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Original article

Antifungal properties of oregano and clove volatile essential oils tested on biodeteriorated archaeological mummified skin

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ABSTRACT

The study of the potential use of essential oils in the field of Cultural Heritage has increased in the last decade due to their demonstrated antimicrobial potential and absence of toxicity. This work aimed at assessing, for the first time, the volatile antifungal activity of two essential oils (EOs) (*Origanum vulgare* and *Syzygium aromaticum*) against fungi isolated from biodeteriorated archaeological mummified skin. The object of study was a mummified *Mustela frenata* from the Tiahuanaco culture of Bolivia. The fungi found on the archaeological animal's skin were preliminarily isolated and identified by DNA-based analysis. Subsequently, the volatile essential oil biocidal activity was evaluated on parchments inoculated with the isolated fungi (*Aspergillus tabacinus, Aspegillus tennesseensis,* and *Trichoderma longibrachiatum*). Vapour tests were conducted in Petri dishes and parchment specimens to assess the fungicidal effect of volatile components of EOs. The volatile EOs used in this study showed antifungal activity against the tested fungi. The oregano showed to be the most effective EO in the *in vitro* and parchment tests for the three fungal strains. The use of EOs, particularly oregano EO, represents a promising green strategy for the sustainable conservation of organic-based cultural assets.

1. Introduction

Commercial biocides of chemical origin have proven to be the most widely used materials for eliminating fungal microorganisms in preserving works of art. However, various investigations warn about their use due to their toxicity since they can cause damage to the Ecosystems, the object surfaces, and the personnel who use them [1]. Indeed, the European Union intervened in biocide regulation to reduce and control its use [1]. Due to this important fact, researchers focus on evaluating alternative products and methods to minimize the abovementioned risks.

Essential oils (EOs) comprise a green alternative to using biocides of chemical origin due to their toxic-free, sustainable, and innovative characteristics to safeguard the environment and a good biocidal power [2]. EOs from aromatic plants are known for their bactericidal, fungicidal, virucidal, and insecticidal properties [3]. EOs are composed of diverse bioactive volatile compounds like monoterpenes, sesquiterpenes, terpene alcohols, terpenoids, and odorants [4]. EOs exert their antimicrobial activities due to different mechanisms, such as fluidifying the membrane structure, increasing its permeability, respiration inhibition, and cell lysis [5–8].

EOs are frequently used in several fields, such as food, cosmetics, pharmaceutical industries, and even integrative medicine and aromatherapy [9–12].

EOs have also been studied in the Cultural Heritage field for biodeterioration control. Their efficacy has been proven in numerous *in vitro* studies with biodeteriorating agents isolated from mural paintings, wood artefacts [13], paper documents [14–16], oil paintings [17,18], historical textiles [19], and stone sculptures [20]. Yet and to the best of our knowledge, this is the first study on applying EOs in mummified materials. Despite the inexistence of

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literature on the application of these natural biocides in mummified materials, fungal biodeterioration has been reported in this type of organic material, such as in several mummies of the Capuchin Catacombs in Palermo, Italy [21], in Chinchorro, Chile [22] and another mummy located in the Archaeological Museum of Zagreb, Croatia [23].

It is worth mentioning that artistic objects like mummified materials are extremely fragile, so it has been necessary to search for non-invasive control systems to avoid deterioration.

More studies have been done in this direction [25,18,24]. Authors have shown the insecticidal effectiveness of EOs in the vapour phase within a glass container to treat wood artwork reproduction [26]. Another study showed the effective use of aerosol 0.1% *Artemisia absinthium* EO (dissolved in ethanol 70%) to remove fungi from paper material without altering its pH or colour [27]. Oregano and cloves EOs have also proven to have an interesting contactless biocidal effect on isolated bacteria and fungi from Oil painting [28].

Among the different existing EOs in the market, oregano (*Origanum vulgare*) and clove (*Syzygium aromaticum*) have shown promising results as natural biocides in numerous studies. Diverse authors have demonstrated their antifungal effectiveness against isolated fungi from artistic materials [18,20,26,29,30,31]. Oregano and clove EO's composition is mainly carvacrol and eugenol, respectively [25]. These EOs have been selected in this study because their phenolic components are considered the most effective (among all the different existing EOs) to eliminate microorganisms demonstrating its antifungal efficacy [33,24,25]. This study assessed the effectiveness of two EOs (oregano and clove) in vapour against fungi isolated from a biodeteriorated archaeological mummified skin.

2. Research aim

The present research was carried out to test the antifungal properties of oregano (*Origanum vulgare*) and clove (*Syzygium aromaticum*) volatile essential oils into biodeteriorated archaeological mummified skin.

This study focused on the case study of fragile and biodeteriorated skin from a Tiwanaku mustelid mummy. First, microorganisms were isolated from the mustelid and identified by DNA-based analysis. Then the antifungal activity of oregano and clove EOs was evaluated in a volatile contactless way.

3. Materials and methods

3.1. Artwork description and conservation state

Mustela frenata is a small mammal belonging to the Mustelidae family, rodents that live in mountainous environments. The animal was found inside the body of an Aymara ethnic mummy located in Tiahuanaco territory, a pre-Columbian culture established by the Andes – (current countries of Bolivia, Argentina, Peru, and Chile)- presenting both specimens a mummification process due to the Andean climate. Currently, the animal is in the Natural History Museum of Valencia (Spain) warehouses.

The state of conservation of the *M. frenata* body is determined as highly fragile due to its fragmentation into three parts, the dryness and fragility in which the skin is found and the presence of a critical fungal attack visible both on the front and the back. The fungal attack is not presented homogeneously since, in some areas, the whitish strains may be more attached to the organic matter, being especially detrimental in some areas of the obverse's legs, tail, and abdomen (Fig. 1).

Fig. 1. *Mustela frenata.*

3.2. Sampling method

Based on the visual fungal attack, 14 sampling areas were chosen, including seven on the front and seven on the back of the specimen (Fig. 2). Non-invasive sterile cotton swabs in physiologic saline solution were used for the sampling. Then each cotton swad was transferred into Petri dishes with Sabouraud chloramphenicol agar (Scharlab SL. Barcelona, Spain) for fungi cultivation. Finally, Petri dishes were incubated for five days at 28 °C.

3.3. Fungal isolation and molecular identification

Based on their morphological characteristics, fungal colonies were selected and isolated from each sampling point into a new single Petri dish with Sabouraud chloramphenicol agar (Scharlab SL, Barcelona, Spain) and incubated at 28 °C. Three fungal colonies displaying different colours and textures were isolated and selected for further identification. Identification was first carried out by optical microscopy (Leica DM750 microscope with MC170 HD camera incorporation) based on morphological characteristics and dichotomous keys identification and then by DNA-based analysis.

Genomic DNA from pure cultures was extracted from all three isolates by scraping the mycelium from the plates and transferring it to 1.5 ml Eppendorf tubes containing 350 µl of lysing buffer (TNE) and glass beads. The mixture was then shaken in a vortex for 10 min. The DNA was purified by phenol/chloroform extraction and ethanol precipitation. Amplifications of fungal internal transcribed spacer (ITS) regions were attempted using the ITS1 (5≠TCC GTA GGT GAA CCT GCG G) and ITS4 (5≠TCC TCC GCT TAT TGA TAT GC-3́) primers. The PCR reactions were performed in a BioRad thermal cycler (BioRad, Hercules, CA, USA) using the following thermocycling program: 2 min denaturing step at 95 °C, followed by 35 cycles of denaturing (95 °C for 15 s), annealing (55 °C for 15 s) and elongation (72 °C for 2 min). A final elongation step of 10 min at 72 °C was added at the end. The amplification products were purified and sequenced by STAB VIDA Sequencing Services (Lisbon, Portugal).

The DNA sequences obtained were edited using Bioedit v7.0.5.3 software (Technelysium, Tewantin, Australia). After quality control, a similarity search was performed using the BLASTn (Basic Local Alignment Search Tool) algorithm from the NCBI (National Centre for Biotechnology Information, http://www.ncbi.nlm.nih.gov/). The sequences of the identified isolates were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers ON935798, ON93579, and ON935800.

3.4. Skin biofilm formation

Biofilm formation tests on simulated skin models were conducted to evaluate the capability of the isolated fungi to potential growth and therefore cause biodeteriotation on archaeological mummified skin. Sheep parchment (Artepergamino SL. Moncada, Spain) was selected to simulate the mummified animal skin.

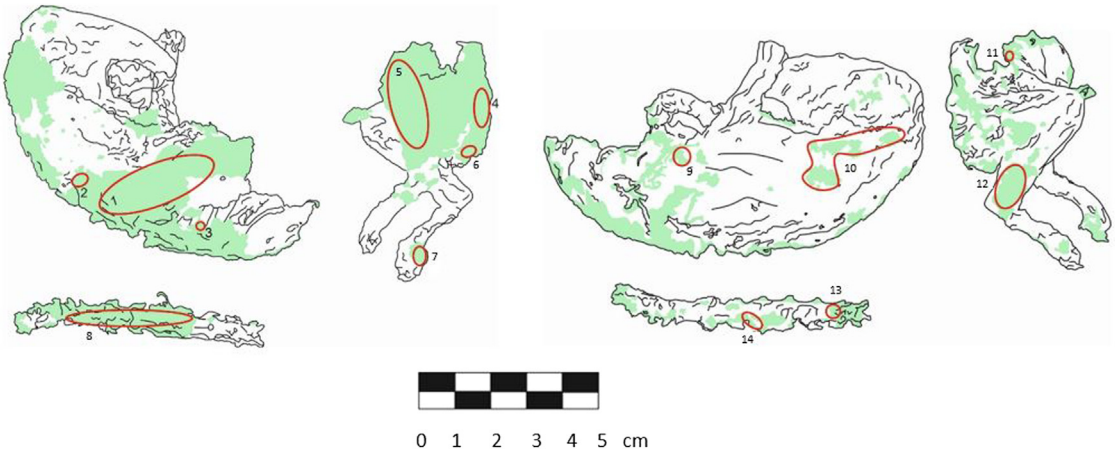

Fig. 2. Scheme of the mummified *Mustela frenata* with sampling points marked as red circles. Green stains represent fungal colonization on the archaeological specimen's obverse (left) and reverse (right) sides.

Parchment fragments of 2 × 3 cm were prepared and sterilized by formaldehyde gas exposure for 48 h following the protocol described by Sanmartín et al. [35]. After sterilization, each fragment was placed on the Sabouraud chloramphenicol agar Petri dish (one per Petri dish).

Fungal suspensions were prepared by adding 5 ml sterile saline solution to the Sabouraud chloramphenicol agar Petri dish with isolated fungus and shaking it with gentle movements for 5 min [24]. The resulting hyphal fragments and spores were collected and transferred to a sterile tube. An aliquot of 200 µl of each fungal suspension was applied to the centre of the surface of each parchment fragment. On each parchment, only one type of isolated fungi was added, and each test was done in triplicate. Plates were incubated for three to seven days at 28 °C. These plates were also representative of fungal growth-like control (blank test).

3.5. Essential oils

In this study, the commercially available EOs used were oregano (*Origanum vulgare* from MARNYS®, Cartagena, Spain) and clove (*Syzygium aromaticum* from Soria Natural, Garray, Spain). EOs were diluted in 0.5% Tween 20, according to Del Río Oliver [32]. Three concentrations were carried out at 100%, 75%, and 50% in the Petri dish assay, using a single 100% pure solution in the parchment assay. Ethanol 70% was used as a positive control, as suggested by Borrego et al. [34].

3.5.1. Essential oils vapour tests in Petri dish

To evaluate the selected EOs volatile inhibition properties on the isolated fungi, first, a contactless Petri dishes test was conducted. One 6 × 6 mm square cut from each 7-day-old fungi culture was plated in the middle of one Sabouraud chloramphenicol agar Petri dish. Next, plates were overturned, and 200 µl of EOs test solution or the positive control solution (ethanol 70%) were placed on the lids of the Petri dishes separately. Then, the Petri dishes were sealed by Parafilm to prevent the vapour from scape. To evaluate the fungicidal or fungistatic effect of the volatile EOs, the protocol of Puškárová et al. was followed [24]. Plates were incubated in reverse for seven days at 28 °C. After those seven days, all the lids were changed for a new one (without any treatment solution) and incubated again for another seven days (total of 14 days) at 28 °C. Tests were performed in triplicate, and blank test controls were simultaneously run without EOs (Fig. 3).

According to Puškárová et al., the EOs concentration that completely prevented visible fungal growth in the first 7-day experiment with EOs and allowed a revival of fungal growth during the second 7-day experiment without EOs (total of 14 days) was identified as a fungistatic effect [24]. EOs concentration that completely prevented visible fungal growth on the first 7-day experiment with EOs and the second 7-day experiment without the EOs (total of 14 days) was identified as having a fungicidal effect.

Also, fungal inhibition was evaluated by the naked eye following the Gatti et al. scale: [+] for medium inhibition (approximately one-third of the Petri dish), [++] for high inhibition (approximately the half of the Petri dish), and [+++] for total inhibition [18]. Regarding the inhibition scale degrees, see Table S1.

3.5.2. Essential oil vapour tests in parchment specimens

To evaluate the EOs vapour inhibition properties on skin materials that simulate the mummified animal skin, sheep parchment was used (Artepergamino SL. Moncada, Spain). Thirty-eight sheep fragments of 2 × 3 cm were prepared. To sterilize them, formaldehyde gas exposure for 48 h was used following the protocol of Sanmartín et al. [35]. After sterilization, each fragment was placed on the Sabouraud chloramphenicol agar Petri dish.

Fungal suspensions were prepared by adding 5 ml sterile saline solution to the Sabouraud chloramphenicol agar Petri dish with isolated fungus and shaking it with gentle movements for 5 min [24]. The resulting hyphal fragments and spores were collected and transferred to a sterile tube. 200 µl of each fungal suspension was applied to the centre of the surface of each parchment fragment. Next, plates were overturned, and 200 µl of 100% pure EOs test solution or the control solution (ethanol 70%) were placed on the lids of the Petri dishes separately. Then the Petri dishes were sealed by Parafilm to avoid the vapour escape. Plates were incubated in reverse for 24 days at 28 °C (Fig. 4)

Each test was performed in triplicate. And controls were simultaneously run without EOs.

4. Results and discussion

4.1. Morphological and molecular characterization of mummified skin biodeteriogens

The cultivable part of the patina that covered the mummy was isolated from the *M. frenata* skin and identified through microscopy and molecular biology investigations. From the 14 sample areas chosen, three different morphological types of fungi were isolated (Fig. 5). First, morphological fungi inspection was conducted by macroscopic agar plate observation and optical microscopy and then by molecular biology sequencing. The fungi

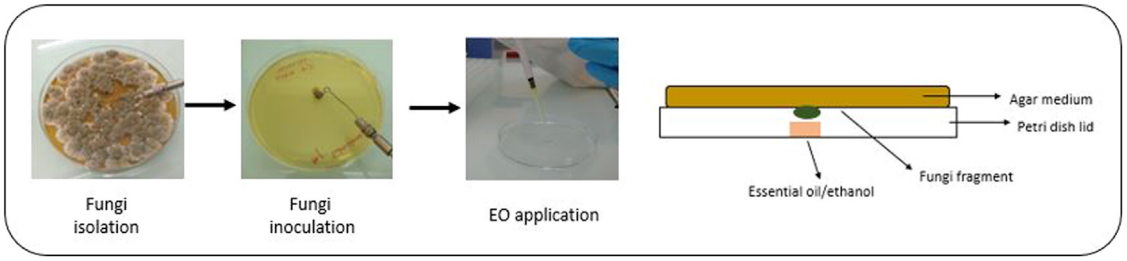

Fig. 3. Essential oils vapour tests in Petri dishes.

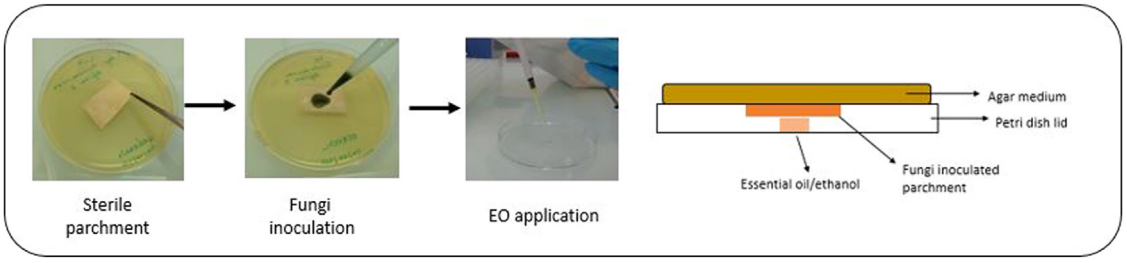

Fig. 4. Essential oil vapour tests in parchment specimens.

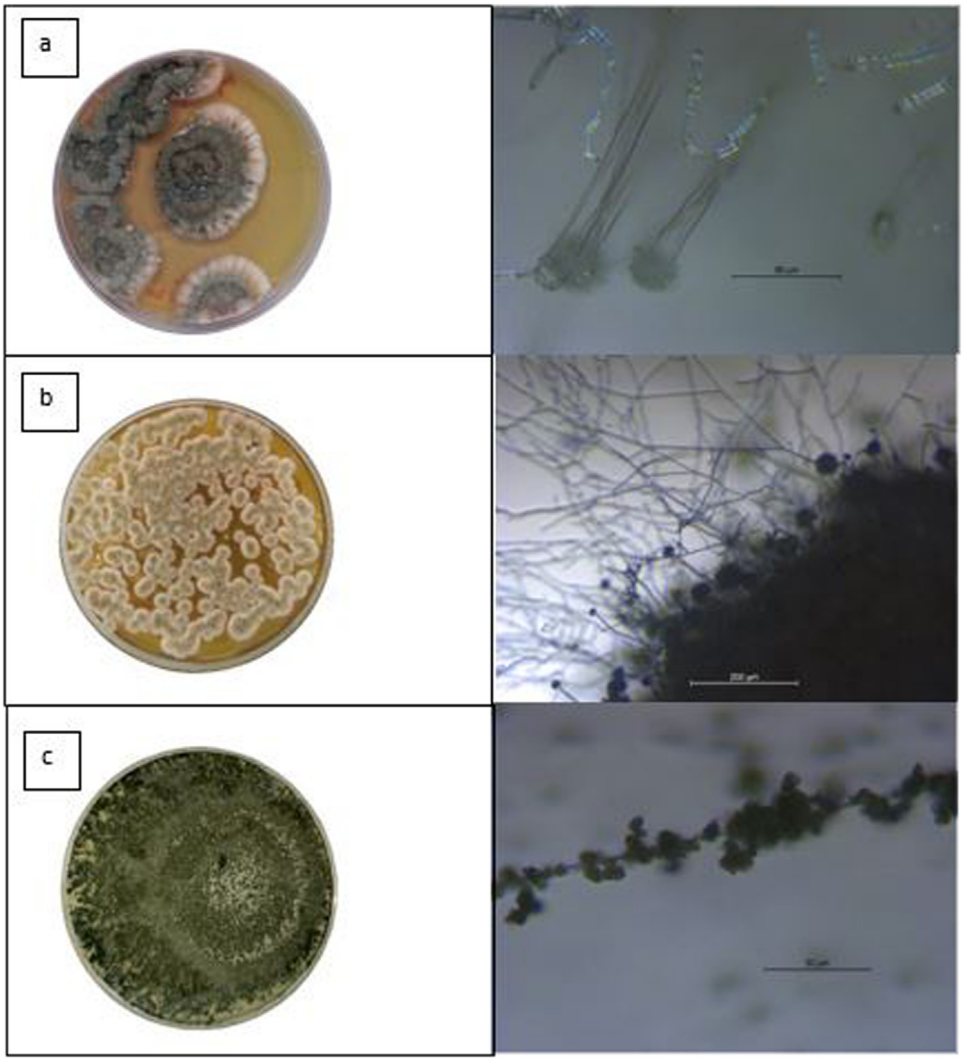

Fig. 5. Microbial strains isolated from the *Mustela frenata*. Morphological Petri dish characteristic (left) and optical microscopy (right) of *Aspergillus tabacinus* (a), *Aspergillus tennesseensis* (b), and *Trichoderma longibrachiatum* (c).

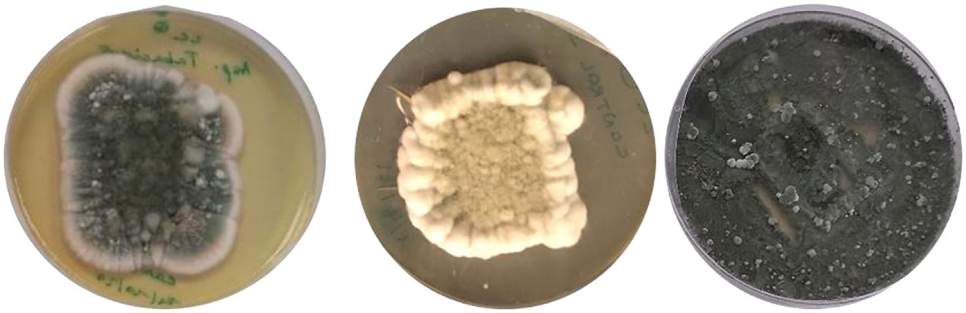

Fig. 6. *A. tabacinus* (left), *A. tennesseensis* (middle) and *T. longibrachiatum* (right) fungal growth on parchment specimen.

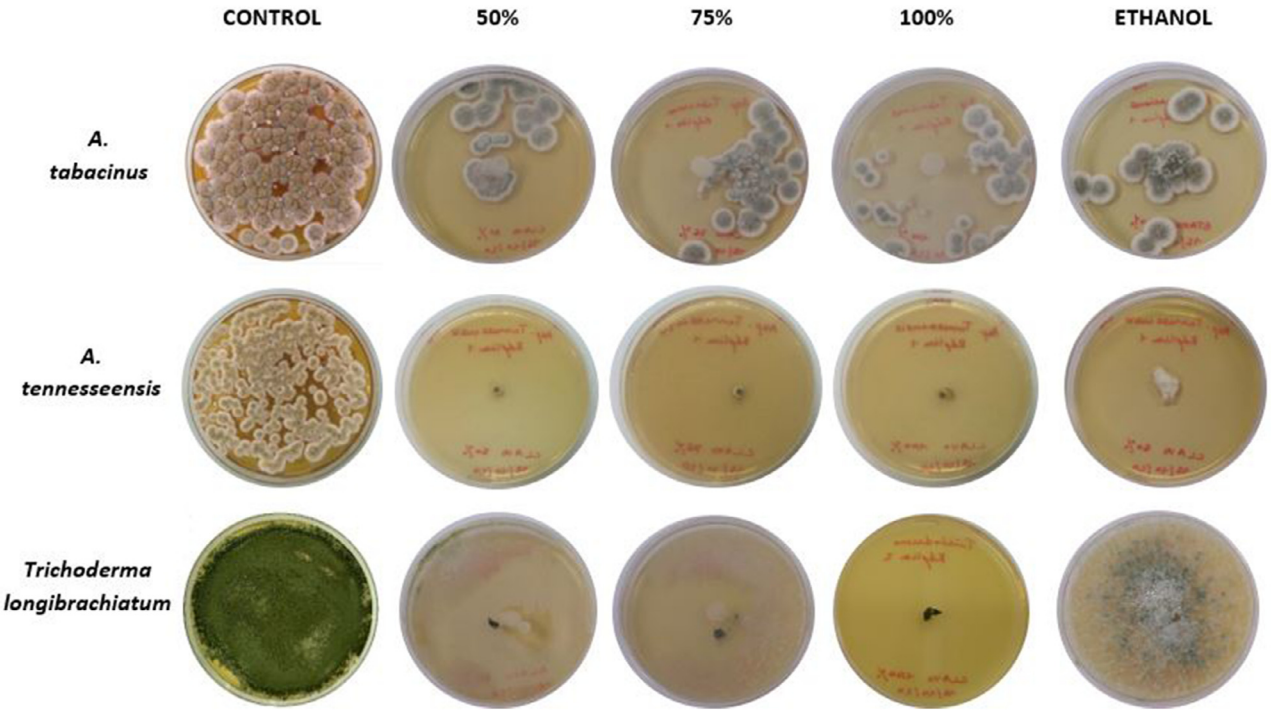

Fig. 7. Inhibition of fungal growth after seven days of incubation with clove essential oil vapour at different concentrations. *A. tabacinus, A. tennesseensis, T. longibrachiatum* on a Sabouraud agar Petri dish.

were subsequently identified as *Aspergillus tabacinus* (100% similarity in nucleotide identity with accessions NR_135361.1; Fig. 5a), *Aspergillus tennesseensis* (99% similarity in nucleotide identity with accessions NR_1354471.1; Fig. 5b) and *Trichoderma longibrachiatum* (99% similarity in nucleotide identity with accessions NR_120298.1; Fig. 5c).

Aspergillus sp. is a fungus frequently found affecting Cultural Heritage materials [36], been isolated in relation to mummies in the Spanish National Archaeological Museum, in the mummies deposit of Argentina's Museo de la Plata, and in textiles present in pre-Columbian Tlayacapan mummies [37–39].

A. tabacinus and *A. tennesseensis* belong to the Versicolor section of the *Aspergillus* genus. Little is known about this section compared to other fungi of the same family. The fungi of the Versicolor section are characterized by slow growth, been isolated in various environments such as indoor environments, food, terrestrial soil, saline water, seaweed, and insect bodies such as moths and mantises [40–42].

On the other hand, *Trichoderma* is a fast-growing genus also found in terrestrial environments where decomposing organic matter such as Wood or cellulosic elements is found [43]. The only case related to the skin has been documented in the Tlayacapan mummies, specifically in the mummified tissue [39].

Even though *Aspergillus* sp. and *Trichoderma* sp. are fungi that affect Cultural Heritage materials and mummified skin materials [37,39]. To the best of our knowledge, this is the first time the three isolated fungi species have been identified affecting an organic artwork such as mummified skin.

4.2. Fungal growth on skin simulated models

A parchment biofilm formation test was done to evaluate the biodeterioration potential of the isolated fungi. The three isolated fungi showed their capacity to grow and form a biofilm over the sterile parchment specimens used as skin-simulated models (Fig. 6). The complete cover of the parchment specimen by the fungi took seven days after inoculation except for *Trichoderma longibrachiatum*, which showed fungal growth and skin biofilm formation three days after fungi inoculation.

4.3. Assessment for antifungal activity of volatile EOs in Petri dish

As detailed in Fig. S1 and Table 1, oregano EO vapour at 100% and 75% concentrations showed total inhibition against the three tested fungi at 7- and 14-day experiments (fungicidal effect). The fungistatic effect was observed for the lowest tested concentration

Table 1
Antifungal activity of different concentrations of volatile EOs in Petri dish.

	Oregano						Clove						Ethanol	
	100%		75%		50%		100%		75%		50%		70%	
	Days													
	7	14	7	14	7	14	7	14	7	14	7	14	7	14
Aspergillus tabacinus														
Replica 1	+++	+++	+++	+++	+++	+	+	-	++	+	+	-	+	-
Replica 2	+++	+++	+++	+++	+++	+	+	-	++	++	+	-	+	-
Replica 3	+++	-	+++	+++	+++	+++	++	+	+	+	++	+	++	-
Aspergillus tennesseensis														
Replica 1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Replica 2	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	++	+
Replica 3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++
Trichoderma longibrachiatum														
Replica 1	+++	+++	+++	+++	+++	++	+++	+++	+	-	++	-	+	-
Replica 2	+++	+++	+++	+++	+++	+++	+++	+++	++	-	+++	-	+	-
Replica 3	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	++	-	+	-

+++ Total inhibition.
++ High inhibition (half of the Petri dish).
+ Medium inhibition (one third of the Petri dish).
- Absence of inhibition (fungal growth throughout the plate)

Table 2
Antifungal activity of volatile EOs in parchment specimens.

	Oregano 100%				Clove 100%				Ethanol 70%			
	Days											
	7	11	18	24	7	11	18	24	7	11	18	24
Aspergillus tabacinus												
Replica 1	+++	+++	+++	+++	+++	++	++	++	+++	+++	++	++
Replica 2	++	++	++	++	+++	++	++	++	+++	+++	+++	+++
Replica 3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++
Aspergillus tennesseensis												
Replica 1	+++	+++	+++	+++	+++	+++	+++	+++	+	-	-	-
Replica 2	+++	+++	+++	+++	+++	+++	+++	+++	+	-	-	-
Replica 3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Trichoderma longibrachiatum												
Replica 1	+++	+++	+++	+++	-	-	-	-	-	-	-	-
Replica 2	+	+	+	+	-	-	-	-	-	-	-	-
Replica 3	++	++	++	++	-	-	-	-	-	-	-	-

+++ Total inhibition.
++ High inhibition (half of the Petri dish).
+ Medium inhibition (one-third of the Petri dish).
- Absence of inhibition (fungal growth throughout the plate).

(50%) except in the case of *T. longibrachiatum* which also showed total inhibition.

However, clove's EO vapour showed lower fungicidal power compared to oregano. Showing only fungicidal effect against *A. tennesseensis* at the three tested concentrations and against *T. longibrachiatum* at a concentration of 100%. Instead, 75% and 50% of clove's EO vapour show alteration of *T. longibrachiatum* sporulating capability (see Fig. 7). Clove's EO vapour did not show a fungistatic or fungicidal effect against *Aspergillus tabacinus*.

The control trial, 70% ethanol vapour, did not show growth inhibition capacity to prevent fungal growth in any of the three fungi tested in the Petri dish.

Our results agree with other authors in that the essential oils tested in their trials proved to be a good alternative against microbial agents, with oregano being one of the most fungicidal essential oils. On the other hand, clove is fungicidal and fungistatic only against some microbial strains [24]. Other tests conducted by Del Río Oliver against isolated fungi in mural painting demonstrate the efficacy of oregano essential oil against all isolated fungi. Clove, for its part, proved to be much less effective, providing the worst results to the investigation [32]. According to the tests carried out by Gatti et al., they show that both the essential oil of oregano and that of clove show inhibitory effects against the growth of most of the fungi tested on oil paints, particularly the volatile components of oregano [18].

4.4. Assessment for antifungal activity of volatile EOs in parchment specimens

Volatile oregano EO 100% concentration on parchment specimens did show total inhibition up to 24 days of *A. tennesseensis* and (2 of the three replicas) of *A. tabacinus.* However, oregano volatile EO *against T. longibrachiatum* showed a lower growth inhibition (see Table 2 and Fig. 1S) even though it was able to prevent its sporulation. Similar results were obtained by Benkovičová et al. showing that oregano EOs has the strongest microbial activity on fungi biodeteriorated sandstone, whitewood, and paper [44].

Volatile clove EO 100% concentration, on the other hand, produces a total inhibition of A. *tennesseensis* for up to 24 days in all replicas and a total or high inhibition against *A. tabacinus*. None has been able to inhibit the growth of *T. Longibrachiatum* (Table 2). Volatile 70% ethanol did only prevent the growth of *A. tabacinus*.

The three tested vapours showed for *A. tabacinus* absence of the sporulation present in the control, remaining in this way until the end of the experiment.

5. Conclusions

The research has expanded the use of natural biocides in an inconspicuous field of conservation of archaeological materials of an organic nature, being unprecedented and providing new data with which to continue working in the future.

It has been verified the fungicidal and fungistatic potential of the oregano and clove essential oils in the vapour phase in different concentrations against *A. tennesseensis, A. tabacinus, and Trichoderma longibrachiatum,* the cultivable part of the patina that covered the mummified skin. Tested EOs present a better biocidal effect than 70% vaporized ethanol in most cases. On the other hand, of the two essential oils analysed, oregano has shown to be the most effective in the *in vitro* test and the parchment test on the three tested fungi. Also been, clove EO in the vapour phase very effective against *A. tennesseensis*. Of the three evaluated fungi, *A. tennesseensis* was the most sensitive to the volatile clove and oregano EOs because they prevented fungal growth from the first day of treatment to the last without registering any fungal growth. Finally, the volatile EOs treatments carried out altered not only the growth potential of the tested fungi but also their sporulating capability. However, these tests have all been done *in vitro* and on the cultivable part of the fungi covering the mummified skin. Therefore, further investigation must be done to develop an effective volatile EOs application method to "*in vivo*" threat the original biodeteriorated *M. frenata* specimen confirming the effectiveness and the lack of undesirable effects and, therefore, their safe use for further archaeological biodeteriorated similar pieces. Also, when evaluating the mummy's disinfection treatment, the non-cultivable part of the biofilm on the mummy's skin must be considered, as biofilms are generally complex microbial communities formed by cultivable and non-cultivable microorganisms.

The results of this research can help move forward to greener, safe, and contactless conservation treatments of biodeteriorated archaeological mummified cultural assets. Contactless biocidal treatments are particularly interesting on fragile specimens (like deconsolidated pieces). Avoiding potential problems due to EOs' reaction with the artistic surface (including, for example, colour alteration or solvent effect). Using plant-derived biocides instead of synthetic biocides can also favour a circular economy and environmental sustainability.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.culher.2023.02.006.

References

[1] M. Veneranda, L. Blanco-Zubiaguirre, G. Roselli, G. Di Girolami, K. Castro, J.M. Madariaga, Evaluating the exploitability of several essential oils constituents as a novel biological treatment against cultural heritage biocolonization, Microchem. J. 138 (2018) 1–6, doi:10.1016/j.microc.2017.12.019.

[2] K. Pietrzak, A. Otlewska, D. Danielewicz, K. Dybka, D. Pangallo, L. Kraková, A. Puškárová, M. Bučková, V. Scholtz, M. Ďurovič, B. Surma-Ślusarska, K. Demnerová, B. Gutarowska, Disinfection of archival documents using thyme essential oil, silver nanoparticles misting and low temperature plasma, J. Cult. Herit. 24 (2017) 69–77, doi:10.1016/j.culher.2016.10.011.

[3] M.R. Fidanza, G. Caneva, Natural biocides for the conservation of stone cultural heritage: a review, J. Cult. Herit. 38 (2019) 271–286, doi:10.1016/j.culher.2019.01.005.

[4] S. Angulo-Milhem, M. Verriele, M. Nicolas, F. Thevenet, Indoor use of essential oils: emission rates, exposure time and impact on air quality, Atmos. Environ. 244 (August 2020) (2021), doi:10.1016/j.atmosenv.2020.117863.

[5] S.D. Cox, C.M. Mann, J.L. Markham, H.C. Bell, J.E. Gustafson, J.R. Warmington, S.G. Wyllie, The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil), J. Appl. Microbiol. 88 (1) (2000) 170–175, doi:10.1046/j.1365-2672.2000.00943.x.

[6] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, Biological effects of essential oils – a review, Food Chem. Toxicol. 46 (2) (2008) 446–475, doi:10.1016/j.fct.2007.09.106.

[7] K. Hąc-Wydro, K. Szydło, The influence of environmentally friendly pesticide – Eucalyptol – alone and in combination with terpinen-4-ol – on model bacterial membranes, Colloids Surf. B 146 (2016) 918–923, doi:10.1016/j.colsurfb.2016.07.044.

[8] B.A. Behbahani, M. Noshad, F. Falah, Cumin essential oil: phytochemical analysis, antimicrobial activity and investigation of its mechanism of action through scanning electron microscopy, Microb. Pathog. 136 (August) (2019) 103716, doi:10.1016/j.micpath.2019.103716.

[9] J.L. Ríos, M.C. Recio, Medicinal plants and antimicrobial activity, J. Ethnopharmacol. 100 (1–2) (2005) 80–84, doi:10.1016/j.jep.2005.04.025.

[10] S. Chouhan, K. Sharma, S. Guleria, Antimicrobial activity of some essential oils—present status and future perspectives, Medicines 4 (3) (2017) 58, doi:10.3390/medicines4030058.

[11] D. Predoi, S.L. Iconaru, N. Buton, M.L. Badea, L. Marutescu, Antimicrobial activity of new materials based on lavender and basil essential oils and hydroxyapatite, Nanomaterials 8 (5) (2018) 291, doi:10.3390/nano8050291.

[12] K. Kozics, M. Bučková, A. Puškárová, V. Kalászová, T. Cabicarová, D. Pangallo, The effect of ten essential oils on several cutaneous drug-resistant microorganisms and their cyto/genotoxic and antioxidant properties, Molecules 24 (24) (2019) 4570, doi:10.3390/molecules24244570.

[13] N.S. Geweely, H.A. Afifi, D.M. Ibrahim, M.M. Soliman, Inhibitory effect of essential oils on growth and physiological activity of deteriorated fungal species isolated from three archeological objects, Saqqara excavation, Egypt, Geomicrobiol. J. 37 (6) (2020) 520–533, doi:10.1080/01490451.2020.1731021.

[14] L. Campanella, R. Angeloni, F. Cibin, E. Dell'Aglio, F. Grimaldi, R. Reale, M. Vitali, Capsulated essential oil in gel spheres for the protection of cellulosic cultural heritage, Nat. Prod. Res. 35 (1) (2021) 116–123, doi:10.1080/14786419.2019.1616726.

[15] I. Čabalová, B. Češek, O. Mikala, J. Gojný, F. Kačík, T. Tribulová, The influence of selected efficient compounds of essential oils for paper protection, J. Cult. Herit. 37 (0201) (2019) 148–154, doi:10.1016/j.culher.2018.11.002.

[16] S. Borrego-alonso, Los biocidas vegetales en el control del biodeterioro del patrimonio documental. Perspectivas e impacto, Rev. CENIC Ciencias Biol. 46 (3) (2015) 259–269 (PDF) Los biocidas vegetales en el control del biodeterioro del patrimonio documental. Perspectivas e impacto (researchgate.net).

[17] Y. Elsayed, Y. Shabana, The effect of some essential oils on Aspergillus niger and Alternaria alternata infestation in archaeological oil paintings, Mediterr. Archaeol. Archaeom. 18 (3) (2018) 71–87, doi:10.5281/zenodo.1461616.

[18] L. Gatti, F. Troiano, V. Vacchini, F. Cappitelli, A. Balloi, An *in vitro* evaluation of the biocidal effect of oregano and cloves' volatile compounds against microorganisms colonizing an oil painting—a pioneer study, Appl. Sci. 11 (1) (2021) 78, doi:10.3390/app11010078.

[19] K. Matusiak, W. Machnowski, H. Wrzosek, J. Polak, K. Rajkowska, K. Śmigielski, A. Kunicka-Styczyńska, B. Gutarowska, Application of Cinnamomum zeylanicum essential oil in vapour phase for heritage textiles disinfection, Int. Biodeterior. Biodegrad. 131 (2018) 88–96, doi:10.1016/j.ibiod.2017.02.011.

[20] V. Rotolo, G. Barresi, E. Di Carlo, A. Giordano, G. Lombardo, E. Crimi, E. Costa, M. Bruno, F. Palla, Plant extracts as green potential strategies to control the biodeterioration of cultural heritage, Int. J. Conserv. Sci. 7 (Specialissue2) (2016) 839–846.

[21] G. Piñar, D. Piombino-Mascali, F. Maixner, A. Zink, K. Sterflinger, Microbial survey of the mummies from the capuchin catacombs of palermo, italy: biodeterioration risk and contamination of the indoor air, FEMS Microbiol. Ecol. 86 (2) (2013) 341–356, doi:10.1111/1574-6941.12165.

[22] A. DeAraujo, A. Vasanthakumar, M. Sepulveda, V. Standen, B. Arriaza, R. Mitchell, Investigation of the recent microbial degradation of the skin of the Chinchorro mummies of Ancient Chile, J. Cult. Herit. 22 (July) (2016) 999–1005, doi:10.1016/j.culher.2015.11.004.

[23] M. Čavka, A. Glasnović, I. Janković, P.R. Šikanjić, B. Perić, B. Brkljačić, E. Mlinarić-Missoni, J. Škrlin, Microbiological analysis of a mummy from the archeological museum in Zagreb, Coll. Antropol. 34 (3) (2010) 803–805.

[24] A. Puškárová, M. Bučková, L. Kraková, D. Pangallo, K. Kozics, The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells, Sci. Rep. 7 (1) (2017) 1–11, doi:10.1038/s41598-017-08673-9.

[25] M. Stupar, M.L. Grbić, A. Džamić, N. Unković, M. Ristić, A. Jelikić, J. Vukojević, Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects, South Afr. J. Bot. 93 (November 2015) (2014) 118–124, doi:10.1016/j.sajb.2014.03.016.
[26] F. Palla, M. Bruno, F. Mercurio, A. Tantillo, V. Rotolo, Essential oils as natural biocides in conservation of cultural heritage, Molecules 25 (3) (2020) 730, doi:10.3390/molecules25030730.
[27] I. Romano, G. Granata, A. Poli, I. Finore, E. Napoli, C. Geraci, Inhibition of bacterial growth on marble stone of 18th century by treatment of nanoencapsulated essential oils, Int. Biodeterior. Biodegrad. 148 (2020) 104909, doi:10.1016/j.ibiod.2020.104909.
[28] X. Wang, Y. Hu, Z. Zhang, B. Zhang, The application of thymol-loaded chitosan nanoparticles to control the biodeterioration of cultural heritage sites, J. Cult. Herit. 53 (2022) 206–211, doi:10.1016/j.culher.2021.12.002.
[29] Y. Xie, Z. Wang, Q. Huang, D. Zhang, Antifungal activity of several essential oils and major components against wood-rot fungi, Ind. Crops Prod. 108 (January) (2017) 278–285, doi:10.1016/j.indcrop.2017.06.041.
[30] N. Valentín, A. Fazio, Análisis de la incidencia del SARS-CoV-2 en bienes culturales. Sistemas de desinfección. Fundamentos y estrategias de control, Ge-lic (2020) https://www.ge-iic.com/wp-content/uploads/2020/06/Versi%C3%B3n-2.-COVID-Publicac.-Nieves-Valent%C3%ADn-Alejandra-Fazio.pdf.
[31] P. Sanmartín, A. DeAraujo, A. Vasanthakumar, Melding the old with the new: trends in methods used to identify, monitor, and control microorganisms on cultural heritage materials, Microb. Ecol. 76 (1) (2018) 64–80, doi:10.1007/s00248-016-0770-4.
[32] Río Oliver, C. del, Actividad Antifúngica de Tres Aceites Esenciales Contra Hongos Aislados de Pintura Mural, 2019 https://riunet.upv.es:443/handle/10251/129409.
[33] D. Kalemba, A. Kunicka, Antibacterial and antifungal properties of essential oils, Curr. Med. Chem. 10 (10) (2005) 813–829, doi:10.2174/0929867033457719.
[34] S. Borrego, O. Valdés, I. Vivar, P. Lavin, P. Guiamet, P. Battistoni, S. Gómez de Saravia, P. Borges, Essential oils of plants as biocides against microorganisms isolated from Cuban and Argentine documentary heritage, ISRN Microbiol. (2012) 1–7, doi:10.5402/2012/826786.
[35] P. Sanmartín, A. DeAraujo, A. Vasanthakumar, R. Mitchell, Feasibility study involving the search for natural strains of microorganisms capable of degrading graffiti from heritage materials, Int. Biodeterior. Biodegrad. 103 (2015) 186–190, doi:10.1016/j.ibiod.2015.05.010.
[36] G. Caneva, M.P. Nugari, O. Salvadori, La Biología En La Restauración, Editorial Nerea, 2000 Vol. 5.
[37] N. Valentín, Biosensores como sistemas de alarma para detectar riesgos de biodeterioro en restos momificados. Estudios preliminares, Bol. Museo Arqueol. Nacional 33 (2015) 344–354 https://dialnet.unirioja.es/servlet/articulo?codigo=5339758.
[38] D.S. Nitiu, A.C. Mallo, L.A. Elíades, M.N. Saparrat, H.R. Vazquez, Monitoreo de la carga fúngica ambiental y de otros bioaerosoles en un depósito de restos momificados del NOA del Museo de la Plata (Argentina): un estudio de caso, Bol. Soc. Argentina Bot. 50 (4) (2015) 427–436 http://sedici.unlp.edu.ar/bitstream/handle/10915/65230/Documento_completo.pdf-PDFA.pdf?sequence=1&isAllowed=y.
[39] J.L. Ruiz González, E. Bazán Mora, K.E. Barrera Moreno, R. López Martínez, Estudio micológico para la conservación de las momias de Tlayacapan, Morelos, Estud. Antropol. Biol. 18 (2) (2016) 55–67 http://www.revistas.unam.mx/index.php/eab/article/view/56872.
[40] V.M.M. Chaparro, J.R.P. Castiñeira, M.S. Puerto, Estudio de microorganismos causantes de biodeterioro mediante técnicas de biología molecular en el IAPH, PH: Bol. Inst. Andaluz Patrimonio Hist. 21 (84) (2013) 174–187 http://www.iaph.es/revistaph/index.php/revistaph/article/view/3379.
[41] Z. Jurjevic, S.W. Peterson, B.W. Horn, Aspergillus section Versicolores: nine new species and multilocus DNA sequence based phylogeny, IMA Fungus 3 (1) (2012) 59–79.
[42] Z.X. Li, X.F. Wang, G.W. Ren, X.L. Yuan, N. Deng, G.X. Ji, P. Zhang, Prenylated diphenyl ethers from the marine algal-derived endophytic fungus Aspergillus tennesseensis, Molecules 23 (9) (2018) 2368, doi:10.3390/molecules23092368.
[43] D. Acevedo, C. Granados, E.M. Guerrero, Cinética enzimática del bagazo de caña para la producción de glucosa utilizando la enzima trichoderma iongibrachiatum, Inf. Tecnol. 25 (5) (2014) 65–72.
[44] M. Benkovičová, Z. Kisová, M. Bučková, E. Majková, P. Šiffalovič, D. Pangallo, The antifungal properties of super-hydrophobic nanoparticles and essential oils on different material surfaces, Coatings 9 (3) (2019) 176 https://www.researchgate.net/publication/331603711_The_Antifungal_Properties_of_Super-Hydrophobic_Nanoparticles_and_Essential_Oils_on_Different_Material_Surfaces.