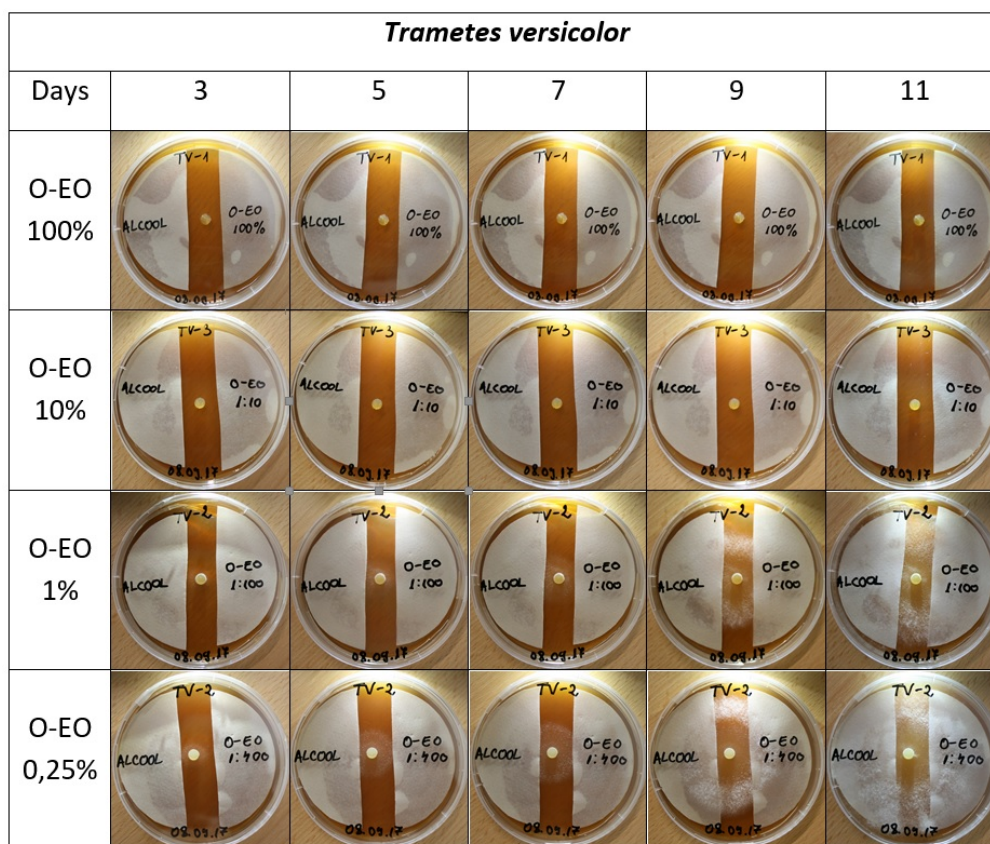


Fig. 4. Assessment of biocidal effect of oregano essential oil O-EO at different concentrations against the white rot fungus *Trametes versicolor* - evolution of fungal development throughout the test (max. 11 days)

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

These demonstrate the potential antifungal effect of T-EO against the brown rot fungus *Postia placenta*, at concentrations starting from above 0.25%.

Considering Figure 4 (O-EO/*Trametes versicolor*), it can be observed that at 0.25% concentration, the fungus did not show a preferential growth, but it developed relatively uniformly, covering the whole surface of 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 7 days), ultimately covering the whole surface of the Petri dish. At 10% and 100% concentrations, the fungus did not develop at all, and the essential oil sterilized the entire Petri dish, a case described as absolute inhibition, corresponding to a growth reduction index of 100% (IRD= abs (100%)). These demonstrate the potential antifungal effect of O-EO against the white rot fungus *Trametes versicolor*, at concentrations starting from above 1%.

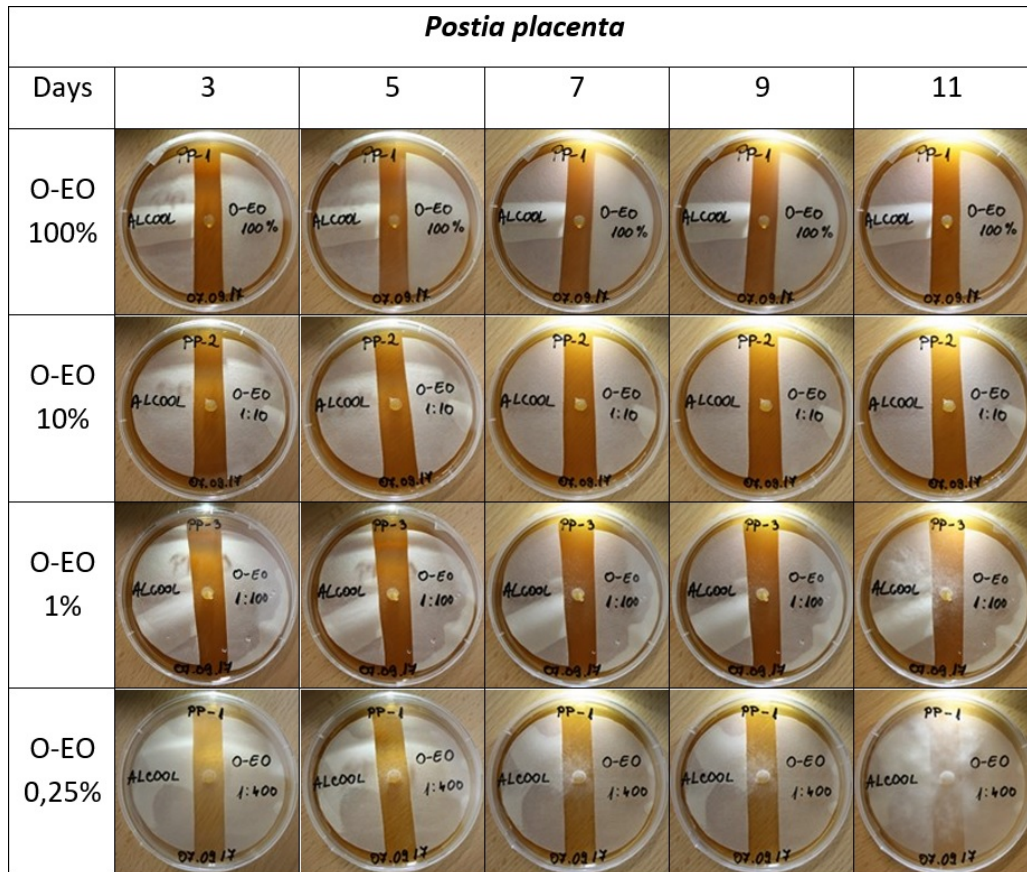


Fig. 5. Assessment of biocidal effect of oregano essential oil O-EO at different concentrations against the brown rot fungus *Postia placenta* - evolution of fungal development throughout the test (max. 11 days)

Considering the pictures in Figure 5 (O-EO/*Postia placenta*), it can be observed that at 0.25% concentration the fungus showed a preferential growth towards the control area in the first part of the test (up to 9 days), ultimately covering the whole surface of the Petri dish. At 1% concentration the fungus presented a clear delay and much slower growth, with clear preference for the control area. At 10% and 100% concentrations, the fungus did not develop at all, and the essential oil sterilized the entire Petri dish, a case described as absolute inhibition, corresponding to a growth reduction

index of 100% (IRD = abs (100%)). These demonstrate the potential antifungal effect of O-EO against the brown rot fungus *Postia placenta*, at concentrations starting from above 0.25%.

Considering Figure 6 (B-EO/*Trametes versicolor*), it can be observed that at 0.25%, 1%, and 10% concentration the fungus did not show a preferential growth, but it developed relatively uniformly, covering the whole surface of the Petri dish after 11 days. At 100% concentrations, the fungus did not develop at all, and the essential oil sterilized the entire Petri dish, a case

described as absolute inhibition, index of 100% (IRD= abs (100%)).
corresponding to a growth reduction

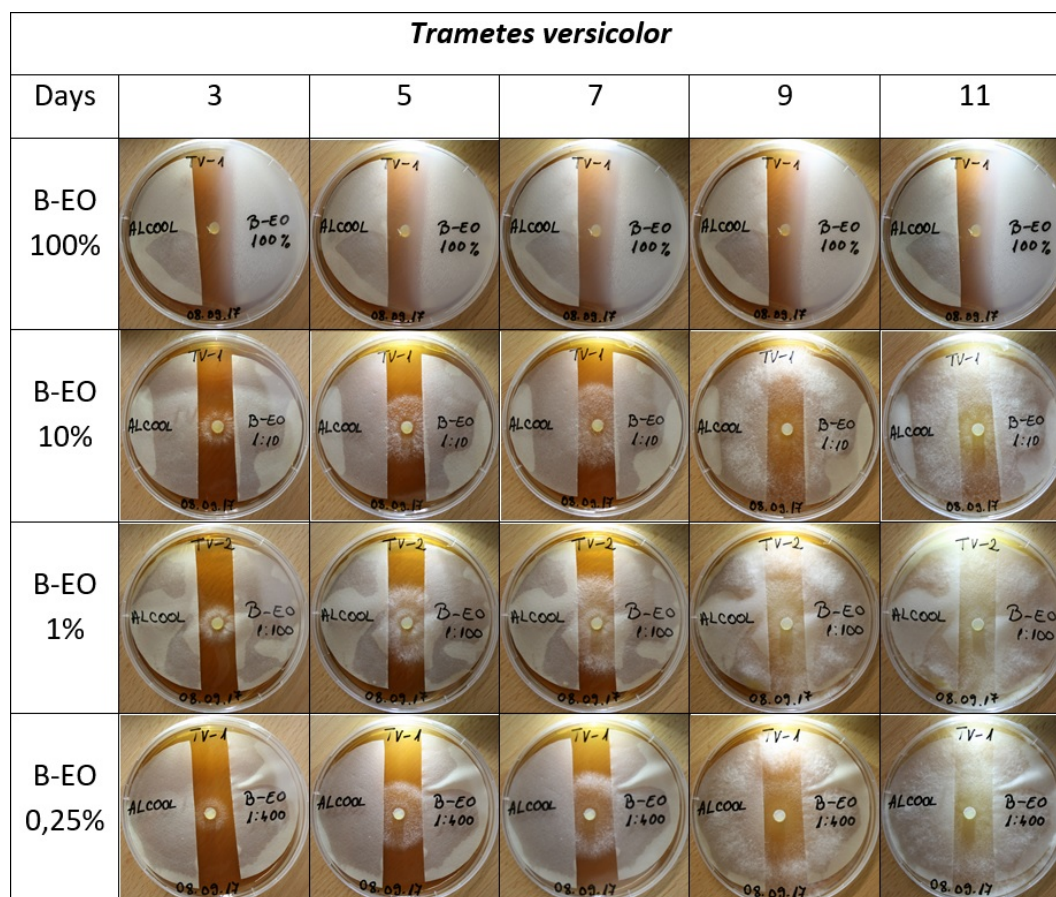


Fig. 6. Assessment of biocidal effect of basil essential oil B-EO at different concentrations against the white rot fungus *Trametes versicolor* - evolution of fungal development throughout the test (max. 11 days)

Considering Figure 7 (B-EO/*Postia placenta*), it can be observed that at 0.25%, 1%, and 10% concentration, the fungus did not show a preferential growth, but it developed relatively uniformly, covering the whole surface of the Petri dish after 11 days. At 100% concentrations, the fungus did not develop at all, and the essential oil sterilized the entire Petri dish, a case described as absolute inhibition, corresponding to a growth reduction

index of 100% (IRD= abs (100%)).

Comparing the results obtained for B-EO in the tests involving the two types of fungi, it can be said that B-EO has a very low efficiency against the brown rot and white rot fungi, only at 100% concentration.

Analyzing the results obtained for T-EO in the tests with the two types of fungi, a higher efficiency against the brown rot fungus compared to the white rot is suggested. This is similar to the case of O-

EO and consistent with the literature [10]. A possible explanation is related to the composition of these EOs, namely the chemical components with recognized antifungal activity (e.g. phenols) and the biochemical processes associated with the biodegradation of wood by white rot and brown rot fungi [17]. The brown rot fungi

are capable of degrading only polysaccharides (cellulose, hemicelluloses), while the white rot fungi can also degrade lignin due to a more complex enzymatic system. As lignin is of polyphenolic structure, it is reasonable to believe that the evolution of these fungi will not be stopped by phenols [2].

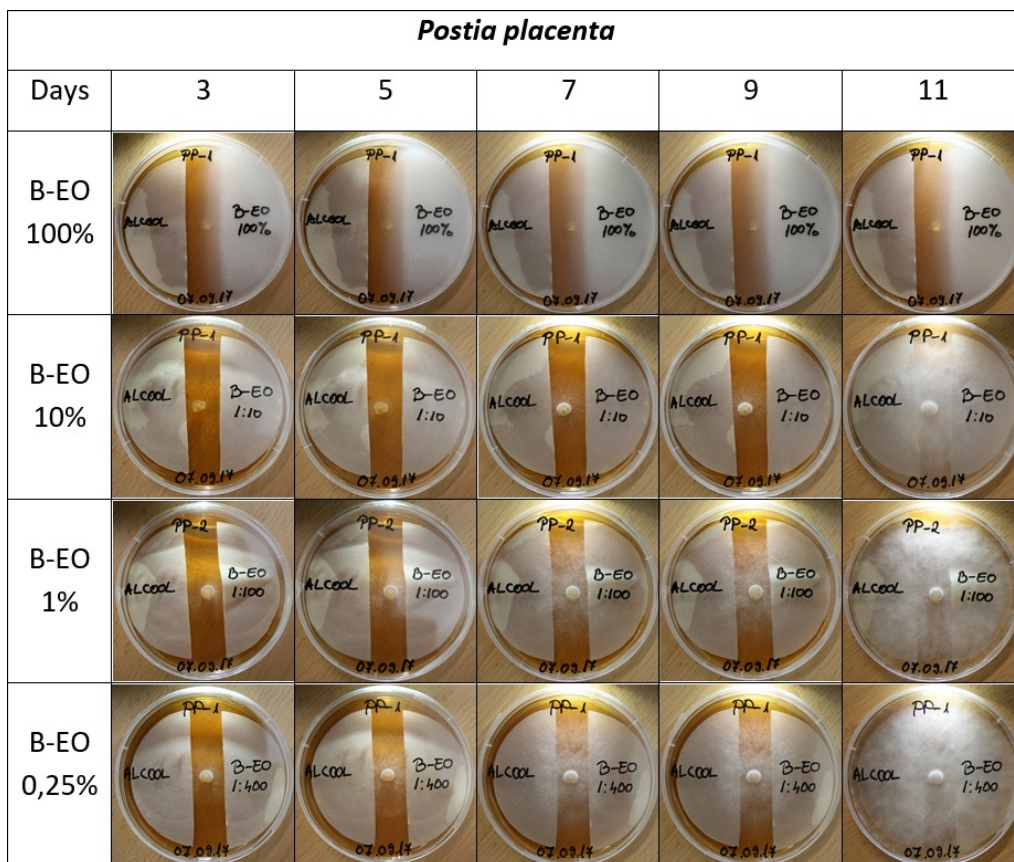


Fig. 7. Assessment of biocidal effect of basil essential oil B-EO at different concentrations against the brown rot fungus *Postia placenta* - evolution of fungal development throughout the test (max. 11 days)

The quantitative results referring to the biocidal-antifungal effect of the three tested essential oils, expressed by the growth reduction index (IRD) values, are presented comparatively in Figure 8 for the brown rot *Postia placenta* and Figure 9

for the white rot fungus *Trametes versicolor*. The graphs refer to the IRD values calculated after 7 days of incubation. This period of time was considered relevant in relation to the development stage of the fungus (where

growth occurred), stage which allowed the maximum radial growth values with differentiation of preferential growth (if the case) and the possibility of measuring the reduction index values (IRD, %).

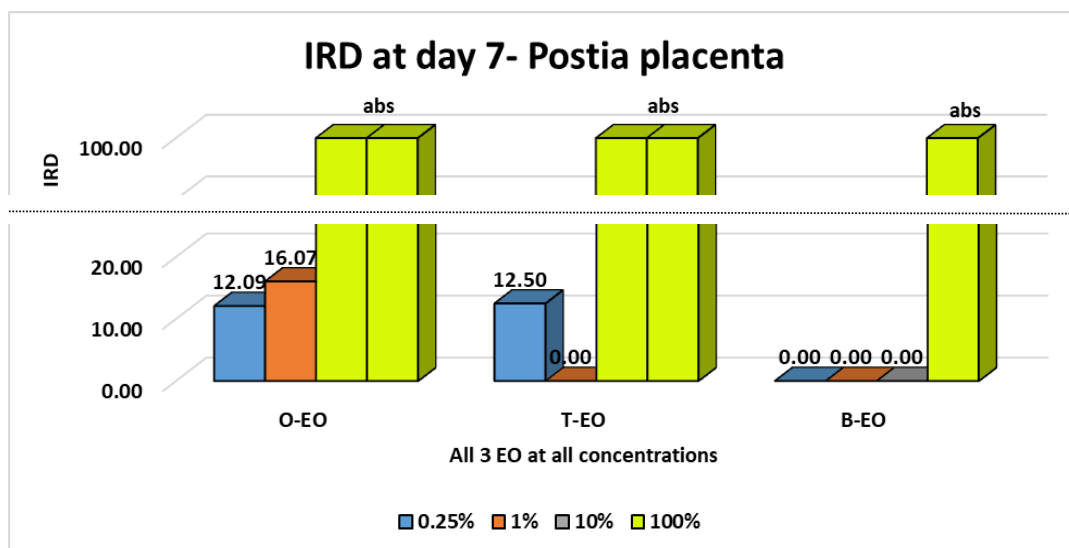


Fig. 8. Growth Reduction Index (IRD) on day 7 for the brown rot fungus *Postia placenta* and all 3 EOs at all concentrations

As shown in Figure 8, the development of brown rot fungus (*Postia placenta*) was totally inhibited (IRD= abs (100%)) by oregano essential oil O-EO at 100% and 10% concentrations. At 1% and 0.25% concentrations a reduction of fungal growth was obtained, corresponding to an IRD value of about 16%, respectively 12%.

Thyme essential oil T-EO totally inhibited the development of the brown rot fungus *Postia placenta* (IRD= abs (100%)) when employed at 10% and 100% concentrations, while no antifungal potential was recorded at 1% concentration. At the lowest concentration of 0.25%, a reduction of fungal growth corresponding to an IRD value of about 12% was obtained.

Basil essential oil B-EO totally inhibited the development of the brown rot fungus

Postia placenta (IRD= abs (100%)) when employed at 100% concentration, while no antifungal potential was recorded at the other concentrations of 10%, 1%, and 0.25%.

Based on the results obtained in this research, comparing the antifungal potential of T-EO, O-EO, and B-EO against the brown rot fungus *Postia placenta*, it seems reasonable to say that B-EO is not a feasible option because of the low efficiency shown only at pure 100% concentration.

The other two essential oils, T-EO and O-EO, are both active at 0.25% concentration and above. Total inhibition was registered at 10% and 100% concentrations for both T-EO and O-EO.

As shown in Figure 9, the development of the white rot fungus (*Trametes versicolor*)

was totally inhibited (IRD= abs (100%)) by oregano essential oil O-EO at 100% and 10% concentrations, while no antifungal potential was recorded at the lower

concentration of 0.25% (IRD=0%). A reduction of fungal growth corresponding to an IRD value of about 24.5% was obtained at 1% concentration.

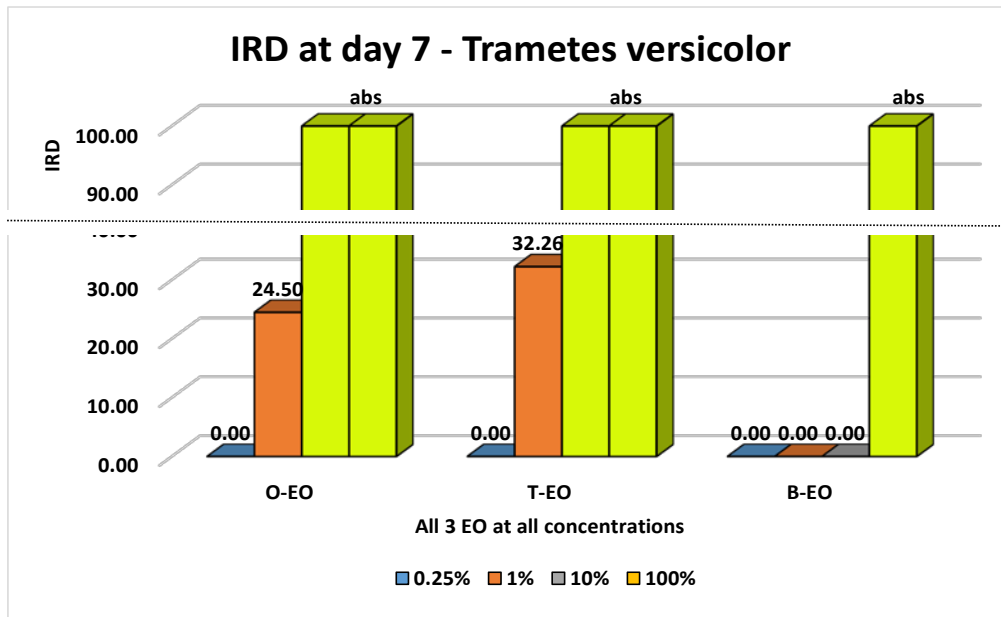


Fig. 9. Growth Reduction Index (IRD) on day 7 for the white rot fungus *Trametes versicolor* and all 3 EOs at all concentrations

Thyme essential oil T-EO totally inhibited the development of the white rot fungus (*Trametes versicolor*) (IRD= abs (100%)) when employed at 10% and 100% concentrations, while no antifungal potential was recorded at the 0.25% concentration. A reduction of fungal growth, corresponding to an IRD value of about 32% was obtained at 1% concentration.

Basil essential oil B-EO totally inhibited the development of the white rot fungus *Trametes versicolor* (IRD= abs (100%)) when employed at 100% concentration, while no antifungal potential was recorded at the other concentrations of 10%, 1%, and 0.25%.

Comparing the antifungal potential of T-EO, O-EO, and B-EO against the white rot fungus *Trametes versicolor* based on the results obtained in this research, it seems reasonable to say that B-EO is not a feasible option, because of the low efficiency shown only at pure 100% concentration.

The other two essential oils, T-EO and O-EO, are both active at concentrations starting from 1% and above. Total inhibition was recorded at 10% and 100% concentrations for both T-EO and O-EO.

5. Conclusions

1. The screening test employed in this

research demonstrated the biocidal antifungal potential of oregano (*Origanum vulgare*) essential oil (O-EO) and thyme (*Thymus vulgaris*) essential oil (T-EO) against the white rot fungus *Trametes versicolor* and the brown rot fungus *Postia placenta*.

2. The antifungal potential of both O-EO and T-EO depends on the concentration and type of rot fungus. Both oregano (*Origanum vulgare*) essential oil (O-EO) and thyme (*Thymus vulgaris*) essential oil (T-EO) demonstrate biocidal effect against the white rot *Trametes versicolor* from 1% concentration and against the brown rot *Postia placenta* from 0.25% concentration.
3. Both O-EO and T-EO seem more active against the brown rot fungus *Postia placenta* than the white rot fungus *Trametes versicolor*, which is in accordance with the literature and related to the biochemical processes associated with the biodegradation of wood by white rot and brown rot fungi.

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