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ESSENTIAL OILS AS POTENTIAL ECOLOGICAL WOOD PRESERVATIVES – A PRELIMINARY TEST ON THYME ESSENTIAL OIL

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ABSTRACT

The paper refers to a laboratory test, designed to allow a quick and reliable evaluation of the potential biocidal effect of essential oils. These are intended to be used as ecological wood preservatives with antifungal properties, for various applications including furniture conservation. The screening tests employed thyme *(Thymus vulgaris)* essential oil at 3 different concentrations and two types of fungi, one white rot *Trametes versicolor* and one brown rot *Postia placenta*. Based on these tests, thyme *(Thymus vulgaris)* essential oil has proven to be a potential active fungicide against the brown and white rot fungi, in a wide range of concentrations.

Key words: wood preservatives, essential oils, screening test, thyme essential oil.

INTRODUCTION

Many of the products with recognized efficiency in wood protection against rot fungi contain heavy metals and/or other toxic and bio-accumulative substances, so that in many countries their use has been banned or restricted because of the health problems they cause and/or the negative impact on the environment. (Panek et al., 2014)

However, wood is susceptible to biodegradation in a relatively short time, depending on wood species (natural durability) and conditions of exposure (use classes), which generates a need for research and development of products that have both biocidal effect against rot fungi and a low toxicity to human, alongside minimal ecological impact. The importance and urgency of identifying such products is even more critical in certain handicrafts and areas where all interventions on wood are made by mankind employing (manual) procedures which keeps the operator in close / direct contact with the artefact (e.g. conservation-restoration of furniture / wood artefacts).

Promoting such products requires several steps, starting with screening tests to assess their biocidal potential and the concentrations range in which they can be efficient (Panek et al, 2014)

Essential oils, as natural products with a complex chemical composition, including various chemicals with fungicidal, insecticidal, antibacterial properties, etc., could be an interesting option for wood protection, with good efficiency and low environmental impact. The potential/ usefulness of essential oils have been proven in various areas: nutrition, pharmaceutics and agriculture (Righi et al., 2010; Gatto et al., 2011; M. Viuda-Martos et al., 2008; Bouaziz et al., 2009; Roby et al., 2013; Roman Pavela, 2005; Janatova et al., 2015; Zabka et al., 2014; Ondřej Mikala, 2015; Silva Bomfim et al., 2015; Munhuweyi et al., 2018), preservation of heritage documents (Borrego et al.,2012) and also in wood protection against molds and rot attack (Wang et.al, 2005; Yang and Clausen, 2007; Singh and Chittenden, 2008; Macías et al., 2005; Zhang et al., 2016; Panek et al., 2014;

Cheng et al., 2008; Zyani et al., 2011; Mohareb et al., 2013; Xie et al., 2017; C.M. de Medeiros et al., 2016).

Screening tests to assess the potential biocidal fungicide effect of a certain product are based, as principle, on the inhibition of fungal development (or lethal effect), on a inoculated culture medium, which is poisoned with the respective product. Poisoning of the medium with the potential biocide to be tested might be achieved in different ways: addition of the product into the liquid medium, spreading the product on the surface of the jellified medium or, most often, diffusion from a reservoir (e.g. blotting paper) containing the tested product (Humar and Pohleven (2007), Vek *et al.* 2013, Delenk *et al.* 2015, Pop and Varodi, 2017). These screening tests have the advantage of providing rapidly (days) preliminary information on the biocidal potential of the tested products, compared to the longer (months) and laborious tests on treated wood (e.g. EN 113).

There are no standard screening tests for biocidal products intended as wood preservatives, so different methods from different areas nutrition, pharmaceutics, medicine and agriculture (Eva Ürgeová and Ľudovít Polívka 2009, Gatto *et al*. 2011, Salima Varona *et al* 2013, Zaida N. Juárez *et al*. 2015, M. Viuda-Martos *et al.* 2008, Neelam Gurnani *et al.* 2016, Najeeb Ullah *et al.* 2016) were taken over and adapted (Humar and Pohleven (2007), Vek *et al.* 2013, Delenk *et al.* 2015).

In previous experiments of the authors, five screening tests were performed and compared using water-soluble biocides with recognized biocidal efficiency (Pop and Varodi, 2017). It has been found that these tests can not be applied to oily products (results from unpublished own research). Thereupon, a new approach was needed for the oily products, and the present paper refers to a screening test for oils, which are introduced directly into the culture medium.

OBJECTIVES

Two main objectives were considered in the research reported in the present paper:

- assessing feasibility of the test method;
- assessment of fungicide potential for one essential oil.

MATERIALS AND METHODS

Wood-destroying fungi

Two types of rot fungi: *Postia placenta* (brown rot) and *Trametes versicolor* (white rot) were used for this experiment.

Essential oils

Only one essential oil was used in this experiment: thyme essential oil *(Thymus vulgaris)*, coded T-EO, which is a commercially product, available, under the label Steaua Divina, in 10 ml bottles. It was used at 3 dilutions (1:10; 1:400 and 1:800).

Screening antifungal test

The method used was the dilution of essential oil directly in the culture medium (test code: VM) Fig. 1.

The MEA culture medium (prepared from 40 g malt extract Standard from ROTH and 20 g agar-agar, purified and free from inhibitors for microbiology, from Merck, dissolved in 1 l distilled water) was sterilized at 121°C for 20 minutes in the autoclave (REYPA AES 75). After sterilization is was allowed to cool down to a temperature of 40–45°C. At this temperature the necessary amount (measured volume) of thyme essential oil (T-EO) was added to the medium and thoroughly dispersed by stirring until homogenization. Then the medium was poured into Petri dishes, which were kept at room temperature, in sterile conditions, for 24 h, which allowed jellification of the medium. After that, the Petri dishes were inoculated with

fungi, placing a circular piece (of about x mm) of active mycelium on medium (inoculum) in the center of each Petri dish.

Three dilutions of thyme essential oil (T-EO) in the MEA medium were tested (1:10; 1:400; 1:800, as volume: volume) and two samples were run in parallel for each dilution. Two control samples with pure, unpoised medium were tested also for each fungus (Fig. 1).

After sealing with para-film, 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%.

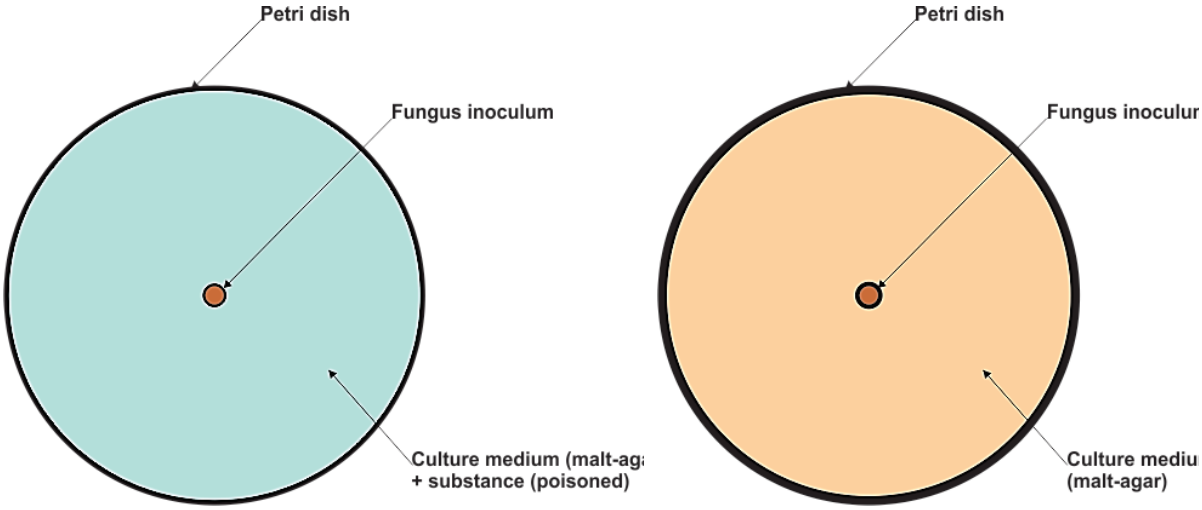

Figure 1: The principle scheme of the VM screening test (left) and control (right)

Monitoring of fungal development was done qualitatively (photographs), at different periods of time after inoculation, namely 3, 5, 7, 9 and 11 days.

RESULTS AND DISCUSSIONS

The results were centralized as photo – cards showing the evolution of the fungus development throughout the test (11 days), for the three concentrations of thyme essential oil (T-EO), comparatively to the control samples. These results are presented in Fig. 2 for the brown rot fungus *Postia placenta* and in Fig.5 for the white rot fungus *Trametes versicolor.*

In Figure 2 it can be seen that the fungus *Postia placenta* has developed in the control samples continuously, smoothly and uniformly, until the entire surface of the Petri dish was covered with mycelium, which proves the viability of the inoculated fungus. In contrast, no development of the inoculum was observed in the dishes containing the T-EO in the medium. For all three dilutions, the lethal effect of thyme essential oil (T-EO) was observed, and the fungus did not develop at all. At the 1:10 dilution (the highest concentration) the T-EO essential oil dispersed in the culture medium endured it with some opacity and changed its normal appearance, while the volatilization of a portion of the essential oil has created a foggy atmosphere in the Petri dish, blurring the transparency of the cover lid. However, it can be clearly seen in Fig.3 that the fungus has not developed at all, in comparison with the control sample. In the case of the other two dilutions (smaller concentrations of T-EO), a small lighter aura could be observed around the inoculum (Fig. 2), which might apparently indicate some mycelium development. However, in Fig. 4 it can be noticed that it is only a discoloration of the culture medium in this area and not a development of the rot fungus.

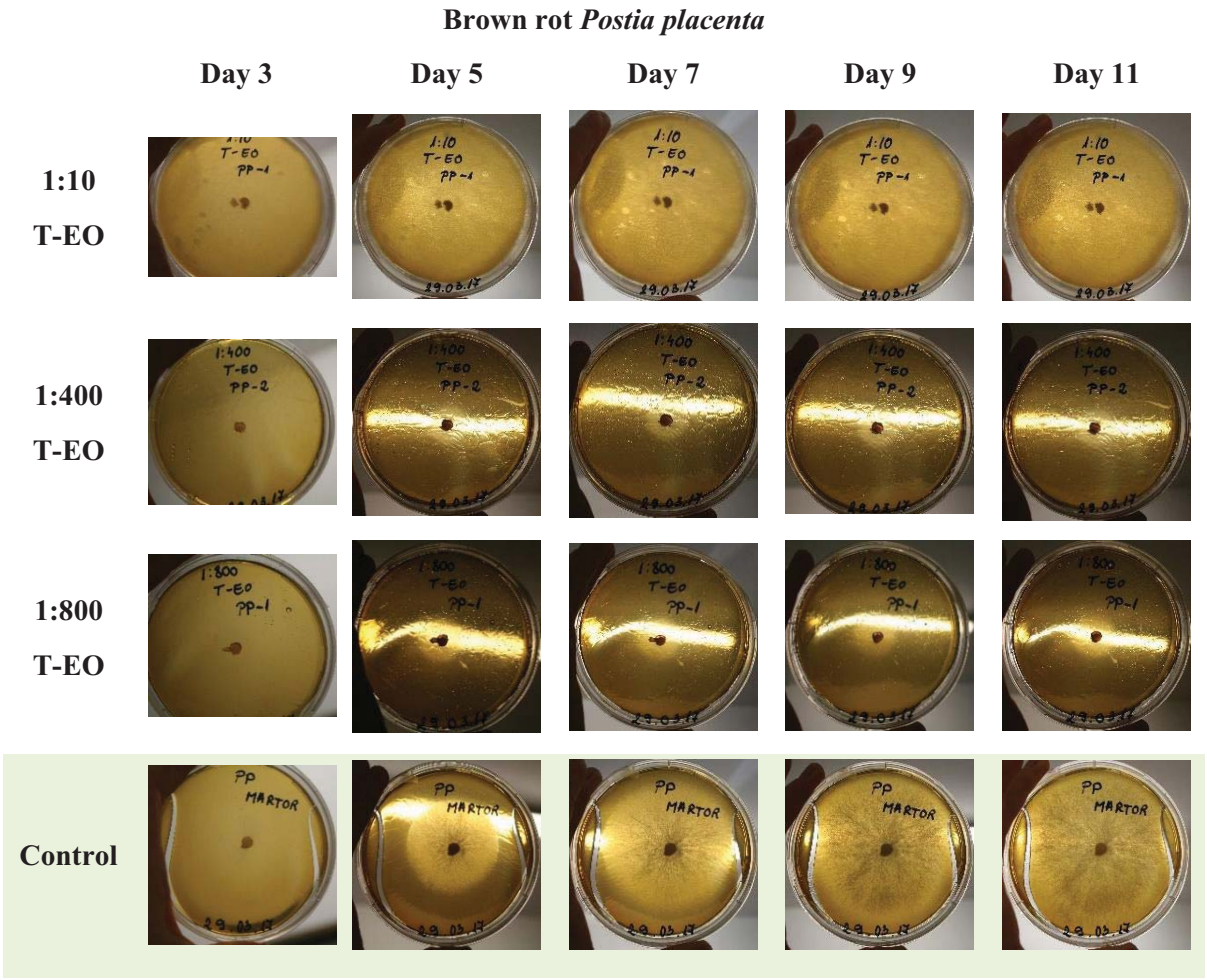

Figure 2: Evolution of brown rot fungus *Postia placenta* during the VM test (11 days): samples with different concentrations of thyme *(Thymus vulgaris)* essential oil (T-EO) compared to control.

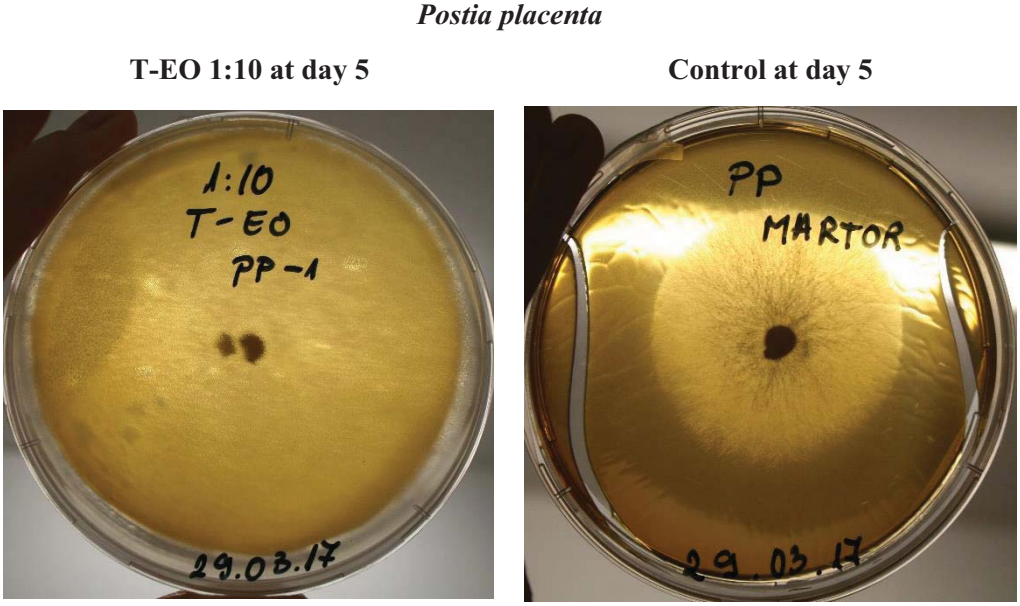

Figure 3: The differences between the appearance of the opacised culture medium with high concentration of T-EO (1:10) and the development of the whitish mycelium of the brown rot fungus *Postia placenta* in the control sample after 5days of incubation.

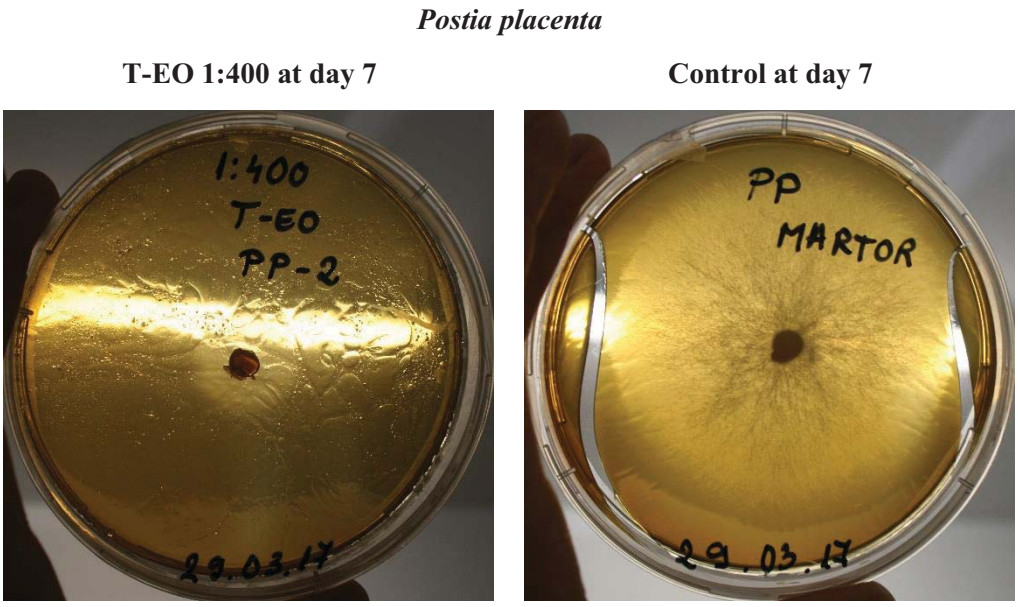

Figure 4: The differences between the appearance of the culture medium with small lighter aura (sample 1:400 T-EO) and the development of mycelium of brown rot fungus *Postia placenta* (control sample), after 7 days of incubation. It can be seen that the aura around the inoculum is only a discoloration of the culture medium.

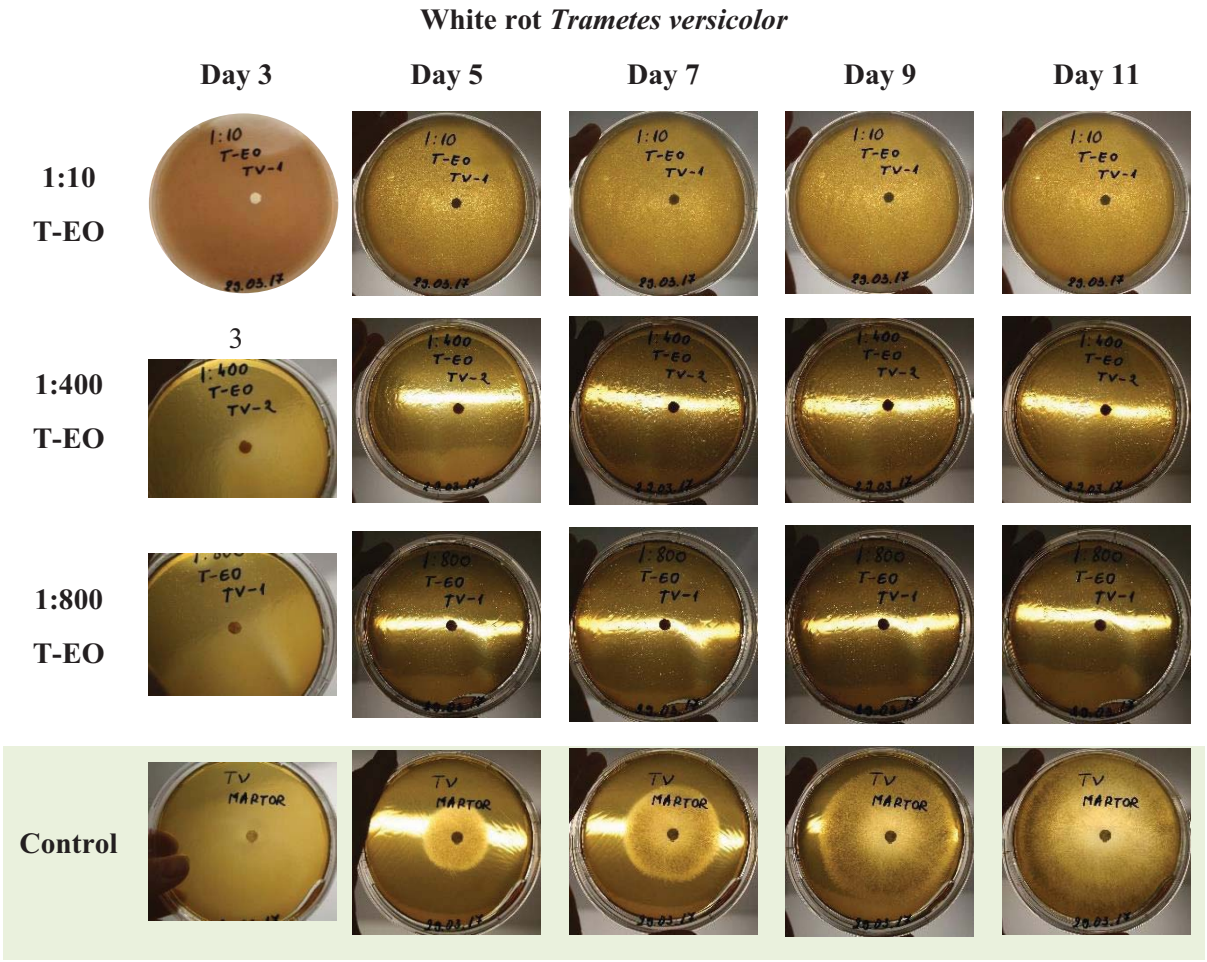

Figure 5: Evolution of white rot fungus *Trametes versicolor* during the VM test (11 days): samples with different concentrations of thyme *(Thymus vulgaris)* essential oil (T-EO) compared to control.

Trametes versicolor

T-EO 1:10 at day 7 **Martor at day 7**

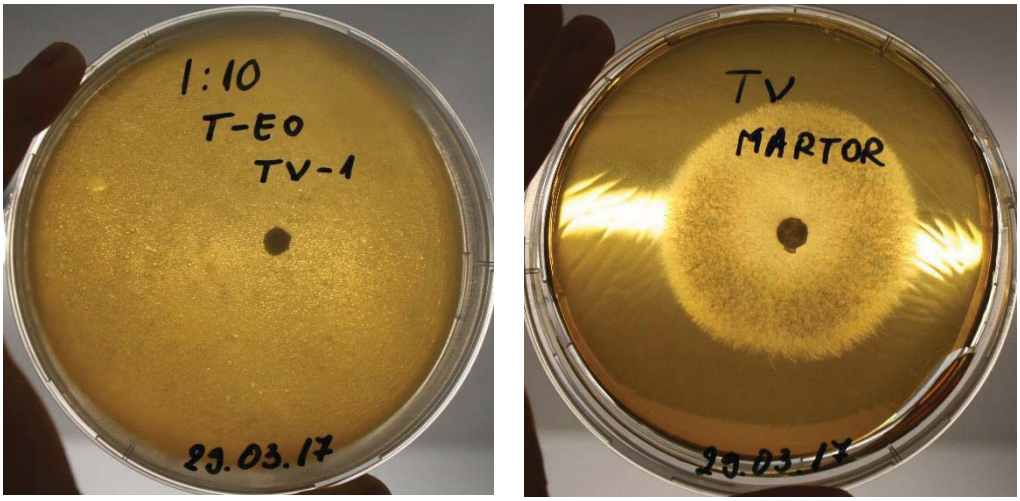

Figure 6: The differences between the appearance of the opacised with high concentration of T-EO (1:10) and the development of the withish mycelium of white rot fungus *Trametes versicolor,* after 7 days of incubation.

Trametes versicolor

T-EO 1:10 at day 7 – inoculum

Figure 7: The aspect of white rot *Trametes versicolor* inoculum after 7 days incubation in the Petri dish with thyme essential oil T-EO (concentration 1:10)

Figure 5 shows comparatively the evolution of white rot fungus *Trametes versicolor* during the 11 days of the VM test for the control sample and the samples with T-EO at the three dilutions (1:10, 1:400, 1:800). Similarly, to the previously presented case, it can be seen that for the control sample the fungus has developed continuously, smoothly and uniformly until the entire surface of the Petri dish was covered with mycelium. For all three dilutions, the lethal effect of thyme *(Thymus vulgaris)* essential oil (T-EO) on the white rot fungus *Trametes versicolor* was observed. The fungus did not develop at all. At the 1:10 dilution (the highest concentration) the thyme essential oil dispersed in the culture medium endured it

opacity and changed its normal appearance, while the volatilization of a portion of the essential oil has created a foggy atmosphere in the Petri dish, making assessment at the first sight more difficult. However, at a closer look it can be clearly seen that the fungus has not developed at all, the aspect being totally different from that of the corresponding control sample (Fig. 6). Moreover, the inoculum gained a white, opaque appearance (Fig. 7) and it is noticed a trend of possible on top development instead of spreading on the available surrounding but poisoned culture medium. This brings to the same conclusion: thyme *(Thymus vulgaris)* essential oil (T-EO) is potentially effective as fungicide against the fungi tested.

CONCLUSIONS

Based on the experiments carried out and the results presented, the following conclusions could be drawn:

- The VM screening test in which thyme *(Thymus vulgaris)* essential oil was dispersed directly in the culture medium proved to be feasible and useful for such oily products.
- Thyme *(Thymus vulgaris)* essential oil has proven to be a potential active fungicide against the brown and white rot fungi.
- The results obtained in this test showed a high efficiency against *Postia placenta* and *Trametes versicolor*, as lethal or total inhibition effect, in a wide area of concentrations in the culture medium.

More research is needed to implement these results on wood treatment.

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