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Thymus Species: A Powerful Tool for Conservation of Historical Art Craft

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Abstract: The application of conventional biocides to manage biodeterioration in cultural heritage is being progressively discouraged because of the hazards they present to human health, the environment, and the possible modification of historical materials. For this reason, research is focused on identifying alternative solutions that are more sustainable, innovative, and eco-friendly. This study aims to analyze the results of recent scientific investigations, identifying the most promising natural substances, highlighting knowledge gaps, and outlining future research directions. We carried out a systematic review of the literature, analysing peer-reviewed articles published between 2010 and 2025 that explore the application of natural biocides to counteract the biodeterioration of materials like stone, wood, textiles, and paper. Among the emerging solutions, essential oils have shown particular promise due to their antimicrobial effectiveness and lower environmental impact. Among the aromatic plants studied, belonging to the Lamiaceae family, *Thymus pulegioides*, *Thymus serpyllum*, *Thymus vulgaris*, *Thymus mastichina*, *Thymus zygis*, and *Thymus capitatus* (syn. *Thymbra capitata*) stand out, with their essential oils demonstrating significant antimicrobial properties. Experimental tests have employed various methodologies, predominantly in vitro studies, with fungi being the most frequently targeted organisms, followed by algae and cyanobacteria. However, the efficacy of these substances has shown considerable variability, mainly due to discrepancies in experimental protocols and dosages, making it challenging to establish clear guidelines. Furthermore, some studies have reported potential undesirable interactions between essential oils and the original materials, which require further investigation. Despite these limitations, the use of natural biocides offers an innovative and promising approach to preserving cultural heritage. Nevertheless, it is crucial for future research to adopt more standardized protocols to systematically evaluate the effectiveness and safety of these substances, ensuring a balance between conservation and sustainability.

Keywords: Biodeterioration; essential oils; *Thymus* species; cultural heritage; biocides.

1. Introduction

Since prehistoric times, humans have left behind a cultural heritage that, due to biological degradation, requires conservation and, in many cases, restoration efforts. In the 21st century, the importance of preserving this heritage has been recognized by scholars, leading to investments in research and education that resulted in the development of many chemical and mechanical solutions to

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counteract biodeterioration. However, the 21st century has also seen an increased focus on environmental issues, which have stimulated numerous analyses on the reliability and sustainability of the substances employed in the preservation and restoration of cultural heritage [1]. The protection of cultural heritage, alongside the enhancement and promotion of the use of these assets, is a key mechanism for increasing the value of the rich historical heritage of our country.

In this context, numerous restoration and conservation operations have been implemented to limit the deterioration of materials. Biodeterioration, which refers to the undesired modifications of various substances primarily induced by microorganisms, is a phenomenon detected in artworks as well as in architectural structures of cultural significance. This process is mainly linked to the spread of microorganisms (fungi, bacteria, lichens, algae) that, individually or in combination, through their metabolic activities, produce acidic substances capable of damaging the chemical-physical structures of materials [2]. Structural degradation includes mechanical damage, such as breaking or reducing the cohesion of substrates, chemical alterations caused by metabolite activity, and aesthetic damage, such as the formation of coatings or encrustations. These phenomena are strongly influenced by the climatic and environmental factors to which the artworks are subjected [3].

In restoration and conservation, synthetic substances have traditionally been used; however, they can pose risks to both humans and the environment, as well as lead to unwanted effects on treated materials. Conventional biocides are classified into four primary categories: pesticides, disinfectants, preservatives, and other antimicrobial products [4]. For example, substances like quaternary ammonium salts, phenols, aldehydes, and alcohols are employed as disinfectants but often provide short-term effects while causing lasting harm to preserved objects [5]. Indeed, a study by Cappitelli *et al*. [6] reported that treatments with benzalkonium chloride (a quaternary ammonium compound) led to irreversible discoloration and the formation of salt efflorescence on frescoes within two years of application. Furthermore, the European Chemicals Agency classifies many of these compounds as hazardous to aquatic life, with LC_{50} values below 1 mg/L, indicating high acute toxicity. Growing environmental concerns and the adverse effects of disinfectants have encouraged the search for alternative biocidal materials. At present, cost-effective biocides with a wide range of action are favored, emphasizing the use of plant-derived natural compounds, including essential oils (EOs), which are typically simple to manage, environmentally stable, and less toxic [7]. Unlike synthetic biocides, which may accumulate and release toxic byproducts over time, essential oils exhibit lower environmental persistence and reduced bioaccumulation potential. Moreover, their volatile nature facilitates degradation, minimizing long-term residues on heritage materials**.**

This study focuses on six aromatic species fitting to the Lamiaceae family and the *Thymus* genus: *Thymus pulegioides* L., *Thymus serpyllum* L., *Thymus zygis* Loefl. ex L., *Thymus vulgaris* L., *Thymus mastichina* L., and *Thymus capitatus* (L.) Hoffmanns. & Link (syn. *Thymbra capitata* (L.) Cav.) [8].

Essential oils possess a large variety of secondary metabolites that influence various biological systems. Their intricate composition may consist of numerous substances from distinct lipid families, such as alcohols, hydrocarbons, aldehydes, esters, phenols, ketones, terpenes and phenolic ethers. Analysis of the essential oils reveals that terpenes are the most abundant components, subdivided into diterpenes, monoterpenes and sesquiterpenes, among others. Generally, the predominant compound determines the primary biological effect of the essential oil, though this effect often arises from the combined action of multiple components [9].

In particular, thymol and carvacrol—two major phenolic compounds in thyme essential oil—have demonstrated minimum inhibitory concentrations (MICs) as low as 50–100 µg/mL against biodeteriogenic fungi such as *Aspergillus niger* and *Penicillium chrysogenum* [10]. Their antifungal action is coupled with antioxidant properties, reducing the oxidative stress that contributes to material degradation.

The essential oils of thyme species are particularly suitable for cultural heritage conservation because of their broad-spectrum antimicrobial activity, low cytotoxicity on human cells, and minimal impact on substrate integrity. Unlike synthetic agents that can alter porosity, color, or cohesion of materials, studies have shown that thyme essential oil treatments do not significantly modify the surface morphology or chromatic parameters of stone and fresco samples [11].

These substances can serve as eco-friendly pesticides, targeting microorganisms via different mechanisms, such as inhibiting growth, disrupting the cytoplasmic membrane, modulating metabolism by affecting enzymatic processes, or influencing enzyme production and function. Certain chemical constituents extracted from essential oils have been analyzed for their potential to hinder biocolonization and enhance heritage preservation, including thymol.

The research project consists of several phases, including plant collection, steam distillation, and the chemical analysis of essential oils. Tests have been conducted to assess biocidal potential, both in vitro in the laboratory and in situ on monuments. For other materials, bibliographic research has been conducted.

This study primarily aims to select *Thymus* plant species, identifying those whose essential oils demonstrate the highest biocidal potential, to develop an eco-friendly biocide suitable for the preservation of stone, wood, textiles, and paper materials.

2. Objective of the Research

In the quest for innovative approaches to combat biodeterioration challenges, scientific research has progressively centred on the application of natural biocides, sourced from microorganisms and various other organisms, which are regarded as safer compared to conventional ones [12]. Extensive and long-anticipated studies are underway to identify sustainable and eco-friendly compounds and techniques capable of mitigating biological deterioration. Despite the increasing interest in this field, a critical assessment of the tests, materials, and methodologies employed within the scope of cultural heritage preservation remains insufficient [11]. In this regard, a report providing an overview of current knowledge and unresolved questions would be a valuable contribution. Therefore, this study aims to examine the existing literature on this topic, highlight the results achieved so far, and assess the potential applications of these substances in the conservation of materials such as textiles and paper.

3. Methodology

The review was conducted using approaches and techniques commonly employed in research related to cultural heritage, with the objective of collecting and analysing studies on the use of natural molecules and compounds with biocidal properties applicable to the preservation of materials such as stone, wood, textiles, and paper.

Articles were identified through a structured search across multiple academic databases, including Web of Science, Scopus, ResearchGate, Google Scholar and PubMed, covering publications from 2010 to 2025. The search strategy incorporated specific keywords, such as: “Thymus essential oil”, “cultural heritage”, “biodegradation”,”biodeterioration” and “green conservation”.

To ensure scientific rigor, inclusion criteria were established: only peer-reviewed articles written in English, reporting experimental data on the biocidal efficacy of essential oils (either in vitro or in situ) applied to cultural heritage materials, were considered. Exclusion criteria included conference abstracts, non-peer-reviewed reports, and studies focused solely on agricultural or food applications.

The selection process was conducted in multiple phases: after initial screening by title and abstract, full texts were reviewed for eligibility. A total of fifty articles met the inclusion criteria and were analysed. The review process followed PRISMA guidelines, and a PRISMA style flow diagram is provided to illustrate the number of records identified, screened, excluded, and included at each stage.

Several research works on the application of essential oils derived from various *Thymus* species were reviewed. Some studies began with plant collection, followed by hydrodistillation or steam distillation and subsequent chemical profiling through gas chromatography (GC). Others utilized commercially available essential oils, applying them either directly or encapsulated in nanocapsules.

Data analysis highlighted considerable variability in the yield and chemical profile of the extracted essential oils, identifying the predominant compounds in the examined species.

This study offers valuable perspectives on the bioactive characteristics of essential oils, currently under investigation for their potential application in preventing biocolonization on culturally and historically significant materials.

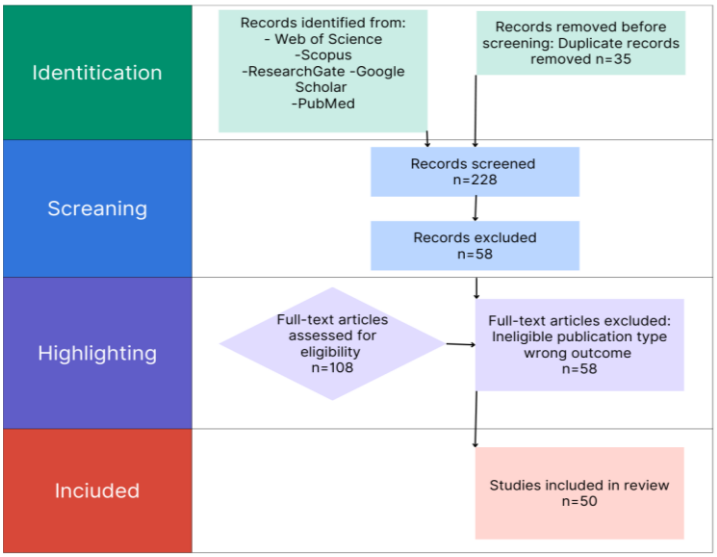

Figure 1. PRISMA flow diagram

4. Cultural Heritage Conservation: Innovative Approaches to Biodeterioration

Biodeterioration refers to a mix of physical and chemical deterioration, accompanied by visual changes, that endangers cultural assets. In this context, the expression “biodegradation” is avoided, since some experts claim it could imply a favorable or advantageous process, such as the conversion of waste materials into more sustainable or functional forms [13].

Thanks to technological advancements, numerous techniques have been developed - often applied in combination - to counteract the growth of harmful microorganisms. These advancements have expanded the range of interventions available to microbiologists and conservators, providing more effective tools for preserving historical materials [14].

In this section, an overview of the main methodologies employed to manage biodeterioration is presented, with an investigation of their strengths and weaknesses. Particular attention is given to the most exposed historical materials, such as outdoor stone surfaces, wooden artifacts, textiles, and papers. This study is necessary because, given the variety of available approaches, it is not always immediately clear to experts what the advantages and disadvantages of each solution might be.

4.1. Application of Thyme Essential Oil on Stone Materials for Conservation and Restoration

Microorganisms responsible for stone deterioration find an ideal environment on already degraded stone surfaces, further promoting microbiological growth. In response to this issue, natural solutions are emerging that can act directly at the cellular level, as an alternative to traditional chemical products, such as those based on quaternary ammonium salts. It is essential to develop new ecological biocides that can replace synthetic ones. Natural products are increasingly gaining attention in restoration, thanks to their safety for the heritage, and the environment for those who use them [6]. Thymus essential oil is the most promising solution among the essential oils. Studies conducted on various cases - including fountains, historic buildings, tombs, churches, and temples in different locations - have demonstrated effectiveness in this area of different species.

Thymus ssp., belonging to the Lamiaceae family, comprises several plants whose properties are widely used in various fields, including the conservation and restoration of stone materials. Among these species, the six main ones - *Thymus pulegioides*, *Thymus serpyllum*, *Thymus vulgaris*, *Thymus mastichina*, *Thymus zygis*, and *Thymbra capitata* - are used in the form of essential oil, known for their beneficial properties, particularly effective in protecting and treating stone surfaces

Thymus species for conservation of historical art craft

Thymbra capitata (syn. *Thymus capitatus*) is an aromatic and medicinal plant, indigenous to the Mediterranean area, known for its significant healing attributes linked to its essential oil. Among these attributes, its antimicrobial activity renders the essential oil of *T. capitata* a promising option for industrial uses, such as a biocidal agent to inhibit and eradicate biological films formed by cyanobacteria and green algae on ancient monuments. In a study by Gagliano Candela *et al.* [15], the composition of *T. capitata* essential oil was examined. The primary compound identified was the phenolic monoterpene carvacrol (73.2%), which exhibited the capability to disrupt the cytoplasmic membrane and hinder microbial development and invasive potential. Nevertheless, the role of minor constituents, such as terpinene and *p*-cymene, should not be overlooked. Pickering emulsion techniques were used to apply this oil onto stone surfaces. The emulsions, containing a 1:3 oil/water ratio and stabilized with 4% kaolinite and Laponite®, minimized oil volatilization, enhancing both application and removal from cultural heritage structures affected by biodeterioration. This formulation enabled the eradication of biodeteriorating agents from treated areas without leaving residues or coatings on the artistic surfaces, with its effectiveness persisting for up to four months. The research carried out by Genova *et al.* [16] examined the cleansing effectiveness of formulations integrated into a hydrogel structure, consisting of a blend of surfactants and polymer-based compounds. These formulations incorporated essential oils (EO) from *Thymus vulgaris*, *Calamintha nepeta* (L.) Savi and *Origanum vulgare* L., as well as their respective main active compounds (EO-AC): thymol, carvacrol, and pulegone. Their impact was assessed on a phototrophic biofilm developing on granite. For comparison, formulations including a blend of the three EOs, the mixture of the three EO-ACs, and Preventol RI-80® (one of the most potent commercial cleaning agents based on quaternary ammonium salts) were also analysed, all integrated into a hydrogel matrix. Additionally, tests were performed using only the hydrogel matrix, distilled water, and Preventol RI-80®, applied with a brush. The effectiveness of the cleaning treatments was assessed immediately following application and later, utilizing colorimetric spectrophotometry to evaluate the occurrence and survival of phototrophic organisms as well as the cleaning efficiency on granite. Among the treatments, *C. nepeta* and its main active compound, pulegone, demonstrated the highest effectiveness, yielding results comparable to uncolonized granite and surpassing those obtained with Preventol RI-80® applied via brush, especially in the final phase of the experiment. This represents the first research where phytochemical substances have been integrated into a hydrogel framework and used to treat phototrophic biofilms in an open-air setting.

Hofbauerová *et al.* [17] assessed the protective as well as antimicrobial properties of essential oils derived from *Origanum vulgare* and *Thymus capitatus*, encapsulated in polymeric nanocapsules (Or-NC and Th-NC), targeting four environmental microorganisms: *Pleurotus eryngii* and *Purpureocillium lilacinum* (fungal strains), *Pseudomonas vancouverensis* and *Flavobacterium* sp. (bacterial strains). Sandstone samples were exposed to these microorganisms either before or following treatment with Or-NC and Th-NC, revealing a dose-dependent response.

The findings indicated that Or-NC and Th-NC displayed strong antimicrobial and anti-biofilm effects, outperforming non-encapsulated essential oils. These eco-friendly and biocompatible nanosystems emerged as potential antimicrobial solutions for preserving sandstone surfaces contaminated by fungal species and bacteria, effectively preventing biofilm development without posing risks to human health.

The study identified these green nanocapsules as a potential solution for protecting and disinfecting sandstone from microbial bio colonization.

Casiglia *et al.* [12] emphasizes the remarkable antimicrobial properties of wild thyme essential oil, indicating its potential as a natural solution for safeguarding historical artworks from microbial deterioration. The essential oil, obtained from *Thymus capitatus* naturally growing in northern Sicily, was extracted through hydrodistillation of aerial parts harvested at various growth phases. Its chemical profile was examined through gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), leading to the identification of fifty-five compounds. The extracted oil was notably rich in carvacrol (81.2–14.2%), γ-terpinene (34.4–2.6%), and *p*-cymene (22.8–5.0%). The yield and composition of the oil were found to fluctuate based on the plant's vegetative development. The ideal harvesting period, determined by phenol concentration, was identified as either during or immediately preceding full bloom. The analyzed oils (Tc2 and Tc3) exhibited notable antibacterial activity against

Bacillus subtilis and *Staphylococcus epidermidis* and excellent antifungal efficacy against *Fusarium oxysporum* and *Aspergillus niger*.

Fidanza et al. [18] reviewed the declining use of traditional biocides for stone monuments due to health, environmental, and material compatibility concerns. They highlight ongoing research into eco-friendly alternatives to mitigate biodeterioration.

Analyzing studies from 1986 to 2018, the authors examined 61 natural substances, primarily essential oils and plant-based compounds, with *in vitro* tests being the most common. Fungi were the most studied organisms, followed by cyanobacteria and algae.

The results showed varying efficacy, inconsistent protocols, and limited discussion of substrate interactions, emphasizing the need for standardized methodologies. The authors consider this research area promising but requiring further study.

Geweely et al. [19] assessed the antifungal properties of 12 essential oils (including thyme, clove, and geranium) against 16 fungal species extracted from archaeological artifacts from the Saqqara shops in Egypt, with a particular focus on mural painting stone. Various essential oils were analysed, and their minimum inhibitory concentration (MIC) was determined. The most potent oils were thyme (MIC 0.25-0.75 µl/ml), followed by clove (MIC 0.25-1 µl/ml) and geranium (MIC 0.5-1 µl/ml). The primary active constituents were p-cymene and thymol in thyme, while triacetin and eugenol were identified in clove oil.

Aspergillus niger proved to be the most resistant fungus, whereas *Fusarium oxysporum* and *Penicillium citrinium* displayed the highest sensitivity. The fungi responsible for the deterioration of archaeological marble include *Aspergillus oryzae, A. flavus, A. niger, Penicillium oxalicum, Rhizopus oryzae* and *Fusarium oxysporum*. Among all of these, *Rhizopus oryzae* (8.6%) was the most frequently detected species on the mural painting stone.

The study conducted by Hegab *et al.* [20] aimed to identify specific medicinal plants as potential candidates for antifungal applications. These botanical specimens were sourced from Egyptian herbal traditions and classified accordingly. The crude oil was obtained through steam distillation. Various fungal strains were isolated from three distinct historical structures in Old Cairo (the Yusuf Agha Al-Heen Mosque, the Tameem Alrassafy Mosque, and the Azbak Al-Yusufi Madrasa), which exhibited signs of biological degradation on their limestone surfaces. The antifungal potential of crude oil extracts from cloves, pepper, and thyme was examined against these isolated fungi. The possible disinfectant impact of these extracts on limestone samples was also explored.

The efficiency of the treatment using these natural extracts was assessed through controlled laboratory experiments. The findings indicate that this approach could be an effective solution in conserving study historic stone structures.

The research conducted by Levinskaite *et al.* [21] examined fungal proliferation in moisture-damaged structures within the historic center of Vilnius. The study focused on mold on walls and in the air, testing their sensitivity to six chemical biocides and seven essential oils. Contamination was more prevalent on wallpapered surfaces, with *Penicillium chrysogenum* and *Aspergillus versicolor* being the most frequently detected fungal species. Among the chemical biocides, Biosheen and Boramon demonstrated the highest effectiveness, while clove oil exhibited the strongest antifungal potential among the tested essential oils. The findings indicate that essential oils, particularly clove oil, may be an effective alternative for sanitizing surfaces affected by fungi.

In the research by Mironescu *et al.* [22], six essential oils—including common thyme (*Thymus vulgaris*), wild thyme (*Thymus serpyllum*), pine, juniper, fennel, and fir—were extracted and examined through GC-MS analysis. These oils underwent testing to determine their antifungal properties against four mold strains belonging to the genera *Aureobasidium, Penicillium,* and *Alternaria*, which had been isolated from surfaces. The effectiveness of their antifungal action was assessed using the antibiogram method.

The findings revealed that the antifungal effectiveness of the oils varied, depending on their composition and the mold species. Among the oils, creeping thyme (*Thymus serpyllum*), common thyme (*Thymus vulgaris*), and fennel showed the strongest fungicidal effects against all tested molds. These oils' antifungal potency is attributed to their alcohol-based components, such as borneol, cineole, thymol, cymene, carvacrol, and α-terpineol in thyme; thymol and carvacrol in wild thyme; and anethole and estragole in fennel.

Pine oil exhibited lower antifungal activity, with *Penicillium* and *Aureobasidium* species showing resistance to it, while *Alternaria* species were more susceptible. Juniper and fir oils had little to no inhibitory effect on the molds.

The study concluded that essential oils from thyme (both common and wild) and fennel were the most effective in combating fungal growth, with pine oil following. Fir and juniper oils had minimal to no impact on mold spores. The study recommends using thyme oil, or wild thyme oil, as antifungal agents in biocide formulations for treating building surfaces [23]. The study examined the inhibitory impact of three essential oils (*Thymus vulgaris*, geranium, and clove) on various physiological aspects of three destructive fungal species (*Rhizopus oryzae, Aspergillus niger,* and *A. flavus*), extracted from archaeological artifacts unearthed at Egypt, Saqqara, Giza. The oils were evaluated for their influence on factors such as dry weight, enzymatic activities (protease, amylase, and cellulase), polysaccharide levels, nitrogen concentration, and citric acid production.

Thyme essential oil demonstrated the highest effectiveness, exhibiting the strongest inhibition of all physiological functions in the three fungal species, with clove and geranium oils following in potency. Specifically, thyme oil markedly suppressed the proliferation of *Aspergillus niger* and entirely halted the growth of *A. flavus* and *Rhizopus oryzae*.

The study also evaluated the impact of the essential oils on color changes in three artificially aged models representing archaeological objects (e.g., ceramics and limestone). Thyme oil demonstrated the most acceptable color difference and the highest antimicrobial activity, establishing it as a promising ecological treatment for the conservation of archaeological objects. The study highlights the potential of essential oils, particularly thyme oil, as a safe and eco-friendly method for protecting cultural heritage from microbial damage. This approach offers low toxicity to humans and the environment while preserving the aesthetic and physical qualities of archaeological artifacts.

Romano *et al.* [24] explored the use of nanocapsules (NCs) loaded with essential oils (EOs) from *Thymus capitatus* (thyme) and *Origanum vulgare* (oregano) to combat bacterial growth on the marble stone of an 18th-century church altar. The study specifically targeted *Kocuria rhizophila* and *Escherichia coli*, assessing the antimicrobial potential of EO-loaded nanocapsules (EO-NCs).

Treatment with EO-NCs effectively inhibited bacterial growth on pre-inoculated stone surfaces, as confirmed by agar disk contact tests. SEM-EDX analysis showed that the application did not alter the stone's structure, ensuring its suitability for heritage conservation. Among the tested formulations, EO-NCs containing thyme EO and a biodegradable polymer (poly(ε-caprolactone)) exhibited the highest antimicrobial activity, surpassing the effectiveness of NCs loaded with oregano EO or hexane.

This study represents the first application of eco-friendly polymeric nanocapsules loaded with essential oils for inhibiting both gram-positive and gram-negative bacteria on cultural heritage materials. The findings suggest that these nanosystems could serve as a viable alternative to traditional chemical biocides. Additionally, they could be integrated with commercial restoration products (e.g., consolidants) to enhance their protective efficacy, offering a sustainable approach to cultural heritage preservation.

Macedo-Arantes *et al.* [25] investigates the encapsulation of essential oils (EOs) as an alternative strategy for controlled release, improving their stability and reducing interaction with cultural heritage materials. Seven EOs from aromatic plants (*Lavandula stoechas* ssp. *luisieri* (Rozeira) Rozeira, *Calamintha nepeta* (L.) Savi, *Rosmarinus officinalis* L., *Lavandula viridis* L'Hér., *Thymus vulgaris, Thymus mastichina* and *Salvia officinalis* L.) were tested for their antimicrobial properties against bacteria, yeasts and fungi previously isolated from colonized artworks. Free EOs from *C. nepeta* and *L. stoechas* ssp. *luisieri* demonstrated broad-spectrum antimicrobial activity, with strong effects against *Cladosporium* spp., *Aspergillus niger, Rhodotorula* sp., *Penicillium* spp. and *Arthrobacter* sp. The EO of *Thymus vulgaris*, encapsulated through co-precipitation with β-cyclodextrin (β-CD), retained effective antifungal activity against *Penicillium brevicompactum* and *Fusarium oxysporum*, with an encapsulation efficiency exceeding 50%. The results highlight that EOs from *L. stoechas* ssp. *luisieri* and *C. nepeta* offer an eco-friendly and biodegradable alternative to synthetic biocides, which are often toxic and promote microbial resistance. Encapsulation with β-CD is a promising method to address the limitations of free EOs (volatility and chemical instability), providing a sustainable approach to preserving cultural heritage artifacts.

Prieto *et al.* [26] conducted a comprehensive laboratory and field study over two decades to assess the potential effects of herbicide treatments on the construction materials of the Roman Wall of Lugo, a UNESCO World Heritage site. The study examined various maintenances, including herbicides such as sulphosate, glyphosate, oxyfluorfen and glufosinate-ammonium, sand physical methods like infrared and controlled burning, which were applied to different sections of the wall. The researchers also tested three essential oils— *Thymus vulgaris*, *Thymus zygis* Loefl. ex L., and *Origanum vulgare* L. as potential eco-friendly alternatives. The research focused on evaluating mineralogical changes (using X-ray diffraction) and visible physical alterations, such as colour shifts and saline residue deposits, caused by these treatments. In the initial trials (1998/1999), physical and glyphosate treatments induced modifications in vermiculite clay minerals within the schist, while physical treatments also affected kaolinite. However, these changes were not highly noticeable in terms of colour alterations. Oxyfluorfen did not alter the mineral composition of the construction materials but slightly darkened the granite and increased chloride, nitrate, and sulphate deposits. The final stage of the study revealed that only *Thymus zygis* essential oil caused a perceptible increase in lightness and a reduction in the yellow component of the granite. Overall, the study assessed the impact of these treatments on the masonry and concluded that physical methods involving extreme temperatures (above 800°C) were unsuitable for conservation, as they caused irreversible damage to the schist. Herbicidal treatments, such as oxyfluorfen, left salt residues, but no major harm was observed beyond minor colour variations, which were not a concern due to the already heterogeneous appearance of the wall. The study underscored the importance of adopting environmentally friendly and low-risk treatments, particularly in public spaces surrounding the Roman wall. It recommended *Thymus vulgaris* and *Origanum vulgare* essential oils as the most suitable alternatives, as they did not induce mineralogical alterations, salt accumulation, or significant color changes, making them viable for long-term conservation efforts.

Mateus *et al.* [27] tested EOs from five plants (*Lavandula viridis, Foeniculum vulgare* Mill., *Mentha pulegium* L., *Helichrysum stoechas* (L.) Moench., and *Thymus mastichina*) collected from the Convent of Christ in Tomar to assess their antimicrobial properties against microorganisms found on historic materials. The results showed that not all EOs displayed the same level of effectiveness. For instance, *Helichrysum stoechas* exhibited no antifungal or antibacterial effects, while *Thymus mastichina* was active solely against bacteria. Conversely, EOs from *Lavandula viridis, Foeniculum vulgare* and *Mentha pulegium*, demonstrated both antifungal and antibacterial activity, with increasing potency at higher concentrations. At a 20% dilution, these EOs yielded encouraging results, achieving efficacy rates of up to 63% against bacteria and 37% against specific fungi, such as those from the genera *Aureobasidium, Cladosporium* and *Penicillium*. Despite these promising findings, the authors emphasized that EO biocidal performance is influenced by multiple factors, such as concentration, microbial species, and experimental methodologies, making direct comparisons with other studies difficult. This research represents a significant initial step toward employing EOs as natural alternatives to synthetic biocides for cultural heritage preservation. However, additional studies are necessary to evaluate the compatibility of EOs with historical materials, their environmental impact, and the longevity of their protective effects under real-world conservation settings. Preliminary findings suggest that EOs from *Mentha pulegium, Thymus mastichina, Lavandula viridis* and *Foeniculum vulgare* could provide a sustainable and viable substitute for conventional synthetic biocides.

A recent study Long *et al.* [28] addresses the challenge of stone building degradation caused by algae, which are pioneer plants. The lack of effective algaecides for preventing algae growth on stone structures calls for ecological methods to tackle this issue. The study investigated the application of aqueous and alcoholic extracts from four plant leaves (*Syzygium aromaticum* (L.) Merr. & L.M.Perry, *Cinnamomum cassia* Siebold, *Pogostemon cablin* (Blanco) Benth. and *Thymus mongolicus* (Ronniger) Ronniger) to synthesize silver nanoparticles (AgNPs). By refining the synthesis conditions, the researchers efficiently generated nanoparticles and assessed their algaecidal efficacy through laboratory and field experiments. The findings revealed that algae exposed to AgNPs derived from the alcoholic extract of *Cinnamomum cassia* leaves exhibited approximately three times lower chlorophyll-a levels, with an algae removal efficiency of 71.34%. Furthermore, the application of AgNPs led to stone fragments colonized by algae regaining a color comparable to non-colonized

surfaces, demonstrating successful algae elimination. The research confirms that silver nanoparticles from plant extracts represent a promising eco-friendly algaecide approach for protecting stone buildings.

In the case of algae growth contributing to stone corrosion in YunfengTunpu, where *Chlorella sp.* and *Chlorococcus sp.* are dominant, spherical silver nanoparticles were successfully synthesized. The study demonstrated both in laboratory and field tests that these nanoparticles significantly reduced the chlorophyll-a content in biomass and algae. The effectiveness of the AgNPs in inhibiting algae growth depended on the plant extract used, the concentration of AgNPs, and the exposure time. For instance, the AgNPs synthesized from *Cinnamomum cassia* extracts were more effective in algae removal than those from *Thymus mongolicus*. Overall, the study highlights the potential of plant-synthesized silver nanoparticles as a non-toxic, effective alternative to traditional algaecides for preserving delicate cultural heritage structures.

The recent study by Mateus *et al.* [29] focuses on the uses of essential oils (EOs) obtained from aromatic plants as natural alternatives to synthetic biocides to combat microbial deterioration in mural paintings, a major risk to build heritage. The research evaluated the potency of essential oils extracted from four aromatic plants—fennel, thyme, green lavender and pennyroyal,—against microorganisms isolated from mural paintings in the Casa de Moscadim, an 18th-century manor house in Portugal. Isolated microorganisms included bacteria such as *Bacillus mobilis* and *Bacillus wiedmannii*, and fungi such as *Cladosporium cladosporioides* and *Penicillium brevicompactum*. Fennel essential oil demonstrated the strongest antifungal activity, outperforming the effectiveness of the commercial biocide Biotina T® in certain cases. Essential oils from pennyroyal and green lavender also showed notable inhibitory effects. Tests using the microatmosphere technique revealed that EOs could be applied non-invasively, maintaining the integrity of mural surfaces without causing damage. These results indicate that essential oils, particularly fennel EO, offer a promising ecological and sustainable substitute for synthetic biocides in cultural heritage conservation. The study also highlights that EO mixtures may have synergistic effects, further enhancing their antimicrobial efficacy.

Rinaldi *et al.* [30] investigated the impact of essential oils (EOs) on phototrophic biofilms responsible for the deterioration of stone cultural heritage. The study compared the antimicrobial properties essential oils of *Thymus vulgaris* and *Lavandula angustifolia* Mill, with the former—rich in thymol—proving more effective in inhibiting biofilm growth, particularly when incorporated into an alginate hydrogel. This encapsulation technique allowed for the use of a lower EO concentration (0.1% v/v) while preserving its antimicrobial activity and minimizing the volatility of terpenic compounds. The developed method enhances application on vertical surfaces and improves biofilm removal, presenting a sustainable alternative to conventional chemical treatments.

Rotolo *et al*. [31] studied the biodeterioration of mosaic tiles in the "House of Leda" at the Greco-Roman site of Solunto, Sicily. The study highlighted the presence of a thick biofilm that significantly altered the mortar, leading to the detachment of mosaic tesserae. A comprehensive microbial analysis was conducted using microscopy (O.M., C.L.S.M.), molecular biology techniques (analysis and DNA sequencing), and in vitro culturing (Sabouraud and Nutrient media). The biofilm was found to contain fungi (*Alternaria*, *Aspergillus*), bacteria (*Bacillus*), green algae (*Chlorella*), and cyanobacteria (*Chroococcus)*. To control microbial colonization, the antimicrobial activity of two essential oils, *Origanum vulgare* and *Thymus vulgaris*, was tested through in vitro agar diffusion methods, followed by ex situ and in situ applications. This study aims to develop and implement eco-friendly biocides as an alternative to traditional chemical treatments, offering a sustainable approach with lower environmental and health risks.

4.2. Application of Thyme Essential Oil on Wood Materials for Conservation and Restoration

Essential oils from thyme (*Thymus vulgaris*) are widely used for the restoration and conservation of wooden artifacts due to their antimicrobial and antifungal properties. Their ability to inhibit fungal and bacterial growth makes them effective tools for protecting organic materials like wood, which are often susceptible to biodeterioration. Particularly, thyme essential oil is valued for its versatility, sustainability, and compatibility with delicate substrates, offering an eco-friendly alternative to traditional chemical preservatives.

The study by Benkovičová *et al.* [32] investigated the *in vitro* antifungal properties of essential oils (EOs), superhydrophobic nanoparticles (SHNPs) and their combinations (SHNP/EO). SHNPs were mixed with various concentrations of three essential oils— *Thymus vulgaris* (thyme), *Thuja plicata* Donn ex D. Don (arborvitae) and *Origanum vulgare* (oregano) and applied to different surfaces, including white wood, to analyze their protective and antifungal properties.

The tests included measuring the reflectivity ratio (Rr) to assess color changes, while digital microscopy was used to study the mold colonization area and its penetration into the analyzed materials. The results showed that SHNPs alone provided a balanced effect in inhibiting mold growth. However, the antifungal effectiveness of essential oils, whether used alone or combined with SHNPs, depended on the type of essential oil and the treated material. EO/SHNP mixtures were particularly effective in protecting wood, although some molds, such as *Aspergillus fumigatus*, demonstrated the ability to penetrate substrates, indicating the need for further optimization. The study concluded that the combined use of essential oils and superhydrophobic nanoparticles represents a promising approach for wood protection, promoting the essential oils as sustainable adjuvants for surface preservation.

The study by Salem *et al.* [33] investigated the antifungal properties of two extracts (ethyl ether extract of ripe *Pinus rigida* Mill. heartwood and *Schinus terebinthifolius* Raddi fruits) and two essential oils (*Origanum majorana* L. leaves and *Thymus vulgaris*) against the fungi *Aspergillus niger* and *Trichoderma harzianum*. The results showed that *P. rigida* essential oil and *O. majorana* extract had the highest activity against both fungi and were selected for application on four wood species: *Fagus sylvatica, Acacia saligna, Pinus rigida* and *Juglans nigra*.

The effect of the treatments on the wood structure was examined using SEM, FTIR, and colorimetry.

O. majorana essential oil demonstrated the best results, effectively penetrating the wood and strengthening its structure without significantly altering its properties. In contrast, the *P. rigida* extract caused undesirable color changes, making it less suitable for wood applications.

In conclusion, *O. majorana* essential oil proved to be the most promising as a bio-fungicide for wood protection, preserving its structural integrity. The study suggests that future research could explore combining these natural materials with natural polymers to further enhance their antifungal and consolidating properties.

The study by Casiglia *et al.* [12] highlights the issue of biodeterioration caused by bacteria and fungi, which significantly affects historical art and craftworks in archives, museums, and private collections. These microorganisms degrade natural organic materials like fibers, wood, dyes, and even stone objects, leading to physical, chemical, mechanical, and aesthetic damage. The study focuses on the antifungal and antibacterial properties of wild thyme (*Thymus capitatus*) essential oil as a natural alternative for combating such microorganisms.

The essential oil was obtained via hydrodistillation from the aerial parts of *T. capitatus* collected at different growth stages in northern Sicily. Its composition was analysed using GC-MS (Gas chromatography and Mass Spectrometry). The results showed that the oil belongs to the carvacrol chemotype, and its composition varied depending on the harvesting time. The oil demonstrated strong antimicrobial activity against *Aspergillus niger, Bacillus subtilis,* and *Fusarium oxysporum*—common microorganisms that infest historical art objects, libraries and archives. Its effectiveness was comparable to chloramphenicol and ketoconazole, standard antimicrobial agents.The study suggests that *T. capitatus* essential oil has potential applications in protecting and disinfecting museum objects, offering a natural and effective solution for preserving historical artifacts.

The study by Geweely *et al.* [19] investigates the effect of fungal activity on the degradation of cultural heritage, a global concern that makes long-term preservation a complex challenge. The antifungal properties of 12 essential oils (castor, black cumin, clove, cinnamon, garlic, cumin, lavender, geranium, mint, lemongrass, thyme and olive) were evaluated against 16 fungal species isolated from three archaeological objects, including a wooden statue, from the Saqqara storerooms in Egypt. The most frequently identified fungal species, confirmed through molecular analysis, were *Rhizopus oryzae*, *Aspergillus niger* and *A. flavus*.

The minimum inhibitory concentration (MIC) of antifungal effectiveness essential oils were tested at various levels. The most effective oils were thyme (*Thymus vulgaris*), clove and geranium,

respectively. The main active components of thyme were thymol (37.1%) and p-cymene (26.32%), while clove oil contained triacetin (69.36%) and eugenol (28.67%). For geranium, the primary components were α-citronellol (20.62%) and geraniol (14.43%). *Aspergillus niger* was the most resistant species, while *Penicillium citrinum* and *Fusarium oxysporum* were the most susceptible. The study highlights the serious issue of fungal deterioration in archaeological objects, emphasizing that traditional chemical and mechanical disinfection methods can cause erosion, surface damage, and risks to public health and the environment. Therefore, new investigations are needed to protect archaeological objects without harming their surfaces. The main recommendation is the use of essential oils, particularly geranium, thyme and clove, as eco-friendly and promising solutions for cultural heritage preservation, due to their high antifungal activity and low toxicity to humans and the environment.

Geweely *et al.* [23] investigated the suppressive effects of three essential oils— geranium (*Pelargonium graveolens* L'Hér), thyme (*Thymus vulgaris*), and clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry)—on three fungal species (*Rhizopus oryzae Aspergillus niger*, and *A. flavus*) isolated from archaeological objects (including wooden pieces) from the Saqqara excavations in Egypt. Thyme oil was the most effective, completely inhibiting *A. flavus* and *R. oryzae* and significantly reducing the metabolic and growth activities of *A. niger*. Additionally, thyme oil caused the least color change on artificially aged experimental materials, maintaining an aesthetically acceptable appearance. The study highlights the importance of eco-friendly conservation methods to protect cultural heritage from microbial damage, avoiding the risks associated with traditional chemical and mechanical treatments. Thyme oil, due to its high antimicrobial activity and low toxicity, is proposed as a promising of archaeological artifacts. Microbial biodeterioration presents an increasing threat to cultural heritage. Traditional biocides, while effective, carry risks of toxicity to health and the environment. Essential oils (EOs), thanks to their low toxicity and antimicrobial potential, offer a safe and sustainable alternative for controlling microbial growth on heritage objects. However, the volatility of EOs limits their use. Encapsulation of EOs could solve this issue by ensuring controlled release and enhancing their effectiveness as eco-friendly biocides.

In the study by Macedo-Arantes *et al.* [25], seven EOs derived from aromatic herbs (*C. nepeta, L. luisieri, L. viridis, T. mastichina, T. vulgaris, R. officinalis, S. officinalis*) were tested to assess their antimicrobial properties against filamentous fungi, yeasts, and bacteria isolated from colonized artworks. Free EOs, particularly those from *L. luisieri* and *C. nepeta*, demonstrated broad-spectrum antimicrobial activity, effectively inhibiting *A. niger, Cladosporium spp., Penicillium spp., Rhodotorula sp.,* and *Arthrobacter sp*. When encapsulated with β-cyclodextrin (β-CD), the EO from T. vulgaris maintained similar antifungal activity, with an encapsulation efficiency exceeding 50%. According to the results of the research suggest that EOs, especially when encapsulated, can serve as eco-friendly, biodegradable, and non-toxic biocides for the protection of cultural heritage. Encapsulation reduces the volatility and instability of free EOs, preventing unwanted interactions with heritage materials. This approach represents a sustainable substitute for synthetic biocides, which are often toxic and contribute to microbial resistance and the spread of harmful microorganisms.

The study by Sparacello *et al.* [34] investigated aromatic plants such as *Thymus vulgaris* as sources of natural substances with therapeutic properties, widely used in the pharmaceutical and food sectors. Due to the increasing demand for eco-friendly, non-toxic alternatives essential oils (EOs) and hydro-alcoholic solutions (HA) have been suggested for the sustainable preservation of artistic and historical objects. This study focused on counteracting microbial colonization (*Aspergillus sp., Streptomyces sp., Micrococcus sp*.) on the surfaces of wooden artworks, using a polyphasic approach involving *T. vulgaris* EO and HA solutions. HA solutions and the antimicrobial effects of the EOs were initially evaluated using well plate diffusion (WPD) and agar diffusion (ADD) methods. Microbial identification was carried out by optical microscopy, in vitro cultures, and DNA-based molecular biology techniques. The treatment involved the direct removal of microbial patina with an HA solution (monitoring any colorimetric changes on the artwork surface) combined with exposure to the volatile compounds of the EO in a dedicated clean chamber. This study is the first to propose the combined use of plant extracts (EOs and HA solutions) to counteract microbial growth on wooden artworks. The approach proved effective for the Sogo Bò theater artifact (Mali, Africa), which lacked a protective coating on its surface. After the alcohol evaporated, the antimicrobial compounds of *T.*

vulgaris remained on the object's surface, enhancing the activity of the EO's volatile compounds. This eco-friendly conservation strategy, safe for both operators and the environment, offers a sustainable replacement for synthetic biocides in the preservation of cultural heritage. It highlights the potential of plant-derived bioactive substances in the sustainable conservation of historical and artistic assets.

D'Agostino *et al.* [35] conducted a study on the conservation of cultural heritage, particularly focusing on the restoration of wooden artifacts. Traditionally, products not specifically designed for this purpose have been used, but the research explored a more eco-friendly approach by utilizing microcrystalline cellulose derived from almond shells (*Prunus dulcis* Miller), a waste material abundant in Southern Italy. This cellulose, obtained through a simple alkaline treatment, was combined with various binders to create stuccos suitable for wood consolidation.

To provide biocidal properties to the stuccos, useful against fungi and insects that often damage wooden artifacts, essential oil from *Thymus capitatus* was added, known for its antimicrobial properties. The physical and mechanical characteristics of the new materials were tested, particularly through DMA (Dynamic Mechanical Analysis) under flexural stress. The results showed that only stuccos with Aquazol 500 as the binder did not reach the breaking point, maintaining good mechanical properties even with the addition of the essential oil.

The inclusion of the essential oil increased the strain in the samples, reducing their mechanical strength, except in the case of Aquazol 500. Digital microscope analysis revealed that the essential oil did not emulsify well in the mixture, making the samples less homogeneous. Despite this, the study demonstrates that microcrystalline cellulose derived from almond shells, combined with Aquazol 500, is effective as a stucco for wood restoration. Furthermore, the addition of *T. capitatus* essential oil provides antimicrobial features without compromising mechanical performance, paving the way for new eco-friendly reintegration protocols in cultural heritage conservation.

Pop *et al.* [36] investigated essential oils as natural wood preservatives with antifungal properties. In a laboratory study, basil, oregano, and thyme essential oils were tested at different concentrations against two wood-decaying fungi: *Postia placenta* (brown rot) and *Trametes versicolor* (white rot). The results showed that thyme and oregano oils exhibited strong biocidal activity, being more effective against brown rot, while basil oil had minimal impact. The study highlights the potential of essential oils as eco-friendly alternatives to synthetic wood preservatives.

Russo *et al.* [37] analysed biodeterioration, a complex process involving interactions between micro/macrobiological systems and inorganic/organic materials, leading to chemical and physical changes linked to the metabolic functions of organisms. Counteracting microbial deterioration requires a careful approach, including an understanding of the changes and the use of methods that respect the artwork, human health, and the environment. Traditionally, physical, mechanical, and chemical methods, including synthetic biocides, have been used, but these have limitations due to their toxicity to operators and environmental impact. Researchers are increasingly focusing on natural alternatives, such as essential oils (EOs), which offer safe and eco-friendly solutions. These oils, extracted from plants, contain natural chemical substances (e.g., alkaloids, phenols, flavonoids) with antimicrobial properties, useful for preventing the biodeterioration of cultural heritage. Russo et al.'s review focuses on the use of EOs in restoration processes, highlighting their advantages in terms of sustainability and human safety. Cultural heritage, of immeasurable historical and social value, is subject to deterioration caused by natural, anthropogenic, and biodeteriogenic factors (e.g., fungi, bacteria). These microorganisms not only damage artworks but may also pose a threat to the health of operators and visitors. The use of commercial chemical biocides is becoming less favored due to their toxicity, driving research toward green solutions like EOs. EOs are considered a valid substitute for synthetic biocides due to their low toxicity, ease of use, and environmental sustainability. However, further studies are needed to evaluate their effectiveness in relation to environmental factors (light, temperature, humidity) and to develop standardized protocols. Additionally, critical aspects such as the volatility of EOs, their concentration, and the possibility of using them in mixtures to enhance their effectiveness must be considered.

Although research on EOs is particularly active in Europe (Italy, Portugal, Serbia, etc.), greater attention is needed globally, considering the different climatic and geographical conditions in which cultural assets are located. The use of green substances protects not only cultural heritage but also preserves human health by reducing exposure to pathogens and toxic substances.

4.3. Application of Thyme Essential Oil on Textile and Paper Materials for Conservation and Restoration

Essential oils are increasingly studied for their potential use in cultural heritage preservation, particularly for the disinfestation and protection of organic artifacts such as paper, textiles, and leather. Among these, thyme essential oil (*Thymus vulgaris*) stands out for its proven antimicrobial and antifungal properties, making it a promising option for treating surfaces prone to biological degradation. The integration of essential oils with innovative technologies, such as superhydrophobic nanoparticles (SHNPs), offers new perspectives for enhancing the protection and conservation of materials.

Benkovičová *et al.* [32] conducted a study to assess the *in vitro* antifungal activity of superhydrophobic nanoparticles (SHNPs), essential oils (EOs), and their combinations (SHNPs/EOs). A thin layer of SHNPs was applied in combination with different concentrations of three essential oils: Giant Thuja (*Thuja plicata*), Oregano (*Origanum vulgare* L.), and Thyme (*Thymus vulgaris*). The mixtures were spread on various surfaces, including paper. The antifungal and protective properties of SHNP and EO combinations were then assessed.

To determine the colour changes of the substrates, the Rr parameter (reflectance ratio) was measured. Digital microscopy was employed to evaluate the mold colonization area and their penetration into the analysed materials. Surprisingly, the use of SHNPs alone exhibited an effective balance in preventing mold growth on treated surfaces. From the perspective of material preservation, the use of essential oils as adjuncts in surface protection is highly promoted. The results demonstrated that the antifungal effect of essential oils, either alone or in combination with SHNPs, depends on both the type of essential oil used and the treated material.

Regarding colour changes, the application of essential oils shifted colour saturation toward orange-yellow tones, while SHNPs made the colour lighter due to enhanced Rayleigh scattering in the blue part of the visible spectrum.

This study highlighted the potential use of SHNPs, alone or in combination with essential oils, for the conservation of indoor building materials or furniture against mold colonization. The experimental use of high fungal concentrations, which are rarely encountered in normal indoor environments, further supports their antifungal effectiveness. In the future, it will be interesting to test their inhibitory effect on the growth of specific microbial communities.

Lavin *et al.* [38] studied the impact of the fungi *Scopulariopsis* sp. and *Fusarium* sp. on paper degradation, analyzing their ability to adhere to surfaces, form biofilms, and produce foxing-like brownish spots. These microorganisms alter the physicochemical properties of materials by producing pigments and acids, causing both structural and aesthetic damage to documents.

Scanning electron microscopy (SEM) confirmed biofilm formation and the production of extracellular polymeric substances (EPS), demonstrating their deterioration potential. Additionally, a pH decrease of one unit was observed in contaminated samples, contributing to cellulose degradation.

The study also assessed the effectiveness of essential oils from thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) against these fungi using the micro atmosphere method. The results showed that the volatile compounds of these oils exhibit antifungal properties, providing a sustainable and safe alternative for protecting documentary heritage in museums, archives, and libraries.

Casiglia *et al.* [12] analyzed the biodeterioration of organic historical artifacts, such as paper and textile fibers, caused by bacteria and fungi—a widespread issue in archives, libraries, and museums. These microorganisms degrade natural organic materials, compromising their physical and chemical structure and causing aesthetic alterations such as discoloration and stains. The study highlighted the high antimicrobial activity of wild thyme (*Thymus capitatus*) essential oil, suggesting its use as a natural alternative for protecting organic artifacts susceptible to biodeterioration. The oil, extracted from plants collected along the northern coast of Sicily, belongs to the carvacrol chemotype, with its composition varying depending on the harvesting period. The essential oil demonstrated strong antifungal and antibacterial activity against *Aspergillus niger*, *Bacillus subtilis* and, *Fusarium oxysporum*, microorganisms frequently responsible for the deterioration of paper and fibers. Its

efficacy was found to be comparable to that of chemical preservatives such as chloramphenicol and ketoconazole, making it a promising agent for the disinfestation and preservation of paper-based and textile materials. The oil was extracted through hydrodistillation and characterized using gas chromatography (GC) and GC-mass spectrometry, confirming the presence of bioactive compounds with potential applications in protecting documentary and textile heritage from microbial attacks. This offers a sustainable and environmentally friendly solution for cultural heritage conservation.

Bosco *et al.* [39] investigated the control of microbial contamination in indoor spaces like libraries and archives, where microorganisms can damage paper documents and pose health hazards to staff. Essential oils (EOs), recognized for their antimicrobial properties and widely used in the pharmaceutical and food industries, were evaluated for their ability to safeguard water-damaged paper documents and enhance indoor air quality. Among the oils tested, *Thymus vulgaris* essential oil was identified as the most potent against microorganisms that affect paper, such as *Staphylococcus epidermidis, Rhodotorula mucilaginosa*, and *Alternaria alternata.* The oil showed strong inhibitory effects on these microbes, with particularly high effectiveness when applied immediately after lyophilization. For example, the oil inhibited *S. epidermidis* and *R. mucilaginosa* growth at very low concentration, and it was able to halt the growth of *A. alternata* at a significant rate, especially in its non-sporulated form. It also proved effective when used on a contaminated book cover. The study demonstrated that EOs can delay or completely stop the growth of biodeteriorating microorganisms on paper, under both water-independent and water-related contamination scenarios. While this shows promising potential for preserving documents and treating multiple items at once, further research is needed to apply these findings in real-world archival settings.

A compelling study by D'Agostino *et al.* [40] examined the chemical composition of *Thymus vulgaris* essential oil (EO) to assess its biological activity against microorganisms affecting Tholu Bommalu, traditional Indian leather puppets preserved at the International Puppets Museum Antonio Pasqualino in Palermo, Italy. The EO's chemical profile was determined using GC–MS with both polar and apolar columns. The research aimed to evaluate the antimicrobial potential of *Thymus vulgaris* and *Crithmum maritimum* L. EOs in vapor phase for disinfecting heritage leather puppets. Leather artifacts contaminated with various bacterial strains were subjected to the essential oils under vacuum and static evaporation conditions. The results indicated that vapor-phase application of the essential oils was an effective strategy for sanitizing natural leather, eradicating microorganisms in a short period. A 50% solution of *T. vulgaris* EO demonstrated outstanding antibacterial activity against the isolated strains using both methods, though the vacuum technique facilitated faster biocide exposure. Additionally, the antimicrobial properties of *Crithmum maritimum* EO were analysed and found to be significantly less effective against the tested microorganisms. The study investigated the yield, composition, and antimicrobial effects of both essential oils. *T. vulgaris* EO was abundant in monoterpenes such as p-cymene (35.96%), terpinen-4-ol (10.29%), γ-terpinene (8.85%), and thymol (25.38%), whereas *C. maritimum* EO was primarily composed of myrcene (13.66%), *p*-cymene (11.67%), *β*-phellandrene (6.57%), and thymol acetate (14.38%). Additionally, the research suggested implementing a vacuum chamber to optimize the disinfection process. In vapor phase, *T. vulgaris* EO exhibited strong antibacterial effects, forming inhibition halos up to 33 mm, with colonies treated under vacuum showing substantial reductions compared to those exposed to ambient conditions. In contrast, *C. maritimum* EO failed to produce inhibition halos. The findings indicate the potential application of commercial plant-derived essential oils as eco-friendly biocides, offering a safer alternative to conventional toxic chemicals in cultural heritage conservation.

Boniek *et al.* [41] carried out research to tackle fungal proliferation and biodeterioration in cultural heritage artworks, focusing on an engraving attributed to the Dutch artist Rembrandt. The study aimed to detect fungal communities present on the piece, which exhibited noticeable signs of degradation. Microbiological analyses, including culturing techniques and molecular biology approaches, were employed to identify the fungi, with particular attention to *Cladoposporium spinulosum*, a metabolically active species. The research evaluated environmentally friendly antifungal treatments, such as essential oils (EOs) extracted from *Melaleuca alternifolia* (Maiden & Betche) Cheel, *Curcuma longa* L. and *Thymus vulgaris*, for their effectiveness in controlling fungal growth. Laboratory tests demonstrated that the EOs suppressed fungal strain development, with *T. vulgaris* and *M. alternifolia* exhibiting low minimum inhibitory concentration levels. Exposing fungal cultures to

anaerobic conditions for 35 days successfully eradicated them. The study proposed that the combination of an oxygen-deprived environment and essential oils offers a non-invasive, eco-conscious alternative to traditional chemical antifungal treatments for the conservation of artworks. The study also explored the biochemical mechanisms behind the antifungal properties of EOs, including their effects on fungal cell membranes, mitochondrial function, and gene expression. The researchers concluded that using an anaerobic atmosphere and EOs could control fungal growth, minimize aesthetic and structural damage to artworks, and prevent fungal colonization. However, further testing is necessary to assess the potential impact on the artwork, such as any bio-receptivity or staining caused by EO application. The study recommends using steam EO from *C. longa*, *M. alternifolia*, and *T. vulgaris*, alongside nitrogen gas to control fungal growth, with temperature and humidity conditions carefully controlled. Overall, these eco-friendly alternatives offer a promising solution for fungal management in cultural heritage conservation, preserving the integrity of valuable artworks like Rembrandt's engraving.

El-Molla *et al.* [42] concentrated in this study on the formulation and isolation of sustainable essential oils from natural origins and their application in treating bleached cotton fabrics. The objective was to provide a pleasant scent and antibacterial properties, making them suitable for medical textiles. The essential oils from lavender, thyme, and clove were extracted and combined with β-cyclodextrin inclusion compounds. These molecules can form inclusion complexes with essential oils, fitting into the hydrophobic cavity of the β-cyclodextrin molecule. The essential oils were applied to cotton fabrics through a dry method at low temperatures, followed by a thermo-fixing process. Various techniques, such as IR spectroscopy, GC-MS, and scanning electron microscopy (SEM), were used to analyze the structure of the oils. The antibacterial activity of the treated fabrics against *Escherichia coli* and *Staphylococcus aureus* was evaluated, showing that the fabrics exhibited significant antibacterial properties. The study revealed that the treated fabrics maintained a strong fragrance for up to 50 days. After sixty days or five washing, the fragrance weakened to a medium level. The novelty of this research lies in its ability to produce medical textiles that are economically feasible, while achieving the dual goals of providing antibacterial properties and a pleasant scent. The application of microcapsules with essential oils on cotton fabrics via the exhaustion method demonstrated promising results for functional medical textiles creation.

Pietrzak *et al.* [43] examined the efficiency of three decontamination techniques—thyme essential oil vapor treatment, silver nanoparticle spraying, and low-temperature plasma—utilizing both culture-based methods and RNA sequencing analysis. The study also investigated the impact of these approaches on the structural and visual characteristics of paper from historical books with different degrees of microbial contamination. The findings revealed that all disinfection techniques exhibited bacteriostatic and fungistatic effects, with silver nanoparticle spraying proving to be the most efficient at restricting bacterial proliferation, followed by low-temperature plasma and thyme essential oil. However, the antifungal efficacy of these methods was lower, with thyme essential oil demonstrating a broader fungicidal spectrum compared to the other two treatments. The success of disinfection depended on the extent of microbial contamination in the books and the susceptibility of the microorganisms to the active substances. Higher decontamination efficiency was recorded in books with severe microbial contamination. RNA concentration emerged as a reliable indicator of antimicrobial activity, showing a notable decrease following treatment with low-temperature plasma and thyme essential oil, while silver nanoparticles resulted in a more moderate reduction. Regarding the structural and visual integrity of the paper, the decontamination processes did not cause significant alterations but had beneficial effects on books with greater microbial infestation. The study suggests that low-temperature plasma, thyme essential oil, and silver nanoparticles could be microbiostatic alternatives to conventional treatments. Decontamination strategies should be tailored based on the specific microorganisms present in the books to maximize their efficacy. In conclusion, the tested approaches had minimal influence on the physical properties of paper in books with low contamination but proved advantageous for those with higher levels of microbial presence. Further studies are necessary to evaluate the applicability of these techniques to other historical materials, including parchment, leather, inked manuscripts, prints, wax-coated items, and photographs.

Menicucci *et al.* [44] examine the deterioration processes affecting historical documents and artworks preserved in library collections, often triggered by “biodeteriogens” thriving in these settings.

With increasing attention to safe and advanced preservation methods, plant-based solutions, particularly essential oils (EOs), have gained recognition for their potential role in safeguarding cultural heritage. The paper presents a literature review of research on EOs and their key components in the protection of paper-based heritage objects, outlining both their advantages and limitations. Traditionally, fragrant plants and their extracts have been utilized to protect books, but recent studies stress the importance of a scientific approach to evaluating EOs. This involves chemical profiling through techniques such as GC-MS to unravel the complex composition of these plant-derived compounds. EOs are regarded as a distinctive and adaptable source of bioactive substances, offering a promising alternative to the hazardous chemicals still widely used in conservation. Recent advancements have led to the development of EO-infused formulations that provide long-lasting effectiveness and controlled dispersion, demonstrating significant potential for future applications. The paper also stresses that indirect treatments, where EOs do not directly contact the materials, are preferable to avoid potential damage. However, more research is necessary to define the safety thresholds for using EOs in paper conservation, ensuring that they are both non-invasive and non-toxic. These two aspects—safety and non-toxicity—should be the focus of future studies in the field of cultural heritage preservation.

Gümüştekin *et al.* [45] conducted research investigating the dyeing potential and antimicrobial effects of extracts derived from thyme (*Thymus vulgaris*) and mint (*Mentha spicata* L.) plants. The study involved treating cotton and wool fabrics using different mordanting techniques—pre-mordant, meta-mordant, post-mordant, and non-mordant processes—with $AlK(SO_4)_2$, $CuSO_4$, and $FeSO_4$ as mordants. The shade and durability of the dyed textiles were assessed based on resistance to rubbing, washing, and light exposure, utilizing a colour analysis spectrophotometer. The highest K/S value was recorded with $FeSO_4$ using the meta-mordant technique on wool fibres dyed with thyme extract. The antibacterial effectiveness of the dyed fabrics was evaluated through the disk diffusion technique, revealing that textiles dyed with $CuSO_4$ exhibited the strongest antimicrobial properties among the mordants tested. The antimicrobial performance of the dyed materials suggests their potential application in healthcare environments, contributing to improved hygiene standards. The study verified that mint and thyme extracts are reliable natural dyes for textile applications. The research included extracting pigments from dried and powdered mint and thyme, followed by dyeing forty samples of wool and cotton fabric. The stability of the colours was analysed in terms of resistance to rubbing, washing, and light exposure. Furthermore, the antimicrobial potential of the dyed textiles was examined. Findings indicated that $CuSO_4$ mordant enhanced antibacterial properties, making these textiles well-suited for medical and hygiene-related uses. In conclusion, the research established that thyme and mint extracts can function as sustainable and efficient substitutes for synthetic dyes in textile manufacturing, offering high-quality coloration, strong fabric adhesion, and durability. These plant-based dyes hold significant value for both environmental sustainability and the textile industry.

The study by Othman *et al.* [46] investigated the antifungal efficacy of five essential oils and nine powdered plant extracts against four common fungal species (*Aspergillus versicolor, A. flavus, P. purpurogenum* and *Penicillium sp*.), which were isolated from archaeological artifacts made of linen and papyrus. The results showed that essential oils were more effective than powdered extracts, with citronella and thyme being the most potent. The fungicidal concentrations and minimum inhibitory were determined, and the chemical composition were analyzed using gas chromatography-mass spectrometry (GC-MS). Tests conducted on deteriorated samples demonstrated that both oils could eliminate the fungi, with exposure times ranging from two to 14 weeks depending on the treated material. Analyses using scanning electron microscopy (SEM), FTIR spectroscopy, and colorimetry confirmed the treatment's effectiveness, suggesting that essential oils could serve as potential antifungal agents for disinfecting biodeteriorated papyrus and linen artifacts. However, further research is needed to ensure their practical application in preventive conservation.

Walentowska and J. Foksowicz-Flaczyk [47] investigated the effectiveness of thyme essential oil as a natural biocide to enhance the resistance of lignocellulosic fabrics against bacteria and mold. The study demonstrated that thyme essential oil, applied at 8% concentration in methanol to mixed linen-cotton and pure linen fabrics, exhibited high antibacterial and antifungal activity, without mold formation or significant loss of fabric strength. Additionally, the application of this eco-friendly

biocide provided antimicrobial barrier properties to the fabrics containing natural fibers during the finishing process.

5. Chemical constituents

The chemical composition of essential oils obtained from the utilized *Thymus* taxa has been investigated although in several cases it is missing. The major compounds (> 3%) occurring in the chemical composition of the essential oils are reported in Table 1 and their structures are depicted in Figure 2. The chemical composition of essential oils is influenced by various internal and external factors, such as plant species, geographical location, climate conditions, harvesting time, and extraction method, leading to variations in their properties and applications.

As for *Thymus capitatus* (syn. *Thymbra capitata*) the oil composition of the different accessions seems quite homogeneous; carvacrol is always, by far, the main constituent followed by minor quantity of *p*-cymene, *y*-terpinene and *β*-caryophyllene. Only two essential oils of *T. mastichina* have been chemically investigated. The first one showed as main products *p*-cymene (24%), 1,8-cineole (15%) and thymol (12%), whereas the second one showed to be very rich in 1,8-cineole (56%) and camphor (13%). The essential oil of *T. serpyllum* was shown to be very rich in oxygenated monoterpenes whereas no investigation was reported for *T. pulegioides*. The main constituents of the different accessions of *T. vulgaris* were, in almost all cases, thymol and *p*-cymene, although the occurrence of carvacrol and linalool, as main metabolites, has also been reported. Finally, the essential oil of *T. zygis* was particularly shown rich in thymol (49%) and *p*-cymene (19%).

Table 1. Main components (>3%) of *Thymus* species essential oils from different origins previously reported in literature.

Taxa	P.	Origins	Main components (%)	Ref.
T. capitatus	a.p.	Sicily (Italy)	carvacrol (81), *p*-cymene (5.0)	[12]
T. capitatus	a.p.	Sicily (Italy)	carvacrol (81), *p*-cymene (5.0)	[35]
T. capitatus	c	Flora s.r.l., Lorenzana, Italy and Esperia S.p.a., Milan, Italy	carvacrol (70), *p*-cymene (9), *y*-terpinene (8)	[17]
T. capitata	a.p.	Malta	carvacrol (73), *y*-terpinene (7), *p*-cymene (4)	[15]
T. capitatus	a.p	Sicily (Italy)	carvacrol (73), *p*-cymene (7), *β*-caryophyllene (6)	[24]
T. mastichina	c	Ervitas Catitas (Portugal)	*p*-cymene (24), 1,8-cineole (15), thymol (12), γ-terpinene (7) linalool (4)	[25]
T. mastichina	a.p.	Alentejo, Portugal	no composition	[27]
T. mastichina	a.p.	D'Alenguadiana Company (Alentejo, Portugal)	no composition	[29]
T. mastichina	a.p.	D'Alenguadiana Company located in Cortes-Sines, Mértola, Alentejo, harvested in Canais do Guadiana	1,8-cineole (56), camphor (13), borneol (7), camphene (6), *α*-pinene (5), *β*-pinene (3)	[5]
T. pulegioides	n.s.	n.s.	no composition	[21]
T. serpyllum	a.p.	n.s.	borneol, cineole, tymol, cymol, carvacrol, *α*-terpineol, *β*-caryophyllene, myrcene, caffeic acid, rosmarinic acid	[22]
T. vulgaris	a.p.	Daran, Karaman, Turkey	*p*-cymene (36), thymol (25), terpinen-4-ol (10), *α*-terpinene (9), linalool (6), caryophyllene (4)	[40]
T. vulgaris	n.s	n.s.	thymol (37), *p*-cymene (20), terpinyl acetate (16), carvacrol (10), bisabolene (6).	[42]

T. vulgaris	c	Esencias Martínez Lozano (Murcia, Spain)	thymol (46), *p*-cimene (16), *y*-terpinene (10), linalool (5), carvacrol (4)	[16]
T. vulgaris	n.s	n.s.	thymol (37), *p*-cymene (26), *α*-pinene (11), *α*-myrcene (9)	[19]
T. vulgaris	a.p.	Natural Oils Department, National Research Center, Dokki, Egypt	no composition	[23]
T. vulgaris	n.s	Tokat, Türkiye	no composition	[45]
T. vulgaris	c	dōTERRA International LLC company (Utah, USA)	methyl thujate; carvacrol, thymol; thyme, thymol, *p*-cymene, *γ*-terpinene	[32]
T. vulgaris	c	Laszlo Aromaterapia, Minas Gerais, Brazil.	thymol (47), *p*-cymene (40), linalool (4), *α*-pinene (3)	[41]
T. vulgaris	r	n.s.	no composition	[44]
T. vulgaris	c	n.s.	20-54% thymol, no other composition	[20]
T. vulgaris	c	Food Industry Research, Havana Cuba	no composition	[38]
T. vulgaris	n.s	Provided by a local producer of biological aromatic plants, Ervitas Catitas (Portugal).	*p*-cymene (24), 1,8-cineole (15), thymol (12), *γ*-terpinene (7), linalool (4)	[25]
T. vulgaris	n.s.	Sekem Company, Egypt	*m*-thymol (44), *o*-cymene (20), *o*-thymol (10), carvacrol methyl ether (6)	[46]
T. vulgaris	n.s.	n.s.	carvacrol (65), *p*-cymene (11), thymol (8)	[11]
T. vulgaris	c	Doterra, Pleasant Grove, USA	phenols (60), thymol (<54%), no other composition	[43]
T. vulgaris	n.s.	Steaua Divina	no composition	[36]
T. vulgaris	n.s.	Esencias Martínez Lozano, Murcia, Spain	linalool (81), *a*-terpinene (8), *β*- myrcene (4), limonene (3)	[26]
T. vulgaris	a.p.	n.s.	tymol, carvacrol, *p*-cimene, *α* pinene, *β*-pinene, camfene, *γ*-terpinene, mircene, limonene, carene, triciclene, fenchene, p-mentane, mentene, sabinene, terpinolene, *β*-fellandrene, bornyl acetate, linalyl acetat, nerol, citronellol	[22]
T. vulgaris.	c	Sarandrea Marco & C. s. r.l. (Collepardo, FR, Italy)	thymol (70), linalool (12), cymene (7), menthol (4)	[30]
T. vulgaris.	c	n.s.	no composition	[31]
T. vulgaris	a.p.	n.s.	no composition	[24]
T. vulgaris.	c	National Research Center, Cairo, Egypt	carvacrol (9), terpinen-4-ol (7), *γ*-terpinene (6), estragole (5), L-camphor (5), linalool (5), *β*-caryophyllene (4), *p*-cymene (4), α-terpinene (4), 5-isopropyl 2-methylanisole (3), *α*-pinene (3), 3-thujene (3)	[33]
T. vulgaris	c	EPOSrl., Milano	carvacrol (65), thymol (8), *p*-cymene (11).	[34]
T. vulgaris	c	Avicenna-Oil Company, Wroclaw, Poland (Member of IFEAT)	thymol (32), *p*-cymene (25), carvacrol (4), borneol (3)	[47]
T. zygis	n.s.	Esencias Martínez Lozano, Murcia, Spain	thymol (49), *p*-cymene (19), *y*-terpinene (8), linalool (5)	[26]

P. = parts; a.p. = aerial parts; c = commercial; n.s. = not specified; r = review

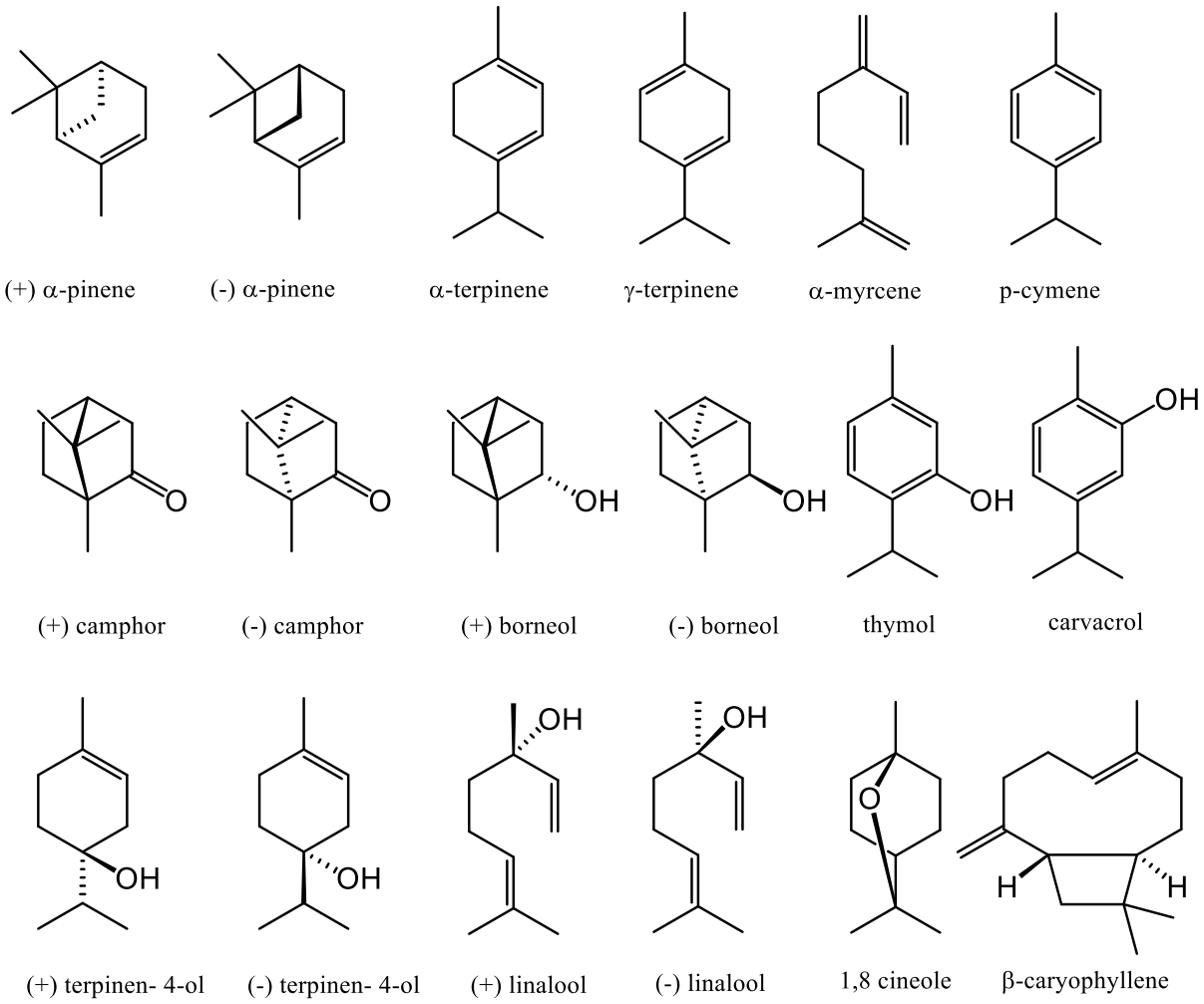

Figure 2. Main metabolites occurring in the essential oils of *Thymus* species

6. Comparative Evaluation of *Thymus* Essential Oils and Other Natural Biocides

Thymus essential oils (EOs) are among the most extensively studied natural biocides for applications in the field of cultural heritage conservation. However, it is important to compare them with other commonly used plant-derived sources, such as *Origanum vulgare* (oregano) and *Syzygium aromaticum* (clove), in order to highlight their distinctive advantages.

From a chemical perspective, *Thymus* EOs are characterized by high concentrations of thymol and carvacrol, two phenolic compounds known for their strong antimicrobial activity against both bacteria and fungi. In comparison, oregano essential oil also contains significant amounts of carvacrol but lower levels of thymol, whereas clove essential oil is dominated by eugenol, a phenolic compound with more selective and predominantly antifungal activity [48].

A unique advantage of *Thymus* EOs lies in the versatility of their chemical profiles across different species within the genus, allowing the modulation of biocidal activity depending on the composition (e.g., higher thymol or carvacrol content depending on the selected species). This feature provides greater flexibility in treatment selection compared to essential oils with a single dominant component, such as eugenol in clove oil.

In terms of application, *Thymus* EOs have demonstrated good compatibility with historical materials such as stone, wood, and paper, without causing significant chromatic or mechanical alterations even after prolonged exposure. This result has been less consistent in some studies involving oregano and clove oils, which may leave more persistent residues or induce color changes [49].

Moreover, the antimicrobial activity of *Thymus* EOs operates through multiple mechanisms of action—including disruption of the cell membrane, enzymatic inhibition, and interference with protein synthesis—reducing the risk of resistance development compared to isolated monoterpenic compounds [50].

Finally, compared to oregano and clove oils, *Thymus* EOs exhibit lower residual cytotoxicity and a more neutral, less persistent olfactory profile, a relevant factor for application on artifacts displayed in enclosed or publicly accessible spaces.

These characteristics make *Thymus* essential oils particularly suitable for use in the conservation of cultural heritage, positioning them as one of the most balanced options in terms of antimicrobial efficacy, material safety, and environmental sustainability.

7. Conclusion

This review provides an innovative perspective on the use of natural biocides, particularly on *Thymus* essential oils, in the preservation of cultural heritage. The demonstrated antimicrobial efficacy, combined with their lower environmental impact, confirms the potential of these compounds as a sustainable alternative to conventional biocides. However, the study also highlights significant limitations: the variability of experimental outcomes, the lack of standardized protocols, and the possible chemical interactions with original materials represent noteworthy challenges. Therefore, future research should prioritize the development of standardized methodologies, the long-term assessment of material interactions, and the evaluation of compatibility across different substrates, to ensure the safe and effective use of these natural biocides while balancing sustainability with the preservation of cultural integrity.

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