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Review

Eco-Sustainable Approaches to Prevent and/or Eradicate Fungal Biodeterioration on Easel Painting

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Abstract: Eliminating and controlling fungal biodeterioration is one of the most important challenges of easel painting conservation. Historically, the pathologies produced by biodeterioration agents had been treated with non-specific products or with biocides specially designed for conservation but risky for human health or the environment due to their toxicity. In recent years, the number of research that studied more respectful solutions for the disinfection of paintings has increased, contributing to society's efforts to achieve the Sustainable Development Goals (SDGs). Here, an overview of the biodeterioration issues of the easel paintings is presented, critically analyzing chemical and eco-sustainable approaches to prevent or eradicate biodeterioration. Concretely, Essential Oils and light radiations are studied in comparison with the most used chemical biocides in the field, including acids, alcohols, and quaternary ammonium salts. This review describes those strategies' biocidal mechanisms, efficiency, and reported applications *in vitro* assays on plates, mockups, and real scale. Benefits and drawbacks are evaluated, including workability, easel painting material alterations, health risks, and environmental sustainability. This review shows innovative and eco-friendly methods from an easel painting conservation perspective, detecting its challenges and opportunities to develop biocontrol strategies to substitute traditional chemical products.

Keywords: biodeterioration; fungi; prevention; biocidal methods; easel paintings; essential oils; light radiations

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1. Introduction

Cultural Heritage (CH) materials are susceptible to being complexly damaged physically, chemically, and aesthetically by the growing and metabolic activities of living beings, as investigators know as biodeterioration [1]. Many studies have been performed concerning vegetal or microbiological biodeterioration on inorganic materials, such as mural paintings [2–5], stone buildings [6–9], or stone sculptures [10–14]. Easel painting biodeterioration is a less-studied topic, although, in the last ten years, there has been more interest in their biological control and prevention [15–20].

Different investigations worldwide conclude that issues such as climate change and global warming contribute to damaging historical buildings, archaeological sites, museums, and collections physically, chemically, or biologically [21–23]. In this way, new analysis and control methodologies are recently being discussed and developed [24,25]. Concerning biodeterioration, climate change phenomena such as seasonal variations, heavy rain, and global warming raise the risk of microbiological growth in tangible CH due to increased moisture and temperature (Figure 1) [22,26,27]. Due to this biodeterioration damage, biocides, which are generally toxic for humans and the environment, are frequently required.

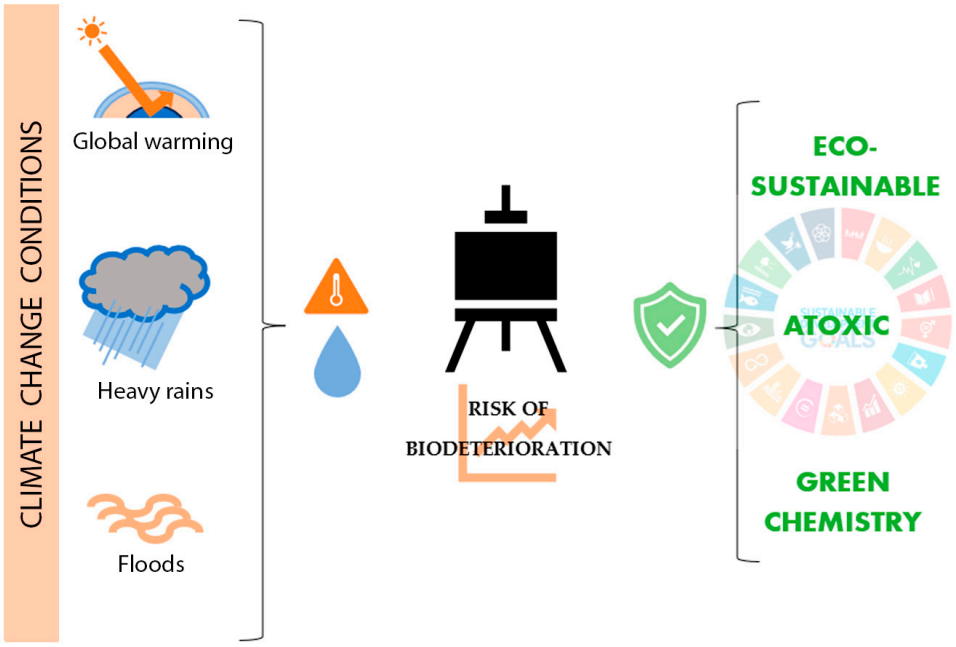


Figure 1. Influence of climate change on easel painting biodeterioration and current trends on its treatment.

Investigating new eco-sustainable and atoxic solutions for CH biodeterioration found broad support in the Sustainable Development Goals (SDGs). SDGs are a group of 17 objectives and 169 targets approved by the United Nations that aim to achieve a fair, healthy, and equal society [28]. Between these 17 objectives, CH plays an important part in SDG No. 11.4, which recognizes it as a fundamental part of human relations and their necessity to be protected. Furthermore, SDGs establish issues directly related to Good Health and Well-Being (SDG No. 4), Climate Action (SDG No. 13), Life Below Water (SDG No. 14), and Life on Land (SDG No. 15) [29]. In this context of promoting new, eco-sustainable, and non-toxic solutions for society's needs, CH conservation should be an active part (Figure 1). Applied to CH conservation, Turo and Medeghini, 2021 [30] defined Sustainability as "all actions aimed at developing protective materials and/or analytical methodologies that fall into the green chemistry family". Green chemistry, moreover, is understood as products or methodologies that do not use substances that are dangerous to humans or the environment [31,32]. In recent years, different investigations reported the benefits of natural origin biocides as Essential oils (EOs) or new physical biocide approaches, such as light radiations as antimicrobial treatments in CH, exported from other disciplines such as medicine, pharmacy, or food industry.

Although easel paintings refer to other supports such as stone [33], metal [34], glass [35], or other techniques, such as gum or proteinaceous binders [36], oil on canvas or wood is the most popular. Due to their historical popularity and their susceptibility to biodeterioration, this paper aims to overview the state of the art of EOs and light radiations as treatment solutions for this type of artwork conservation. In addition, this review analyzes critically their possibilities and future perspectives based on their application in other CH materials or other fields of knowledge (Figure 2).

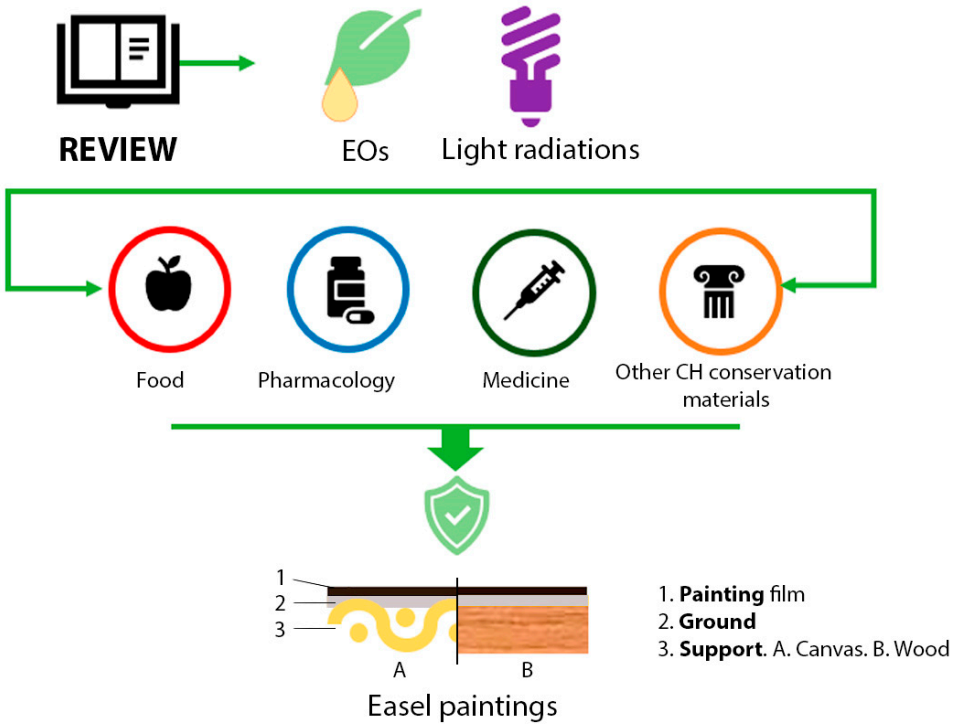


Figure 2. Graphical representation of the investigation aim. Essential oils (EOs); Cultural Heritage (CH).

The bibliography research for this investigation was based on a Boolean search on Scopus, Web of Science, and Google Scholar, using different formulas with the following keywords: "essential oils", "light radiations", "Cultural Heritage", "easel paintings", "oil on canvas painting", "biodeterioration", "antifungal", "disinfection". Critical analysis was performed using the SWOT system (Strengths, Weaknesses, Opportunities, and Threats). The investigation search included all the published papers until December 2023.

2. Fungal Biodeterioration of Easel Paintings

Historical easel paintings have a mixture of materials and layers, increasing their conservation complexity. Materials such as cellulose on the support, rabbit skin glue, egg yolk, linseed oil, or varnishes on the polychromy are some organic materials that can compound an easel painting, although the painting layer also contains inorganic pigments [37,38]. These organic materials can be degraded by fungi if the environmental conditions are favorable (Figure 3). More common fungi are mesophiles, whose ideal conditions revolve around 20–30 °C and a Relative Humidity (RH) higher than 70%, and pH conditions between 4 and 6 [18,20,39–41].

In the specific literature about fungal biodeterioration of easel painting, there are many fungal families that can grow on this kind of surface, of which the most prevalent are exposed in Table 1. All those fungi are mostly from the phylum *Ascomycota*, although there are fungi from the phylum *Basidiomycota* or *Mucoromycota*, which are three types of filamentous fungi. Filamentous fungi are formed by hyphae—large cell filaments—that generally have walls between them and are called coenocytic hyphae. Depending on their function, this kind of fungi has two different types of structures: vegetative mycelium, which penetrates the substrate and absorbs nutrients, and aerial mycelium and fruitful bodies, which are responsible for reproductive function [42] (Figure 4).

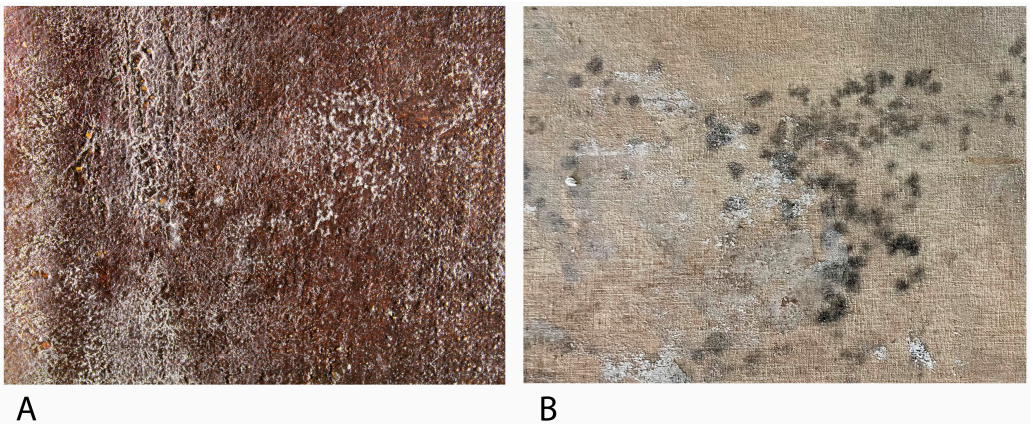


Figure 3. (**A**) Filamentous fungi growth on the obverse a Bartolomé Mongrell's oil on canvas painting (Museu Municipal d'Alzira, València, Spain). (**B**) Fungal growth on the reverse of Santos Juanes Church's (València, Spain) oil on canvas marouflage.

Table 1. Most common fungal species on easel painting.

Species	Genus	Order	Phylum	References
Aerobasidium pullulans	*Aerobasidium*	Dothideales	*Ascomycota*	[38,43–48]
Alternaria alternata	*Alternaria*	Pleosporales	*Ascomycota*	[38,45,47–50]
Aspergillus clavatus	*Aspergillus*	Eurotiales	*Ascomycota*	[38]
Aspergillus flavus	*Aspergillus*	Eurotiales	*Ascomycota*	[45,46,51,52]
Aspergillus fumigatus	*Aspergillus*	Eurotiales	*Ascomycota*	[48]
Aspergillus niger	*Apergillus*	Eurotiales	*Ascomycota*	[43,44,47–51,53,54]
Aspergillus sp.	*Apergillus*	Eurotiales	*Ascomycota*	[41,55]
Aspergillus versicolor	*Aspergillus*	Eurotiales	*Ascomycota*	[43,45,48,50]
Bjerkandera adusta	*Bjerkandera*	Polyporales	*Basidiomycota*	[38]
Chaetonium globosum	*Chaetonium*	Sordariales	*Ascomycota*	[44,47,48,52,56]
Cladosporium cladosporioides	*Cladosporium*	Capnodiales	*Ascomycota*	[43–46,48,50]
Cladosporium sp.	*Cladosporium*	Capnodiales	*Ascomycota*	[41]
Filobasidium magnum *Mucor* spp.	*Filobasidium* *Mucor*	Filobasidiales Mucorales	*Basidiomycota* *Mucoromycota*	[38,41,45]
Penicillium chrysogenum	*Penicillium*	Eurotiales	*Ascomycota*	[20,38,43–45,50,52–54]
Penicillium sp.	*Penicillium*	Eurotiales	*Ascomycota*	[20,41]

The development of fungal strains damages easel painting materials in different ways, causing a multitude of pathologies depending on the substrate and the strain [57]. Generally, fungal growth produces acidification, chromatic changes, structural damage across chemical mechanisms—liberation of fatty acids and enzymatic action— and mycelium growth [19] (Figure 5). Microbial deterioration usually affects the painting support in the first instance, reaching and colonizing the pictorial layers in later phases [58]. The organic nature of these materials presents a large carbon source for microorganisms, which makes them susceptible to biological attack. In addition, the presence of glues or coating pastes applied as sizing treatments increases that susceptibility. Aesthetical damages are generally related to pigment production. *Ascomycota* strains such as *A. niger* produces azanigerones B and C—yellow [59]—or other colors from pale yellow to brown [60]. *Cladosporium* sp. and *Fusarium* sp. produce anhydrofusarubin—red [61,62]—or *A. versicolor* produces asperversin—yellow [63]. Red-orange, β-carotene, is the most produced pigment by *Mucoromycota* as a preventive measure for stress oxidative damages [64].

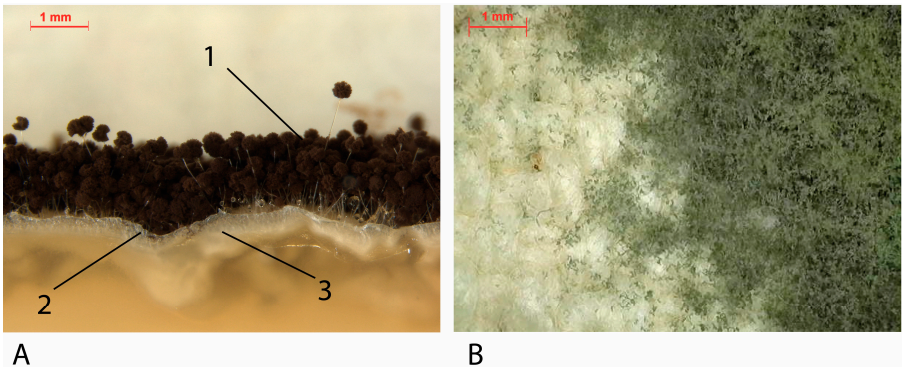


Figure 4. (**A**) Cross-section of *A. niger* cultivated in agar. (**1**) Fruitful bodies (dark parts): spores-production structures. (**2**) Aerial mycelium: white hyphae on surface. (**3**). Vegetative mycelium: white hyphae into the agar medium. (**B**) *Penicillium* spp. fruitful bodies and areal mycelium on a cotton canvas mock-up.

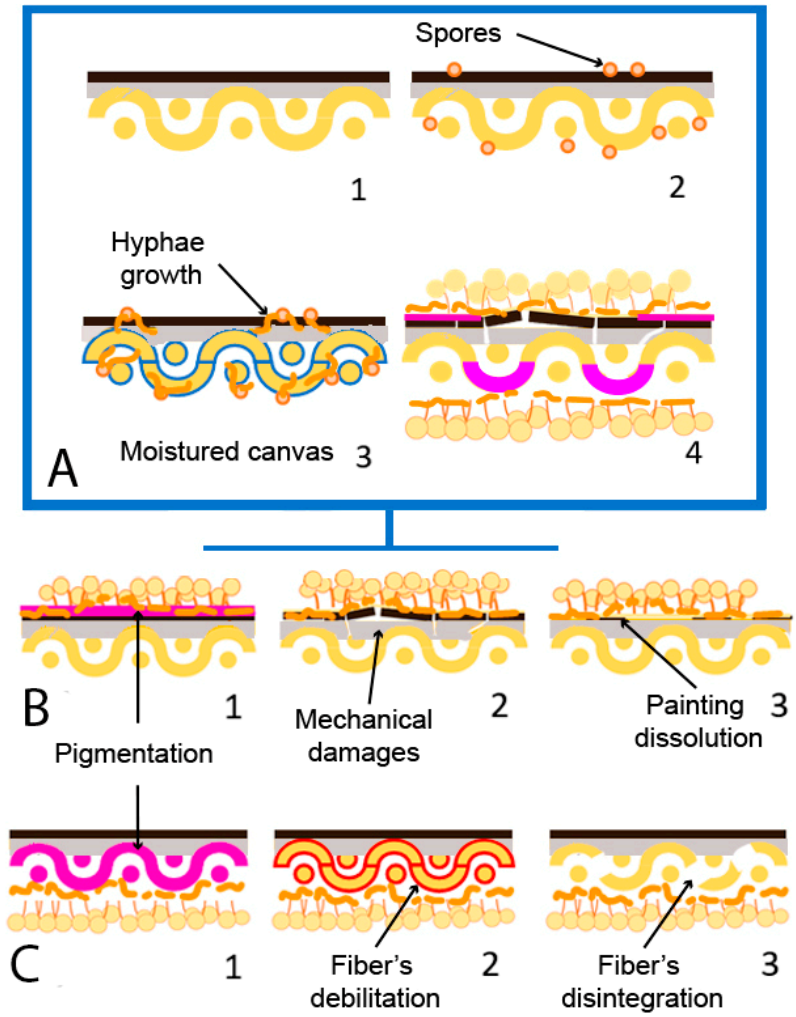


Figure 5. Fungal biodeterioration mechanism and damages on canvas painting (adapted from Poyatos et al., 2018 [19]). (**A**) (**1**) Non-biodeteriorated oil on canvas painting; (**2**) presence of fungal spores (orange) on the surface; (**3**) moisture of canvas (blue) due to RH increase and fungal development. (**4**) Biodeteriorated painting. (**B**) Damages on painting strata; (**1**) Pigmentation (pink); (**2**) mechanical damage of painting film; (**3**) dissolution of painting film. (**C**) Damages on canvas support; (**1**) pigmentation (pink); (**2**) fiber's debilitation (red); (**3**) fiber's disintegration.

2.1. Fungal Biodeterioration of Wood on Easel Paintings

Wood was the most important painting support until canvas popularization in the 16th century. It presents well-known biodeterioration problems due to its use in different industries or as a construction material [65]. Wood fungal biodeterioration can be classified as white rot, brown rot (exclusively by *Basidiomycota*), and soft rot (generally *Ascomycota*), depending on the produced damages [66,67]. Brown rot fungi feed on cellulose and produce brown pigmentation and structural damage on wood, while white rot fungi also feed on lignin and produce discoloration [68]. Soft rot fungi can produce lignin and cellulose, penetrating the surface at various centimeters, producing cavities and erosion [67]. Recently, Afifi et al., 2023 [69] detected biodeterioration damages produced by *Aspergillus* sp., such as discoloration or hyphae penetration into the wooden support of mock-ups based on a historical stucco (Sultan al-Ashraf Qaytbay Mausoleum, Egypt).

2.2. Fungal Biodeterioration of Textile Fibers on Easel Paintings

Starting in the 17th century, textile fibers began to expand as the main artistic support due to their lower cost or technical characteristics, such as the possibility of producing larger and lighter formats, the contribution of texture, or the ease of transport and storage [19,70]. The historical textiles usually used are made of natural fiber fabrics, such as cotton, hemp, linen, or jute. These fabrics' main component is cellulose, which comes from the plant fibers from which the materials are obtained [58,71]. For example, cotton comprises seed fibers, while flax or jute are made from phloem fibers [58]. The main component of the cell walls of tissues of plant origin is cellulose. Cellulose is a polysaccharide formed by a crystalline region with great resistance to biodeterioration and an amorphous region easily attacked by microorganisms, in which the biological attack usually begins [72]. The fungal deterioration of tissues happens mainly due to the production capacity of intracellular and extracellular hydrolytic enzymes and organic acids, using natural fibers as a carbon source for their growth and development. These enzymes are cellulases, breaking cellulose's intramolecular bonds and obtaining glucose molecules [71,72]. That is, they decrease the degree of polymerization of the cellulose molecules [73]. This can cause fiber breakage and thinning and, therefore, a decrease in the individual resistance of the fibers and the textile [72].

In addition, organic acids produce minerals for some phototrophs and have a wide variety of pigments, being the first alteration of fungal biodeterioration and complex to eliminate. Therefore, the amount of cellulose in the tissues will be decisive regarding their vulnerability to being colonized by micro-organisms. Of the fabrics widely used as pictorial textile supports, cotton is the material that has the highest amount of cellulose (90%), followed by linen (80%), hemp (77%), and finally, jute (60%) [58,71]. Factors such as composition or fiber and the structural and chemical characteristics of the textile influence the risk of biodeterioration. Increasing the amount of cellulose—in addition to pectins or pentoses—on the textile composition will increase the biodeterioration susceptibility, too. However, some substances are present in textiles that few microorganisms cannot metabolize, such as lignin [71,74]. Structurally and chemically, the cellulose chains' length, degree of polymerization and crystallinity, the fibers' orientation or the threads' thickness, and their chemical, photochemical, and mechanical degradation must be considered [19,71,75,76].

2.3. Fungal Biodeterioration of Oil Binders in Easel Paintings

On polychromies, fungal biodeterioration affects the chemical and physical stability: while fatty acids acidify the surface, esterases and lipases disaggregate the pictorial layer, damaging the oil medium and producing resistance loss, fracture, and detachments in extreme cases [19,77,78]. Therefore, enzyme liberation supposes triacylglycerols hydrolyzation by lipases and the aqueous part hydrolyzation by esterases, favoring the mycelium growth, which produces microcracks [54,79]. As ideal conditions for fungal growth—high RH—are also prejudicial for easel paintings because of the different mechanical behavior between layers [80], a synergy between biodeterioration agents and other types of alteration

is caused, such as moisture, dust, or environmental contaminants [81]. Contrary to support, pictorial films have more factors in the biodeterioration mechanisms, such as pigments. Paintings with Pb, Zn, Cr, or Cd are more resistant to fungal biodeterioration, while earth pigments are more susceptible [82,83]. Different investigations studied the effect of fungal strains isolated from easel paintings. Salvador et al., 2017 [15] analyzed the biodeterioration of different Giorgio Marini (1836–1905) portraits conserved in museums or private collections in Évora, Portugal. Strains of *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp., and *Mucor* sp. were isolated, attributing their growth to the presence of proteinaceous binders and inadequate climate conditions. Even in outdoor environments, Poyatos-Jiménez et al., 2021 [41] evaluated the biodeterioration of a collection of paintings placed on different Cloisters of Quito, Ecuador. In their study, authors isolated strains of *Aspergillus* sp., *Mucor* sp., *Cladosporium* sp., or *Penicillium* sp. They attributed their biodeterioration pathologies to the semi-tropical environment where the paintings are exhibited.

3. Chemicals Biocides Frequently Used in Easel Painting

Biocides are, according to the European Regulation No. 52/2012—which controls their commercialization—a chemical substance used to control organisms that are damaging to persons, animals, or objects. On CH, most used biocides are organic or inorganic compounds, such as phenols, acids, quaternary ammonium salts (QACs), alcohols (ethanol) or nitrogen-containing compounds, and inorganic compounds like urea derivatives, heavy metals salts, and, recently, metal nanoparticles (NPs) (Table 2) [84]. Depending on their structure, biocides have different biological mechanisms affecting target organisms, but most do not present long-term effectiveness and help new micro-organisms proliferate thanks to microbial resistance. Furthermore, products with these compounds can damage artistic materials. Chemical products used as biocides are divided into oxidative (oxidizing the cell structure) and non-oxidative (inhibiting vital enzyme production), being the second group the most used on CH conservation [85,86].

The biocide mechanism of phenol compounds is characterized by disrupting the fungal cell membrane by ergosterol enzyme inhibition and an oxidative action, supposing the increase in ROS (reactive oxygen species) levels and cell stress [87,88]. The boric acid antifungal mechanism disrupts oxidative metabolism, decreasing ergosterol synthesis and affecting the hyphae by its growth inhibition [89]. On the other hand, low molecular weight QAC biocide activity resides on the cationic immobilization because of the combination of the QAC's positive charge with the negative charge of the fungal cell, producing disorder of charges, the membrane cell disruption, cytoplasmatic compounds liberation and cell lysis [90,91]. Koestler et al., 1993 [92] reported the use of orthophenylphenol (Lysol, Reckitt Benckiser, Parsippany, NJ, USA), inorganic compounds such as sulfuryl fluoride (Vikane, Douglas Products, Liberty, MO, USA), and QAC mixtures (BioMet 66, M&T Chemicals Inc., Rahway, NJ, USA) in North American museums in the last years of the 20th century, although nowadays they are not used. Their study analyses the negative effects of these products on paintings, demonstrating that these materials produced gloss, color, or topography changes, in contrast with gas N, which was also tested.

Table 2. Description of most used chemical biocide on oil on canvas conservation treatments on consulted bibliography [85,93–98].

Biocidal Group	Biocide	Composition	Mechanisms of Action	Treatment Location	Use
Alcohol	Ethanol	CH_3CH_2OH	Non-oxidative/Oxidative Cell membrane damage ROS increase	Reverse/Obverse	Disinfection
QACs Azoles	Biotin R	N-octyl-isothiazolinone (3%–5%) þ 3-iodopro–p-2-ynyl N-butylcarbamate (10%–25%)	Non-oxidative Cationic immobilization Ergosterol biosynthesis inhibition	Reverse/Obverse	Disinfection
QACs Azoles Acids	Biotin T	di-n-decyl-dimethylammoniumchloride 40%–60%, 2-N-octil-2H-isotiazol-3-one 7%–10%, Isopropanol 15%–20%, formic acid (1%–2.5%)	Non-oxidative Cationic immobilization Ergosterol biosynthesis inhibition	Reverse/Obverse	Disinfection
QACs Alcohol	Preventol RI50/RI80	Alkyl dimethyl benzyl ammonium Chloride; (50%), isopropyl alcohol (2%)/Alkyl dimethyl benzyl ammonium chloride; (80%), isopropyl alcohol (2%)	Non-oxidative Cationic immobilization Ergosterol biosynthesis inhibition	Reverse	Prevention/Disinfection
Phenols	Preventol ON	Sodium-2-phenylphenolate	Non-oxidative/Oxidative Ergosterol production inhibition and ROS increase	Obverse	Prevention
Metal NP's's	ZnO NP"s	ZnO	Oxidative ROS increase	Obverse	Prevention
Acids	Boric acids	H_3BO_3	Non-oxidative Ergosterol biosynthesis inhibition	Support	Prevention/Disinfection

In addition, authors such as Di Vito et al., 2022 [93] reflected the traditional use of QAC products, such as Biotin R, Biotin®T (CTS, Vicenza, Italy), Preventol RI50, or Preventol RI80 (Lanxess, Cologne, Germany). Villarquide, 2005 [94] reported using 3% Preventol RI50 for canvas reverse treatment. Villarquide, 2005 [94] also reported the use of phenol liquids derivates for reverse disinfection in different concentrations: pentachlorophenol (Mystox LPL, Catomance, Nottingham, UK) 5% on White Spirit, orthophenylphenol (Lysol, Reckitt Benckiser, Parsippany, NJ, USA) 0.5%–1% on distilled water, and dichlorophen (Permicide, Athea Laboratories®, Milwaukee, WI, USA). In terms of preservation, Romero-Noguera et al., 2020 [95] tested the efficacy of the addition of some biocides on colophony, sandarac, and Manila copal varnishes using biocides reported by Caneva et al., 1998; Nugari and Salvadori, 2003; and Salthammer and Schieweck, 2006 [99–101]: benzalkonium chloride, orthophenyl phenol (Preventol ON, Lanxess, Cologne, Germany), and tributyltin naphthenate (Metatin N, Acima Chemical Industries, Buchs, Switzerland). Spreading and incorporating the biocides into the varnish were the methodologies followed and had positive results on the tested fungal strains (*Pennicilium chrysogenum*, *Chrysonilia sitophila*, and *Phoma herbarum*), with different efficiencies depending on the biocide, the varnish and the method of application. Boric acid is applied in industrial wood as a protective measure, being more effective against brown rot fungi than white rot, as reported by Estevez-Fregoso et al., 2021 [102]. In CH, boric acid is used as a curation treatment for canvas conservation, as Guillén et al. reported in 2011 [103].

Although the ethanol biocide mechanism needs to be clarified, it is reported that it can also damage the membrane cell and coagulate proteins, supposing cell lysis [104].

Furthermore, it is more efficient when mixed with water (70% for total antifungal activity on paper samples), which can be related to the protein coagulate/denaturation effect. However, surfaces cleaned with ethanol are faster recolonized, probably because of their non-sporicidal activity and the spread of spores during the cleaning process [104,105]. Calvo, 2002 [106] reported the traditional use of ethanol for textile support disinfection.

Abbas et al., 2012 [107] analyzed the antifungal capacities of inorganic compounds, such as heavy metals compounds: mercury (II) chloride ($HgCl_2$), copper (II) acetate ($Cu(CH_3COO)_2$), cadmium (II) chloride ($CdCl_2$); and urea ($(NH_2)_2CO$) being more effective the inorganic salts of heavy metals. In recent years, there have been experiences of using TiO_2 and ZnO nanoparticles [96,108] on coating materials applied to paintings or cellulose materials, such as paper or wood [109,110], as a green solution for biological growth preservation. Franco-Castillo et al., 2021 [111] reported that the antifungal activities of ZnO and TiO_2 nanoparticles are their photocatalytic capacities, with some differences. Generally, both can create ROS from O_2 or H_2O molecules in the atmosphere, such as superoxide anions radicals, hydroxide radicals, and hydrogen peroxide, which can kill micro-organisms across oxidative reactions on the membrane cell. For these reactions, and the difference between both, TiO_2 nanomaterials need UV radiation or solar light, while ZnO nanoparticles are effective even in dark conditions.

In addition to the biocides mentioned above, some authors suggest using azoles [97]. These antifungals avoid ergosterol biosynthesis in the fungal membrane cell, inhibiting the enzyme P-450 sterol 14α-demethylase. On organic materials conservation, such as easel paintings, Sameño Puerto, 2018 [97] reports that the most used antifungal products are azole derivates or mixtures, such as Biotin T—in combination with QAC's—Biotin R and Rocima GT (Lanxess, Cologne, Germany)—added to QAC's and formaldehyde [112], on cellulosic materials.

The problem of some of the traditional biocides used in easel painting conservation and restoration processes and exposed in this chapter is their toxicity, which is noted by the safe use recommendations in each Safety Data Sheet. Although the use of Personal Protective Equipment is necessary for all curative treatments—even when natural biocides are being used—and the conventional biocides concentrations generally are low, the toxicity for humans, non-target organisms, and the environment opened in the last 10 years, new investigation lines for the study and use of green biocides on CH conservation [113–115]. In European Regulation No. 528/2012 [116], the use of QACs is permitted in concentrations between 100 and 250 ppm (mL/L) and, higher than 6%, can be prejudicial for skin, eyes, or mucosa [70]. Other products, such as azole derivates, have been reported as a risk for aquatic forms of life because of the river's contamination [117], while phenols can damage humans in different ways, producing acute or chronic injuries [118]. The high toxicity of heavy metals, such as copper or mercury, on humans or sea organisms has also been reported [119,120]. Recently, an interesting investigation published by Casorri et al., 2023 [98] reviewed the literature on the toxicity of different chemical biocide products used in CH conservation, pointing out natural biocides as good alternatives due to synthetic biocide's health risks. In addition, the high hygroscopicity of textile fibers of easel paintings makes it impossible to apply treatments in the liquid phase. This can lead to excessive wetting and penetration and an increase in the dimensions of the textile, creating such harmful alterations as the detachment of the pictorial layers. For this reason, fungal disinfection on textile supports is usually applied with dry treatments or gelled solutions [121]. The characteristics and needs of pictorial textile support is among the first factors to consider in searching for effective and harmless alternatives.

4. Essential Oils as a Green Biocide

EOs are liquids and volatile organic compounds (low molecular content) extracted from plant regions such as flowers, peels, stems, or seeds. EOs result from the secondary plant metabolism participating in different biological mechanisms of the plant [122]. They have different functions in defense plant activity, such as antimicrobial mechanisms. Gen-

erally, EOs contain 20 to more than 100 single components, of which only 2 to 4 are in high concentrations (20%–70%) [123,124]. Mono (C10) and sesquiterpenes (C15), such as carbohydrates, phenols, alcohols, ethers, aldehydes, and ketones, are their main components, being 80%–90% of the EOs. However, each has different structures, defining the EO peculiarities [123,125,126]. More than 150 types of EOs can be extracted from 30,000 different plants, varying in composition depending on the species, the organ, and the season [125], and are used in multiple fields, such as medicine, agroalimentary, or pharmacological industries because of their antimicrobial, antitumor, antioxidant, antidiabetic, or insecticidal capacities [123,127,128]. EOs can be extracted by steam distillation, solvent extraction, cohobation, and maceration, although there are more effective methods, such as supercritical fluid, microwave-assisted extraction, and ultrasound-assisted extraction [122,128].

EO components such as hydrocarbons, alcohols, phenols, lactones, esters, or aldehydes are responsible for antimicrobial activities, probably acting each component in its own way [129]. The antifungal mechanism of EOs can be influenced by the lipophilic nature and low molecular weight of terpenes/terpenoids, allowing cell membrane disruption, sporulation inhibition, and germination [130]. The cell membrane and cell wall are the main structural elements of fungal cells and are composed mainly of ergosterol, chitin, b-glucans, and mannan, respectively. EO compounds can inhibit and stop the production of these substances, which causes cell disruption and death. Furthermore, EOs affect one of the most critical cell organelles, the mitochondria, where ATP biosynthesis and the respiration process succeed and coagulate the cytoplasm. EOs can damage this organelle, producing a variation in the biological activity and causing cell death (Figure 6) [126,130–136].

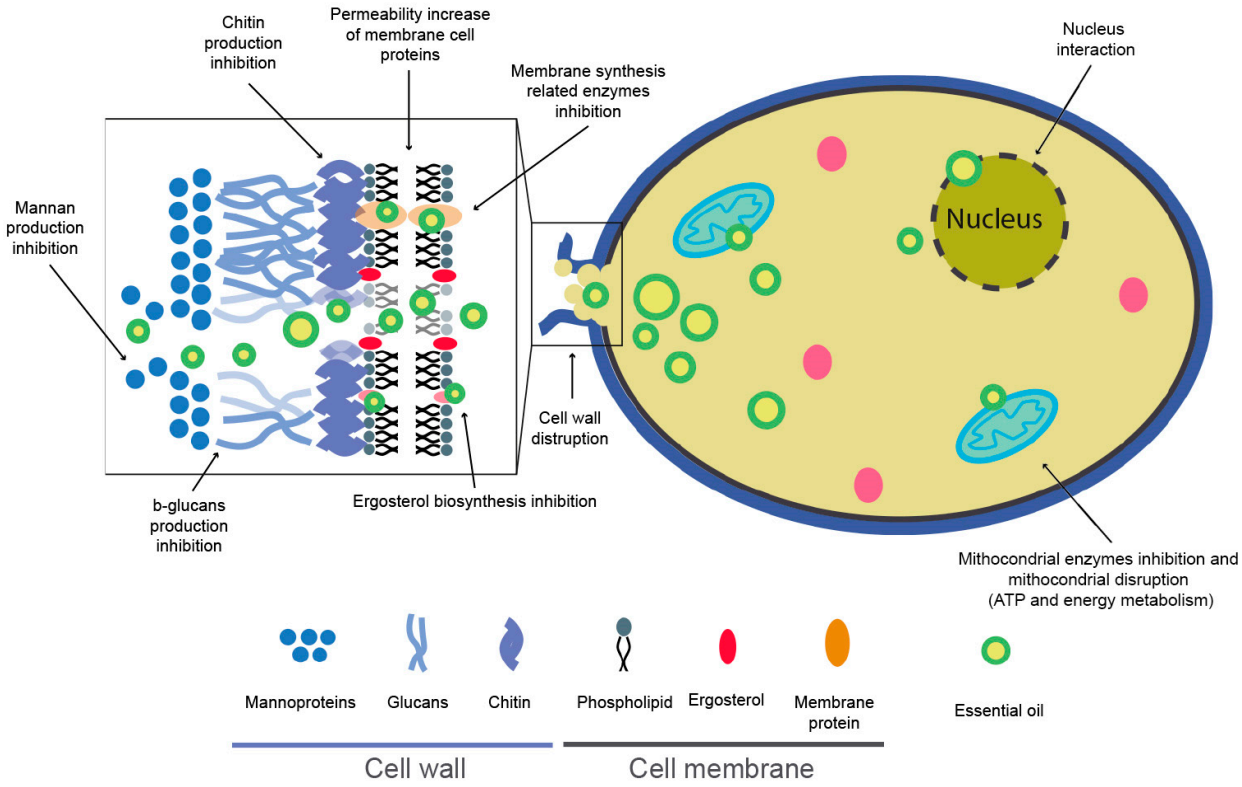


Figure 6. Schematic representation of possible essential oils (EOs) biocide mechanism [123,126,127,130,131,134–136].

EO toxicity and its current legislation are complex and discussed further in the critical comparison section. The reason is that although the EO toxicity is considered low to moderate in mammalians (LD_{50}: 800–3000 mg·kg^{-1} in rodents) [136], there are components of EOs that are toxic also in small (LD50 0.1–1 g/kg [137]) [138] and huge doses (lethal doses of 350 g to a 70 kg adult [137]). In this way, it is reported that some components, such

as carvacrol, pulegone, safrole, psoralen, bergapten, thujone, camphor, pinocamphone, and cinnamaldehyde, can produce skin sensitization, dermatitis, or neurological or endocrine issues [137–140]. Therefore, EO safety is based on their chemical structure and dose, and in comparison, chemical biocides used in conservation are a less hazardous alternative [114].

4.1. EOs Application on Easel Painting Conservation

The use of essential oils in easel painting conservation treatments is emerging, and there are few references. For this reason, this chapter overviews different investigations about the study of EO application on easel paintings and related materials, including wood, textiles, and polychromies (Table 3). Recent references, according to the use of EOs in easel paintings, are generally *in vitro* studies on plates or mock-ups [49,55,141,142] and, in just one, applied the EOs *in vivo* [47].

Table 3. Use of EO on easel painting conservation.

EOs	Action and Application Method	Microbiological Assays	Fungal Strain	Reference
O. majorana [n.a.] *C. camphora* [n.a.] *S. aromaticum* [n.a.] *O. basilicum* [n.a.]	Prevention Spraying	*In vitro* on plates and on oil on canvas painting mock-ups	*A. alternata* *A. niger*	[49]
M. didyma [n.a.] (Hy and EO) *M. fistulosa* [n.a.] (Hy and EO) *C. zeylanicum* [a] *C. aurantium* [n.a.] (Hy)	Prevention/Curation Spraying o/w emulsion	*In vitro* and reverse treatment of original painting	*A. alternata* *A. niger* *A. pullulans* *C. globosum* *C. cladosporioides* *P. citrinum*	[47]
M. alternifolia [b] *R. officinalis* [n.a] *T. vulgaris* [n.a]	Prevention Additive	*In vitro* on lining adhesive mock-ups	*A. niger* *P. chrysogenum* *C. globosum*	[70]
S. aromaticum [c] *O. vulgare* [d]	Prevention Vapor	*In vitro* on plates	*P. chrysogenum* *Penicillium* sp. *C. foveolata* *A. versicolor* *C. parahalotolerans* *Trichocomaceae* *Chaetomiaceae*	[141]
P. nigrum [ebfg] *C. annuum* [ebfg] *C. verum* [ebfg] *Z. officinale* [ebfg] *C. camphora* [ebfg]	Prevention Vapor	*In vitro* test on oil on canvas painting and cotton canvas mock-ups	*A. niger* *A. flavus* *C. halotolerans* *N. goegapense*	[143]
T. vulgaris [n.a.] *S. aromaticum* [n.a.] *P. odorantissimum* [n.a.]	Prevention	*In vitro* test on plates and sprayed on painting mock-ups	*Aspergillus* sp.	[55]
E. caryophyllata [c]	Curation	*In vitro* test on wood mock-ups	n.a.	[144]
C. nardus [n.a.]	Prevention Additives	*In vitro* test on adhesive film and oil-on-wood mock-ups	*A. niger*	[142]
E. camaldulensis [b] *P. rigida* [h]	Prevention	*In vitro* test wood mock-ups	*A. alternata* *F. subglutinans* *C. globosum* *A. niger* *Trichoderma viride*	[145]

EOs extraction plant parts. n.a.: Non-available; a: bark; b: leaves; c: buds; d: flowers; e: seeds; f: steams; g: fruits; h: wood.

Elsayed and Sabana, 2018 [49] tested the effect of EOs extracted from *Origanum majorana*, *Cinnamomum camphora*, *Syzygium aromaticum*, and *Ocimum basilicum* (Elnekity, Mansoura, Egypt) at 20%–60% for plates assays and 40%–80% on mock-ups against stock strains

of *A. alternata*, and *A. niger* (Plant Pathology Department, Mansoura University, Egypt). In their investigation, the authors used the poisoned food method as an *in vitro* antifungal test (20%–60% EO concentration) on plates and mock-ups, simulating archaeological oil paintings (40%–80% EO concentration). The best *in vitro* plate results were achieved by the highest concentration in all essential oils and strains: *S. aromaticum* and *C. camphora* against *A. alternata* at 60% obtained 76.00% and 74.44% of inhibition, respectively. In comparison, at the same concentration, both EOs against *A.* niger achieved 61.89% and 74.78% of inhibition. The authors sprayed the different EOs on the surface of mock-ups as a preventive treatment. After the inoculation and incubation of both strains, the best result was achieved with *C. camphora* (80%) obtaining an inhibition of 98% on *A. alternata* and 95.6% on *A. niger*. All essential oils at 80% concentration achieved <90% of spores' mortality.

Gatti et al., 2021 [141] tested *S. aromaticum* (buds, Fitomedical, Binasco, Italy) and *Origanum vulgare* EOs (flowers, Fitomedical, Binasco, Italy) diluted with ethanol 70% at a 2:1 ratio against fungal strains isolated from a Giovanni Maria Mossa's oil on canvas painting: *P. chrysogenum*, *Penicillium* sp., *Cephalotheca foveolata*, *A. versicolor*, *Cladosporium parahalotolerans*, and other fungi from Trichocomaceae and Chaetomiaceae families. Using an *in vitro* test on agar plates, the authors tested the effect of both EOs in contact (disk diffusion assay) and contactless (vapor phase, overturning the agar plate, and adding the EOs on the lid). In the contact test, both EOs showed high antifungal activity against *P. chrysoenum*, *C. foveolata*, *Cephaloteca* sp., *A. versicolor*, and Chaetomiaceae. However, *O. vulgare* EO performed better in the rest of the strains. Because of that, this EO was chosen for the contactless test. Due to the high antifungal activity of the *O. vulgare* volatile compounds, the inhibition was higher than the contact test on the same strains (*P. chrysogenum*, *Aspergillus* sp., *A. versicolor*, and *C. foveolata*).

Furthermore, Martin Rey, 2017 [70] studied the incorporation of *Melaleuca alternifolia* (leaves), *Rosmarinus officinalis*, and *Thymus vulgaris* EOs and *R. officinalis* and *T. vulgaris* extracts (Manuel Riesgo SA, Madrid, Spain) as conservative substances into synthetic and semisynthetic adhesive mixtures (Plextol B500, Synthomer plc, London, UK, Klucel G, CTS España, Getafe, Spain) for canvas reinforcement. In the study, the author inoculated different biocide-adhesive dried films (7% of EOs concentration) with *A. niger*, *P. crysogenum*, and *C. globosum* from a stock collection. Regarding antifungal abilities, all EOs and extracts tested in the study showed fungal growth total inhibition after four weeks of incubation. Similarly, Lee et al., 2018 [142] tested the addition of *Cymbopogon nardus* EO (Anshin camphor oil, Ltd., Hsinchu, Taiwan) on a gelatin solution, a very common adhesive used in oil on canvas consolidation. At 0.5% and 1%, the EO inclusion achieved the total inhibition of a stock *A. niger* strain (Colección Española de Cultivos Tipo, Valencia, Spain) after four weeks of incubation on adhesive films and tung oil-on-wood mock-ups treated with the adhesive mixture. However, as the authors reported, the results obtained on the mock-up test are influenced by the antifungal effect of tung oil.

In the research of Minotti et al., 2022 [47], the authors develop a treatment methodology for "Il Silenzo", a Jacopo Zucchi oil painting conserved at the Uffizi Museum. In their study, the authors test EOs and Hydrolates (Hys) from *Monarda didyma* and *Monarda fistulosa* (Dept. of Agricultural and Food Sciences, University of Bologna, Italy), EO from *Cinnamomum zeylanicum* (bark, PRANAROM International, Ghislenghien, Belgium), and Hys from *Citrus aurantium* (Erboristeria Magentina s.r.l., Turin, Italy). Fungal strains were isolated from the painting and identified as: *A. alternata*, *A. niger*, *A. pullulans*, *C. globosum*, *C. cladosporiorides* and *P. citrinum*. To improve the hydrophilicity of the EO, the treatment was performed using an EO–Hy oil in water (o/w) emulsion. The authors used a broth microdilution susceptibility test to determine that *C. zeylanicum* EO and *C. aurantium* Hy achieved the best performance. The highest Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC) were 0.008% v/v and 0.125% v/v and 6.25% v/v and 3.12% v/v, respectively, on all strains tested. Zucchi's painting was applied by spraying the o/w emulsion, where the oil phase was composed of the EO–Hy mixture.

Elsayed et al., 2023 [143] tested self-extracted essential oils from seeds, leaves, stems, or fruits of *Piper nigrum*, *Capsicum annuum*, *Cinnamomum verum*, *Zingiber officinale*, and *C. camphora* on *A. niger*, *A. flavus*, *C. halotolerans*, and *N. goegapense* isolated from different objects of Egyptian CH, among which were three oil paintings. Using *in vitro* contactless assay on oil on canvas painting mock-ups, the authors reported that *P. nigrum* L. EO achieved the most important inhibition against *A. flavus* (98.81%), *C. halotolerans* (97.68%), and *N. goegapense* (99.85%), while EO of *Z. officinale* achieve the best results against *A. niger* (98.21%). The same results were achieved in terms of spore death.

On an oil-on-wood painting from the Saqqara excavation (Giza, Egypt), Geweely et al., 2019 [55] studied 12 different EOs (Natural Oils Department, National Research Center in Dokki, Egypt) as antifungal agents at concentrations of 0.125 µL/mL, 0.25 µL/mL, 0.5 µL/mL, 0.75 µL/mL, 1 µL/mL, using a similar *in vitro* methodology to Elsayed and Shabana, 2018. All EOs were tested on 16 species of fungi isolated from the studied objects. *Aspergillus* sp. was the genera with the highest frequency of occurrence. They conclude that the best EOs for the inhibition of the growth of tested fungi are *T. vulgaris* (MIC: 0.25–0.75 µL/mL), *S. aromaticum* (MIC: 0.5–1.0 µL/mL), and *Pelargonium odorantissimum* (geranium) (MIC: 0.5–1.0 µL/mL), improving their antifungal activity when increasing its concentration.

On wood, Pop et al., 2020 [144] tested *Eugenia caryophyllata* (buds, Steaua Divina Ilfov, Romania) EO applied by brush at 5%–10% in ethanol on samples of *Fagus sylvatica* wood. At the same concentrations as Diffusit S (Wolman, Sinzheim, Germany) at 10% and Biotin T (CTS, Vicenza, Italy) at 5%, *E. cayophyllata* EO achieved the best results. However, any biocide achieves total inhibition after 23 days. Although it is not a CH-related investigation, some interesting conclusions about wood protection can be attached from Salem et al., 2016 [145]. In this paper, the authors test the efficacy of self-extracted *Eucalyptus camaldulensis* (leaves) and *Pinus rigida* (wood) EOs on the vapor phase (at concentrations of 5000 ppm, 2500 ppm, 1250 ppm, 625 ppm, 312.5 ppm, and 156.25 ppm) as prevention treatment against *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *Trichoderma viride* on *Pinus sylvestris*, *Pinus rigida* and *Fagacea sylvativa* wood samples. Authors find a complete inhibition of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride* using *P. rigida* EO at 5000 ppm, while all strains grew completely at 156.5 ppm except for *C. globosum. E. camaldulensis* EO just reduced the growth of *C. globosum* at 5000 pm and 156.25 ppm and *F. subglutinans* at 5000 ppm. No inhibition effect was found in *A. niger* and *T. viride* with *E. camaldulensis* EO.

4.2. Issues about the Effect of EOs on Easel Painting Materials

Due to the aesthetical, historical, or symbolic values of CH, there are different requirements for any material used in its conservation. Some of them are compatibility with artistic materials, reversibility—or retractability—and chemical, physical, and aging stability [146]. Their volatility and hydrophobicity make EO substances difficult to apply, although they are good alternatives for air control in their vapor phase [147,148]. As a hydrophobic material, it is difficult to obtain a stable mixture with water, the most used and preferred solvent in any conservation treatment, especially for cleaning purposes. Furthermore, their use on CH artifacts can pose a risk to their preservation in terms of binders and pigment solubility during the application or chromatic changes due to aging [141,149]. In the easel painting conservation-related bibliography, authors tested material properties such as mechanics, chemical stability, or colorimetry before and after EO treatments and artificial aging.

In terms of colorimetric changes, the study of Elsayed and Sabana, 2018 [49] determined no significant changes in non-biodeteriorated mock-ups (black, white, blue, red, yellow) treated with 80%–100% EO concentration after thermal aging. Color changes were appreciated in biodeteriorated mock-ups because of the fungal remains. In the same way, Minotti et al., 2022 [47] pointed out no changes in color on oil-on-canvas mock-ups after aging but some variations on tempera ones. Martin Rey, 2017 [70] reported in her inves-

tigation changes in the color of dried adhesive samples mixed with *Cympopogon* sp. and *Mentha* sp. EO.

Furthermore, Veneranda et al., 2018 [149] reported in an interesting paper that different EO constituents, such as eugenol, thymol, and cinnamaldehyde, were yellowing after two months of aging in sunlight and UV light, added to volumetric mass density changes in the case of cinnamaldehyde. Although non-molecular changes were detected in this study by Fourier Transformed Infrared (FTIR) in eugenol and thymol, truxillic and truxinic acids as photo-dimerization products appeared on the cinnamaldehyde spectra. The vapor phase methodology employed by Elsayed et al., 2023 [143] in their study showed non-significant changes in color values on non-biocolonized mock-ups. Furthermore, the use of *P. nigrum* L. EO vapor does not compromise the molecular structure of paintings, as was demonstrated with their FTIR analysis.

Martín Rey, 2017 [70], Lee et al., 2018 [142], and Doménech-Carbó et al., 2015 [150] reported the effect on the mechanical properties of EO-adhesive mixtures tested. First, the addition of EOs on Plextol–Klucel mixtures supposes a barrier effect, improving their moisture-loss stability and their reversibility due to the Tg reduction and their ductility increase. Furthermore, after their thermal aging, the adhesive mixture's flexibility increased by 5% [70]. In the case of gelatin-based adhesive, the addition of *Cymbopogon nardus* EO and glycerol suppose an increase in viscosity—although lower than synthetic alternatives—and adhesion, in addition to the reduction of the Elastic modulus [142]. However, these effects are due to a synergy between EOs and glycerol [150].

Regarding mechanical properties, the food industry contributes interesting investigations about natural polymer–EO mixtures for edible coatings. Although these materials are not applied to artistic materials, they can be a reference for the mechanical responses of similar mixtures applied to easel painting. For example, Ganeson et al., 2022 [151] studied the functionality of edible films using cinnamon oil-gelatin emulsion and reported a decrease in their tensile strain and an increase in Young's modulus. The authors explain this phenomenon as the result of increased interactions between the EO–surfactant and the polymeric network of gelatin.

5. Light Radiations as a Green Physical Biocide

Although chemical methods have been the most used to disinfect cultural heritage, other treatments, such as physical ones, have also been used. Physical methods produce a biocidal effect by altering the molecules of biodeteriogenic organisms, fracturing their chemical bonds, and causing modifications in the essential biopolymers and their enzymes. Within this typology of disinfection treatments, we can make two distinctions: the systems that are based on the application of electrical and electromagnetic radiation, such as ultraviolet or gamma radiation, and those that are based on the modification of physical parameters, such as pressure or temperature [71]. The overall advantages of all of them include the simplicity of application, their harmlessness to the environment and their users, and the fact that the development of genetically resistant populations is rare, as can happen with chemical biocides [103]. On the other hand, light radiation is currently widely used in treatments in other areas of study to stop biological propagation. It is also beginning to be applied within the conservation of historical monuments [152,153].

The application of electromagnetic radiation, such as gamma or ultraviolet (UV) radiation, has been extended for a multitude of actions during recent decades. These radiations have opened a new perspective as a disinfection treatment for fungi, molds, and insects that are harmful to the health of archivists, librarians, and restorers within the conservation of cultural heritage due to the inactivation capacity of nucleic acids of organisms [152] Electromagnetic sources, such as gamma, Rötgen, and UV radiation, have an energy range of weak interaction (10^2 to 10^7 electronvolts), which limits their penetration into materials, being one of the biggest drawbacks of this type of treatment, in addition to their activity limited to the exposure time [154].

UV lighting is one of the most used radiations as an antimicrobial system in sterilizing different compounds, materials, and spaces, such as water and air, food, hospitals, microbiological laboratories, etc. [71,155,156]. Many studies demonstrate the infeasibility of propagation and metabolic activity, such as cyanobacteria, microalgae, and plants, after exposure to UV radiation. [157]. The biocidal capacity of UV lighting is directly related to its absorption by the nucleic acids of organisms. The dimers formed between the adjacent thymines of the polynucleotide chains of DNA and damage to the DNA/RNA are the main photoproducts of treating microorganisms exposed to UV radiation, which is summarized in chemical alterations that do not allow the development of biodeterogens. The microbiological inactivation capacity of this lighting will depend on several factors directly related to the radiation, such as its intensity, wavelength, and penetration. Other factors are the distance at which the object to be disinfected is located, environmental conditions, or the sensitivity of microorganisms to illumination. UV radiation is divided into four categories depending on its wavelength: UV-A (315 nm–380 nm), UV-B (280 nm–315 nm), UV-C (200 nm–280 nm), UV vacuum (100–200 nm), with the categories with lower values being those with the highest energy and lowest penetration [158].

The biocidal power of UV occurs in intensities between 200 nm and 300 nm. In the UV-C category, its maximum effectiveness is between 230 nm and 275 nm, with 254 nm of UV usually used for sterilization [156,159–161]. This radiation is considered dangerous for human health, as it is a potential cause of dermatitis, skin cancer, burns, peeling, etc. Been one of the main causes of its disuse in the treatment of heritage objects. Therefore, the use of protective equipment is essential [156]. Likewise, although it has great antimicrobial power at these intensities, its penetration is limited, making it impossible to inactivate the organisms that have penetrated deeply into the interior of the materials. In addition, exposure to ultraviolet lighting can cause chemical alterations, mainly in protein, cellulosic materials, and pigments, and aesthetic damage, such as chromatic changes and yellowing [71].

The devastating consequences generated in the health, economy, and education of human society due to the 2019 (COVID-19) pandemic caused by SARS-CoV-2 encouraged research into disinfection systems for pathogens that are harmless to humans. Although 254 nm UV-C lighting is a good microorganism disinfection system, due to its harmful nature to human health, it is considered inadequate radiation for continuous application in indoor areas with an accumulation of people, such as schools, shops, offices, health centers, and hospitals [162]. Therefore, the challenge researchers face is implementing UV-C light in inhabited spaces [163]. Far-UVC illumination (200–230 nm) is being investigated as one of the alternatives to UV-C (254 nm). By presenting a penetration of the cells of microorganisms like UV-C (254 nm), its disinfection power is similar. Although its greatest advantage is its lower penetration into the skin's cellular barriers, its harmlessness to mammals [162,164].

Researchers such as Buonanno et al., 2013 [165] et al. and Woods et al., 2015 [166] were pioneers in the study of Far-UVC (207–222 nm) as a substitute for skin antiseptics and the prevention of surgical infections. Determining that it is less harmful lighting than UV-B for the skin due to its limited penetration into human cells [165,166]. With the 2020 epidemic and the need to decontaminate areas contaminated by SARS-CoV-2, many researchers focused on verifying its effectiveness against the virus, among others, and its applicability in inhabited areas, concluding in its adequate viability [156,163,167]. Although the research being carried out around this lighting focuses mainly on the inactivation of bacteria and viruses, some of the lamps most currently used by different researchers such as Buonanno et al., 2020 [163], Narita et al., 2020 [156] or Panzures et al., 2023 [164], such as the Care222® Far-UVC Excimer Ultraviolet Germicidal Irradiation (UVGI) lamp, confirm its effectiveness against yeasts and fungi such as *Candida* spp., *Penicillium expansum*, and *A. niger* [168].

5.1. The Application of Light Radiation on Cultural Heritage

As indicated by Caneva et al., 2000, the chemical alterations UV lighting can cause in cellulosic and protein materials such as pigments mean that their use has been mainly

reduced to stone, caves, and monuments [155,169]. In recent years, several researchers have used light radiation as a treatment against the proliferation of different organisms (Figure 7 and Table 4) [153,155,157,169–179]. Pfendler et al. 2019 [155] have proven that 254 nm UV illumination can eliminate mycelia of four fungi (*Penicillium* sp., *Geomyces* sp., *Rhizomucor* sp., *Engydonyium album*) of the six studied species found in French and Swiss caves, going so far as to affirm that it is a more economical, efficient, fast, and environmentally friendly treatment than chemical biocides [86,177]. Despite the little current information regarding the use of this treatment in easel paintings, authors such as Reverter-Carrión et al., 2018 [180] confirmed its efficiency for the disinfection of fungi recurrently present in paintings on canvas such as *A. niger*, although presenting greater resistance probably due to the UV absorption capacity of the melanin present in this fungus. Likewise, Borderie et al., 2014 [157] confirmed the suitability of applying UV-C treatment to eliminate other organisms, such as algae, without damaging prehistoric paintings composed of mineral pigments.

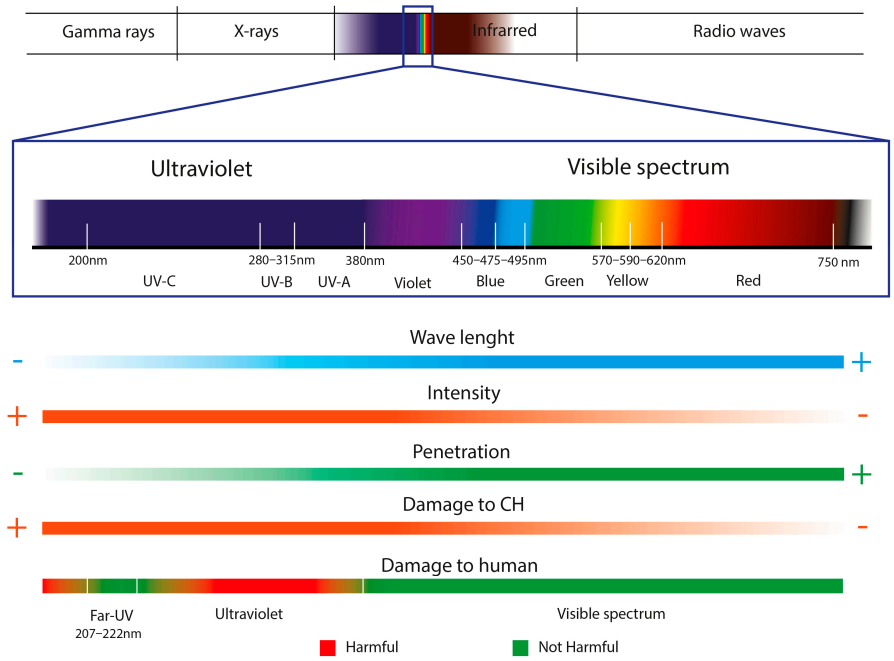


Figure 7. Characteristics of light radiation used as a disinfectant treatment in Cultural Heritage (CH).

Likewise, the less energetic wavelengths within the UVR range (UV-A/UV-B) are also being tested for treating heritage monuments, mostly cyanobacteria [153,181,182]. Although UV-B rays have a lower energy power than UVC, the damage they cause is similar. Within the RUV range, UV-B (280–315 nm) includes the range of wavelengths most harmful to humans, which can cause everything from sunburn to skin cancer [182]. Pozo-Antonio and Sanmartín, 2018 [153] tested a treatment based on UV-B irradiation and the subsequent application of benzalkonium chloride on granite colonized by green algae and filamentous cyanobacteria, obtaining ideal results for the eradication of biofouling in granite, similar to those obtained with UV-C. The studies carried out on the application of UV-B in fungi are quite limited. For example, Selbmann et al., 2011 [183] studied the resistance of two Antarctic fungi, *Cryomyces antarcticus* and *Cryomyces minteri*, verifying their great resistance to this lighting due to the high resistance of Antarctic fungal strains. On the other hand, UV-A (315–380 nm) can also interfere with the cellular processes of organisms such as cyanobacteria [181]. Castenholz and García-Pichel, 2012 [182] indicate that UV-A presents similar effects to UV-B but with milder consequences, the main effects of the damage caused by radiation being the production of reactive oxygen that is also emitted by blue and violet waves.

During the last decade, it has been observed that outdoor lighting in stone monuments has biostatic effects on algae and cyanobacteria [184]. According to Mendez et al., 2023 [184], to minimize the impact that the lighting of monuments can have on animals, humans, and plants, this lighting should focus mainly on the yellow part of the spectrum (570–620 nm), using the smallest possible amount of blue light (380–495 nm), that is, low-energy lighting. A clear example of treatment using low-energy radiation is the Nerja Cave in Malaga (Spain), in which green lighting (500–555 nm) was applied to reduce biofilms composed of cyanobacteria and red and green algae [185]. Blue light (380–495 nm) has also been used in the Roman Catacombs of St. Callistus and Domitilla in Rome (Italy) to reduce bacterial biofilms [184,186,187]. Researchers like Sanmartín et al., 2021 [179] have gone further and combined radiation with low energy wavelengths and UVR. Specifically, the combination of UV-A and UV-B with red LED (670 nm) has been studied as a treatment for subaerial biofilms formed on the granite facades of the Xelmírez Palace and the Monastery of San Martiño de Pinario (Santiago de Compostela, Spain). Obtaining disparate results depends on the species of cyanobacteria and algae and the combination of radiation. Achieving stimulation of the proliferation of *Isocystis* sp. and *Stichococcus bacillaris*. Preventing the growth of *Bracteacoccus minor*, as with the application of UV-A with red LED [179].

5.2. Issues about the Effect of Light Radiations on the Textile of Paintings

In addition to its low penetration, as indicated by Caneva et al., 2000 [71], the most problematic disinfection treatment with light radiation (UV-C) is its chemical interaction with artistic materials. The most vulnerable pieces are those composed of pigments, cellulosic, and protein materials, such as easel paints [71]. Vaillant, 2003 [188] describes how UVR can precisely damage cellulosic supports, indicating that its action can cause the photolysis of cellulose molecules, their photo-oxidation, and the breaking of the chains, reducing the degree of polymerization and increasing reducing groups in the fabrics of paintings and paper works. Alterations may lead to modifications in these materials, such as increased hygroscopicity or decreased mechanical resistance.

As it is not a commonly used technique, there are no significant references to the degradation caused by UV-C disinfection in artistic fabrics. Despite this, in different industries, the study of the resistance to high-energy radiation of materials such as fabrics is recurrent, which allows us to deduce the degradation that canvas paint could suffer [189]. As Vaillant, 2003 [188] indicated, and Cuden et al., 2023 [189] reaffirmed, photodegradation is the main damage mechanism that UV produces in polymers due to its high energy concerning visible illumination, causing physicochemical modifications. The reduction in molecular weight caused by photodegradation can cause changes in physical properties such as extensibility and resistance and aesthetic changes such as color, texture, or brightness alterations [190]. It is also essential to know that the higher the energy of the UVR, the greater the degradation will be since it will more easily break the chemical bonds of the textile fibers [189]. These modifications are not immediately observable but develop over time, dependent on the radiation exposure conditions [189,191].

Another application of UV radiation that shows us its great degradation power on materials is aging regulations, which, for example, are frequently used to test the durability of materials. Regulations such as "ASTM G154-16: Standard Practice for Operating Fluorescent Ultraviolet (UV) Lamp Apparatus for Exposure of Nonmetallic Materials" [192] and "UNE 48-251-92: Paints and varnishes: Accelerated aging test. Method of exposure to alternating cycles of ultraviolet light and condensation" [193] establish the UV-A and UV-B lighting conditions applicable to materials for artificial aging. These regulations determine that the different wavelengths and intensities applied to materials can create a significant difference in their aging, in addition to the fact that the alterations are produced by repetitive exposure to said radiation.

Table 4. Use of light radiations on Cultural Heritage (CH) materials conservation.

Light Radiations	Intensity/Exposition	CH Material/Country	Action	Microbiological Assays	Microorganism Strain	Reference
UV-C (254 nm)	85 ergs mm^2 S^{-1}/1, 3, 10, 15, 20, 30 min and 1, 6, 12, 18, 24, 36, 48 h	Sun Temple of Konark/India	Disinfection	*In vitro* test in Petri dishes	*Tolypothrix byssoidea* [b]	[170]
	0, 30, 60, 180 min (suspensions) 3, 6, 9 h (stones)	Domme, Laugerie-Haute and Jovelle caves/France	Disinfection	*In vitro* test in suspensions and in calcareous stones mock-ups	*Klebsormidium flaccidum* [a] *Chlorophyceae* *Diniphyceae* [a]	[172]
	180 kJ m^{-2}/periods of being on (30 min) and off (15 min) for 12 h	Moidons cave/France	Disinfection	*In situ*	*C. minutissima* *Bracteacoccus* sp. [a] *Scenedesmus* sp. [a]	[157]
	150 and 300 kJ m^2/4 and 8 h	Moidons cave/France	Disinfection and bleaching	*In vitro* test in suspensions and *in situ*	*Chlorella minutissima* [a]	[173]
	150 kJ m^{-2}/4 h	Moidons cave/France	Disinfection and bleaching	*In vitro* and *in situ*	Green algae	[169]
	0, 1, 2, 5, 10, 15 min	Stone buildings/n.a	Disinfection	*In vitro* test in Petri dishes	*Nostoc* sp. [b]	[174]
	4800 kJ m^{-2}/62 h	Prehistoric caves/n.a	Disinfection	*In vitro* test in Petri dishes and limestone mock-ups	*Bracteacoccus* sp. [a] *Phormidium* sp. *Verticillium* sp. [b] *Bryum argenteum* [d] *Bryum capillare* [d] *Barbula convolute* [d]	[175]
	30 kJ m^{-2}/14 h	Moidons cave/France	Bleaching	*In vitro* in Petri dishes and *in situ*	*Chlorella* sp. [a]	[177]
	646 kJ m^{-2}/14 h	Monument Vicherey church/France	Disinfection	*In situ*	Algae and cyanobacteria	[194]
	0, 2, 4, 6, 8, 10, 20, 30 kJ m^{-2}/corresponding to 2.6, 5.2, 7.8, 10.4, 13, 26, and 39 min	Moidons cave/France	Disinfection	*In vitro* test in suspensions and Petri dishes	*Chlorella vulgaris* [a]	[178]
	2, 10, 20 and 30 kJ m^{-2}/2.6, 13, 26 and 39 min	Lascaux cave La Glacière cave Vicherey church/France	Disinfection	*In vitro* in suspension and Petri dishes and limestone mock-ups	*Geomyces* sp. [c] *Rhizomucor* sp. [c] *Penicillium bilaiae* [c] *Engyodontium album* [c] *Ochroconis lascauxensis* [c]	[155]
UV-A/B/C + benzalkonium chloride (3% H_2O)	48 h	Granite of Rodas/Spain	Disinfection	Tests in naturally granite slab	*Sporotetras polydermatica* [a]	[153]

Table 4. *Cont.*

Light Radiations	Intensity/Exposition	CH Material/Country	Action	Microbiological Assays	Microorganism Strain	Reference
Red LED + UV-A/B	Daylight 16 h + darkness 4 h + different lights 4 h	Palace of Xelmírez and the Monastery of San Martiño Pinario/Spain	Disinfection	*In vitro* test in Petri dishes	*Bracteacoccus minor* [a] *Pseudomuriella aurantica* [a] *Stichococcus bacillaris* [a] *Chlamydomons* sp. [a] *Isocystis* sp. [b] *Chlorella* sp. [a] *Aphanocapsa* sp. [b]	[179]
Red light (630–650 nm) Blue light (460–480 nm)	25 h	Christian catacombs of St. Domitilla and St. Callistus/Italia	Prevention	*In vitro* test in suspension	*Eucapsis* sp. [b] *Scytonema julianum* [b] *Leptolyngbya* sp. [b] *Symphyonemosis* sp. [b]	[171]
LED light + red, yellow, green and blue cellophane	21 days with 12 h of darkness and 12 of light	Historical granite buildings/Spain	Prevention	*In vitro* test in suspensions	*Bracteacoccus* sp. [a] *Chroococcus* sp. [b] *Isocystis* sp. [b] *Pseudocapsa dubia* [a]	[176]

Cultural Heritage and countries. n.a.: Non-available. Microorganism strain. a: Algae; b: cyanobacteria; c: fungi; d: moss.

6. Critical Comparison: Can EOs and Light Radiations Be a Good Alternative?

A modification of the SWOT analysis has been used to theoretically guarantee that EOs and/or light radiations can be a good alternative to traditional chemical biocides. In addition, their actual situation across the specific literature was analyzed. Thanks to this methodology, a picture of the real possibilities of these materials on easel painting conservation can be taken (Figures 8 and 9).

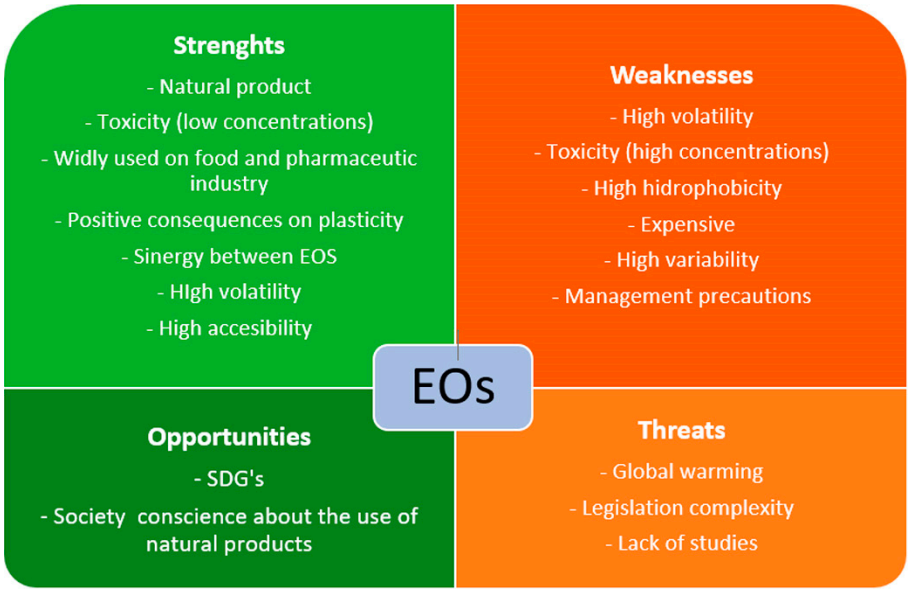


Figure 8. Schematic representation of essential oils (EOs) SWOT analysis. Sustainable Development Goals (SDGs).

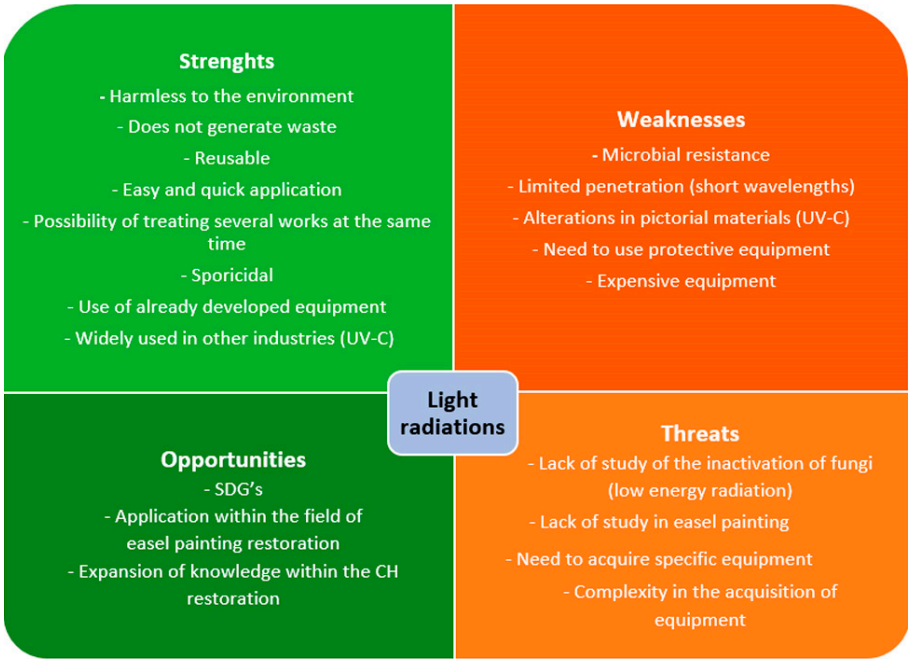


Figure 9. Schematic representation of light radiations SWOT analysis. Sustainable Development Goals (SDGs); Cultural Heritage (CH).

One of the most appreciable strengths of EOs is their natural origin. EOs are plant substances and have different functions, as explained before. In general, society wants to consume products as naturally as possible [195], and the tendency for conservation

treatments has been similar in recent years. The use of "Green Chemistry" in conservation promotes the reduction and substitution of chemicals by eco-sustainable products, and in this way, EOs can be suitable substitutes for chemical biocides [1,196].

In contrast, one weakness of EOs is their high variability in terms of antimicrobial activity, which depends directly on the plant species, the origin region, the season of pick up, the part of the plant where EOs are extracted (leaves, buds, roots…), or even the method of EO extraction [197]. Furthermore, their effectiveness depends on the fungal species they treat. However, in front of this weakness, EOs can synergize with other EOs, achieving better biocide results than separately [198,199]. The hydrophobicity of EO compounds could be a weakness in easel painting conservation. As mentioned above, these inconveniences are related to their use in dilution with solvents for cleaning purposes or as preventive material in adhesive mixtures. Following "Green Chemistry" recommendations, restorers are used to choose water as a solvent in any treatment, and the inclusion of an oil phase supposes the addition of surfactants and low- or high-energy instruments to obtain an emulsion [200]. This is not always possible because of the adverse effects of some surfactants on painting surfaces. In addition, EOs as preservatives generally need encapsulation to improve their useful lives [201]. The high volatility of EOs can be a weakness and a strength. As an additive in an adhesive or a cleaning system, EOs will require a reduction of volatility to increase the mixture's shelf-life. However, its high volatility is important in air cleaning or contactless treatments [141,147,202].

As Isman, 2020 [203] reported, EO price and availability depend on each other, which can be considered a weakness. EO commercialization has some issues that affect their cost due to the requirement of expensive production methods and the low yield of raw materials [204]. For example, 450 kg of *Narcisus poeticus* flowers yields between 350 g and 1 kg of EOs, depending on the extraction method [205]. As with any product, the large-produced, widely-used EOs are cheaper than those less used or demanded [203]. Taking, for example, *Biotin T* (CTS) and *Origanum compacum*, *T. vulgaris*, *M. alternifolia*, or *S. aromaticum* EOs (PRANAROM), the price range around 0.057 €/g for Biotin T, and around 1.30–1.60 €/g, 3.30–4.50 €/g, 0.60–0.70 €/g and 0.60–1.20 €/g for each EO, respectively (October 2023). In terms of availability, EOs can be found easily in pharmacies—although generally in small doses—while conservation biocides need to be ordered from specialized distributors, spending generally more time.

The biggest threats to EOs are probably legislation and regulation complexity related to their toxicity and global warming. The toxicity of EOs plays a two-sided role, and their concentration and appropriate management are the key [206], as also reported in Safety Data Sheets or leaflets. In other industries, such as pharmacy or food science, EOs are being applied in low concentrations as a treatment against fungal growth, among other utilities [207]. In those areas, products such as fruit are coated with EO edible films [208], or EO-based drugs are being produced [209]. In those applications for human consumption or health care, the safety of some EOs was proven and is considered GRAS (Generally Recognized as Safe) by the USA Food and Drugs Administration (FDA).

Furthermore, Institutions such as the European Medicinal Agency recognize EOs as pharmaceutical ingredients, subjecting them to different controls [210]. As was mentioned before, some studies warn about the toxicity of EOs, and legislation concerning EOs is complex insofar as there are no standardized controls, and they depend on their use [211]. In Europe, synthetic and natural biocides share the Regulation n° 528/2012. This Regulation establishes the possibility of increasing the number of commercially accepted biocides, above all natural ones that substitute synthetic ones, although it is a costly process [212,213].

Recently, there were different polemics about including some EOs as toxic in a revision of Regulation No. 1272/2008 [214] about the classification, labeling, and packaging of substances and mixtures. This revision aimed to consider it as "More than one constituent substance" (MOCS). As a result, EOs would have been considered toxic if one of their components had been considered this way. Finally, in October 2023, the European Parliament approved excluding that change from the final revision, considering [215]. Internationally,

the World Health Organization (WHO) or the Food and Agriculture Organization (FAO) of the United Nations regulates the use of EOs, highlighting the WHO "Essential Medicine and Health Products Information Portal", guidelines and volumes [211]. As Neagu et al. 2023 [210] reported, the FDA regulates the use of EOs as cleaners, fragrances, or drugs, restricting their sales with any curative or prevention intention. For this reason, the present investigation considers that low-toxicity EOs in small doses are a strength. Still, the caution of their management in pure for their dilution for being used in a conservation treatment must be considered as a weakness or even a threat.

As plants derivate, the effect of global warming and climate change on plant cultivation is critical for their production. This phenomenon can change the EO properties due to changes in some plant development [216]. Studies such as Laftouhi et al., 2023 [217] show the changes in the content of primary and secondary metabolites of *R. officinalis* EOs from Taounate, Morocco due to T^a or water variations; Cautela et al., 2021 [218] also demonstrate the effect of the drought of *Citrus Bergamia* EOs production in Calabria, Italy. However, the effect of EO production on the environment should be considered during fertilization, irrigation, and extraction to reduce their impact on nature and human health [219]. Apart from that, regarding material mechanics, their role as plasticizers is a strength, improving the elasticity of film-forming substances, such as adhesives used in easel painting conservation.

Opportunities for EOs in easel painting conservation are related to their natural origin and biocide properties. Today, society's conscience about the importance of natural, sustainable products linked to the SDGs plays an important role in the industry, and restorers require effective, non-toxic solutions, as was mentioned above. Furthermore, the use of EOs can be a good option for dynamizing the circular economy, especially in the case of citrus, whose major component is Limonene, a high antimicrobial agent [220,221]. It is well-known that the EOs extracted from citrus are in the peel and seeds of the fruits, supposing their reuse for EOs extraction is a good way to reduce plant waste [222,223]. As plant derivates, EOs can contribute to climate change if they are cultivated in a responsible way. For this, ecological cultivation and using green EO extraction systems must be considered to reduce the environmental impact [224–226]. In this way, EOs can be a good opportunity to improve CO_2 footprint reduction, promoting km0 cultivation, extraction, and consumption.

On the other hand, as indicated in Figure 9, one of the most notable points of the disinfection treatment with light radiation is its harmlessness to the environment. As artificial lighting is replicated from natural radiation, it does not produce pollution or generate waste when produced, respecting the environment [194]. In this way, it supports the SDGs, highlighting 12.4 "Climate action", to which the implementation of UV would contribute since the use of chemical compounds would be reduced [29].

Likewise, unlike traditional chemical biocides with a single use, since lamps generate radiation, they can be reapplied multiple times without acquiring new equipment over a long period. For example, the UNE 48-251-92 regulation indicates that under conditions of use, the useful life of UV-A/UV-B lamps can reach 1600 to 1800 h.

On the other hand, applying the treatment with light radiation is simple for the user since it is unnecessary to carry out dissolutions or gelations, as in the case of chemical biocides. The affected parts or areas must be exposed to lighting for the required time. It can also treat multiple works or large, colonized surfaces in each treatment cycle. Furthermore, as it is a controlled treatment, only the affected area is treated without interfering with the environment [157].

Light radiation (UV-C) is commonly used for disinfection in different areas, such as clinical and food. For this reason, the equipment used for disinfection has already been designed, developed, and tested [71,156,194]. Therefore, developing new equipment for use in CH is not necessary; only its adaptation is.

The most notable strength of light radiation in CH, mostly UV-C, as an organism inactivation treatment is its negative effects on the viability and metabolic activity of

organisms such as plants, microalgae, cyanobacteria, and fungi [155,157]. Likewise, authors such as Pfendler et al., 2018 [194] have verified that UV-C, besides achieving the inactivation of fungal mycelia, is also sporicidal and can be applied preventively and curatively. Also, this light has interesting effects on restoring monuments, caves, or sculptures, such as the bleaching of biofilms [227], which could also create unwanted decoloration of the tissues.

Likewise, one of the drawbacks that must be considered regarding the effectiveness of radiation treatment, specifically UV-C, is that fungi that contain pigments such as melanin can make them resistant to inactivation [194]. The low penetration of short wavelength radiation, which is most used in the inactivation of micro-organisms (UV-C) [87], can be a serious weakness due to the impossibility of eliminating internal colonization. The physicochemical and mechanical alterations caused by the different radiations when applied repeatedly to tissues can be one of the disadvantages of this treatment [71]. By modifying the characteristics of the tissues, they could become more prone to subsequent colonization due, for example, to the possible increase in sensitivity to humidity caused by the breakdown of cellulose bonds.

Non-visible radiation, such as UV, can also cause alterations in human health, causing damage to the skin and eyesight, except for Far-UV lighting and visible radiation that are not harmful to mammalian cells [162]. Therefore, using Personal Protective Equipment (PPE), such as protective glasses and clothing opaque to UV penetration, is essential. Applicants' exposure to this radiation should be as little as possible, as indicated by the UNE-EN ISO: 15858 regulations [228]. Although the durability of the lamps is extensive, their acquisition is only affordable for some restoration teams. For example, the equipment currently being created, such as the Care222® Far-UVC Excimer Ultraviolet Germicidal Irradiation (UVGI) lamp (222 nm), is complicated to acquire, and its cost is high.

One of its greatest threats is the lack of knowledge regarding disinfection treatment using light radiation in easel painting. The applications of light radiations are mostly reduced to the treatment of monumental heritage [179]. Likewise, the research has mostly analyzed its effectiveness against organisms other than fungi. And the research on fungi has yet to focus on fungal organisms from easel paintings. Therefore, whether these radiations effectively inactivate fungal mycelia and spores from easel paintings is unknown.

We can also identify the opportunities this new research line can offer. Treatment with light radiation for disinfection fungal agents in textile pictorial supports has yet to be studied, offering the possibility of opening a new line of research. It is an opportunity to develop an ecological, healthy, and non-harmful treatment for easel paintings, providing new knowledge to the conservation of painting on canvas and other CH objects.

7. Conclusions

Biodeterioration is a complex damage combination that includes aesthetical, physical, and chemical alterations due to biological activities, which can contribute to the loss of material and immaterial values of easel paintings. As was explained in the introduction of this investigation, climate change and global warming increase the risk of biodeterioration, and this challenge requires eco-suitable and non-toxic solutions. EOs are natural-origin substances with great biocidal activity, although their application in real cases is scarce. After analyzing the benefits and drawbacks of most used traditional and alternative biocides for easel painting conservation, it can be concluded that EOs are a good alternative to investigate further (Table 5). On the other hand, disinfection using light radiation is another ecological alternative. Despite this, they present significant problems in terms of penetration and alteration of the materials, so it is possible that in addition to causing damage to the tissues, they do not completely inactivate the organisms in the works. Therefore, a good alternative would be radiation with longer wavelengths, greater penetration, and lower intensity. Likewise, arduous research is necessary to verify the effectiveness of these radiations against the fungi usually present in paintings on canvas. EOs and light radiations are shown here as interesting eco-friendly methods for easel painting biocontrol. Further research will allow them to substitute traditional chemical products.

Table 5. Analysis of benefits and drawbacks of most commonly used biocides and alternative biocides.

Biocide Family	Biocide	Application	Benefits		Drawbacks		Economical Cost
			Water Solubility	MIC/MFC	Toxicity	Risks	
Alcohol	Ethanol	Mechanically	Very high	Very high	Low	Moderate–High	Low
QACs—Azoles	Biotin T	Mechanically Additive	High High	Low Low	High High	Moderate–High Moderate–High	Moderate
QACs—Alcohol	Preventol RI50/RI80	Mechanically	High	Low	High	Moderate–High	Moderate
Phenol	Preventol ON	Mechanically	High	Low	High	Moderate–High	Moderate
Metal NPs	ZnO NPs	Brush/spray	High	Very low	Low	Low	High
Natural biocides	Essential oils	Spray Additive In vapor	Low Low Low	Very low Very low Very low	Low Low Low	Low–High	High

Risks refer to the painting's sensibility, specifically with the biocide solvent. MIC/MFC depends on fungal strain and Eos. Toxicity is also related to concentration.

8. Perspectives

EO's perspectives in easel painting conservation can include encapsulation, air diffusion, or nanoemulsions. Regarding prevention, EO encapsulation has some advantages compared to non-encapsulated ones. Its hydrophobicity, volatility, risk of binders solubilization, or colorimetric changes can be reduced, in addition to the increase of protection against degradation (UV light or oxidation) [128,229–231]. In addition, different treatments for easel painting conservation can be good alternatives for including encapsulated EOs as antifungal substances (e.g., varnishing). Due to the effectivity of their volatile compounds, EOs can be investigated as indoor air quality control agents for museum rooms or storage, taking as reference studies such as Díaz-Alonso et al., 2021 [147]. Regarding curation, using EO nanoemulsions or nano gels for fungal strain removal, adapted from other CH conservation disciplines, explored by Minotti et al., 2022 [47], is a potential investigation line. In this way, the versatility of emulsions or gels in terms of applicability and their well-known use in painting conservation cleaning treatments [232,233] could allow restorers to control the EO's use to eliminate fungal contamination.

On the other hand, light radiation within easel painting conservation offers promising possibilities against biological colonization, although more investigations are required. The greatest challenge in applying these treatments is determining each light's exposure times and fungal inhibition. Regarding fungal inactivation, wavelengths with less penetration but greater energy could play a fundamental role in paralyzing superficial fungal attacks, such as Far-UV or UV-C, commonly used for macro and microorganisms [156,234]. In canvas paintings with more developed fungal damage and greater fungal penetration, applying treatments with greater penetration, such as visible radiation or UV-A/B, would be necessary. One of the most beneficial options for human health would be using radiation that is not harmful to the skin and eyes, such as visible lighting or Far-UV [163], which would allow the application of the disinfection treatment without the need for specific protective equipment. In addition to being a curative treatment, determining the same parameters for the inactivation of fungal spores could open the doors to preventive treatments that would minimize biological development in works exposed to favorable climatic conditions. It must be considered that the recurrent application of light treatments such as UV can accelerate the aging of materials such as cellulose [189].

Future perspectives on both research lines must be focused on reducing risks, improving economic and environmental sustainability, and increasing easel painting compatibilities, biocide long-term effects, and efficacy.

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