Combined Testing Approach to Evaluate the Antifungal Efficiency of Clove (*Eugenia caryophyllata*) Essential Oil for Potential Application in Wood Conservation

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The efficiency of clove (*Eugenia caryophyllata*) essential oil (C-EO) for the curative antifungal treatment of historic wood was investigated in comparison with two classical biocide products: a boron-based preservative (Diffusit S) and a formulation containing quaternary ammonium salts and isothiazole (Biotin T). A combined approach was adopted that consisted of implementation of C-EO in a practical case study on a degraded beech (*Fagus sylvatica*) wood artifact and evaluation of the treatment efficacy *via* an original laboratory mycological test. Small samples, extracted from the degraded wood material before and after curative treatments, were placed as inoculum on sterile culture medium and incubated for periodic monitoring of the emerging fungal growth for 140 d. Direct observation was supplemented with digital quantification of the fungal coverage area *via* ImageJ software and calculation of the absolute and relative indices of fungal development reduction. The results indicated that the C-EO solutions at both tested concentrations (10%, 5%) were more efficient than the considered reference products at similar concentrations (Diffusit S (10%) and Biotin T (5%)) for curative antifungal treatment. However, none of the treatments applied entirely prevented reactivation of the severe and complex fungal attack, which was highlighted by the mycological tests conducted on the control samples.

Keywords: Essential oils; Historic wood; Curative treatment; Antifungal; Mycological test; ImageJ

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INTRODUCTION

Wood is an organic material with an intrinsic biodegradation resistance characterised by its natural durability. This varies across wood species, between heartwood and sapwood, and depends on the types of biotic agents active in different environmental conditions. Active biotic agents, which include bacteria, fungi, and insects, can cause serious aesthetic and functional problems and impede wood preservation (Fabri 2012) in different environmental conditions. Bacteria and fungi generally require a moist environment to successfully attack wood. The relationship between moisture in wood and fungi development was recently reviewed by Brischke and Alfredsen (2020).

Many mould fungi are involved in surface discolouration and the weathering process; however, they live on the surface of wood and do not affect the strength properties of wood (Lie *et al.* 2019; Lie 2019). In contrast, wood-decaying fungi, which belong to three major groups (brown rot, white rot (*Basidiomycetes*), and soft rot (*Ascomycetes*, *Deuteromycetes*)), cause serious damage to wood structure at macroscopic, microscopic,

and chemical levels by specific mechanisms of degradation (Fackler and Schwanninger 2012; Brischke *et al*. 2014; Walsh-Korb and Avérous 2019). These fungi produce different enzymes that are capable of selectively decomposing cellulose, hemicelluloses, and lignin, which results in specific patterns of wood tissue degradation (Broda and Mazela 2016).

Biodeterioration caused by fungal attack represents a serious concern for wood cultural heritage (Sterflinger 2010; Irbe *et al.* 2012; Alfieri *et al.* 2016; Sabatini *et al.* 2018). Large microbial and fungal diversity are characteristic of old wood artifacts and cultural heritage (CH) sites (Kim *et al*. 2016), which has been determined by classical culture-based methods (Held and Blanchette 2017; Lee *et al.* 2018; Sabatini *et al.* 2018) and by modern molecular techniques and genetic and biochemical methods (Sterflinger 2010; Held and Blanchette 2017; Adamiak *et al*. 2018). Newly available systemic methods called "omics" techniques that can be used to assess CH biodeterioration were recently reviewed (Gutarowska 2020), and the importance of understanding the mechanisms of biodeterioration by microbial communities in selecting appropriate strategies for the restoration and maintenance of CH was highlighted.

As the use of biocides is among the most effective methods of controlling microorganisms for cultural heritage conservation (Kakakhel *et al.* 2019), identifying solutions for the efficient preventive and curative bioprotection of wood with reduced toxicity to humans and environmental impact are important challenges for researchers. A new generation of "green" products from natural resources have been considered as alternatives to classical biocides. These include a large group of essential oils (EOs) from selected aromatic plants, such as *Eugenia caryophyllata*, *Lavandula angustifolia*, *Melaleuca alternafolia*, *Origanum compactum*, *Ocimum basilicum*, *Pelargonium graveolens*, *Rosmarinus officinalis*, *Satureja hortensis*, *Salvia officinalis*, *Thymus vulgaris* (Zyani *et al*. 2011; Pánek *et al*. 2014; Stupar *et al.* 2014; Bahmani and Schmidt 2018; Kakakhel *et al*. 2019), or other extracts from vegetal sources (Ashmawy *et al*. 2020), which have been investigated for their antifungal activity and potential for wood preservation with some promising results.

Several diverse laboratory mycological methods are currently employed to assess the antifungal effect and compare the efficiency of different products, including EOs. Screening tests on a culture medium, which are typically based on a disc diffusion technique or agar (medium) dilution method and rarely based on vapour exposure (Zyani *et al.* 2011), are usually run as a first step for the evaluation of antifungal potential. Tests on treated material (wood) are generally a second step. These are either standardised or adapted mycological tests that examine the fungal development and/or mass loss (for decay fungi) following inoculation with individual fungi and incubation for certain periods of time in a controlled environment. When examining anti-mould effects, mixtures of spores of different fungi might be employed. Depending on the test employed, qualitative and/or quantitative results are obtained. When fungal growth is evaluated, conventional subjective rating schemes (Bahmany and Schmidt 2018; Lee *et al.* 2018; Lie *et al*. 2019) or calculated growth inhibition indices (Zyani *et al.* 2011; Pánek *et al.* 2014; Xie *et al.* 2017) are typically used to express the results. The percentage mass loss caused by fungal decay and the mass loss relative to reference are usually calculated in mini-block tests adapted from SR EN 113 (1997) or similar methods (Pánek *et al.* 2014; Lee *et al*. 2018).

Several complementary investigation methods, such as X-ray radiography, computer tomography associated with ImageJ quantification, and micro imaging *via* optical microscopy and scanning electron microscopy (SEM), have been applied to assess fungal development and associated structural degradation phenomena (Broda and Mazela

2016; Held and Blanchette 2017; Alfieri and Correa 2018; Alfieri *et al*. 2020). In addition, Fourier transform infrared (FTIR) investigation, which requires smaller sample sizes, has been employed to highlight chemical changes due to fungal and bacterial biodegradation (Fackler and Schwanninger 2012; Gelbrich *et al*. 2012).

Clove essential oil (C-EO) extracted from *Eugenia caryophyllata* is an EO that shows potential for use in wood bioprotection. The antifungal properties of C-EO were demonstrated by several researchers who employed different testing methods (*e.g.*, screening tests on culture mediums and mini-block tests on wood) and different fungal strains, including white rot (*e.g.*, *Trametes hirsute* and *Trametes versicolor*), brown rot (*e.g.*, *Laetiporus sulphureus* and *Coniophora puteana*), and mould fungi (*e.g.*, *Aspergillus niger*, *Penicillium brevicompactum*, *Alternaria alternata*, and *Chaetomium globosum*), all of which are important in wood biodegradation (Pánek *et al.* 2014; de Medeiros *et al*. 2016; Reinprecht and Vidholdová 2017; Xie *et al.* 2017). The antifungal activity of C-EO is due to its main chemical component, eugenol, which is a phenolic product found in proportions of 67% to 78% in the complex chemical composition of C-EO (Borrego *et al*. 2012; Xie *et al*. 2017).

However, research that focuses on the potential of C-EO or EOs in general for wood CH protection and considers the diversity of fungal strains involved in the respective situations and their interaction in wood degradation is rather limited. The *in vitro* antifungal activity of five essential oils from plants (*Eugenia caryophyllata*, *Origanum compactum*, *Ocimum basilicaum*, *Thymus vulgaris*, and *Melaleuca alternafolia*), including C-EO, against five wood decay fungi (four white rot: *Thielavia hyalocarpa*, *Penicillium commune*, *Penicillium chrysogenum*, and *Penicillium expansum*; one brown rot: *Cladosporium cladosporioides*) isolated from the degraded wood of an old house at the Medina of Fez (Maroc) was reported by Zyani *et al.* (2011). Disc diffusion, agar dilution, and vapour phase tests were performed, all of which found that the essential oils of *O. compactum* and *E. caryophyllata* demonstrated the broadest antifungal spectrum (active against all five fungi tested) and the highest antifungal activity.

Previously published research proved the antifungal activity of C-EO against brown rot (*Postia placenta*, *Serpulla lacrymans*) and white rot (*Trametes versicolor*) fungi *via* screening diffusion tests on the culture medium (Pop *et al.* 2018, Reinprecht *et al.* 2019). Further research that has yet to be published employed original dedicated tests on sound (naturally infected) and degraded wood material that highlighted the potential of C-EO as a green alternative protection system for use in wood cultural heritage conservation in preventive and curative antifungal treatments. These promising results inspired the present study, which was motivated by the need for such treatments in current wood conservation practices. Notably, real situations in conservation practice are far more complex in terms of fungal diversity than any test performed with individual fungi, which should be considered in testing potential alternative products.

RESEARCH AIM AND OBJECTIVES

This study aimed to investigate the efficacy of C-EO for the curative antifungal treatment of degraded wood from historic objects for conservation purposes. Therefore, a combined approach was adopted, which consisted of the evaluation of C-EO in a practical case study on a degraded wood artefact and the determination of the treatment efficacy by laboratory mycological tests specially designed for this purpose. Small samples were

extracted from the degraded wood material before and after curative treatments, placed as inoculum on a sterile culture medium, and incubated to evaluate fugal development. The procedure was performed to compare the antifungal efficiency of C-EO to that of two classical biocidal products currently employed in wood conservation practice and considered as references in this research. Further, a digital method of fungal growth quantification based on imageJ software was employed to allow better interpretation of the results.

EXPERIMENTAL

Antifungal Products

Clove (*Eugenia caryophyllata)* essential oil available on the Romanian market under the label of "Steaua Divina" was tested in this research as a potential alternative green antifungal product (Steaua Divina, Ilfov, Romania). Two treatment solutions, referred to as C-EO 5% and C-EO 10% were prepared by dilution of the original product (100%) with ethyl alcohol (96%) (S.C. Chemical Company S.A., Iaşi, Romania) at volumetric ratios of 5:100 and 10:100, respectively. The actual concentrations were 4.8% and respectively 9.1%, as volumetric percentages.

The Diffusit S (boron-based preservative) produced by BASF Wolman GmbH (Sinzheim, Germany) was provided by Abies Transylvania SRL. The Biotin T (based on 2-octyl-2H-isothiazole (OIT) and quaternary ammonium salts as a water dilutable concentrated liquid) was obtained from CTS (Centrali Superfici Trattamento, Vicenza, Italy). Diffusit S was prepared as water solution with a 10% concentration, whereas Biotin T was prepared as a 5% concentration water solution (as related to the original concentrated products).

Wood Material - Case Study

For analysis, old wood material with clear evidence of fungal degradation that required curative treatment was necessary. This material was provided by a conservation case study of a museum artifact, which was a wooden saw horse (Fig. 1a) from the collection of the open-air ASTRA Museum of Traditional Folk Civilisation in Sibiu, Romania. This artifact was conserved in a restoration camp in 2019. This case study was selected as relevant for the purpose of this research due to the advanced biodegradation of the wood material (mostly as result of a combined fungal attack) and its structure (four similar elements), which allowed the implementation and comparison of different treatments.

This kind of utilitarian object, used to support wood being sawed, would have likely been present in most rural households in its time period of origin. The object was a rack consisting of a pair of X-shaped legs made of two cross-lapped elements that were joined by a horizontal crosspiece with tenon fasteners on each end. This artisanal object was made from beech wood (*Fagus sylvatica* L.) that was roughly processed in with approximate squared cross-section.

Because the saw horse was made from a non-durable wood species and displayed for a long time period outdoors (under a roof but in direct contact with soil), the initial state of the artifact was poor and represented a worst case scenario for risk of fungi attack, which cannot always be avoided in ethnographic open-air museums. The biotic and abiotic factors damaged the appearance and integrity of the wood material (Fig. 1b to Fig. 1e). Advanced

decay areas were identified on all the elements, and evidence of deterioration, erosion, and insect attack was evident on the bottom part of the legs. The whole surface was weathered and rough; deep cracks, fissures, and ruptures in wood were present, and dirt depots were present.

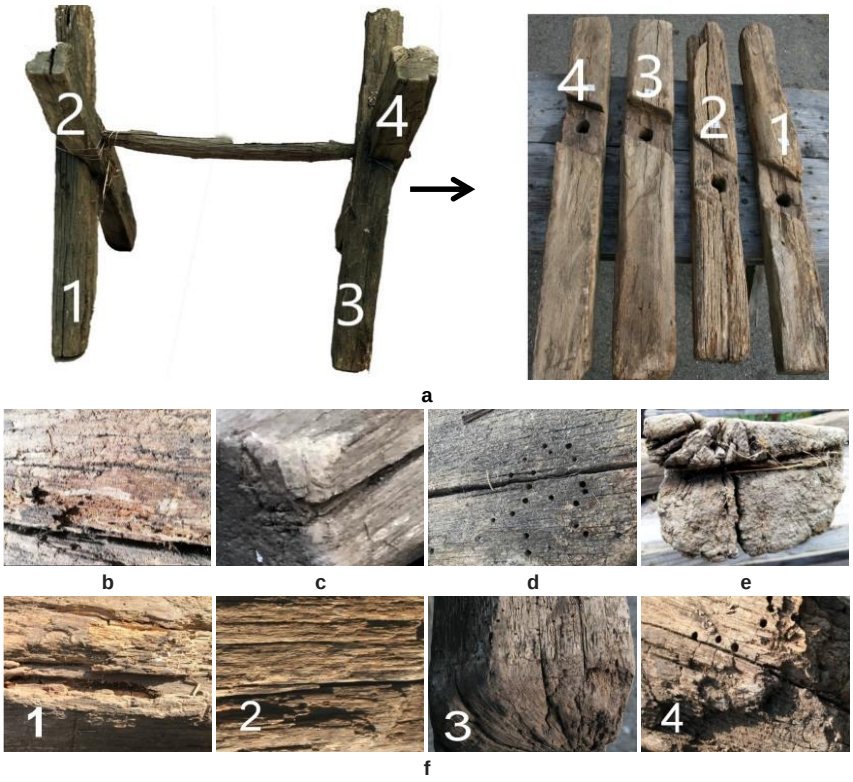

Fig. 1. Case study: saw horse made of beech (*Fagus sylvatica*) wood from a museum collection: **a.** general initial aspect and the four constitutive elements coded 1 through 4 after dismantling and cleaning; **b** through **e** illustration of the precarious initial conservation state of wood material due to advanced complex degradation: fragilisation by brown rot and insects (**b**); deep cracks, weathered surface, dirt depots (**c**); insects atttack and cracking (**d**); biological degradation, cracking and errosion of areas in direct soil contact (**e**); (**f**) details of biological degradation causing frailness of the wood material for all the elements coded 1 through 4

The object was dismantled, and all the elements were thoroughly cleaned. After cleaning, the biological degradation was more visible, and large areas of frail wood were widespread on all four main constitutive elements (coded 1 to 4) (Fig. 1f). However, the elements 1 and 2 exhibited more prominent degradation. Whitish and brownish zones, which likely corresponded to white and brown rot, respectively, heavily degraded areas at the bottom of the legs with a possible contribution of soft rot and surface discoloration by fungi and insects damage were evident, which highlighted a complex and advanced biological degradation that required curative treatments.

Antifungal Treatments and Sampling

Based on the analysis of the state of the wood, active conservation was implemented. This included, as a key step, the antifungal treatment, meant to stop the fungal attack if active, or to prevent its activation in high humidity conditions.

Before any treatment, several small samples (with cross-sections of approximately 2 mm^2 to 15 mm^2 and lengths of up to 15 mm to 20 mm) were randomly extracted from the areas that clearly exhibited different forms of fungal degradation (especially zones with indications of brown rot and white rot) from all four constitutive elements. These were employed as controls for the existing fungal infection or active attack in the mycological tests.

Next, the four constitutive elements (1 to 4) were each treated differently (Table 1) with the reference products of Diffusit S (10%) and Biotin T (5%), and 10% and 5% C-EO solutions. Equal amounts of 300 mL solutions were applied on each element, by repeated brushing on the whole surface, insisting on the most damaged areas. The alcoholic solutions of C-EO were more readily absorbed than the aqueous solutions of Biotin T and Diffusit. The aim was to compare the efficiency of C-EO to that of the specified reference products when applied in similar conditions (concentration, amount, and application procedure). Elements 1 and 2, which exhibited stronger degradation, were chosen to be treated with the higher concentration (10%) solutions, and elements 3 and 4 were treated with solutions of 5% concentration. From the two pairs of elements (1 and 2; 3 and 4) the elements that exhibited more prominent degradation than the other element of their respective pair were chosen to be treated with the essential oil solutions (element 2 with C-EO 10% and element 4 with C-EO 5%). Sampling from each element was repeated after these treatments (Fig. 2).

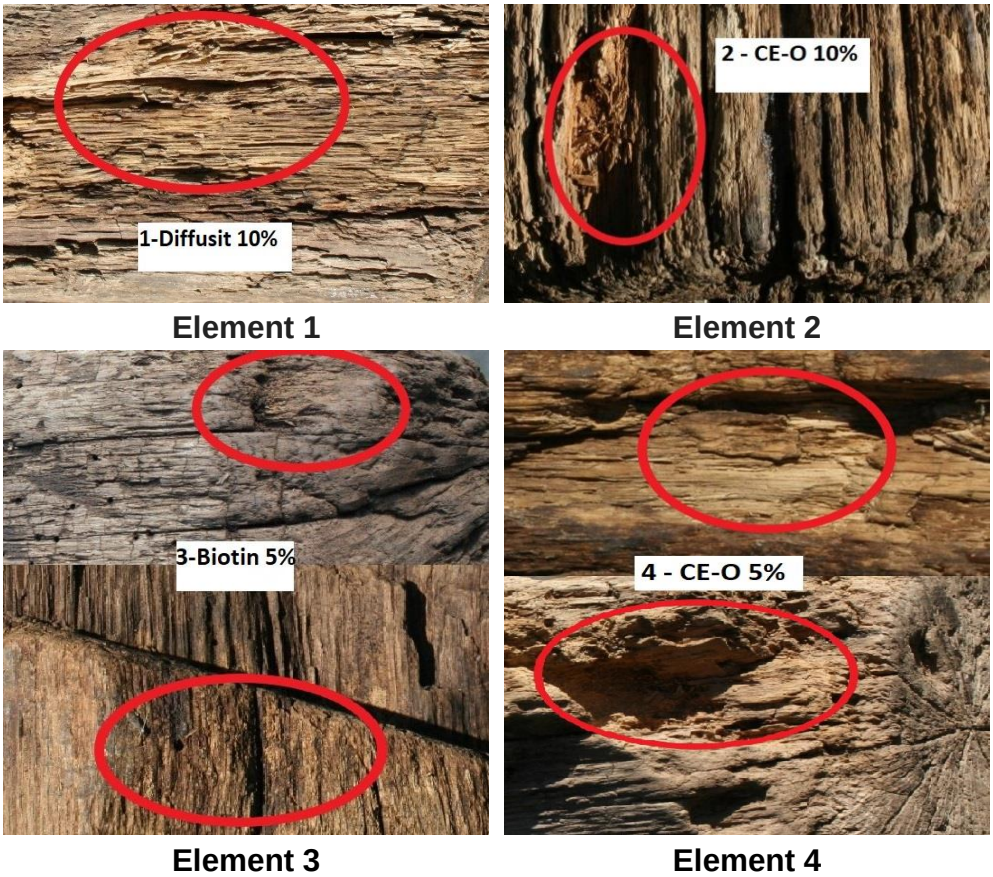

Fig. 2. Sampling map after antifungal treatments of the four elements 1 to 4 with the different tested products

Table 1. Antifungal Curative Treatments Applied to Constitutive Elements (1 to 4)

Wood Element	Product Applied	Code of Treatment
Element 1	Diffusit S 10%	1- Diffusit 10%
Element 2	Clove essential oil 10%	2- C-EO 10%
Element 3	Biotin T 5 %	3- Biotin 5%
Element 4	Clove essential oil 5%	4- C-EO 5%

Mycological Tests

Procedure

The mycological tests employed in this study were designed to be representative of real situations that require efficient curative antifungal treatments to stop an active or reactivated fungal attack. The tests were performed on MEAA (malt extract, agar-agar) culture medium (prepared from 40 g of malt extract Carl Roth GmbH + Co KG (Karlsruhe, Germany) (and 20 g of agar-agar ROTH for 1 L of distilled water), sterilised at 121 °C for 20 min, and poured into sterile (single-use) Petri dishes. The small extracted samples (untreated controls and treated samples) were placed directly on the sterile culture medium in Petri dishes, which were then sealed with parafilm and incubated at 22 °C and 70% relative humidity (RH) in a Climacell (BMT Medical Technology S.R.O., Brno-Zábrdovice, Czech Republic). Because the test was run in sterile conditions, any fungal development could only have originated from the wood samples (serving as the only inoculum), and the effectiveness of the treatments was reflected in the reduction or cessation of fungal growth. Fungal development was periodically observed, and its in-time evolution was monitored and documented by photos for 140 d.

Because this research was a case study on a museum artefact, sampling was limited. Two small samples (one resembling brown rot and one resembling white rot) were placed in each Petri dish. Four Petri dishes were prepared for control samples (coded C1 to C4), whereas two or three plates were prepared for each of the four treatments.

Digital quantification of fungal development and indices calculation

To allow better interpretation of the results, a digital method based on ImageJ (National Institutes of Health, ImageJ bundled with 64-bit Java 1.8.0_112, Bethesda, MD, USA) software was applied to quantify the area of mycelium growth. Digital, high quality pictures (1878 pixels × 1870 pixels) of the test plates (Petri dishes) were employed for this purpose. The zones covered with mycelium were individually delimited *via* the free contour tool, and their areas were computed by the software, which considered the scale set by the known diameter of the Petri dishes. The total area covered by the mycelium (summation of all individual areas) was evaluated in relation to the whole area of the plate (Petri dish). A quantitative indicator of fungal development, the fungal coverage area (FCA), was calculated with Eq. 1 and expressed as a percentage (%),

$$\mathrm{FCA}\ (\%) = \frac{A_\mathrm{m}}{A_0} \times 100 \quad (1)$$

where A_m is total area (mm^2) covered by the mycelium and A_0 is total area (mm^2) of the plate (Petri dish). Based on the FCA values, the absolute and relative indices of fungal development reduction (IRD, %) were calculated with Eq. 2 and Eq. 3, respectively, to highlight and compare the antifungal activity of the tested products:

$$\mathrm{IRD_{abs}}\ (\%) = \frac{(\mathrm{FCA_{control}} - \mathrm{FCA_{treated}})}{\mathrm{FCA_{control}}} \times 100 \quad (2)$$

$$\mathrm{IRD_{rel}}\,(\%) = \frac{(\mathrm{FCA_{reference}} - \mathrm{FCA_{C-EO}})}{\mathrm{FCA_{reference}}} \times 100 \quad (3)$$

The interpretation of FCA values and the related calculated indices should be correlated with the images. For instance, there were cases where full plate coverage by mycelium (FCA = 100%) was reached in early stages of the test and remained constant, but the fungi continued to develop (*e.g.*, thicker mycelium, sporulation). The IRD values should be considered also less relevant for testing periods that exceeded the time needed for 100% fungal coverage for the control or reference products.

RESULTS AND DISCUSSIONS

Figures 3 (control samples) and 4 (treated samples) (one plate for each variant) show the fungal development that emerged for the control and treated samples.

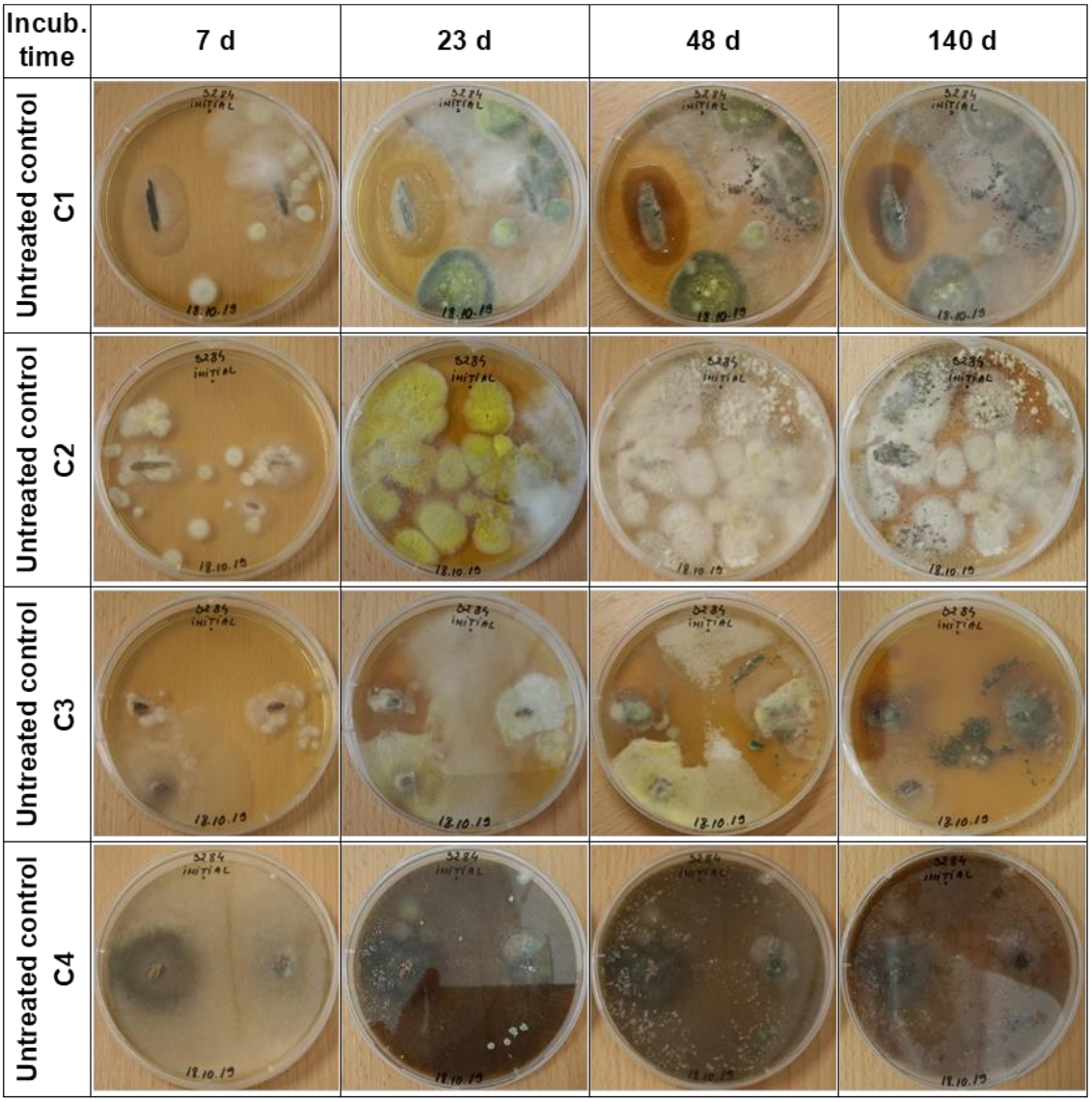

Fig. 3. In-time evolution of the fungal development from the control samples extracted from degraded areas of the four beech (*Fagus sylvatica*) wood elements, which was indicative of a diverse active or activated fungal attack

The specimens are shown after 7 d, 23 d, 48 d, and 140 d of incubation. These qualitative results are complemented by the quantitative results expressed by the average fungal coverage area values (FCA, %), based on which the diagram of dynamic of fungal development (Fig. 5) was drawn.

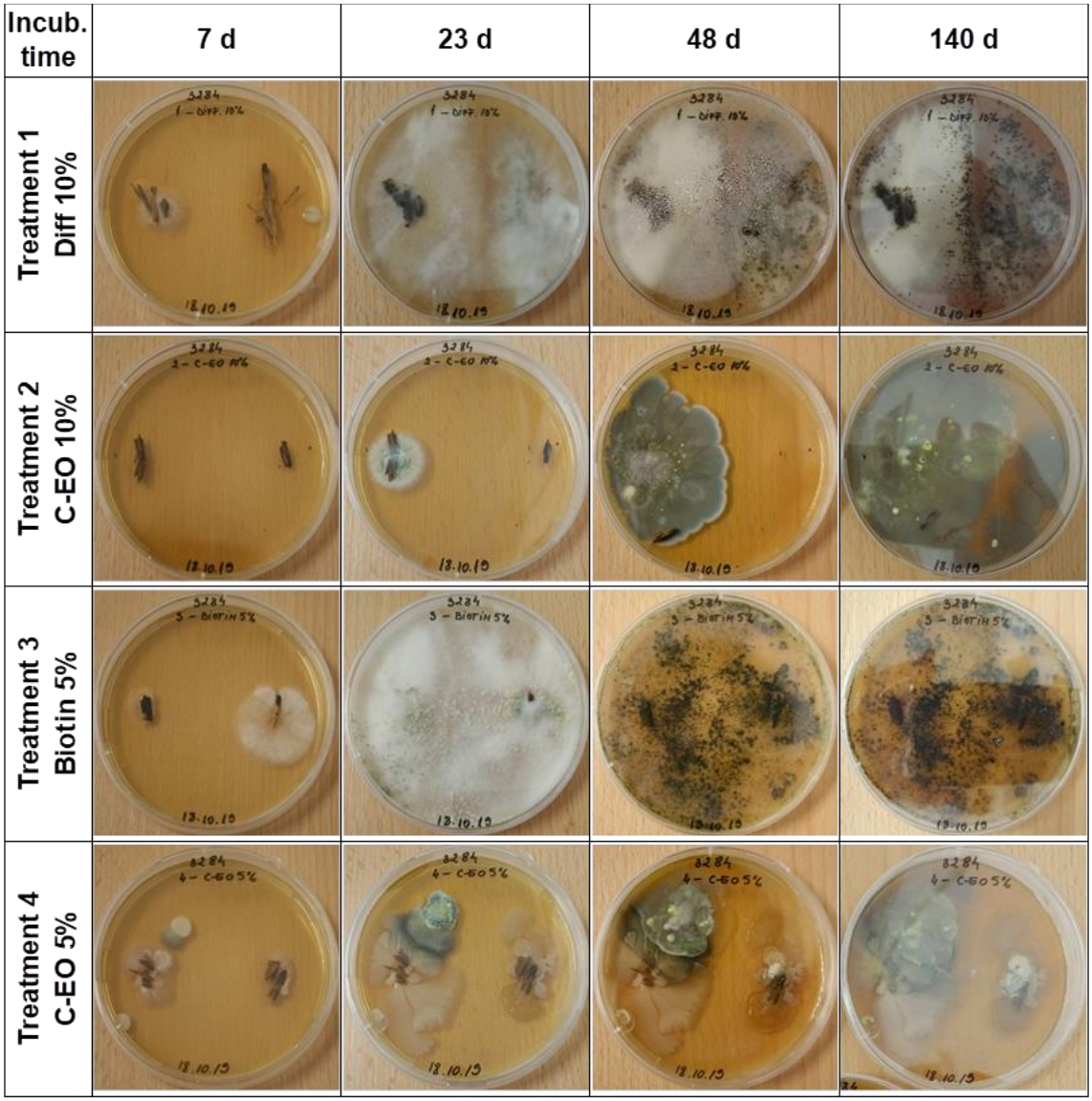

Fig. 4. Comparative in-time evolution of fungal development from samples extracted from degraded beech wood (*Fagus sylvatica*) after curative antifungal treatments with the reference products (Diffusit S (10% - treatment 1), Biotin T (5% - treatment 3), and C-EO solutions (10% - treatment 2 and 5% - treatment 4) at similar concentrations

For the control samples (Fig. 3), there was rapid development (clearly visible after 7 d of incubation) of a diverse fungal attack for all samples (FCA of 38.7% to 100% for C1 to C4, with an average of 60.4%), which indicated a severe contamination of the wood and the need for efficient treatments. Further, there were remarkable differences among the 4 control plates in terms of type of fungi and their speed of development. A complex fungal attack was expected from visual assessment of wood degradation, but this test highlighted a much larger diversity and an important contribution of different types of moulds, which

generally develop faster than the decay fungi (Alfieri *et al.* 2016; Bahmani and Schmidt 2018). A diverse fungal community involved in wood degradation and present in diverse historic sites has been reported in the literature (Sterflinger 2010; Irbe 2012; Alfieri *et al.* 2016; Kim *et al.* 2016; Lee *et al.* 2018; Sabatini *et al.* 2018; Sanmartín *et al.* 2018; Zhang *et al.* 2019). In addition, non-uniformity of fungal decay in wood generates large variability (even within small areas) when sampling from decayed wood (Fackler and Schwanninger 2012; Brischke and Alfredsen 2020).

A dynamic process of fungal growth continued as time increased, and in addition to an increase in FCA (average of 88.1% after 23 d and 97.9% after 140 d), different specific patterns that suggested succession, interactions, and physiological development of the fungi involved (*e.g.*, color changes and sporulation) were observed. These results are characteristic of wood fungal colonisation and degradation (Morris 2011; Ottosson *et al.* 2014; Alfieri *et al.* 2016; Hiscox *et al.* 2018).

The treatment with Diffusit S (10%) delayed fungal development (compared to the untreated control samples), which was evident from the fact that a limited fungal growth was visible after 7 d of incubation (FCA 7.5%). However, the fungal growth then developed rapidly, and mycelium almost entirely covered the whole surface of the plate in 23 d (FCA 95.4%) (Fig. 4 - Treatment 1). Further development was evident in mycelium thickening and sporulation, as seen in the images after 48 d and 140 d of incubation.

Treatment with C-EO (10%) was more efficient, as no fungal growth was detected before 23 days of incubation, at which point a limited fungal growth was visible (FCA = 4.4%). This evolved more slowly over time (Fig. 4 - Treatment 2), as the corresponding FCA values after 48 d and 140 d of incubation were 27.2% and 77.6%, respectively.

For the treatment with Biotin T (5%), the fungal growth after 7 d of incubation (FCA 18.1%) was the highest of all the tested treatments but lower than that of the untreated controls. It evolved rapidly, and the mycelium covered the whole surface of the culture medium in 23 d (FCA 100%). Further development was evident in mycelium thickening and sporulation that was visible after 48 d and 140 d of incubation (Fig. 4 -Treatment 3).

Compared to Biotin T 5%, the treatment with C-EO 5% (Fig. 4 - Treatment 4) was more efficient, as it resulted in reduced fungal growth (FCA 6.7%) after 7 d of incubation. The FCA value was slightly lower than the corresponding value registered for the samples treated with Diffusit S 10% (FCA = 7.5%). The fungal growth for the samples treated with C-EO 5% evolved more slowly over time than the samples treated with Biotin T 5%.

The comparative dynamics of fungal growth for the untreated control samples and the samples curatively treated with C-EO (5%, 10%) and the corresponding reference products (Biotin T 5% and Diffusit S 10%) are shown in Fig. 5. The curves clearly show that none of the treatments applied entirely stopped the severe fungal attack for the heavily degraded beech wood considered in this research, but those with C-EO solutions provided better protection than the corresponding reference products.

Further, though an expected difference in efficiency of C-EO 10% and C-EO 5% (better results for C-EO 10%) was observed for the first 48 d of incubation, the difference decreased over time, as the FCA values were quite similar after 140 d (approximately 78%). This may have been due to a partial volatilisation of C-EO, which could generate an atmosphere inhibitive of fungal development in the closed environments of the sealed Petri dishes. Such an effect was observed on other mini-block tests for alcoholic C-EO solutions of 10% in unpublished research. This was in accordance with literature reports of an antifungal inhibitory effect in the vapour phase of several essential oils, of which C-EO was ranked as the second most effective antifungal oil of the 16 EOs tested and the one

with the broadest antifungal spectrum (Zyani *et al.* 2011). In addition, different EOs were successfully tested for the vapour disinfection of archival documents (Pietrzak *et al.* 2017) or textile heritage artefacts (Matusiak *et al.* 2018).

Moreover, the visibly increased degradation of element 2 (treated with C-EO 10%) relative to element 4 (treated with C-EO 5%) was likely due to differences in their fungal colonisation (Fig. 3 highlights the large variety in colonisation among the different control samples), which might have resulted in specific patterns of fungal development, succession, and competition aspects that contributed to the unexpected result of C-EO exhibiting similar results for both concentrations tested (5% and 10%) after long time exposure. More research is needed to clarify this result and verify the presented hypotheses.

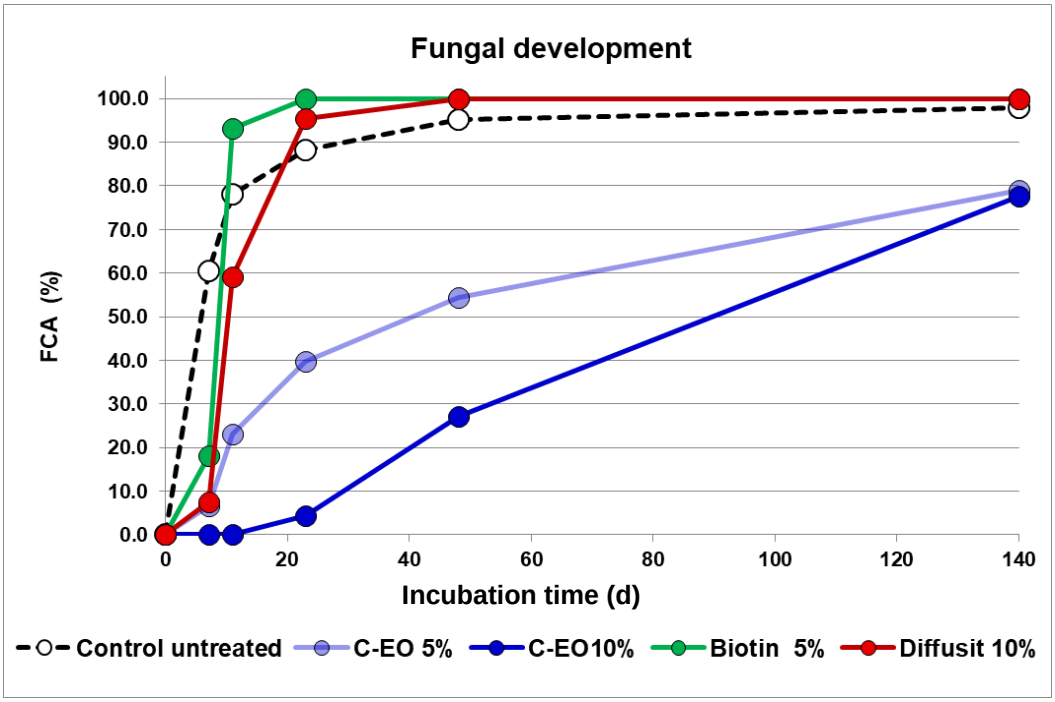

Fig. 5. Comparative dynamics of fungal development for the control and treated samples (4 different treatments: C-EO 5%, C-EO 10%, Biotin T 5%, and Diffusit S 10%) of degraded beech (*Fagus sylvatica*) wood

To better highlight the curative antifungal efficiency of C-EO solutions in comparison with Diffusit S and Biotin T at similar concentrations, the indices of reduction of fungal growth (*IRD*, %) are plotted in Fig. 6.

The value of IRD_{abs} compared treated wood with the untreated control, so that any positive value above zero indicated antifungal activity, higher values represented improved efficiency, and a maximum value of 100% indicated total inhibition or cessation of fungal development. The value of IRD_{rel} compared the antifungal efficiency of treatments with C-EO solutions (10% or 5%) with that of the reference treatments with Diffusit S (10%) and Biotin T (5%). A null value of IRD_{rel} indicated similar activity, as any positive value indicated higher antifungal activity for the C-EO solutions than for the reference treatments (higher values were indicative of better performance), and a maximum value of 100% indicated that C-EO totally inhibited or stopped fungal growth.

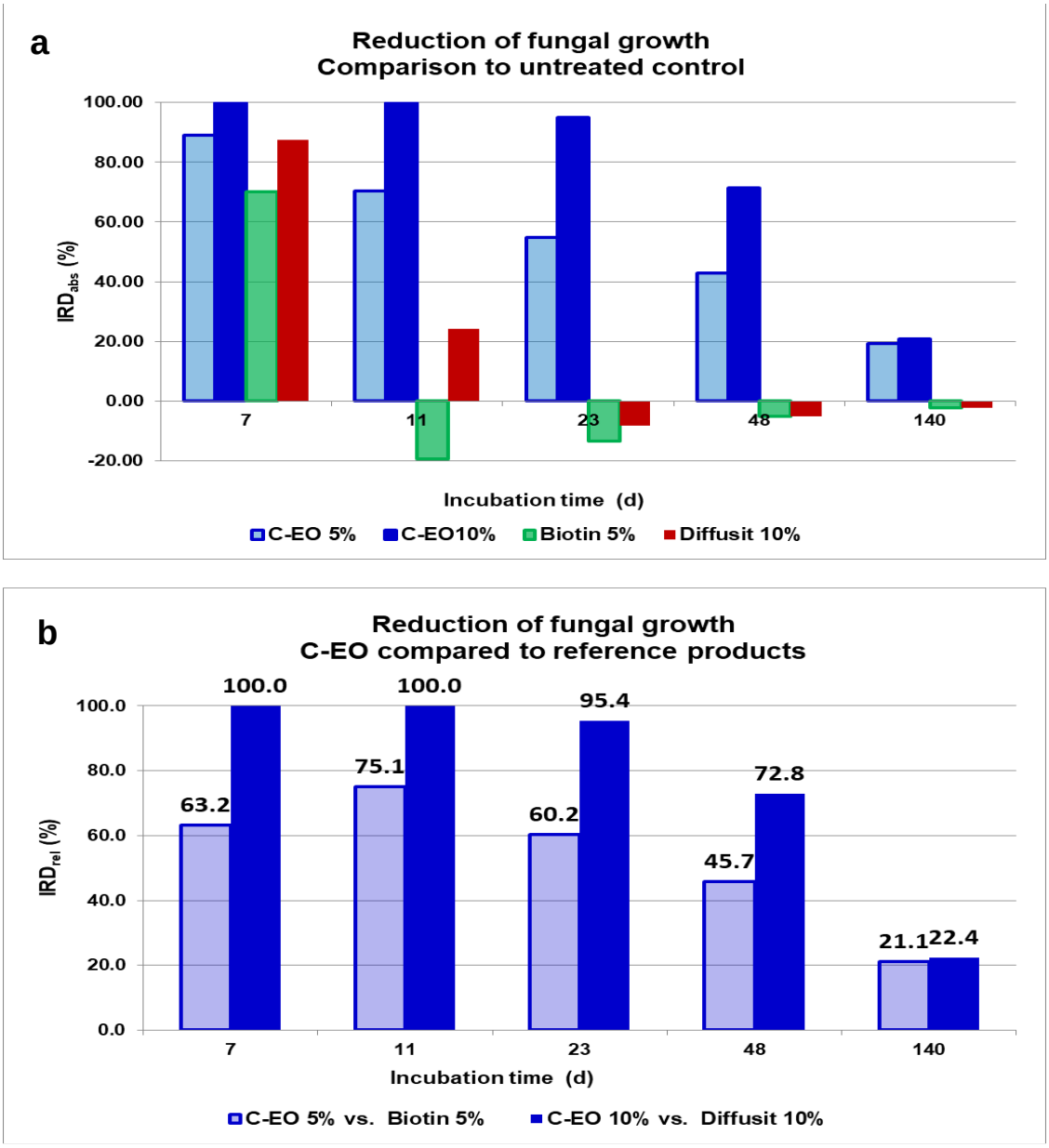

Fig. 6. Comparative efficiency of C-EO (10% and 5%) and the corresponding reference products at similar concentrations (Diffusit S (10%), Biotin T (5%)) in the curative antifungal treatment of degraded beech (*Fagus sylvatica*), which was determined by the indices of reduction of fungal development: (a) IRD_{abs} treatments *versus* control samples; (b) IRD_{rel} C-EO compared to the reference products

Accordingly, both the IRD_{abs} and IRD_{rel} values in Fig. 6 indicated that the C-EO solutions at both tested concentrations (10% and 5%) were more effective than the considered reference products at similar concentrations (Diffusit S (10%) and Biotin T (5%)) for the curative treatment of heavily degraded beech wood. However, though there was a visible declining trend in the calculated values of these indices for the treatments with C-EO solutions as incubation time increased, this trend does not necessarily represent a reduction in efficiency but is rather a product of the calculation method. Because almost total mycelium coverage of the plates (FCA = 95% to 100%) was reached rapidly (23 d to 48 d) for the untreated controls and the reference treatments, the further evolution of the

fungal development was no longer reflected in the FCA value, which remained constant. Therefore, the indices calculated based on those FCA values should be considered with care. More research and complementary investigation methods to evaluate the antifungal protection effects are necessary and should be considered in the future.

CONCLUSION

1. The efficacy of clove (*Eugenia caryophyllata*) essential oil (CE-O) for curative antifungal treatment of old degraded wood from historic objects was investigated and compared to that of two classical biocide products (Diffusit S (boron-based preservative) and Biotin T (organic formulation based on 2-octyl-2H-isothiazole (OIT) and quaternary ammonium salts).
2. The experimental results clearly indicated that C-EO solutions at both tested concentrations (10% and 5%) were more effective than the reference products at similar concentrations (Diffusit S (10%) and Biotin T (5%)) for the curative treatment of heavily degraded beech (*Fagus sylvatica*) wood. The relative indices of reduction of fungal growth show that C-EO 5% was more efficient than Biotin 5% with a percentage varying in the range of 75.1 to 45.7% in the first 48 days of the test. At the same time, C-EO 10% was more efficient than Diffusit 10% with a percentage varying in the range of 100.0 to 72.8% in the first 48 days of the test.
3. However, none of the treatments applied entirely stopped or prevented reactivation of the severe and complex fungal attack, which was highlighted by the mycological tests on control samples.
4. The results are encouraging for the potential of C-EO as an alternative green antifungal protection system for application in historic wood conservation. However, aspects related to the C-EO volatilisation and inhibitory effects on fungi development have to be considered in further research.
5. The combined testing approach employed in this research could become a useful tool, adding new capability to the current testing methods for novel products intended as alternative antifungal systems for historic wood conservation.

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