SCIENTIFIC CULTURE
www.sci-cult.com

SCIENTIFIC CULTURE, Vol. 9, No. 3, (2023), pp. 113-125
Open Access. Online & Print

DOI: 10.5281/zenodo.8244949

ANALYTICAL ASSESSMENT OF SOME ESSENTIAL OILS AGAINST COMMON FUNGI ISOLATED FROM EGYPTIAN HERITAGE PART I: TEXTILES AND OIL PAINTINGS

Yosr Elsayed[1]*, Yasser Shabana[2], Hemdan Elmitwalli[1], Younes Rashad[3], Prasad Sreenivasaprasad[4], and Nabil Mabrouk[1]

[1]Conservation Department, Faculty of Archaeology, Damietta University, Damietta, Egypt
[2]Plant Pathology Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt
[3]Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City, Egypt
[4]Department of Life Sciences and Institute of Biomedical and Environmental Science and Technology, University of Bedfordshire, Luton, LU1 3JU, UK

Received: 28/07/2023
Accepted: 28/08/2023
Corresponding author: Y. Elsayed (yea00@du.edu.eg)

ABSTRACT

Based on the results of our previous work, the essential oils (EOs) of black pepper, red pepper, cinnamon, ginger, and camphor proved the top maximum efficiency among the fifty tested EOs against the eighteen tested fungi. The present study aims to assay the antifungal activities and the positive/negative effects of these five EOs against *A. niger, A. flavus, C. halotolerans, and N. goegapense*, as well as the prevalent deteriorating fungi isolated from the historical oil paintings and textiles in the Agricultural Museum in Dokki, Giza. The vapor technique was used to disinfect the fungi-contaminated mock-ups of oil painting, cotton, and wool fabrics using the plate method. Count of spores, macro and microscopic stereo and SEM techniques, FTIR spectroscopy, and colorimetric measurements were used in assessment. The results revealed the efficient antifungal activities of all tested EOs against the selected fungi, varying from the highest (99.85%) for black pepper EO against *N. goegapense* to the lowest (71.65%) for camphor EO against *N. goegapense*. It proved that the EO of black pepper was the most potent and efficient EO among the tested ones against *A. flavus, C. halotolerans, and N. goegapense*. Regarding *A. niger,* the study revealed that the EO of ginger proved the most potent and efficient EO against it. The vapor phase method proved a very efficient and non-destructive technique against the infected mock-ups. In some cases, it eradicated up to ~100% of fungi without any notable changes in the properties of the treated mock-ups. A small amount of black pepper EO was advised to be vapored in all closed display and storage cases in the museums as a preventive technique to inhibit the fungal growth of heritage materials.

KEYWORDS: Bio-deterioration, conservation, essential oils, fungi, cultural heritage, paintings, SEM, FTIR, textiles

1. INTRODUCTION

Cultural heritage is significant in our life, providing an identity and sense of belonging to individuals, communities, and nations and keeping us attached to our religion, traditions, and beliefs. Egypt's rich and varied cultural heritage extends over thousands of years. However, this cultural heritage is subjected to bio-deterioration by different deteriorating fungi and bacteria, resulting in severe damage, representing the main reason for this concern after detailed investigation and analysis (Abdel-Kareem et al., 2021; Gouda et al., 2023; Mazzoli et al., 2018). Due to their ability to produce a diverse set of lytic enzymes and their low requirement of humidity, fungi are qualified to decay or stain different types of heritage materials. They have a wide range of enzymatic activities and the ability to grow at relatively low water activity levels, enabling them to inhabit, change and/or decay various organic and inorganic materials used in heritage objects indoors or outdoors as well (Görs et al., 2007; Sterflinger, 2010).

As for historical textiles, the main degrading agent is fungi due to their secretion of cellulolytic enzymes and organic acids, affecting the textile's extensibility, appearance, and color (Cappitelli et al., 2020; Omar et al., 2019). In addition, the fungal mycelia penetrate the textile fibers making them difficult to be removed (Kavkler et al., 2015). Paintings and painted surfaces have a complex constitution, with a mix of inorganic and organic materials arranged in thin layers of diverse characters and functions. The textile support of canvas paintings provides the main organic material for fungal and bacterial growth and can support microbiological alteration processes in various environmental conditions (Caneva et al., 2000). Fungi can cause a spoiling and/or staining of paintings and enzymatically degrade the organic paint binders (Mesquita et al., 2009; Sterflinger, 2010).

Various methods can be used to disinfect heritage materials from deteriorating fungi, including physical methods, such as deep freezing, UV radiation, gamma radiation, heat shock, and controlled atmospheres; chemical methods, such as treating with ethanol (70%), thymol, ethylene oxide, metal nanoparticles, or fumigation with pesticide gases. However, each method has disadvantages that may affect the chemical composition of the archaeological heritage, or it may be toxic or harmful to humans (Castillo et al., 2019). Therefore, recent research has focused on the green methods of heritage conservation as a safe and eco-friendly alternative method (Elsayed et al., 2023; Palla et al., 2020; Stupar et al., 2014).

There is great concern about the use of natural substances, such as plant extracts and essences and oils (EOs). However, their use has already been verified in mummification techniques in ancient Egypt (4500 and 3350 BC) owing to their wide spectrum of biological activities against microorganisms (Martín-Rey et al., 2023; Rasooli et al., 2006). They have a wide range of applications in ethnomedicine, preservation, food flavoring, fragrances, perfume industries, etc. Therefore, considerable attention has been paid to their biological effects (Bakkali et al., 2008; Christian et al., 2023; Rashad et al., 2020). Recently, EOs have been applied against insects and microorganisms isolated from different artifacts, but the reports of the assessment of their impact on the physical and chemical characteristics of the artifacts are still limited (Othman et al., 2020; Sadiki et al., 2017; Stupar et al., 2014; Vicenço et al., 2021). Few studies applied EOs and plant extracts in the field of heritage conservation, mainly to protect paper and papyrus documents (Borrego et al., 2016; Guiamet et al., 2008; Noshyutta et al., 2016) and heritage textiles and papyrus (Matusiak et al., 2018; Othman et al., 2020), and wood (Antonelli et al., 2020). Other studies used an efficient mixture of EOs (such as oregano, lemongrass, and peppermint in a ratio of 1:1:1) against some fungi (Tomić et al., 2023).

EOs are concentrated volatile aromatic liquids derived from natural plants and can be extracted from their flowers, leaves, seeds, peels, branches, bark, wood, roots, underground stems, gums, or oily resin (Soliman et al., 2022). They are very complex natural mixtures containing about 20–60 components at quite different concentrations. Each EO is a complex mixture made up primarily of alcohols, esters, aldehydes, oxides, phenols, coumarins, ethers, ketones, acids, and other ingredients. Additionally, EOs are characterized by a strong odor and are mainly composed of two or three major components at fairly high concentrations (20–70%) (Bakkali et al., 2008; Camele et al., 2021). They are usually obtained by steam or hydrodistillation of different parts from natural plants. Still, after the industrial revolution, these materials were industrially developed, and in parallel, they began to be replaced by biocides processed in the laboratory, being mostly very harmful to the human organism (Martín-Rey et al., 2023). Previous studies reported the chemical composition of the five tested EOs according to the Gas Chromatography-Mass Spectroscopy GC-MS (Shi et al., 2013; Menon & Padmakumari, 2005; Murbach Teles Andrade et al., 2014; Wesołowska et al., 2015).

In a previous study conducted by the authors (Mabrouk et al., 2023), fifty EOs were tested for their antifungal potential effects against five prevalent deteriorating fungi, based on a survey study in different archaeological sites and museums in Egypt, namely *A. niger, A. flavus, A. ochraceopetaliformis, C. halotoler-*

ans, and N. goegapense. Based on the results of our previous study, five EOs (black pepper, red pepper, cinnamon, ginger, and camphor) proved the highest antifungal activities against the tested fungi cultivated on a sterilized PDA medium in Petri dishes. The present study aims to 1) evaluate the application of the five EOs against the four prevailing fungi isolated from the painting and textile objects located at the Agricultural Museums (*A. niger, A. flavus, C. halotolerans, and N. goegapense*), cultivated on simulated fabric and painting mock-ups in Petri dishes; 2) assay the impacts (either positive or negative) of the EOs on the properties of the simulated mock-ups of oil paintings and textile fabrics. The study presented a new material (EO of black pepper) recommended to be used in vapor phase as a very efficient and completely safe to heritage materials.

2. MATERIALS AND METHODS

2.1 *Case study heritage objects*

The case study heritage objects included five textile objects (cotton embroidered prayer carpet No. 24 from 18th century, cotton embroidered Indian textile No. 26 from 18th century, cotton embroidered Jilbab "robe", woolen Coptic embroidered textile fragment No. 347/2, woolen embroidered Coptic textile fragment No. 329/4,) No. 224/2 from 17th) and four oil paintings (WELSH PONEY painting canvas by the Egyptian painter Mohammed Hassan in 1934, FRUIT TREES painting on canvas by the Italian painter Amelia Casonato in ~1930, FLOWERS PAINT painting on canvas by by the Egyptian painter Ali Eldeeb in 1939, and COWS AND BUFFALOS painting on canvas by the Turkish painter Hedyat Shirazi in ~1930. All case study heritage textiles and paintings are displayed in the Egyptian Agricultural Museum in Giza, Egypt. These objects were selected because they suffered many deterioration and biodeterioration phenomena due to the inappropriate minor and major display environment (Abdel-Kareem et al., 2022; Amin, 2018). All these selected objects were in bad need of treatment. Among these archaeological textiles and paintings, two textile objects and two painting objects were selected as detailed undermentioned examples (Fig. 1).

Object 1 (Fig. 1a) was a fragment of wool textile decorated with a dyeing method. It had a stripe that ran across the width of the piece of textile by means of dyeing of reddish-brown color. The ornamentation was branches of plants, flowers, and various living creatures; Some were human drawings of people or animals. No Arabic writings were found, which made its attribution to the Islamic era difficult; it was attributed to the Coptic tapestry of the 7th century. It was displayed in the Museum of Heritage Collections with No. 347/2. Object 2 (Fig. 1b) was a Coptic tapestry fragment, dated back to the 7th-8th centuries, divided into two longitudinal stripes of wool and cotton fibers, decorated in dark green, which were in a poor state of preservation. The texture of the decoration consisted of geometric shapes made of lozenges, resulting from the intersection of two narrow bands, representing a broken rib (zigzag). Within each rhombus, an axis of a flower of six petals was drawn. No Arabic writings were found, which made its attribution to the Islamic era difficult. It was displayed in the Museum of Heritage Collections with No. 329/4.

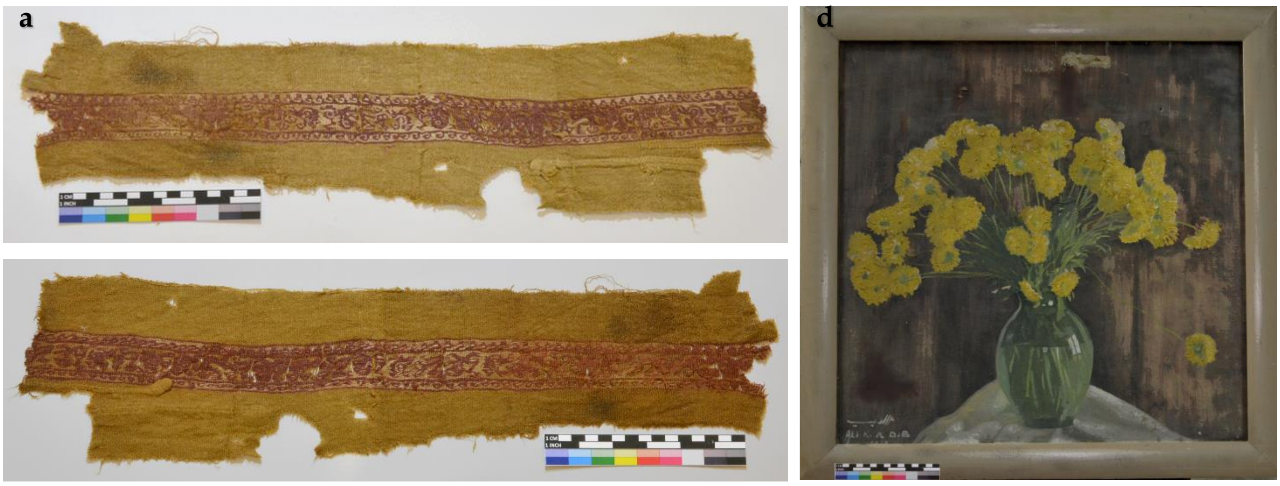


Figure 1. Some of the case-study archaeological textiles and oil paintings; a. object 1, b. object 2, c. object 3, d. object 4

Object 3 (Fig. 1c) was a painting on canvas named "WELSH PONEY", painted in 1934 by the Egyptian painter Mohammed Hassan (1892–1961). Its size was 105 x 78 cm, and its topic was the WELSH PONEY, a breed of small horse popular as a child's or an adult's mount; a hardy breed that developed in Welsh mountains. The painting suffered severe dirt and different solid stains and spots. It was displayed in the Museum of Heritage Collections. Additionally, Object 4 (Fig. 1d) was a painting on canvas named "FLOWERS PAINT", painted in 1939 by the Egyptian painter Ali Eldeeb (1909 – 1997). Its size was 73 x 73 cm, and its topic was the flowers painting, with some similarities to the famous one by Van Gogh.

2.2 *Preparation of mock-ups*

The oil painting and textile mock-ups were prepared to simulate the historic paintings and textiles in the Agricultural Museums in Giza. The oil painting mock-ups were prepared according to the most common and traditional recipes of historic oil paintings detailed in some pieces of literature (Elsayed, 2019; Luke, 1988; Walter & de Viguerie, 2018). A linen fabric support (Egyptian Company for Textile Industry, Egypt) was coated by a white ground layer of animal glue and white lead (local market, Egypt). The ground layer was brushed by a paint layer of color pigments in linseed oil (Winsor & Newton, USA), then dried at room temperature at ~25±2 °C for a month. The textile mock-ups were prepared from plain cotton (Mahalla Co., Egypt) and sheep wool (Wools Golden Tex Company, Egypt) fabric by boiling in water and soap for 30 min. to eliminate any fiber additives, dried, ironed, and cut into 3×5 cm (ASTM- D629, 1999, (Abd Elhameed et al., 2021; Abdel-Kareem, 2010; Abdel-Kareem, 2015; Matusiak et al., 2018). After preparation, oil painting and textile mock-ups were aged at 105±1 °C in an oven (Hot air oven, Binder, ED115, Germany) for 357 h. (Barbera et al., 2022; Elsayed & Shabana, 2018; Yong-hua et al., 2012).

2.3 *Case study fungi and essential oils (EOs)*

Based on the results of our previous work (Mabrouk et al., 2023), four case study fungi (*A. niger, A. flavus, C. halotolerans, and N. goegapense*) were the prevalent deteriorating fungi isolated from the surveyed objects in the Agricultural Museums. Thus, these four fungi would be tested for their sensitivity against the most efficient tested EOs (*piper nigrum* L. (black pepper), *capsicum annuum* L. (red pepper), *cinnamomum verum* J. Presl. (cinnamon), *Zingiber officinale* Roscoe (L.) H. Karst. (ginger), and *cinnamomum camphora* (L.) J. Presl. (camphor), that proved the top maximum efficiency among the fifty tested EOs against the eighteen tested fungi. The tested EOs were extracted from their different plant parts according to (Charles & Simon, 1990), as mentioned in our previous work (Mabrouk et al., 2023).

2.4 *Screening of EOs for their antifungal activity*

A set of 1.5×5 cm paintings, cotton, and woolen fabric mock-ups was dipped into the broth culture of each fungus for 30s (Salvador et al., 2017). Then, these mock-ups were placed on a sterile glass slide in sterile Petri dishes lined with sterile filter papers moistened with distilled water. Segments of each painting, cotton, and woolen fabric mock-ups were separately immersed in the fungal suspension ($24×10^6$ spore mL^{-1} for *A. niger* HYNYY3, $21×10^6$ spore mL^{-1} for *A. flavus* HYNYY2, $27×10^6$ spore mL^{-1} for *C. halotolerans* HYNYY1, and $31×10^6$ spore mL^{-1} for *N. goegapense* HYNYY4) for 30 s. in a sterilized Petri dish, one mock-up was fitted on a sterilized glass slide over sterilized filter papers moistened with sterilized water. The Petri dishes were incubated at 26±2 °C for two weeks until fungal growth appeared on the surface of the mock-ups.

For each mock-up, a volume of 100 µl of the pure essential oil was applied onto two 10mm diameter sterile blank filter discs (Advantec 49006010, Toyo Kaisha, Ltd. Japan) and placed in the Petri dish lid (Lopez et al., 2005). Dishes were sealed with Parafilm® to avoid the evaporation of the volatile solutions and incubated at 28±2C for five days (Lopez et al., 2005; Matusiak et al., 2018; Othman et al., 2020). Petri dishes with non-infested mock-ups (only vapored with each EO) were used as a positive control to assay any probable effect on the properties of mock-ups. Other non-infested and no-vapored mock-ups were used as negative controls. After that, mock-ups were removed for variant methods of assay and finally soaked in 30 ml of sterile water containing 0.5% (v/v) of Tween-20, then shaken for 10 min. on a rotary shaker at 100 rpm to help release the fungal spores. The number of spores/ml was counted with a haemacytometer. The tests were performed in triplicate (Elsayed & Shabana, 2018; Shabana et al., 2000).

2.5 *Macro and microscopic study*

Stereo microscope with integrated camera (LABOMED stereo zoom trinocular head microscope - model Luxeo 6Z, Made in the U.S.A) and photography camera (Sony Alpha a7 III mirrorless digital camera with Sony FE 90mm f/2.8 macro G OSS lens) were used in capturing the surface features of mock-ups. The scanning electron microscopy (SEM, JEOL JSM-6510 LV, JEOL Ltd., Japan, AC-CEL_20KV, MAG 4300, SIGNAL SEI, WD10mm) was used to identify the microbiological features of mock-ups after biodeterioration with the selected fungi and the effects of the selected EOs after treatment. Mycelial plugs (1×1×3 mm) were fixed in phosphate-buffered 3% glutaraldehyde at pH 6.8 and dehydrated in graded series of acetone. Later, samples were dried with CO_2 using acetone as an intermediate fluid. The pieces of agar were sputter-coated with plutonium by JFC-1600 auto-fine coater (Elamin et al., 2018; Elsayed & Shabana, 2018).

2.6 *Colorimetric measurements*

One of the main principles of heritage conservation is to preserve the properties of heritage materials. Therefore, it is very important to assess the properties of the mock-ups before and after treatment with EOs. A spectrophotometer (PCE-CSM 2, Germany) was used to measure the fabric colors and each color of the paintings. The color specifications were CIE Lab, wavelength range: 400-700nm, light source: D65, observer angle: 10°, measuring aperture: Φ4mm). The mock-ups were characterized by means of the L*, a*, and b* uniform color space (CIELAB). The data reported were based on an average of three measurements for each color and calculated for the CIE Lab 1976 color space (Mecklenburg et al., 2013).

2.7 *Fourier transform infrared spectroscopy (FTIR)*

Mock-ups were examined by the Fourier transform infrared spectroscopy (FTIR) to assay any probable changes in the mock-ups' structure. Analysis was performed using a reflection spectrophotometer Nicolet IS50 FTIR (Thermo Scientific, USA) equipped with an ATR (attenuated total reflectance) detector in the range of 600-4000 cm^{-1}, with a resolution of 4cm^{-1}.

3. RESULTS AND DISCUSSION

3.1 *Results of antifungal activity*

The results of the antifungal activity of the selected five EOs against the selected fungi (Fig. 2, Table 1) revealed that all EOs proved variant growth inhibition against all fungi compared to the untreated controls. The EO of black pepper proved the most potent and efficient antifungal activity among the tested EOs

(98.81%, 97.68%, 99.85%) against *A. flavus, C. halotolerans, and N. goegapense,* respectively. This result is in agreement with the other findings that reported the efficiency of black pepper EO in the growth inhibition of *A. flavus.* It affected the cell membrane of *A. flavus* and destroyed the membrane structure of the cell at different concentrations, causing the cell membrane to lose its integrity (Bratitsi et al., 2018; Zhang et al., 2021). Other studies confirmed the efficiency of black pepper EO in the growth inhibition of *A. niger* and its moderate antibacterial activities (Singh et al., 2004; Sultana et al., 2022). This efficacy illustrated using black pepper EO in medicine and food protection much time ago (Bi et al., 2019; Steinhaus & Schieberle, 2005).

The EO of ginger proved the most potent and efficient among the tested EOs against *A. niger; i*t showed more efficiency than black pepper EO in the case of *A. niger.* The efficiency of ginger was reported either in the form of EO or plant extract against many tested fungi due to its efficient constituent, monoterpene, which disrupts fungal membrane integrity (Hasan et al., 2012; Othman et al., 2020). Moreover, it is potent against bacteria and various human diseases (Hunt et al., 2013; Mickienė et al., 2011).

Regarding the viability of spores, black pepper EO resulted in the highest number of dead spores in the case of *N. goegapense* (1364 killed and 2 live, 99.85%) and a very high number of dead spores (95.70%, 98.81%, 97.68%) in the case of *A. nigar, A. flavus, C. halotolerans,* respectively. The EO of ginger resulted in the highest number of dead spores in the case of *A. nigar*, (1450 killed and 25 live, 98.28%), very acceptable results (91.91% and 96.62%) against *C. halotolerans* and *N. goegapense,* and moderate result *(82.94%)* against *A. flavus.*

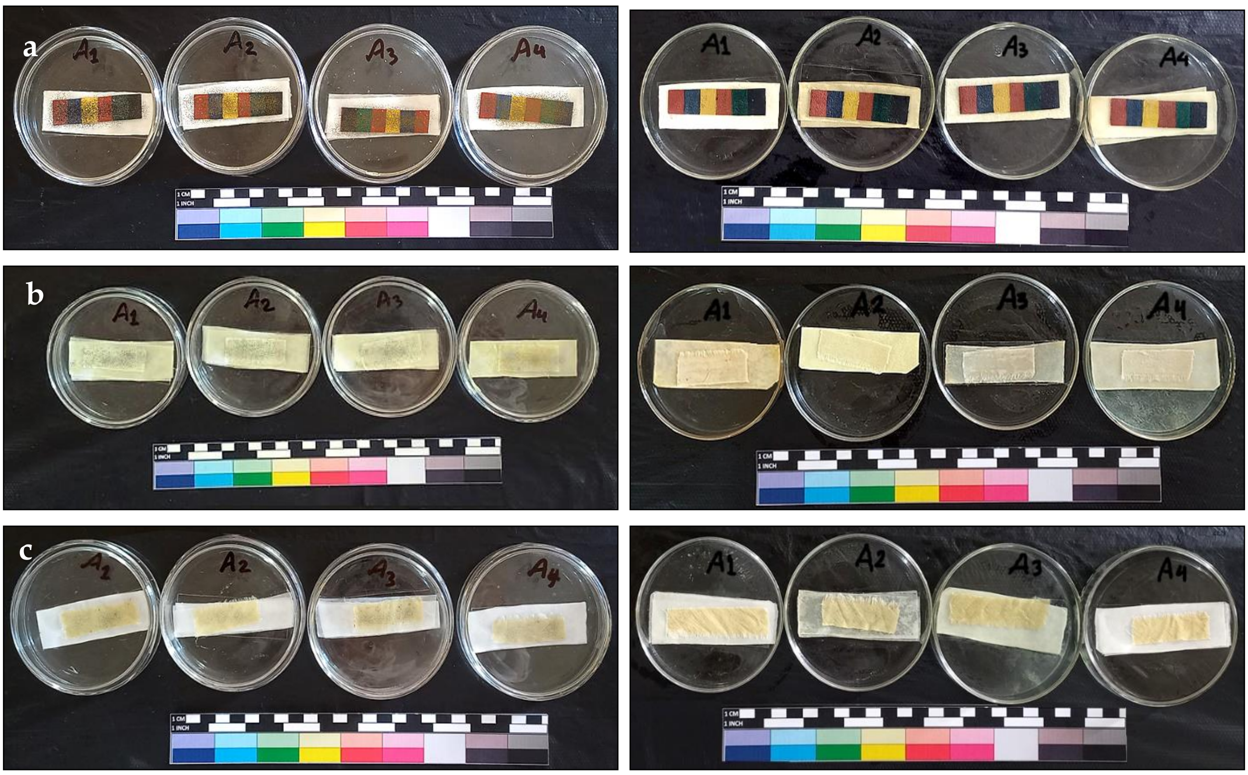


Figure 2. Graphs of the optimum antifungal activity of black pepper oil against the four selected fungi; a. oil paintings mock-ups, b. cotton fabric mock-ups, c. woollen fabric mock-ups. A1, A2, A3, A4 (A. nigar, A. flavus, C. halotolerans, and N. goegapense) respectively. Left (before treatment), Right (after treatment).

Table 1. The average no. of dead and live spores obtained from painting, cotton, and wool mock-ups affected by the tested EOs against A. niger, A. flavus, C. halotolerans, and N. goegapense.

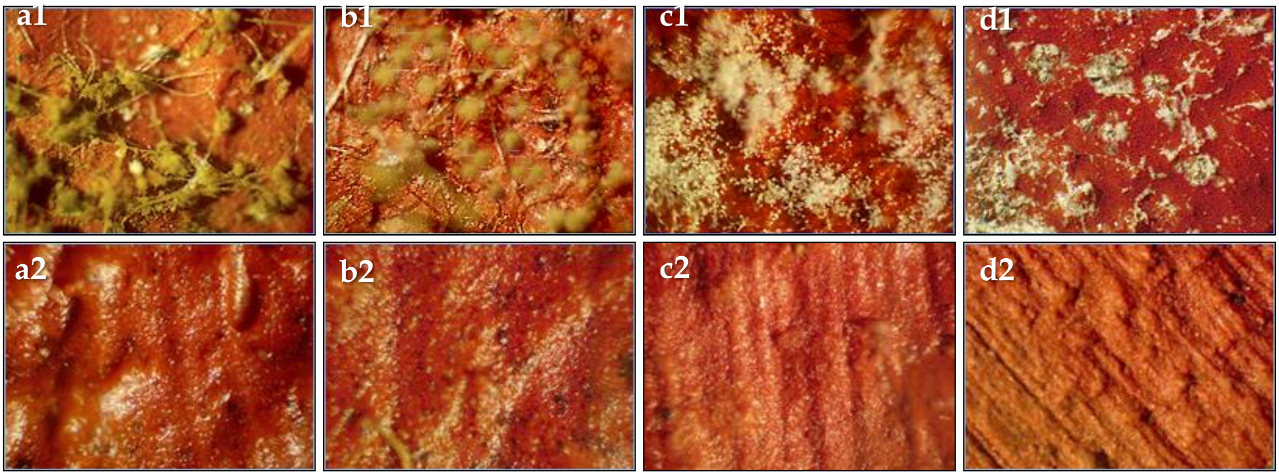
	A. niger			*A. flavus*			*C. halotolerans*			*N. goegapense*		
	No. Dead spores	No. Live spores	% Dead spores	No. Dead spores	No. Live spores	% Dead spores	No. Dead spores	No. Live spores	% Dead spores	No. Dead spores	No. Live spores	% Dead spores
Black pepper	1345.00	54.00	95.70	1421.00	16.00	98.81	1421.00	33.00	97.68	1366.00	2.00	99.85
Ginger	1360.00	25.00	98.28	1385.00	232.00	82.94	1385.00	112.00	91.91	1332.00	45.00	96.62
Red pepper	1258.00	136.00	88.76	1437.00	217.00	82.75	1437.00	82.00	94.29	1456.00	354.00	75.69
Cinnamon	1382.00	175.00	86.93	1384.00	326.00	76.41	1384.00	165.00	88.08	1339.00	97.00	92.76
Camphor	1178.00	108.00	91.36	1320.00	163.00	86.16	1320.00	155.00	88.26	1143.00	324.00	71.65

3.2 Results of SEM and stereo microscopy

The results of the stereo and SEM microscopy of the oil painting and textile fabric mock-ups before and after treatment with the five EOs (Fig. 3, Fig. 4) revealed that all oils affected the fungal growth of all treated fungi in a different way. The stereo micrographs of black pepper oil proved that it had the optimum antifungal activity against *A. flavus, C. halotolerans,* and *N. goegapense*, while ginger EO proved the optimum antifungal activity against *A. niger* (Sultana et al., 2022; Zhang et al., 2021).

The SEM painting micrographs of *A. niger* (untreated mock-ups) showed a cluster of normal unaffected conidia with smooth surfaces, while the treated mock-ups showed a few conidia, and the others were disintegrated. Regarding *A. flavus* (untreated mock-ups), the cluster of normal unaffected conidia with wrinkled surfaces was noted. After treatment, the hyphae were collapsed, twisted, and without cytoplasm. The *C. halotolerans* (untreated mock-ups) showed normal, dense, aggregated, and unaffected hyphae, while the treated mock-ups showed a normal dense hyphal mat carrying normal ellipsoidal conidia. Finally, the *N. goegapense* (untreated mock-ups) showed complete hyphae and conidia, but the treated mock-ups showed a few hyphae that were collapsed with few uncompleted conidia (Elsayed & Shabana, 2018; Noshyutta et al., 2016).

The SEM micrographs of the black pepper EO against *A. nigar*, *A. flavus*, *C. halotolerans*, and *N. goegapense* colonizing the woolen fabric mock-ups revealed the optimum antifungal activities. *A. niger* (untreated) showed normal unaffected clusters of surface-wrinkled conidia and thin conidiophores. *A. niger* (treated) showed a few hyphae with no conidia formed. *A. flavus* (untreated) showed a cluster of normal surface-wrinkled conidia. Additionally, *A. flavus* (treated) showed no hyphae or conidia and remnants of decomposition of the fungus. *C. halotolerans* (untreated) illustrated clusters of ellipsoidal surface-smoothed conidia. *C. halotolerans* (treated) showed few hyphae, and some collapsed. Note that the unknown filaments seem to be a mucilaginous substance. *N. goegapense* (untreated) showed normal smooth septated hyphae and normal conidia. *N. goegapense* (treated) illustrated the hyphae or conidia do not exist. In addition, the fungus' decay leftovers were noticed (Gouda et al., 2023; Matusiak et al., 2018; Othman et al., 2020).



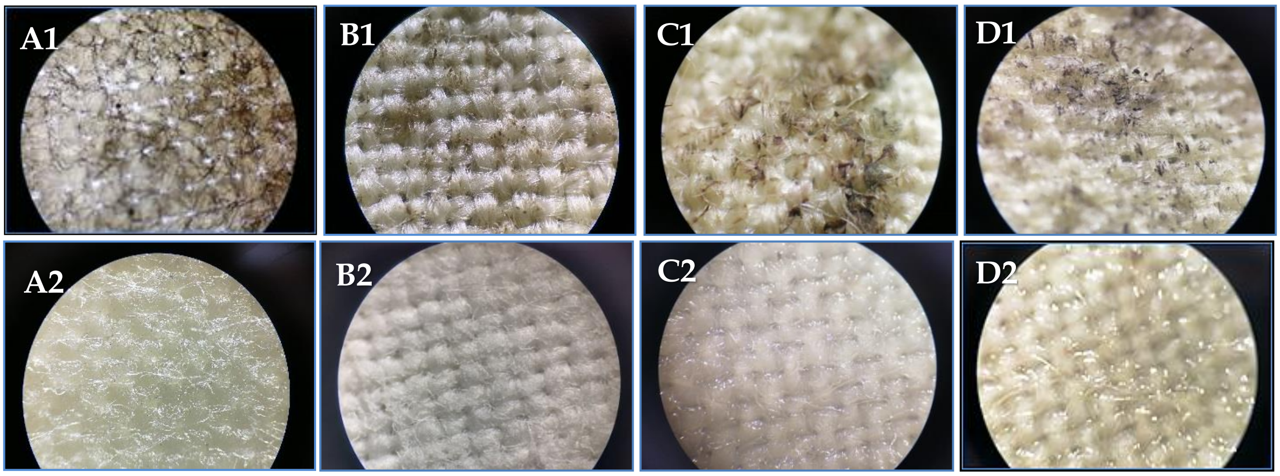


Figure 3. Stereo micrographs (100X) of the antifungal activity of the EO of black pepper against A. niger, A. flavus, C. halotolerans, N. goegapense colonizing the oil painting mock-ups (a., b., c., d.) and the woollen fabric mock-ups (A., B., C., D.) respectively. 1 (untreated control), 2 (treated).

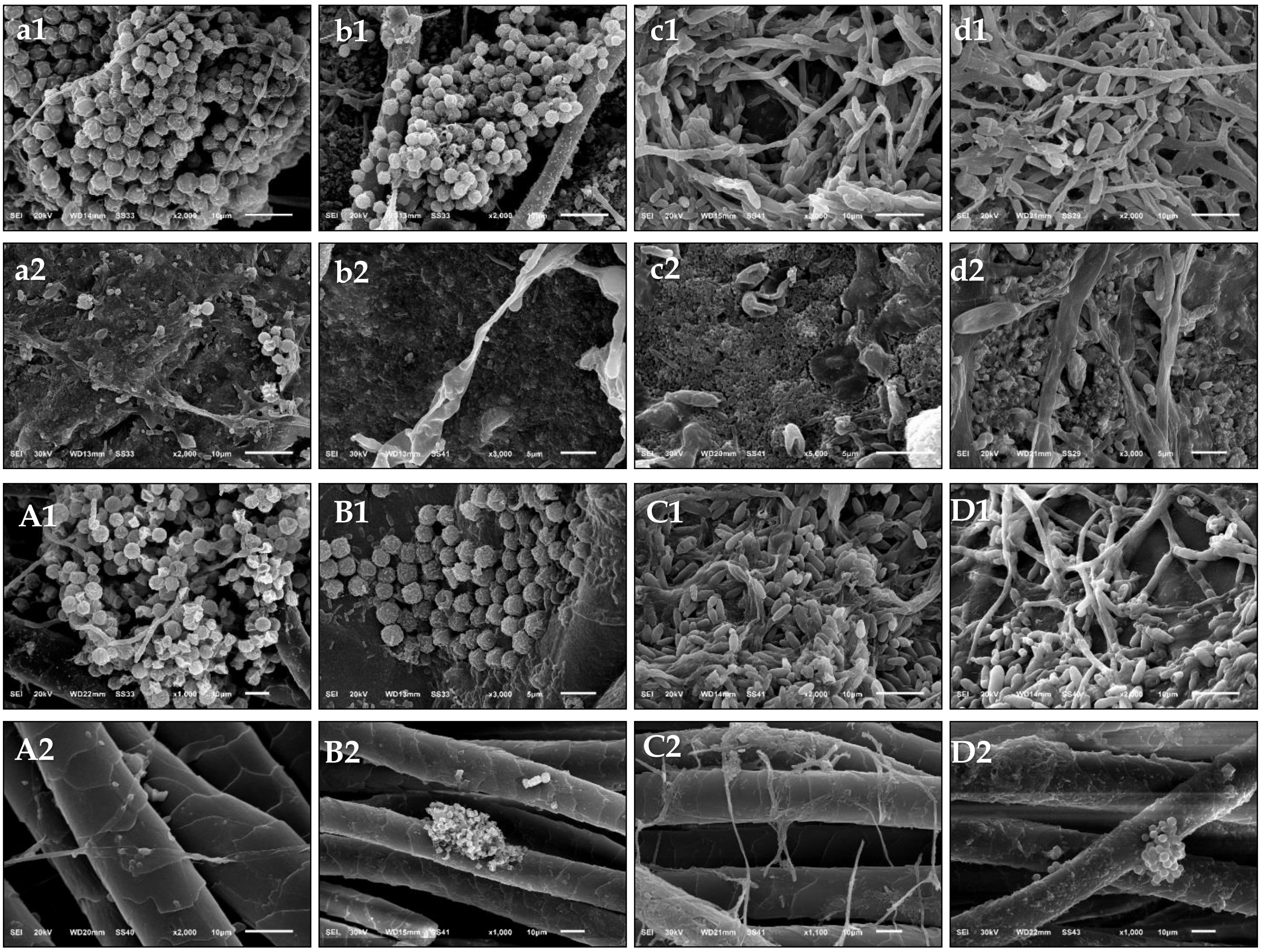


Figure 4. SEM micrographs show the antifungal activity of the EO of black pepper against A. niger, A. flavus, C. halotolerans, N. goegapense colonizing the oil painting mock-ups (a., b., c., d.) and the woollen fabric mock-ups (A., B., C., D.) respectively. 1 (untreated control), 2 (treated).

3.3 Colorimetric measurements

The results of the colorimetric measurements (Table 2) revealed no notable color changes in all negative control mock-ups (EO only). This was clear and almost the same in the five EOs applied to all mock-ups. Although it is considered low, the highest value in color changes was attributed to the EOs of red pepper (ΔE^*= 3.45, 3.55, 4.28) in painting, cotton, and wool, respectively. The lowest one was attributed to camphor (ΔE^*= 1.09, 1.24, 0.92) in painting, cotton, and wool, respectively, and black pepper (ΔE*=.1.04, 1.40, 1.02) in painting, cotton, and wool, respectively. These colorimetric results conclude that the vapor phase technique used in the study had no notable changes in all negative control samples. It is important to clarify if the used method has any negative effect on the samples, away from the negative sure effects of the fungi. On the contrary, the positive control mock-ups (fungi + EO) in all fungi showed deep color changes because of the fungi, not the EO (Elsayed & Shabana, 2018; Othman et al., 2020). Black pepper OE, as the most potent antifungal among the tested EOs, was assayed in all mock-ups infested with the four fungi to clarify if any changes relevant to the interaction between the EO, fungus, and the mock-up could occur. The values were high, especially in cotton mock-ups (ΔE*= 23.92, 27.51, 31.22, 27.67) in *A. niger, A. flavus, C. halotolerans, and N. goegapense*, respectively. The values were high, but they were normal because of the effect of fungi, not the EO. This is well-known that the microbial agent covers the surface of the object and causes many physical changes, besides other chemical and mechanical changes, especially in heritage materials of organic origin (Gouda et al., 2023; Rashad et al., 2020; Sterflinger, 2010).

Table 2. Results of the colorimetric measurements

		Painting				Cotton				Wool			
		ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*
Negative control (EO only)	Black pepper	-0.22	-0.63	-0.8	1.04	-0.48	0.75	1.08	1.40	-0.55	-0.39	-0.76	1.02
	Ginger	-1.15	1.56	1.58	2.50	-1.77	1.18	0.93	2.32	-1.03	1.32	0.16	1.68
	Red pepper	-2.55	1.67	1.62	3.45	-2.26	1.91	1.96	3.55	-2.12	2.05	3.1	4.28
	Cinnamon	-0.22	-0.47	1.03	1.15	-0.74	0.59	0.33	1.00	-0.6	1.05	0.19	1.22
	Camphor	-0.44	-0.55	-0.83	1.09	-0.93	0.48	0.66	1.24	-0.79	0.46	0.1	0.92
Positive control (fungi + Black pepper)	*A. niger*	-11.34	-5.47	-8	14.92	-22.46	7.53	3.29	23.92	-18.39	-3.66	-8.54	20.60
	A. flavus,	-10.45	6.33	-5.58	13.43	-25.45	5.33	-8.99	27.51	-17.98	8.54	-11	22.74
	C. halotolerans	-16.45	8.56	-6.05	19.51	-28	11.02	8.34	31.22	-20.05	5.66	9.76	23.01
	N. goegapense	-10.83	-6.08	11.65	17.03	-24.86	9.53	7.53	27.67	-19.86	8.87	4.53	22.22

3.4 Results of FTIR

The results of the FTIR spectroscopy of the oil painting, cotton, and woolen fabric mock-ups before and after treatment with the black pepper EO as the optimum antifungal EO among the tested (Fig. 5) revealed that the black pepper EO did not cause any significant changes in the molecular and supramolecular structure of mock-ups (cellulose, keratin, pigment, and other impurities included). The results indicated no significant spectral changes after treatment with EO. There was no increase or decrease in the absorbance of the function groups, no new bands related to new functional groups were created in the molecular structure, and no disappeared bands were found. The only small new band appeared at ~1630cm^{-1}, indicating some absorbed excess water particles (Kavkler & Demsar, 2012; Matusiak et al., 2018; Othman et al., 2020). This finding indicated that the vapor of the black pepper EO is completely safe and has no negative effects on the heritage materials if applied in similar cases with the same method.

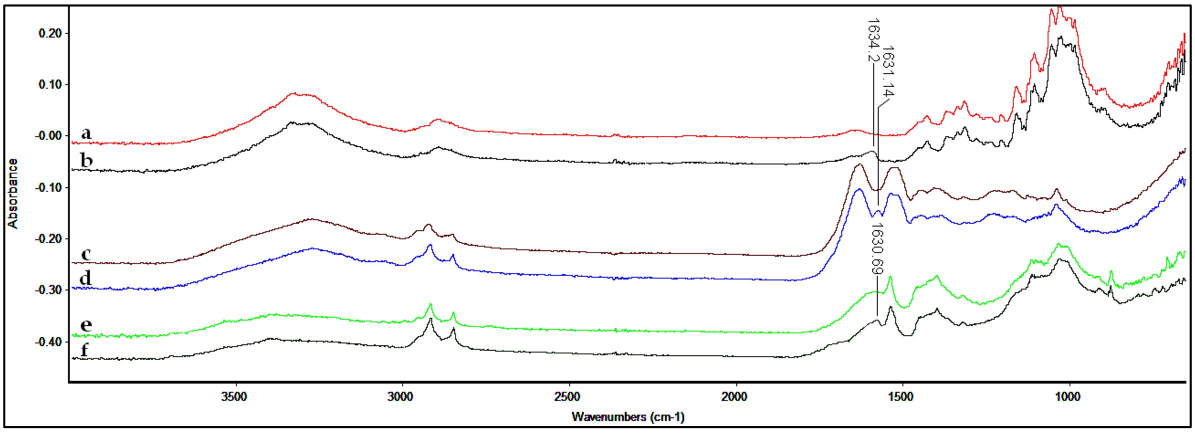


Figure 5. FTIR spectra of the mock-ups before and after treatment with black pepper EO; a. cotton (untreated), b. cotton (treated), c. wool (untreated), d. wool (treated), e. painting (untreated), f. painting (treated).

4. CONCLUSION

The present study proved efficient antifungal activities for all tested EOs against the selected fungi, varying from the highest (99.85%, black pepper EO against *N. goegapense*) to the lowest (71.65%, camphor EO against *N. goegapense*). It recommends using the EO of black pepper to disinfect *A. flavus, C. halotolerans, and N. goegapense*, as it demonstrated the most potent and efficient antifungal activity among the tested EOs against these fungi. Regarding *A. niger,* the study recommends using the EO of ginger, which proved the most potent and efficient antifungal activity against it and showed more efficiency than black pepper in the case of *A. niger*. The vapor phase method showed a very efficient and non-destructive effect against the infected mock-ups. It eradicated up to 99.85% of fungi in some cases without any notable changes in the properties of the treated mock-ups, so it is highly recommended to be applied to the case study infected heritage materials and similar heritage objects in museums. A small amount of black pepper EO is advised to be added in all closed display and storage cases in the museums as a preventive technique for the growth inhibition of fungi. Further research should be conducted to assay the efficiency of the EOs of black pepper and ginger against the common bacteria colonizing the heritage materials. Furthermore, the antifungal activity of some mixed EOs of ginger, cinnamon, camphor, and red pepper could be assayed for probably more efficiency against fungi and bacteria.

Authors contributions: Y.E., N.M.: Heritage objects survey and studies, preparation of the simulated mock-ups; Y.R., P.S.: preparation and antifungal assessment of EOs; Y.S., N.M.: SEM and mock-ups measures; H.E.: writing and reviewing the manuscript.

ACKNOWLEDGEMENTS

This work was funded by the Egyptian Science, Technology, and Innovation Funding Authority (STDF) through project AHRC-STDF, Call 2 - Projects ID 30818 (Egypt–UK Grants).

REFERENCES

Abd Elhameed, M. A. E., Marouf, M., Mohamed, W., and Abdel Wahab, W. (2021). The effect of the accelerated laboratory ageing factors on linen textiles dyed with turmeric dye. *Advanced Research in Conservation Science*, *2*(1), pp. 31-39.

Abdel-Kareem, O. (2010). Monitoring, controlling and prevention of the fungal deterioration of textile artifacts in the museum of Jordanian heritage. *Mediterranean Archaeology and Archaeometry*, *10*(2), pp. 85-96.

Abdel-Kareem, O., Abdel-Rahman, A., Samaha, S. H., and Nasr, H. E. (2021). Conservation processes developed and adapted to clean, disinfect and reinforce a coptic textile object in Egypt. *Mediterranean Archaeology and Archaeometry*, *21*(2), pp. 181-190.

Abdel-Kareem, O. (2015). Preparation of experimental deteriorated dyed textile samples simulated to ancient ones. *International Journal of Conservation Science*, *6*(2), pp. 151-164.

Abdel-Kareem, O., Samaha, S., El-Nagar, K., Essa, D., and Nasr, H. (2022). Monitoring the environmental conditions and their role in deterioration of textiles collection in Museum of Faculty of Archeology, Cairo University, Egypt. *Scientific Culture*, *8*(3), pp. 77-88.

Amin, E. (2018). Technical investigation and conservation of a tapestry textile from the Egyptian textile Museum, Cairo. *Scientific Culture*, *4*(3), 35-46

Antonelli, F., Bartolini, M., Plissonnier, M.-L., Esposito, A., Galotta, G., Ricci, S., Davidde Petriaggi, B., Pedone, C., Di Giovanni, A. and Piazza, S. (2020). Essential oils as alternative biocides for the preservation of waterlogged archaeological wood. *Microorganisms*, *8*(12), p. 2015.

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and chemical toxicology*, *46*(2), pp. 446-475.

Barbera, D., Young, C., Charalambides, M., Taylor, A. C., and Zhang, R. (2022). A methodology for the use of alkyd paint in thermally aged easel painting reconstructions for mechanical testing. *Journal of Cultural heritage*, *55*, pp. 237-244.

Bi, Y., Zhou, G., Pan, D., Wang, Y., Dang, Y., Liu, J., Jiang, M., and Cao, J. (2019). The effect of coating incorporated with black pepper essential oil on the lipid deterioration and aroma quality of Jinhua ham. *Journal of Food Measurement and Characterization*, *13*, pp. 2740-2750.

Borrego, S., Gómez de Saravia, S. G., Valdés, O., Vivar, I., Battistoni, P. A., and Guiamet, P. S. (2016). Biocidal activity of two essential oils on fungi that cause degradation of paper documents. *International Journal of Conservation Science*, *7*, pp. 369-380.

Bratitsi, M., Liritzis, I., Vafiadou, A., Xanthopoulou, V., Palamara, E., Iliopoulos, I. and Zacharias, N. (2018). Critical assessment of chromatic index in archaeological ceramics by Munsell and RGB: novel contribution to characterization and provenance studies. *Mediterranean Archaeology and Archaeometry*, *18*(2), pp. 175-212.

Camele, I., Gruľová, D., and Elshafie, H. S. (2021). Chemical composition and antimicrobial properties of Mentha× piperita cv.'Kristinka'essential oil. Elshafie, S., De Martino, L., and Sofo A., (eds): *Plants*, *10*(8), 1567, pp. 5-18.

Caneva, G., Nugari, M. P., and Salvadori, O. (2000). *La biología en la restauración* (Vol. 5). Editorial Nerea.

Cappitelli, F., Cattò, C., and Villa, F. (2020). The control of cultural heritage microbial deterioration. *Microorganisms*, *8*(10), p. 1542.

Castillo, I. F., Guillén, E. G., de la Fuente, J. M., Silva, F., and Mitchell, S. G. (2019). Preventing fungal growth on heritage paper with antifungal and cellulase inhibiting magnesium oxide nanoparticles. *Journal of Materials Chemistry B*, *7*(41), pp. 6412-6419.

Charles, D. J., and Simon, J. E. (1990). Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *Journal of the American Society for Horticultural Science*, *115*(3), pp. 458-462.

Christian, K. T. R., Carole, D. F. M., Alain, K. Y., Dahouenon-Ahoussi, E., Avlessi, F., Sohounhloue, D., and Simal-Gandara, J. (2023). Essential oils as natural antioxidants for the control of food preservation. *Food Chemistry Advances*, p. 100312.

Elamin, A., Takatori, K., Matsuda, Y., Tsukada, M., and Kirino, F. (2018). Fungal biodeterioration of artificial aged linen textile: Evaluation by microscopic, spectroscopic and viscometric methods. *Mediterranean Archaeology and Archaeometry, 18*(1), pp. 103-120.

Elsayed, Y. (2019). Conservation of a historic panel oil-painting coated with an ancient varnish layer. *Shedet*, *6*(6), pp. 238-256.

Elsayed, Y., El-Kadi, S., El-Rian, M., & Mabrouk, N. (2023). The efficiency of microbial culture extracts as green antimicrobial products against some microorganisms colonizing the historic oil paintings. *Scientific Culture*, *9*(2), pp. 127-143.

Elsayed, Y., and Shabana, Y. (2018). The effect of some essential oils on *Aspergillus Niger* and *Alternaria Alternata* infestation in archaeological oil paintings. *Mediterranean Archaeology and Archaeometry*, *18*(3), pp. 71-87.

Gouda, M., Atiaa, M., and Abdel-Kareem, O. (2023). Investigation and Analysis of Ancient Dyed Textiles. In *Preservation and Restoration Techniques for Ancient Egyptian Textiles*, IGI Global. pp. 93-118.

Guiamet, P. S., de la Paz, N. J., Arenas, P. M., and Gómez de Saravia, S. G. (2008). Differential sensitivity of Bacillus sp. isolated from archive materials to plant extracts. *Pharmacologyonline*, *3*, pp. 649-658.

Görs, S., Schumann, R., Häubner, N., and Karsten, U. (2007). Fungal and algal biomass in biofilms on artificial surfaces quantified by ergosterol and chlorophyll a as biomarkers. *International biodeterioration and biodegradation*, *60*(1), pp. 50-59.

Hasan, H. A., Raauf, A. M. R., Razik, B. M. A., and Hassan, B. A. R. (2012). Chemical composition and antimicrobial activity of the crude extracts isolated from Zingiber officinale by different solvents. *Pharmaceut Anal Acta*, *3*(9), pp. 1-5.

Hunt, R., Dienemann, J., Norton, H. J., Hartley, W., Hudgens, A., Stern, T., and Divine, G. (2013). Aromatherapy as treatment for postoperative nausea: a randomized trial. *Anesthesia and Analgesia*, *117*(3), pp. 597-604.

Kavkler, K., and Demsar, A. (2012). Application of FTIR and Raman spectroscopy to qualitative analysis of structural changes in cellulosic fibres. *Tekstilec*, *55*(1), pp. 19-31.

Kavkler, K., Gunde-Cimerman, N., Zalar, P., and Demšar, A. (2015). Fungal contamination of textile objects preserved in Slovene museums and religious institutions. *International Biodeterioration and Biodegradation*, *97*, pp. 51-59.

Lopez, P., Sanchez, C., Batlle, R., and Nerin, C. (2005). Solid-and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. *Journal of agricultural and food chemistry*, *53*(17), pp. 6939-6946.

Luke, J. T. (1988). A Manual of Painting Materials and Techniques by Mark D. Gottsegen. *Leonardo*, *21*(1), pp. 90-91.

Mabrouk, N., Rashad, Y., Elmitwalli, H., Shabana, Y., Sreenivasaprasad, P., and Elsayed, Y. (2023). Assessment of some green fungicides against fungi isolated from different heritage sites and museums in Egypt. *Scientific Culture*, *9*(3), pp. 101-112.

Martín-Rey, S., Castell-Agustí, M., Vivancos-Ramón, M. V., and Martín-Martínez, J. M. (2023). The essential of essential oils in the preservation of paintings on canvas, and risks of falling into the attractiveness of their essentiality. I*nternational Journal of Human Sciences Research*. 2023, 3(10): pp. 1-8

Matusiak, K., Machnowski, W., Wrzosek, H., Polak, J., Rajkowska, K., Śmigielski, K., Kunicka-Styczyńska, A, and Gutarowska, B. (2018). Application of Cinnamomum zeylanicum essential oil in vapour phase for heritage textiles disinfection. *International Biodeterioration and Biodegradation*, *131*, pp. 88-96.

Mazzoli, R., Giuffrida, M. G., and Pessione, E. (2018). Back to the past:"find the guilty bug—microorganisms involved in the biodeterioration of archeological and historical artifacts". *Applied microbiology and biotechnology*, *102*, pp. 6393-6407.

Mecklenburg, M. F., Charola, A. E., and Koestler, R. J. (2013). New insights into the cleaning of paintings: proceedings from the cleaning 2010 international conference, Universidad Politécnica de Valencia and Museum Conservation Institute.

Menon, A. N., and Padmakumari, K. P. (2005). Studies on essential oil composition of cultivars of black pepper (Piper nigrum L.)—V. *Journal of Essential Oil Research*, *17*(2), pp. 153-155.

Mesquita, N., Portugal, A., Videira, S., Rodríguez-Echeverría, S., Bandeira, A. M. L., Santos, M. J. A., and Freitas, H. (2009). Fungal diversity in ancient documents. A case study on the Archive of the University of Coimbra. *International Biodeterioration and Biodegradation*, *63*(5), pp. 626-629.

Mickienė, R., Ragažinskienė, O., and Bakutis, B. (2011). Antimicrobial activity of Mentha arvensis L. and Zingiber officinale R. essential oils. *biologija*, *57*(2), pp. 92–97.

Murbach Teles Andrade, B. F., Nunes Barbosa, L., da Silva Probst, I., and Fernandes Júnior, A. (2014). Antimicrobial activity of essential oils. *Journal of Essential Oil Research*, *26*(1), pp. 34-40.

Noshyutta, W., Osman, E., and Mansour, M. (2016). An investigation of the biological fungicidal activity of some essential oils used as preservatives for a 19th century Egyptian Coptic cellulosic manuscript. *International Journal of Conservation Science*, *7*(1), pp. 41-56.

Omar, A., Taha, A., and El-Wekeel, F. (2019). Microbial degradation of ancient textiles housed in The Egyptian Textile Museum and methods of its control. *Egyptian Journal of Archaeological and Restoration Studies*, *9*(1), pp. 27-37.

Othman, M., Saada, H., and Matsuda, Y. (2020). Antifungal activity of some plant extracts and essential oils against fungi-infested organic archaeological artefacts. *Archaeometry*, *62*(1), pp. 187-199.

Palla, F., Bruno, M., Mercurio, F., Tantillo, A., and Rotolo, V. (2020). Essential oils as natural biocides in conservation of cultural heritage. *Molecules*, *25*(3), p. 730.

Rashad, Y., Aseel, D., and Hammad, S. (2020). Phenolic compounds against fungal and viral plant diseases. *Plant Phenolics in Sustainable Agriculture: Volume 1*, pp. 201-219.

Rasooli, I., Rezaei, M. B., and Allameh, A. (2006). Growth inhibition and morphological alterations of Aspergillus niger by essential oils from Thymus eriocalyx and Thymus x-porlock. *Food control*, *17*(5), pp. 359-364.

Sadiki, M., El Abed, S., Balouiri, M., Barkai, H., El Bergadi, F. Z., El Farricha, O., and Ibnsouda Koraichi, S. (2017). Combined effect of essential oils against bacteria associated with deterioration of historical wood. *J. Mater. Environ. Sci*, *8*, pp. 594-602.

Salvador, C., Bordalo, R., Silva, M., Rosado, T., Candeias, A., and Caldeira, A. T. (2017). On the conservation of easel paintings: evaluation of microbial contamination and artists materials. *Applied Physics A*, *123*(1), pp. 1-13.

Shabana, Y. M., Elwakil, M. A., and Charudattan, R. (2000). Effect of media, light and pH on growth and spore production by Alternaria eichhorniae, a mycoherbicide agent for waterhyacinth/Wirkung von Medium, Licht und pH auf das Wachstum und die Sporenproduktion von Alternaria eichhorniae, einem Mykoherbizid gegen die Wasserhyazinthe. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection*, pp. 617-626.

Shi, S., Wu, Q., Su, J., Li, C., Zhao, X., Xie, J., Gui, S., Su, Z. and Zeng, H., (2013). Composition analysis of volatile oils from flowers, leaves and branches of Cinnamomum camphora chvar. Borneol in china. *Journal of Essential Oil Research*, *25*(5), pp. 395-401.

Singh, G., Marimuthu, P., Catalan, C., and DeLampasona, M. P. (2004). Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract. *Journal of the Science of Food and Agriculture*, *84*(14), pp. 1878-1884.

Soliman, S. A., Hafez, E. E., Al-Kolaibe, A. M. G., Abdel Razik, E.-S. S., Abd-Ellatif, S., Ibrahim, A. A., and Elshafie, H. S. (2022). Biochemical Characterization, Antifungal Activity, and Relative Gene

Expression of Two Mentha Essential Oils Controlling Fusarium oxysporum, the Causal Agent of Lycopersicon esculentum Root Rot. *Plants*, *11*(2), p. 189.

Steinhaus, M., and Schieberle, P. (2005). Characterization of odorants causing an atypical aroma in white pepper powder (Piper nigrum L.) based on quantitative measurements and orthonasal breakthrough thresholds. *Journal of Agricultural and Food Chemistry*, *53*(15), pp. 6049-6055.

Sterflinger, K. (2010). Fungi: their role in deterioration of cultural heritage. *Fungal biology reviews*, *24*(1-2), pp. 47-55.

Stupar, M., Grbić, M. L., Džamić, A., Unković, N., Ristić, M., Jelikić, A., and Vukojević, J. (2014). Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. *South African Journal of Botany*, *93*, pp. 118-124.

Sultana, R., Islam, M. D., Tanjum, F., Rahman, M. M., Haque, M. A., and Hossain, R. (2022). Antioxidant, Antibacterial and Antifungal Properties of Black Pepper Essential Oil (Piper nigrum Linn) and Molecular Docking and Pharmacokinetic Studies of its' Major Component. *Orient. J. Chem*, *38*, pp. 1554-1560.

Tomić, A., Šovljanski, O., Nikolić, V., Pezo, L., Aćimović, M., Cvetković, M., . . . Markov, S. (2023). Screening of Antifungal Activity of Essential Oils in Controlling Biocontamination of Historical Papers in Archives. *Antibiotics*, *12*(1), p. 103.

Vicenço, C. B., Silvestre, W. P., Lima, T. S., and Pauletti, G. F. (2021). Insecticidal activity of Cinnamomum camphora Ness and Eberm var. linaloolifera Fujita leaf essential oil and linalool against Anticarsia gemmatalis. *Journal of Essential Oil Research*, *33*(6), pp. 601-609.

Walter, P., and de Viguerie, L. (2018). Materials science challenges in paintings. *Nature materials*, *17*(2), pp. 106-109.

Wesołowska, A., Grzeszczuk, M., and Jadczak, D. (2015). GC-MS analysis of essential oils isolated from fruits of chosen hot pepper (Capsicum annuum L.) cultivars. *Folia Pomeranae Universitatis Technologiae Stetinensis*. 320(35)3, pp. 95–108

Yong-hua, R., Bian-xia, L., and Xiao-ning, S. (2012). Research on the Aging of Natural Fiber Textiles. *Advances in Biomedical Engineering*, *9*, 1.

Zhang, C., Zhao, J., Famous, E., Pan, S., Peng, X., and Tian, J. (2021). Antioxidant, hepatoprotective and antifungal activities of black pepper (Piper nigrum L.) essential oil. *Food Chemistry*, *346*, p. 128845.