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Zeng Yang *Editor*

Environmental Science and Technology: Sustainable Development

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Zeng Yang
Editor

Environmental Science and Technology: Sustainable Development

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Editor
Zeng Yang
Department of Environmental Engineering
School of Environmental Science
and Engineering
Qingdao, China

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Preface

The 2022 13th International Conference on Environmental Science and Technology (ICEST 2022) was held as virtual conference during October 21–23, 2022. The Conference has emerged as an exclusive opportunity for the participants from all over the globe to present and discuss the reviews and results in their respective environmental science and technology research areas.

The international conference was divided into three major parts, including keynote speeches, invited speeches, and oral presentations. Over 60 leading environmental and energy researchers, engineers and scientists from numerous countries, including Canada, Croatia, Peru, Portugal, Australia, Philippines, China, etc. Topics included Water and Wastewater Treatment, Environmental Pollution Analysis and Environmental Remediation, Green Technology, Carbon Emission Reduction and Sustainable Development, Water Quality Analysis, Hydrology and Water Resources Planning, Environmental Pollution Control and Chemical Engineering, Energy, Water and Environment. All presentations were oral with ample time given for Questions and Answers at the end of the presentation. One presentation per session was selected as “best presentation” for its generally outstanding quality.

All full papers presented at the 2022 13th International Conference on Environmental Science and Technology (ICEST 2022) have been selected for this volume by solely considering their quality, novelty and the relevance to the conference.

We believe that the conference was of a high and fruitful level, while meeting international standards. The conference series will continue in the future and will again provide an effective platform for further exchange of advanced know-how and knowledge, while also fostering potential international research collaborations in the topics of Environmental Sciences and Technology.

We would like to acknowledge all scientists and administrative staff who have supported ICEST 2022. We are grateful to the presenters and the participants for their thought-provoking contributions. Each individual and institutional support was very important for the success of this conference . Especially we would like to thank the organizing committee for their professional organization and coordination of the peer review of the papers. We hope that it met the expectations. We extend our very best wishes to you wherever you may be around the world.

Zeng Yang
Publication Chair
Shandong University
Jinan, China

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Chapter 10

Essential Oils of Plants as Biocides Against Microorganisms Isolated from Portuguese Convent of Christ in Tomar

Dina M. R. Mateus, Fernando M. C. Costa, and Ricardo P. Triães

Abstract Biodeterioration observed in art and architectural works holding patrimonial value is primarily due to the proliferation of various microorganisms, that through their energy-producing metabolisms lead to the formation of biotic substances that can attack the physicochemical structures of artworks' constitutive materials. In conservation-restoration, different biocides of synthetic origin which exhibit some human and environmental toxicity are traditionally used. Natural resources exploitation awareness and side effects of common disinfectants and inhibitors led to the need for natural compounds with biocidal effects. This work aimed to evaluate the possible use of essential oils (EOs) extracted from endogenous plants as novel conservation products against the biocolonization of cultural heritage materials. The biocidal potential of five EOs extracted from the indigenous plants *Thymus mastichina* (Tm), *Helichrysum stoechas* (Hs), *Mentha pulegium* (Mp), *Foeniculum vulgare* (Fv) and *Lavandula viridis* (Lv) were evaluated. Microorganisms collected at an emblematic site of the country's cultural heritage, Convent of Christ in Tomar, were used to evaluate the biocidal activity of the EOs at concentrations of 2, 10 and 20% (v/v), emulsified in water with 0.2% SDS (m/v). For the tested conditions and comparatively to the commercial biocide Biotin T (1% v/v), it can be concluded that: (i) Hs didn't exhibit fungicidal nor bactericidal activity; (ii) Tm exhibited only bactericidal activity; (iii) in contrast, the other EOs exhibited both fungicidal and bactericidal activity. The results allow considering the use of Tm, Mp, Fv and Lv EOs as a valid alternative to commercial biocides, providing a prospective of application in the field of green conservation of Cultural Heritage.

Keywords Biodeterioration · Built heritage · Essential oils · Green biocides · Plant extracts

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D. M. R. Mateus (✉) · F. M. C. Costa · R. P. Triães
Technology, Restoration and Arts Enhancement Center (Techn&Art), Instituto Politécnico de Tomar, Tomar, Portugal
e-mail: dinamateus@ipt.pt

D. M. R. Mateus
Bioenergy and Applied Biotechnology Lab (BIOTEC.IPT), Instituto Politécnico de Tomar, Tomar, Portugal

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10.1 Introduction

The safeguard of cultural assets is contemporary with the purpose of enhancement and use of cultural heritage. In this protective and forestalling action, various conservation and restoration interventions aim to minimize the degradation of the materials of cultural interest. On the other hand, the twenty-first century brought, along with a touristic boom, environmental concerns which led to many studies on the safety of chemicals used in the Conservation and Restoration of cultural heritage elements (Allsopp 2011).

Biological agents that contribute to deterioration, commonly associated symbiotically in biofilms, comprise fungi and bacteria playing as major culprits in the biodeterioration of organic and inorganic materials (Martin-Sanchez et al. 2012; Mateus et al. 2013; Meng et al. 2017). Given their complex metabolic activities, which include the production of organic and inorganic acids (Allsopp 2011; Sterflinger and Piñar 2013), further convoluted by ecological succession when combined with micro-climatic characteristics, most solutions focus on destroying such microorganisms. Common biocides are produced to eliminate and inhibit the growth of microorganisms, but most biocides can cause damage to heritage artefacts. Biocides such as quaternary ammonium compounds, phenol, aldehydes and alcohol are clear examples of disinfectant biocides, with temporary results and long-term damage to preservation (Mateus et al. 2013; Meng et al. 2017; Zhang et al. 2018). Where current biocide solutions have failed, either due to their environmental hazards or toxicity, the future embraces the need for the development of new materials, including plant essential oils (Mota et al. 2015). These may hold the key to effective conservation of cultural heritage, combining the environmental safety with non-destructive action. At present, marketed biocides have a specific action mechanism that affects cell wall synthesis causing DNA and RNA damage and are highly toxic to humans, even to the touch, leaving conservators at high risk (Varnai et al. 2011; Ashraf et al. 2014). Plants, on the other hand, have lower toxicity and environmental suitability, and can be easily handled.

Natural resources exploitation awareness and side effects of disinfectants and inhibitors led to the need for new materials with biocidal effects. Biocides with a wide spectrum of activity and low cost, favouring natural materials with easy handling, environmental stability and lower toxicities, are currently preferred (Kakakhel et al. 2019). Some characteristics of plants are relatively well known in alternative medicine, the conservation market, however, does not hold such an important economic relevance, hence the lack of research. Research on plants, lichens and mushrooms brought to the biodeterioration field knowledge that such groups can be used as the most promising biocides which are environmentally and ecologically friendly. Plant species such as *Schinus mole*, *Mentha piperita*, *Lavandula angustifolia*, *Rosmarinus officinalis*, *Foeniculum vulgare*, and *Origanum vulgare*, among others, have proven effective antifungal and antibacterial characteristics (Mota et al. 2015; Kakakhel et al. 2019; Jeong et al. 2018; Stupar et al. 2014). Undoubtedly, plant extracts represent a research opportunity.

The idea underlying this study is focused on the use of green biocides in the conservation of cultural heritage, protecting biodiversity, strengthening natural resources and optimizing their use, increasing land productivity and avoiding pollution along the whole life cycle, thus contributing to the achievement of sustainable preservation of cultural heritage. The main objective of this study is to encounter new natural materials extracted from plants that can be used as biocides in the preservation of cultural heritage. The biocidal potential of five EOs extracted from Portugal endogenous plants, namely *Thymus mastielina* (Tm), *Helichrysum stoechas* (Hs), *Mentha pulegium* (Mp), *Foeniculum vulgare* (Fv) and *Lavandula viridis* (Lv), were evaluated. Microorganisms collected from the emblematic site of the country's cultural heritage Convent of Christ in Tomar, Portugal (classified UNESCO world heritage), were used to assess the biocidal activity of the EOs. The use of mixed cultures for in vitro biocidal tests was one of the main innovations of the present study. In the authors' knowledge, only studies with pure isolated cultures are reported in the literature.

10.2 Experimental

10.2.1 Site Description

Convent of Christ is the name usually given to the monument ensemble consisting of the Templar Castle of Tomar (Portugal), the Order of Christ Convent of Rebirth, the conventual wall, the Immaculate Conception Hermitage and the conventual aqueduct. The castle had its foundation in 1160 and comprised the walled village, the yard and the military house situated and the knight's round-shaped Oratorium known as Charola, finished in 1190. This group of spaces, built throughout the centuries, makes the Convent of Christ a grandiose monument complex that earned the UNESCO Heritage of Mankind distinction (Cristo 2022). The Main Cloister (MC) of the Convent of Christ is the masterpiece of the Renaissance convent built by King John III, situated outside the castle walls and surrounding the nave that his father—King Manuel I—used to extend the Templar church. It is a part of the set of four large cloisters in which the formal structure of the conventual spatiality is placed. The MC, adjacent to the conventual church, flanks the Southern façade of the Manueline nave. Its outline is diverse from the rest of the Castilian conventual architecture. Redone in a Mannerist Italian Cinquecento style by Diogo de Torralva, after Castilho's passing, this cloister receives its water from the conventual aqueduct—in a work by Fernandes Torres (Cristo 2022). The MC has a quadrangular shape, measuring approximately 38 m × 43 m and consists of four galleries and 3 floors, the last one comprising a terrace (Fig. 10.1). The material used in its construction was predominantly limestone. In the interior of the cloister and in the architectural elements of the galleries, the rigged stone blocks predominate. The walls of the galleries exhibit irregular mortar masonry, the vaults have limestone staves and keys, and roof is made

Fig. 10.1 South elevation of the Main Cloister of Convent of Christ in Tomar

of brick and plastered. The cloister limestones are similar to the ones found in the region, described as dolomitic limestones from the Jurassic. They are slightly granular, with yellowish, whitish and sometimes greyish colours (Machado 1992). As in the sampling sites (south elevation), most of the elevations reveal intense biological colonization, mainly biofilms and lichens but also mosses and higher plants. The existence of fissures, gaps, and old and recent detachments, as well as differential erosion and limestone concretions, can also be observed.

10.2.2 Microorganism Collection and Characterization

The microbial population to be used in the biocidal evaluation of the EOs was collected by swabbing from pigmented biofilms formed on the limestone walls and columns of the MC (Fig. 10.2). Four samples were collected from different locations selected by visual inspection of the pigmented areas. The swabs were immersed in 9 ml of sterile Ringer's solution and kept sealed at 4 °C for later usage.

Two media were used for isolation and subculture of heterotrophic microorganisms: Tryptic Soy Agar (TSA) (HiMedia) as a specific medium for bacteria; Potato Dextrose Agar (PDA) (HiMedia) supplemented with chlortetracycline for the growth of fungi. Autotrophic organisms were selected with BG-11 media agar.

Heterotrophic microorganisms were grown in an incubator without light and autotrophic microorganisms in an incubator with a photoperiod of natural light, both at a temperature of 22 °C.

Gram stain colouration and tests for the presence of catalase and oxidase were performed for the bacterial isolates. Classification of the fungal isolates was based on their macro and microscopic observation, under a light microscope (Olympus CH30) with particular attention to reproductive structures.

Fig. 10.2 Sample collection from various pigmented biofilms formed on the limestone walls of the Main Cloister

10.2.3 Essential Oils and Preparation

Five essential oils from the Portuguese endogenous plants *Thymus mastichina*, *Helichrysum stoechas*, *Mentha pulegium*, *Foeniculum vulgare* and *Lavandula viridis* were selected for their easy access and reference in the literature to biocidal activity (Fidanza and Caneva 2019; Palla et al. 2020; Marco et al. 2020), or popular belief of antiseptic properties. The EOs were kindly provided by the company Dalenguadiana, located in the Guadiana Valley Natural Park—Corte Sines (Mértola, Portugal). The EOs were produced in organically by hand-harvesting the plants and then by hydro-distilling them in stainless-steel stills. The EOs composition was previously characterized through gas chromatography-mass spectrometry (Baptista et al. 2022). The EOs were tested in three concentrations, 2, 10 and 20% (v/v). Sodium dodecyl sulfate (SDS) (Duchefa Biochemie) was used to emulsify the EOs in sterile deionised water at a fixed final concentration of 0.2% (m/v).

10.2.4 Assessment of Antifungal and Antibacterial Activity of Eos

Evaluations of antibacterial and antifungal activity of the EOs were performed by the well-known agar disk-diffusion method. The tube swabs, kept at 4 °C, were stirred in a vortex for approximately two periods of 30 s. A volume of 200 µl of the microorganism's suspension was then smeared onto agar Petri dishes via the spread plate method. Then, for each tested essential oil, one to three 6 mm filter paper disks (Macherey–Nagel) containing the tested EO were placed on the agar surface. The Petri dishes were incubated at 22 °C for four days, after which the diameters of growth inhibition zones were measured. PDA agar medium, supplemented with chlortetracycline to inhibit the growth of bacteria, was used for the assessment of antifungal activity. TSA agar medium was used to assess bacteria sensibility to the EOs.

The commercial biocide Biotin T (CTS SRL) was selected as the positive control. Biotin T (BT) is a concentrated liquid preparation of the active substances n-octylisothiazolinone and a quaternary ammonium salt. BT is widely used in the preservation of surfaces like stone materials, mortars, frescoes, bricks, etc., from microbiological attacks (Mateus et al. 2013; C.T.S. S.r.l. 2022). BT was used at the concentration recommended by the supplier of 1% (v/v), the dilution was made with sterile deionized water. A solution of DNS (0.2% m/v) in deionized water was used as the negative control. All procedures were conducted under sterile conditions and in triplicate.

10.3 Results and Discussion

10.3.1 Isolation and Characterization of Microorganisms

A total of four fungal species and one bacterial specie were originally isolated from the specimens collected from the limestone supporting walls and pillars of the Main Cloister's of the Convent of Christ. Fungi of the genus *Penicillium* were the main cultivable microorganisms detected, which is in agreement with previous studies (Rosado et al. 2016). In the second round of isolation, the following microorganisms were identified (Fig. 10.3): (i) four fungi, including a specie of the genera *Penicillium*, two species of the genera *Cladosporium* and the black yeast *Aureobasidium pullulans*; (ii) one staphylococcus bacterium (yellow colonies, Gram-negative and catalase and oxidase positive). No autotrophic microorganisms cultivable in the laboratory were detected, despite one of the sampled biofilms being visibly contaminated with green colonization (Fig. 10.2).

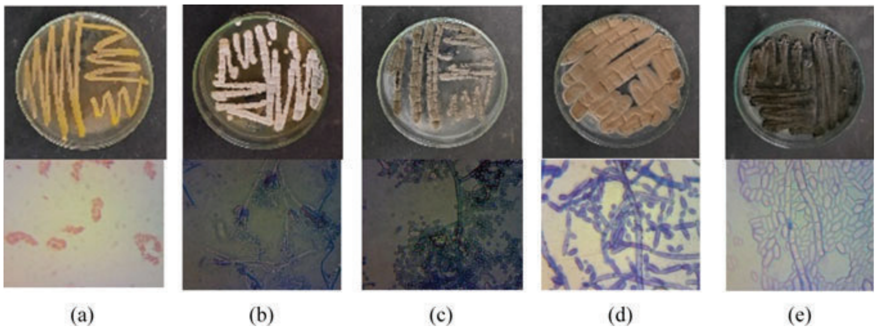


Fig. 10.3 Isolated colonies and their microscopic observation (1000× for bacteria and 400× for fungi): **a** Staphylococcus bacterium; **b** *Penicillium sp.*; **c**, **d** *Cladosporium sp.*; **e** *Aureobasidium pullulans*

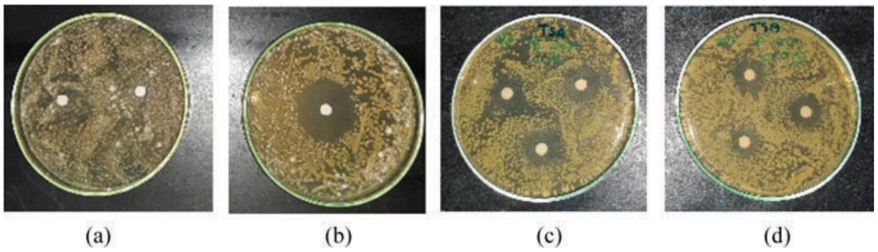
Microbiology identification techniques based on morphological appearance and biochemical characteristics are very useful and have a strong impact due to the ability to cover biotic and abiotic aspects, representing and added-value to characterization of the unseen effects and problems of biodeterioration induced by microbial colonization. Due to the importance of accurate identification of the species, the identification of microflora by molecular biology techniques is foreseen for future works.

10.3.2 Assessment of Antifungal and Antibacterial Activity of Eos

With the goal of setting up green conservation strategies, the antimicrobial activities of *Thymus mastichina*, *Helichrysum stoechas*, *Mentha pulegium*, *Foeniculum vulgare* and *Lavandula viridis* essential oils were assayed in vitro against the mixed population of microorganisms present in the samples collected from the MC. The biodeteriorative action in historic monuments is exerted by several microorganisms, which live symbiotically in biofilms developed on the surface of the monuments' support materials. To evaluate the biocidal activity of the EOs close to real field conditions, it was decided to carry out the biocidal assays with the mixed populations of the microorganisms collected, and in the same proportion of the sampling. Thus, the suspensions of microorganisms collected in the sampling sites were directly used in the biocidal experiments. Assays were carried out in TSA medium for heterotrophic bacteria and in PDA medium for fungi, and this was the only enrichment performed. The use of mixed cultures for in vitro biocidal tests was one of the main innovations of the present study. In the authors' knowledge, only studies with pure isolated cultures are reported in the literature. Biocidal assays with BG11 medium were not performed because autotroph microorganisms cultivable in laboratory were not isolated from the samples collected.

Figures 10.4 and 10.5 show examples of the growth-inhibition-halo diameters.

The diameter of the inhibition halos was used as a measure of the antimicrobial action (sensible >0.6 cm, resistant <0.6 cm). To standardize conditions, and better



(a) (b) (c) (d)

Fig. 10.4 Agar disc diffusion method inhibition halo of growth for antibacterial activity (inoculation on TSA medium): **a** Negative control (DNS aqueous solution, 0.2% m/v); **b** Positive control (BT 1% v/v); **c** Mp EO (20%); **d** Fv EO (20%)

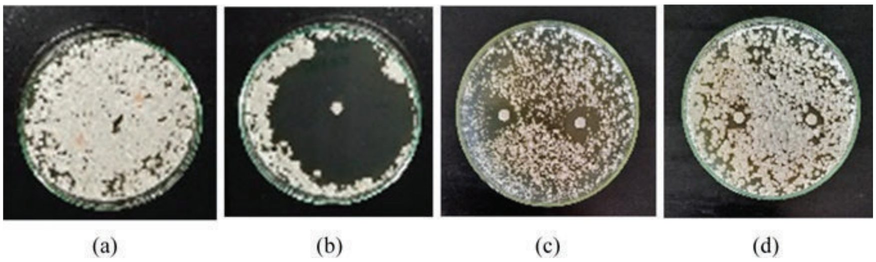


Fig. 10.5 Agar disc diffusion method inhibition halo of growth for antifungal activity (inoculation on PDA medium): **a** Negative control (DNS aqueous solution, 0.2% m/v); **b** Positive control (BT 1% v/v); **c** Mp EO (10%); **d** Fv EO (10%)

compare results from different experiments, the relative diameter of the inhibition halos was calculated. The relative diameter was calculated by dividing the diameter of the inhibition halo by the diameter of the halo of the positive control (BT) of the respective set of experiments. The results obtained for the five EOs are presented in Table 10.1.

The results of the antibacterial assays showed that among the five EOs tested, the only one that did not demonstrate bactericidal activity in any of the concentrations tested (2, 10 and 20%) was the EO obtained from Hs. Lv EO has proven to be the most effective in concentrations of 10 and 20%, with an inhibitory effect corresponding to 36 and 63% of the commercial biocide BT. The aqueous solution of BT was more

Table 10.1 Relative average diameter (±standard deviation) of the EOs inhibition halos, using Biotin T inhibition halos as reference (4.2 ± 1.0 cm for bacterial and 6.7 ± 0.4 cm for fungal assays). Resistant (R) corresponds to absolute inhibition halos <0.6 cm

Biocide	Concentration (v/v %)	Relative inhibition halo (Antibacterial action)	Relative inhibition halo (Antifungal action)
Thymus mastichina (Tm)	2	R	R
	10	0.32 ± 0.04	R
	20	0.44 ± 0.03	R
Helichrysum stoechas (Hs)	2	R	R
	10	R	R
	20	R	R
Mentha pulegium (Mp)	2	R	R
	10	0.31 ± 0.01	0.24 ± 0.01
	20	0.58 ± 0.04	0.37 ± 0.03
Foeniculum vulgare (Fv)	2	0.22 ± 0.05	R
	10	0.27 ± 0.03	0.16 ± 0.01
	20	0.52 ± 0.03	0.30 ± 0.02
Lavandula viridis (Lv)	2	R	R
	10	0.36 ± 0.03	0.18 ± 0.07
	20	0.63 ± 0.12	0.25 ± 0.07

effective against the bacteria colonizing the limestone of the CCT than the EOs. This result is not unexpected due to the high toxicity of the active components of BT (n-octyl-isothiazolinone and a quaternary ammonium salt). Natural biocides are intended to be a compromise solution that allows the control of the biodeterioration of cultural heritage while being more ecological and environmentally friendly.

It could also be observed that for the EOs Tm, Mp, Fv and Lv, and within the range of concentrations tested, the inhibitory effect increases with increasing EO concentration. The EO obtained from Fv was the only one that revealed biocidal activity at the lower concentration (2%).

These results confirm results from previous studies, where *Thymus*, *Mentha*, *Foeniculum* and *Lavandula* EOs were active against bacterial pathogens and human normal microflora species (Roldán et al. 2010). Despite the importance of bacterial species in the biodeterioration of cultural heritage, very few studies address the use of EOs against bacterial species isolated from supporting materials of artworks and architectural heritage (Fidanza and Caneva 2019).

In line with what was observed for bacteria, the aqueous solution of the commercial biocide BT was also more effective against fungi colonizing the CCT limestone than the tested EOs.

Table 10.1 also contains the antifungal activity of the five EOs tested. Tm and Hs EOs did not reveal biocidal activity at any of the concentrations tested. On the other hand, the EOs obtained from Mp, Fv and Lv, despite not showing biocidal activity at a concentration of 2%, showed activity at the concentrations of 10 and 20%.

Mp EO proved to be the most effective, showing an inhibitory effect of 24% and 37% at concentrations of 10 and 20%, respectively. In the literature, a low efficacy for *Menta piperita* against fungus of genera *Alternia*, *Aspergillus, Cladosporium*, *Penicillium* and *Rhizopus* is reported (Levinskaitė and Paškevičius 2013). On the other hand, moderate antifungal activity was reported by Sakr et al.(Sakr et al. 2012) for *Mentha spicata* against fungi of genera *Candida*, *Saccharomyces* and *Lodderomyces*.

Fv EO had an inhibition halo of 16 and 30% at the concentration of 10 and 20%, respectively. Different results were found in the literature for Fv EO. The effect reported in the literature varies from no inhibition or low inhibition against fungi of genera *Candida*, *Lodderomyces* and *Saccharomyces* (Sakr et al. 2012), but high efficacy for the genera *Penicillium*, *Alternaria* and *Epicoccum* (Mironescu and Georgescu 2010).

Lv EO had a moderate fungicide activity of 18% and 25% when compared to BT. The literature reports values from low to high fungicidal efficacy for *Lavandula sp.* (Stupar et al. 2014; Mansour 2013).

The effectiveness of EOs reported in literature is markedly variable, and this can be ascribed to the variability in the methodologies of testing.

In our work, Tm EO did not show fungicide activity against the fungi present in the samples collected (*Penicillium sp.*, *Cladosporium sp.* and *Aureobasidiun pullulans*). Contradictory results were found in the literature for EOs of *Thymus sp.* High biocidal activity of essential oils extracted from the species *Thymus vulgaris* and *Thymus serpyllum* against species of the genera *Penicillium*, *Alternaria* and *Aureobasidium* were reported by Mironescu et al. (2010). However, low efficacy is reported by

Levinskaitè and Paškevičius (2013) for *Thymus pulegioides* against fungus of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium* and *Rhizopus*.

10.4 Conclusions

For the cultivable microorganisms present in the sample collected from the limestone supporting walls and pillars of the Main Cloister's of the Convent of Christ, under the tested conditions and comparatively to the commercial biocide Biotin T (0,1% v/v), it can be concluded that:

(i) *Helichrysum stoechas* essential oil did not exhibit fungicidal nor bactericidal activity.
(ii) *Thymus mastichina* EO did not exhibit fungicidal activity.
(iii) The EOs from *Mentha pulegium*, *Foeniculum vulgare* and *Lavandula viridis* all exhibited fungicidal and bactericidal activity; for aqueous emulsions of these essential oils, it was found that the biocidal efficacy increases with increasing concentration of essential oil.
(iv) For the concentration of 20% (v/v) the EOs of *Thymus mastichina*, *Mentha pulegium*, *Foeniculum vulgare* and *Lavandula viridis* showed high efficacy, respectively 44, 58, 52 and 63%, against the cultivable bacterium present in the collected samples.
(v) For the concentration of 20% (v/v) it can be reported that the EOs obtained from *Mentha pulegium*, *Foeniculum vulgare* and *Lavandula viridis* showed average efficacy, respectively 37, 30 and 25% against the mixed culture of fungi belonging to genera *Penicillium*, *Cladosporium* and *Aureobasidium*.

From this research, it can also be concluded that the biocidal activity depends on the concentration and species of the microorganisms assayed, as well as the method used, which makes the comparison between different studies in the literature difficult. For instance, research reporting higher biocidal activity of EOs is usually associated with use of higher concentrations of essential oils, and sometimes the efficacy is compared with commercial biocides at concentrations lower than the recommended by the producers (Palla et al. 2020).

The results obtained so far must, however, be considered as the first promising step in a novel research field, which discloses new opportunities and stimulates further investigations. In this perspective, more in vitro assays need to be performed to evaluate the inhibition activity of the identified EOs against other cultural heritage biodeteriogens, as well as ecotoxicological tests to evaluate human and environmental toxicity of EOs. Afterwards, experimental studies must be carried out to evaluate the possible interaction between the biocide compounds and cultural heritage materials. Among them, the long-lasting biocidal effect shown by the EOs must be underlined. Although more studies are needed to evaluate their permanence and durability in the thermo-hygrometric conditions of storage and usage, we consider that the natural biocides *Thymus mastichina, Mentha pulegium*, *Foeniculum vulgare* and *Lavandula* *viridis* could be used as a valid alternative to commercial biocides. They provide a perspective of the possible future in the field of green conservation of Cultural Heritage.

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References

Allsopp D (2011) Microbiol Tod 38:150–153

Ashraf M, Ullah S, Ahmad I, Qureshi A, Balkhair K, Rehman M (2014) J Sci Food Agr 94:388–403

Baptista C, Santos S, Amaral M, Silva L (2022) Chemical characterization of essential oils with a biocide base for conservation and restoration. 1st International Proceedings FibEn techn international congress, pp 80-–90. KnE Publishing, Covilhã

C.T.S. S.r.l. (2022). https://www.ctseurope.com/gb/415-biotin-t

Convento de Cristo, Património Cultural, DGPC 2022. http://www.conventocristo.gov.pt/pt/

Fidanza M, Caneva G (2019) J Cult Herit 38:271–286

Jeong S, Lee H, Kim D, Chung Y (2018) Int Biodeter Biodegr 131:19–28

Kakakhel M, Wu F, Gu J, Feng H, Shah K, Wang W (2019) Int Biodeterior Biodegrad 143:104721

Levinskaitė L, Paškevičius A (2013) S. Indoor Built Environ 22:766–775

Machado A (1992) Sedimentary rocks as building materials in the region of Tomar. MSc Thesis Polytechnic Institute of Tomar (in Portuguese)

Mansour M (2013) J Appl Sci Res 3:1917–1930

Marco A, Santos S, Caetano J, Pintado M, Vieira E, Moreira P (2020) Build Environ 167:106459

Martin-Sanchez P, Nováková A, Bastian F, Alabouvette C, Saiz-Jimenez C (2012) Environ Sci 46:3762–3770

Mateus D, Silva R, Costa F, Coroado J (2013) Conserv Patrim 17:11–20

Meng H, Katayama Y, Gu J (2017) Int Biodeter Biodegr 117:78–88

Mironescu M, Georgescu C (2010) Acta Universitatis Cibiniensis. Series e Food Technol 14:41–46

Mota A, Martin M, Arantes S, Lopes V, Bettencourt E, Pombal S, Gomes A, Silva L (2015) Nat Prod Commun 10:673–676

Palla F, Bruno M, Mercurio F, Tantillo A, Rotolo V (2020) Molecules 25:730

Roldán L, Diaz G, Duringer J (2010) Rev Colomb Cienc Pecu 23:451–461

Rosado T, Silva M, Galvão A, Mirão J, Candeias A, Caldeira A (2016) Appl Phys A 122:1012

Sakr A, Ghaly M, Abdel-Haliem M (2012) The efficacy of specific essential oils on yeasts isolated from the royal tomb paintings at tanis, Egypt. Int J Conserv Sci 3:87–92

Sterflinger K, Piñar G (2013) Appl Microbiol Biotechnol 97:9637–9646

Stupar M, Grbić M, Džamić A, Unković N, Ristić M, Jelikić A, Vukojević J (2014) Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. S Afr J Bot 93:118–124

Varnai V, Macan J, Ljubičić A, Prester L, Kanceljak B (2011) Occup Med 61:45–52

Zhang X, Ge Q, Zhu Z, Deng Y, Gu J (2018) Microbiological community of the Royal Palace in Angkor Thom and Beng Mealea of Cambodia by illumina sequencing based on 16S rRNA gene. Int Biodeter Biodegr 134:127–135