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THE EFFECT OF SOME ESSENTIAL OILS ON *ASPERGILLUS NIGER* AND *ALTERNARIA ALTERNATA* INFESTATION IN ARCHAEOLOGICAL OIL PAINTINGS

Yosr Elsayed[1*] and Yasser Shabana[2]

[1]Conservation Department, Faculty of Archaeology, Fayoum University, 63514, Keman Fares, Fayoum, Egypt
[2]Plant Pathology Department, Faculty of Agriculture, Mansoura University, 60 Elgomhorya st., 35516, El-Mansoura, Egypt

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**Corresponding author: Yosr Elsayed (yea00@fayoum.edu.eg)*

ABSTRACT

The main objective of this research is to evaluate using four plant essential oils; marjoram, camphor, clove, and basil as an eco-friendly methods for their antifungal activities against *Aspergillus niger* and *Alternaria alternata* as two of the most common fungi infesting the heritage oil paintings and other heritage objects. The research focuses on: 1) evaluating the efficacy of the four selected essential oils in suppressing the selected fungi cultivated on agar plates. It is found that clove and camphor oils respectively are the most potent oils due to their optimum antifungal activity. Both efficiently inhibited the growth of both fungi especially at higher concentrations. 2) evaluating the using of clove and camphor oils in suppressing the selected fungi cultivated on simulated canvas painting model, camphor oil at concentration 80% (v/v) showed a better antifungal activity than clove for both fungi. Camphor oil had been proven as the most potent antifungal oil and preserving the simulated canvas painting models with no notable side-effects on the properties of painting models.

KEYWORDS: oil painting, heritage, fungi, bio-deterioration, essential oils, *Aspergillus niger*, *Alternaria alternata*.

1. INTRODUCTION

Ancient oil paintings provide us with images that either representing familiar ideas, things, or events or that unfamiliar to our own experience, where we are generally informed, inspired, and pleased by what we can see, but ancient paintings are almost threaten by many deterioration factors that able to put an end for these heritage (Taft, and Mayer, 2000). Bio-deterioration, induced by diverse microbial communities such as fungi, bacteria and lichens, is one of the most important risks threatening the oil painting and resulting in irrevocable damages range between staining and complete destruction (Rajput et al., 2012; Biswas et al., 2013). Fungi have a wide range of enzymatic activities and the ability to grow at relatively low water activity level enabling them to inhabit, change and/or decay various organic and inorganic materials used for objects of cultural heritage in museums' displays or storages, or outdoors (Sterflinger, 1999; Görs et al., 2007). Moreover; fungi are able to release spores, hyphal fragments, toxins and allergens in the aerosol of the indoor cultural heritage affecting the human health at workplaces in museums and archives and causing serious respiratory infections as bronchial irritation and allergy (Krumbein, 2003; Wiszniewska et al., 2009; Di Carlo et al., 2016). Various attempts to conserve deteriorated oil and mural paintings and objects of cultural heritage have been reported (Salama et al., 2016 a, b, 2018; El-Sheikh et al., 2017).

Paintings contain a wide and variant range of biodegradable organic and inorganic components, the support material (canvas, paper, wood, parchment, etc.), organic adhesives (animal glue, fish glue, plant glue, etc.) used in sizing the support and binding the ground layer, and oils used in binding the pigments; may be easily degraded by microorganisms, (Ciferri, 1999; Lopez-Miras et al., 2013), moreover; and if available; the paint varnish is bio-degraded and biodeteriorated easily (Kurowski et al., 2017). They may cause a serious spoiling and/or undesirable staining of the artifacts, in addition; fungi can enzymatically degrade the organic paint binders leading to reduction or detachment of the paint layers (Mesquita et al., 2009; Sterflinger, 2010; Mansour and Salem, 2015). The most common fungi occurring on the ancient oil paintings in museums mostly belong to *Alternaria* sp., *Aspergillus* sp., *Aureobasidium* sp., *Botrytis* sp., *Chaetomium* sp., *Cladosporium* sp., *Eurotium* sp., *Fusarium* sp. *Mucor* sp. *Penicillium* sp., *Rhizopus* sp., *Stemphilium* sp. *Trichoderma* sp., and *Ulocladium* sp. (Pangallo et al., 2009; Seves et al., 1996; Abed el Hamid et al., 2010).

There are many common methods used in controlling biodterioration of heritage paintings, e.g. mechanical methods using brushes, scrapers and scalpels (Ashurst, 1990), physical methods using UV, IR, γ-Rays, X-rays, etc. (Stuart, 2004; Katusim-Razem et al., 2009; Ibrahim et al., 2017) and chemical methods using different biocides such as ethylenebromide, isothiazolinone or benzalkonium chloride, etc. (Blazquez et al., 2000; Martin-Sanchez et al., 2012, Salama et al 2018). The previous methods had been used to disinfect and prevent bio-deterioration in heritage objects, but these methods have had different side-effects and disadvantages such as toxicity for human and objects; higher costs; changing of some properties of heritage objects, not providing a future guarantee against bio-deterioration, etc. (Sterflinger, 2010; Helmi et al., 2011; Noshyutta et al., 2016), so; essential oils (EOs) had been developed as an eco-friendly, efficient, cost-effective, and economic approach for the control of variant types of microorganisms colonization on the cultural heritage objects (Axinte et al., 2011).

EOs are generally extracted from plants by steam distillation and various solvents, these oils are considered a variant complex mixtures of volatile compounds, distinguished by low molecular weight and strong odor (Raut et al., 2014), these volatile compounds have variant ecological activities, acting as antimicrobial materials against microorganisms and herbivores (Bakkali et al., 2008), they had been traditionally used for centuries in treatment of much diseases and infections all over the world (Rios, and Recio, 2005). EOs derived from of plant sources are commonly used as flavouring materials in perfumeries, drinks, food production, pharmaceuticals and cosmetics (Burt, 2004; Hussain et al., 2008; Teixeira et al.,2013). EOs consist of rich ingredients of variant bio-active phyto-compounds such as phenols, quinines, tannins etc. that provide a potent antimicrobial activity (Baka, and Rashad, 2016).

A great number of EOs played an important role as an antimicrobial agents in the field of conservation of cultural heritage. Camphor oil had been proven as a very efficient in inhibition of much fungi growth (Al-Harbi, and Uddin, 2005; Cabello, 2006), also; it is a strong antibacterial due to cinnamaldehyde (Chang et al., 2001). Clove and garlic oils had been proven as a potent antimicrobial materials against different fungal species including *Aspergillus niger* (Borrego et al., 2016), also; tea tree, lavender and thyme oils revealed a strong effect against much common fungi and bacteria infesting ancient documents (Noshyutta et al., 2016). It was also shown that a single treatment of cloves, jojoba and garlic oils was able to effectively kill much fungal species

(Abed el Hamid et al., 2010). Thymol, Rosmarinus officinalis, Origanum vulgare and Lavandula angustifolia showed a strong antifungal activity against much fungi species including *Aspergillus niger* and *Aspergillus ochraceus* (Sakr et al. 2012; Stupar et al., 2014b), moreover; basil, clove, garlic, marjoram, thyme, citronella, coriander, origanum, rosemary, sage and tarragon are generally proven as efficient materials in growth inhibition of much fungi and bacteria tested (Teixeira et al., 2013).

In addition to other fungi and little bacteria; *Alternaria alternata* and *Aspergillus niger* had been proven as the most common fungi infesting most of the investigated oil paintings and other heritage objects in previous conservation studies, both fungi were jointly or separately isolated from many archaeological oil paintings from much museums and private properties, both are of the most common fungi in biodeteriorated oil paintings (Ogbulie, and Obiajuru, 2004; Abed el Hamid et al., 2010; Sterflinger, 2010; Kurowski et al., 2017), mural paintings (Helmi et al., 2011; Veneranda et al., 2018), cellulosic and proteinous textiles in display and storage (Abdel-Kareem, 2010; Błyskal, 2015), cellulosic manuscripts and documentary supports (Borrego et al., 2012; Noshyutta et al., 2016), paper-based photographic collections (Ali et al., 2016) and wood (Stupar et al., 2014). Hence, this work aims to present a new method that addresses the problems and overcomes the drawbacks of the ancient methods which resulted in many side-effects in antifungal treatments of heritage objects, it presents using some eco-friendly and pioneer essential oils in antifungal treatment of ancient oil paintings, these oils might be applicable not only in paintings conservation; but also in all heritage objects in conservation field.

2. MATERIALS AND METHODS

2.1. Essential oils

Four essential oils, namely; marjoram (*Origanum majorana*), camphor (*Cinnamomum camphora*), clove (*Syzygium aromaticum*), and basil (*Ocimum basilicum*) were purchased from Elnekity commercial store for agricultural seeds, spices and medical plants in Mansoura city, Egypt. The four essential oils were directly used without any kind of extraction or treatment, different concentrations of each essential oil were prepared and applied for their antimicrobial activity against the isolated fungi (*A. niger* and *A. alternata*) in both Potato Dextros Agar (PDA) and Simulated Painting Models (SPMs).

2.2. Potato dextros agar PDA and Tween-20

PDA (Sigma-Aldrich, pH; 5.6±0.2 at 25°C, agar; 15 g/L, dextrose; 20 g/L, potato extract; 4 g/L) was used as a medium for germinating the selected fungi (*A. alternata* and *A. niger*). Tween-20 ((Sigma-Aldrich, Germany, lauric acid; ≥40% ''balance primarily myristic, palmitic, and stearic acids'', impurities; ≤3.0% water, CMC; 0.06 mM''20-25°C'', refractive index; n20/D 1.468lit., density; 1.095 g/mL at 25 °C lit., acid number; ≤2.2 mg/g, hydroxyl value; 96‑108 mg/g, transition temp; cloud point 76 °C, HLB; 16.7.

2.3. Fungi

The two case study fungi, *Alternaria alternata* and *Aspergillus niger*, had been selected after deep reviewing much literature on bio-deterioration of oil paintings (Seves et al., 1996; Ciferri, 1999; Knut, 1999; Ogbulie, and Obiajuru, 2004; Romero-Noguera et al. 2008; Doménech-Carbóa et al., 2009; Abed el Hamid et al., 2010; Sterflinger, 2010; Kurowski et al., 2017). Many literatures had no fungal isolation from case study heritage objects, only based on the much previous literatures isolated the studied fungi from canvas and panel paintings, hence; they obtained the selected fungi from the fungi stock of pure culture collections (Seves et al., 1996; Domenech-Carbo et al., 2006; Romero-Noguera, 2010), other authors as well obtained the fungi from the fungi stock for experimental study in the field of conservation of archaeological materials such as paper (Rushdy et la., 2017; Karbowska-Berent et al., 2018), textiles ((Matusiak et al., 2017), stones (Ma et al., 2015; Essa, and Khallaf, 2016), wood (Salem et al., 2016) and parchment (Valentin et al., 1990), the same methodology in the field of microbiology (Carmo et al., 2008; Moghtader, 2013; Phillips et al., 2013; Teixeira et al., 2013; Badawy, and Abdelgaleil, 2014). Both fungi of the present study were obtained from the fungi stock collections of the Plant Pathology Department, Mansoura University, Egypt.

2.4. Simulated painting models SPMs

The SPMs were prepared according to the most common and traditional recipes of ancient oil paintings detailed in some literatures (Gottsegen, 1987; Mayer, 1991; Manzano et al., 2011). The SPMs layers include canvas linen support (purchased from Egyptian Company for Textile Industry, Cairo, Egypt), plain weave structure 1/1, undyed, linear density 16, 194 gm/m2. The white ground layer consists of animal glue and calcium carbonate (purchased from Alwan store, Giza, Egypt), after dissolving the grains of animal glue in warm water; it was mixed to calcium carbonate, then brushed on the canvas after glue sizing, then keep in room temperature for 72 h. at 25±2 °C till dry. The white ground layer then covered by the paint layer consists of commercial linseed-oil paint tubes (purchased from Winsor &

Newton, UK), the white, black, red, blue and yellow color are zincite, carbon black, vermilion, ultramarine and yellow ocher respectively (Gottsegen, 1987; Izzo et al., 2014). After normal drying in room temperature at 25±2 °C for a week; the SPMs were exposed to artificial thermal accelerated aging in a laboratory oven at 105±1 °C in the absence of light for 357 h., according to the ageing protocol of the model canvas paintings; this period result in a SPMs expected to simulate natural aging of canvas painting at 25 °C for 160 years (Seves et al., 2000) (Fig. 1).

Figure 1. Some prepared SPMs after thermal accelerated aging.

2.5. *Antifungal assay*

2.5.1. In vitro antifungal activity on agar plates

The antifungal activity of the above-mentioned four essential oils was evaluated. Suitable volumes of the plant essential oils were separately added to sterilized PDA medium to make four concentrations (20, 40, and 60% v/v) just before solidification; when the medium cooled down to approximately 45°C. Tween-20 was also added at 0.5% (v/v) to all concentrations to help dissolving oils and homogeneity. Discs (5 mm diameter) from 5-days-old culture of *A. niger* and *A. alternata* were placed on the surface of PDA in a 9-cm-diameter plastic Petri dish containing 20 ml medium. The inoculated plates were incubated in the dark at 26±2°C until the fungal growth fully covered the surface of the medium (9 cm diameter) in the control plates (0% oil). Five plates (replicates) were used for each concentration. At the end of the incubation period; the growth diameter of each fungus in all treatments was measured in two diagonal directions. The antifungal activity was expressed as the reduction percentage in the fungal growth diameter against the control treatment (0% oil). The percentage of growth inhibition was calculated using the following equation:

$$X = [(G2/G1) \times 100] - 100$$

Where X was the percentage of reduction in mycelial growth, G1 was the averaged growth of mold fungus in control plates, and G2 was the averaged growth of mold fungus in treated plates (Abdel-Fattah et al., 2007). The most effective oils and concentrations will be selected for further investigations.

2.5.2. In vitro antifungal activity on SPMs

The most effective essential oils obtained from the above experiments of agar plates were tested at the most effective concentrations on pre-infested SPMs for their antifungal activity and probable effect on the SPMs properties. Small pieces of SPMs (4 x 1 cm) were dipped into fungal suspensions (1 x 10^6 propagules/ml for *A. niger* and 1 x 10^5 propagules/ml for *A. alternata*) for 30 s. These infested SPMs were placed on sterile glass slide in the center of 9-cm sterile Petri dishes lined with three sterile filter papers moistened with sterile distilled water. Plates were then incubated in the dark at 26±2°C for two weeks, until the fungal growth appeared on the surface of the SPMs, then; fixed amount of camphor and clove oil emulsions at concentrations of 40, 60 and 80% (v/v) in sterile distilled water while Tween-20 was added at 0.5% (v/v) to all concentrations to help dissolving oils and homogeneity, were sprayed onto fugus-infested SPMs. SPMs with no fungus and oils, kept in Petri dishes as described above, were used as negative control. SPMs with no fungus but sprayed with oils concentration 100%, kept in Petri dishes as described above, were used as reference sample to check if there is any effect on physical and chemical characteristics of the SPMs due to used essential oils. Three replicates were used for each treatment. All plates were then incubated at 26±2°C for another two weeks (Figs 2-3). Sterile distilled water was periodically added to keep the filter paper moistened all the time. After the incubation period; the SPMs were removed and soaked in 30 ml of sterile water containing 0.5% (v/v) of Tween-20, then shaked for 10 min on a rotary shaker at 100 rpm to help release the fungal spores. The number of spores/ml in three replicates was counted with a haemacytometer (Shabana et al., 2000).

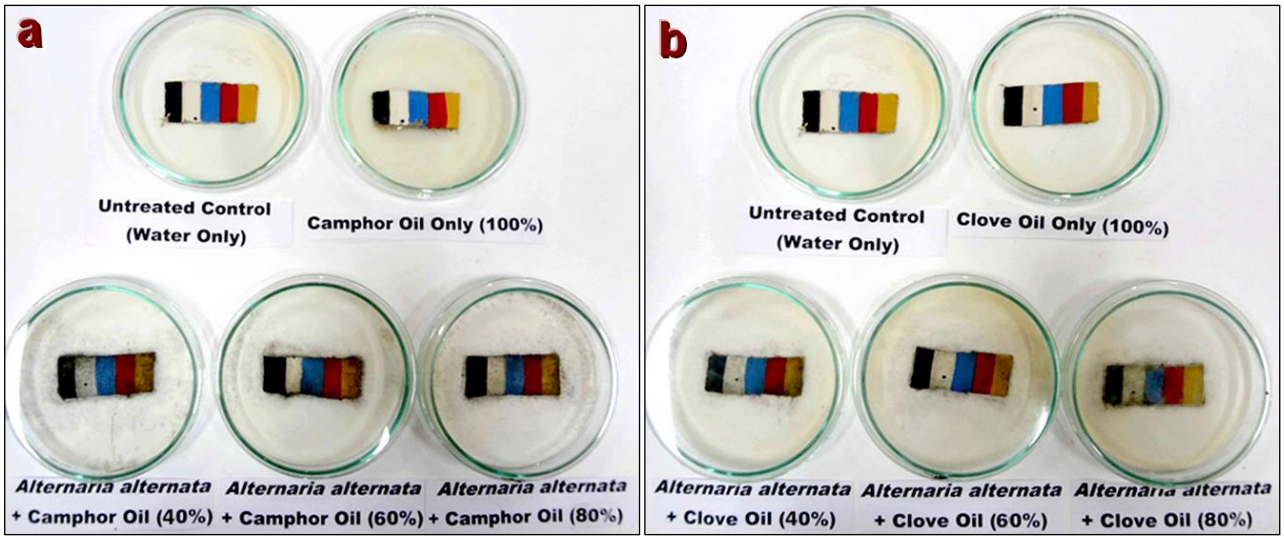


Figure 2. A. alternata pre-infested SPMs; (a) free-oil (control) and camphor oil treated SPMs at different concentrations; (b) free-oil (control) and clove oil treated SPMs at different concentrations, 5 days after oil treatment.

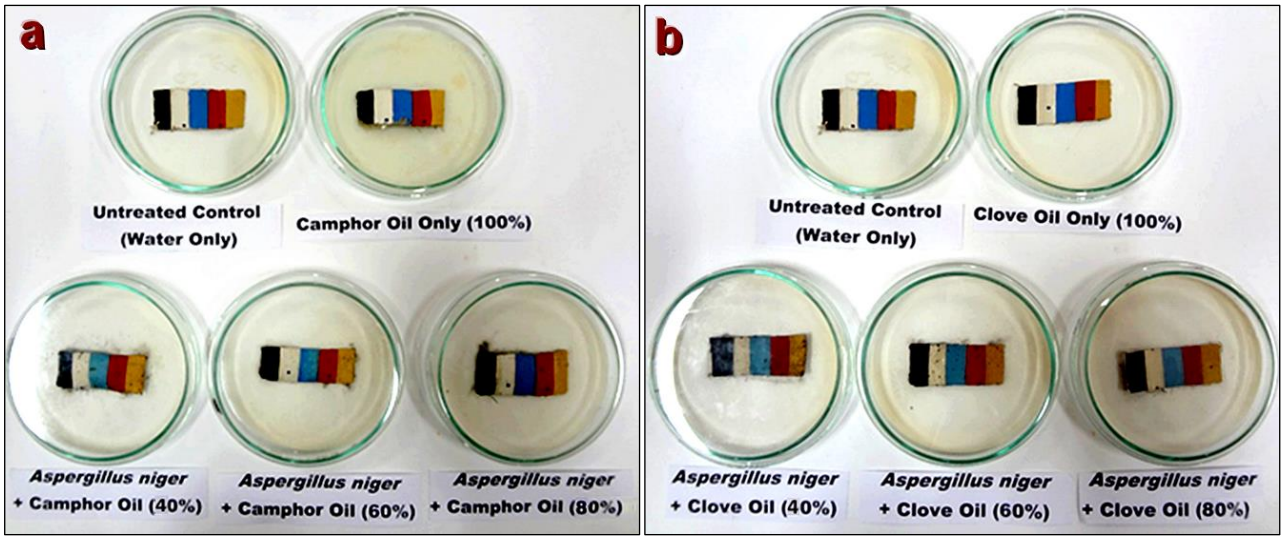


Figure 3. A. niger pre-infested SPMs; (a) free-oil (control) and camphor oil treated SPMs at different concentrations; (b) free-oil (control) and clove oil treated SPMs at different concentrations, 5 days after oil treatment.

2.6. Electron microscopy

The effect of the most inhibitive essential oil at the most effective concentration against the selected heritage-infesting fungi *Aspergillus niger* and *Alternaria alternata* obtained from the above experiments was investigated using scanning electron microscope SEM (JEOL JSM-6510 LV, JEOL Ltd., Japan, ACCEL_20KV, MAG 4300, SIGNAL SEI, WD10mm) and transmission electron microscope TEM (JEOL JEM-2100 TEM, JEOL Ltd., Japan, 80KV). Untreated fungi grown on PDA medium (oil-free control) and those containing camphor oil at 40 and 60% (v/v) were examined by SEM and TEM. Ten days after inoculation; fungal culture segments from the culture plates (1-cm agar discs) were removed and prepared for electron microscopy. Fungal discs from oil-free PDA (untreated control) corresponding to approximately the same locations as those from treated plates were removed and similarly prepared for electron microscopy. Regarding the SPMs; samples with no fungus but sprayed with oils concentration 100% were examined by SEM in comparison to the aged control sample (no fungus and oils) to identify any potential side-effects result from any probable oil activity against the SPM properties.

2.6.1. SEM

In SEM preparation; mycelial plugs (1 × 1 × 3 mm) were fixed in phosphate-buffered 3% glutaraldehyde at pH 6.8, dehydrated in graded series of acetone (25, 50, 75 and 100% for 15 min each). Later on, samples were critical point dried (Polaron CPD 7501, UK) with CO_2 using acetone as an intermediate fluid. The pieces of agar were sputter-coated with plutonium

by JFC-1600 auto-fine coater (Polaron SC7620, UK). Fungal hyphae of both the control and treated treatments were examined by SEM.

2.6.2. TEM

For TEM preparation; the mycelia of studied fungi from the control and the treatments were examined. Samples were fixed in phosphate-buffered 3% glutaraldehyde at pH 6.8, post-fixed in phosphate-buffered 1% Osmium tetroxide and dehydrated in graded series of ethanol following the procedure described by (Alberto et al., 1997) and modified by (Baka, 2014). Mycelial plugs were embedded in plastic resin. Ultra-thin sections were cut with Reichert ultra-microtome, stained with uranyl acetate and lead citrate and examined with TEM.

2.7. Stereo microscope

To examine the surfaces of all treated and un treated control SPMs, and to identify any probable changes in the SPMs due to using oils; and to examine the *A. alternata* and *A. niger* fungus infesting the SPMs; stereo microscope (Olympus CX31 Binocular Halogen Microscope) had been used.

2.8. Statistical analyses

The data were analyzed using the SAS software package (SAS Institute, 2016) using Fisher's LSD and Duncan's new multiple range tests for treatment means comparisons. Data were transformed to square roots to induce homogeneity of variance, and subjected to ANOVA and pairwise contrasts between treatments were performed.

2.9. Colorimetric measurements

The study used colorimeter (Optimatch 3100 Color Spectrophotometer) to measure the different colors of SPMs before and after antifungal treatments to identify any probable color changes might be resulted after oil treatments, the CIE L*,a*,b* color system was used, where L* measures darkness/lightness axis (L*0 dark/L*100 light), a* measures red/green axis (+a* red/-a* green), b* measures yellow/blue axis (+b* yellow/- b* blue), $\Delta L^* = (L^*_1 - L^*_2)$, $\Delta a^* = (a^*_1 - a^*_2)$, and $\Delta b^* = (b^*_1 - b^*_2)$ express the difference between two measures in each axis, $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ express the total color difference between two measures of the three axes (Schanda, 2007; Foster, 2008; Atodiresei et al., 2013; Bratitsi et al., 2018). Three replicas of the five colors in the thermal-aged control SPMs were color-measured, then the average of each three replica was calculated to identify the five original colors of the SPMs, 100% oil-sprayed SPMs were then measured to identify the probable color changes due to oils, finally; 80% oil-sprayed SPMs with fungus were measured to identify the probable changes due to activity of oils against fungus.

3. RESULTS

3.1. In vitro assessment for antifungal activity on agar plates

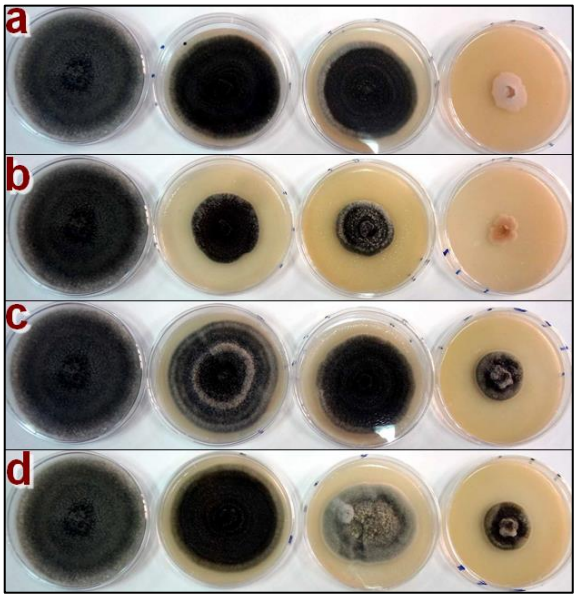
The results of the antifungal activity of the four studied essential oils (concentration 20%, 40%, and 60%) against *A. alternata* and *A. niger* on agar plates (Table 1) revealed that the four oils had a variant inhibitive effects on the growth of both *A. alternata* and *A. niger*, and the increase of oil concentration leads to increase of growth inhibition, hence; decrease of the colony diameter, and concentration 60% (v/v) proved the optimum results. Regarding the *A. alternata;* clove oil and camphor oil were proven to be the optimum essential oils among those used, clove increased the growth inhibition from 0.0% (untreated control) to 76%, and decreased the colony diameter from 9.00 cm (untreated control) to 2.16 cm, while camphor oil increased the growth inhibition from 0.0% (untreated control) to 74.44%, and decreased the colony diameter from 9.00 cm (untreated control) to 2.30 cm, marjoram proved a fair efficacy, basil revealed lower efficacy against growth inhibition of *A. alternata* (Fig. 4).

Regarding the *A. niger;* camphor oil was proven to be the optimum essential oil among those used, it increased the growth inhibition from 0.0% (untreated control) to 74.78%, and decreased the colony diameter from 9.00 cm (untreated control) to 2.27 cm, clove oil revealed an acceptable efficacy, it increased the growth inhibition from 0.0% (untreated control) to 61.89%, and decreased the colony diameter from 9.00 cm (untreated control) to 3.43 cm, marjoram and basil proved a lower efficacy against the growth inhibition of *A. niger* (Fig. 5).

Table 1. Effect of the studied EOs at different concentrations on the mycelial growth of A. alternata and A. niger.

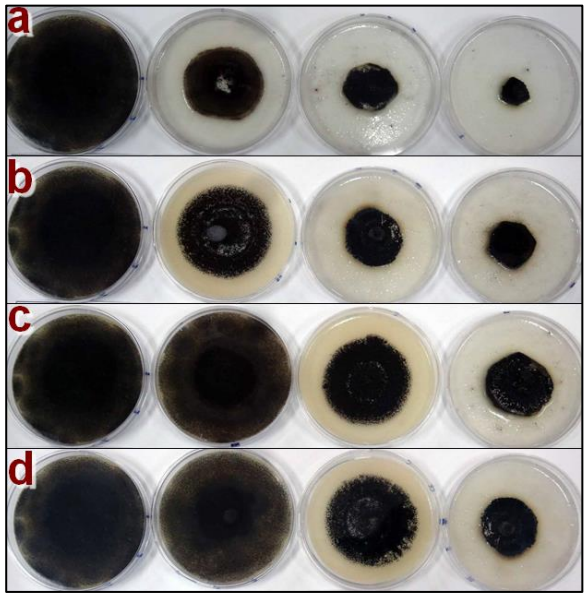
Essential oil	Concentration (%)	*A. alternata* (9 d after inoculation)		*A. niger* (17 d after inoculation)	
		Colony diameter (cm)	Growth inhibition (%)	Colony diameter (cm)	Growth inhibition (%)
Camphor	20	5.77[a] a B	35.89	4.34 a B	51.78
	40	3.56 b C	60.44	3.41 b E	62.11
	60	2.30 b D	74.44	2.27 c F	74.78
Clove	20	7.40 a A	17.78	6.57 a B	27.00
	40	7.01 a A	22.11	4.57 b D	49.22
	60	2.16 c D	76.00	3.43 c E	61.89
Marjoram	20	7.37 a A	18.11	9.00 a A	0.00
	40	7.18 a A	20.22	6.46 b B	28.22
	60	3.59 b C	60.11	5.68 c C	36.89
Basil	20	7.42 a A	17.56	9.00 a A	0.00
	40	6.85 a A	23.89	5.81 b C	35.44
	60	3.74 b C	58.44	4.54 c D	49.56

[a] Values of each oil followed by the same lower case letter or within the whole column (for all oils) followed by same capital letter are not significantly different according to Duncan's multiple range test at P=0.05.



0% 20% 40% 60%

Figure 4. Effect of the EOs at different concentrations on the mycelial growth of A. alternata, 9 days after inoculation (a) camphor, (b) clove, (c) marjoram, (d) basil.



0% 20% 40% 60%

Figure 5. Effect of the EOs at different concentrations on the mycelial growth of A. niger, 15 days after inoculation, (a) camphor, (b) clove, (c) marjoram, (d) basil.

3.1.1. SEM

The SEM micrographs of the morphological characteristics of *A. alternata* showed that the conidia were formed only in the oil-free control fungus (Fig. 6a), but there were little conidia formed in the clove-oil (40%, v/v) treated fungus (Fig. 6b), and no conidia formed in the clove-oil (60%, v/v) treated fungus (Fig. 6c), this means that the oil had prevented the fungus to sporulate. Conidia were oblong and characterized by rough surfaces and one or two projections at one or both ends, possibly the positions of their attachment to the conidiophore and a second conidium along a chain. The SEM examination also revealed that the mycelium in the clove-oil treated plates was shrunk and declined. The SEM micrographs of the morphological characteristics of *A. niger* in the oil-free control fungus (Fig. 7a) revealed that the conidia covered the whole plate and mycelium was difficult to observe by the SEM because of the profuse sporulation of this fungus, the results of the camphor-oil (40%, v/v) treated fungus showed that camphor oil caused some malformation and eruption of the spores of *A. niger* comparing to the

oil-free control fungus (Fig. 7b), the camphor-oil (60%, v/v) treated fungus showed that camphor oil caused much malformation and eruption of the spores (Fig. 7c).

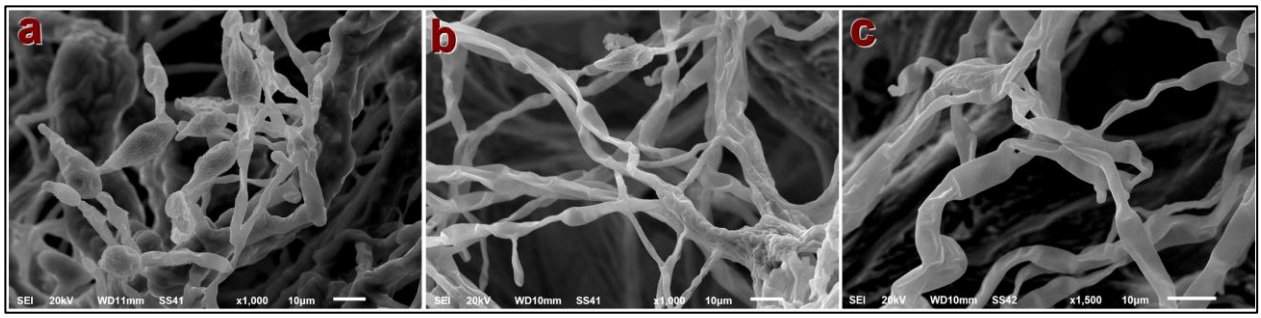


Figure 6. SEM micrographs show the A. alternata of, (a) 0% (untreated control) plate shows the spores (arrow) in the untreated control plate; (b) after treatment of 40% clove oil concentration on the growth of mycelium and few spores in the oil-treated fungus and the rough (verrucose) surface of spores; (c) after treatment of 60% clove oil concentration shows no spores.

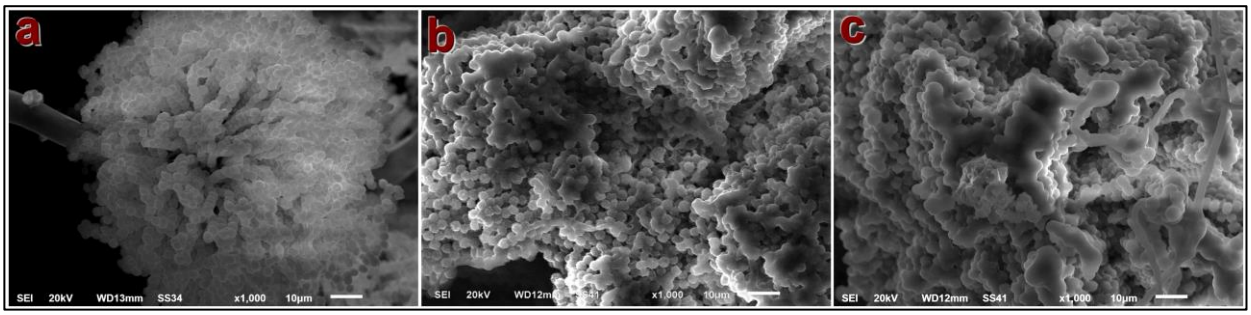


Figure 7. SEM micrographs show the A. niger, (a) 0% (untreated control) plate; (b) after treatment of 40% camphor oil concentration shows fair malformation and burst of the spore head due to camphor oil treatment comparing to the untreated control plate; (c) after treatment of 60% camphor oil concentration shows much malformation and burst of the spore head.

3.1.2. TEM

TEM micrographs of untreated hyphae of *A. alternata* (Fig 8a) revealed a granulated hyphal cytoplasm which contains numerous lipid bodies, a thin cell wall was also observed. After treatment of the hyphae with (60%, v/v) clove oil (Fig 8b); the cytoplasmic contents were seen to be plasmolyzed and collapsed beside the appearance of big vacuoles and electron-lucent materials inside the hyphal cell wall. The exposure of the hypha to clove oil revealed much disintegrated cytoplasmic contents and the occurrence of big vacuoles and electron-lucent materials inside the hyphal cell wall (Fig 8c).

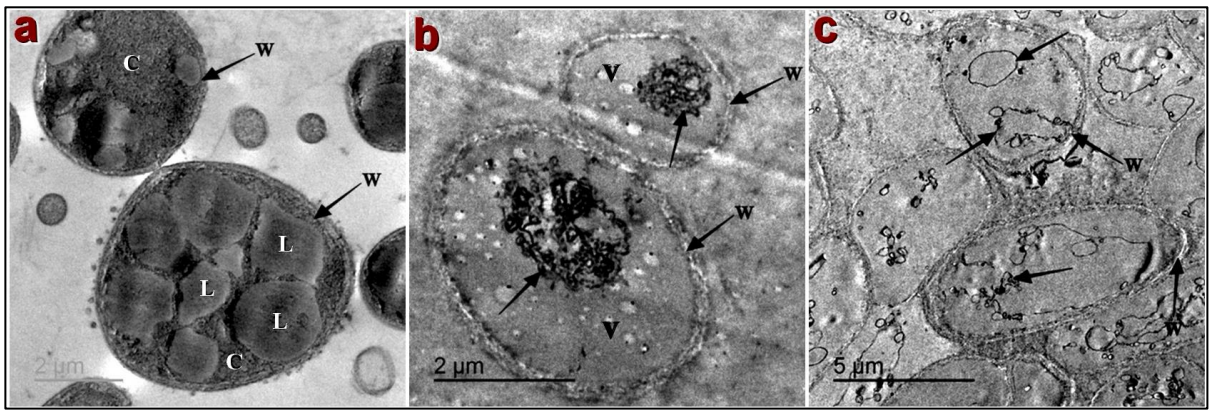


Figure 8. TEM micrographs show the A. alternata, (a) 0% (untreated control) shows numerous big lipid bodies (L) inside a granulated hyphal cytoplasm (C). Note also a thin wall (W) of the hyphal cell, (b) treated hyphae with 40% clove oil shows collapsed and plasmolyzed cytoplasmic contents (arrows) and big vacuole (V) and the appearance of electron-lucent materials inside the wall (W) of the hyphal cell, (c) treated hyphae with 60% clove oil shows much disintegrated cytoplasmic contents (arrows) and the appearance of electron-lucent materials inside the wall (W) of the hyphal cell.

TEM micrographs of untreated hyphae of *A. niger* (Fig 9a) exhibited many vacuoles containing electron-dense bodies inside a uniform and smooth cytoplasm, an integrated cell plasma membrane and an electron-lucent cell wall. When the hyphae were treated with 40% camphor oil (Fig 9b); the hyphal cell showed a granulated cytoplasm, an undulated plasma membrane and a thick wall. In addition, two types of vacuoles were detected, those containing electron-dense bodies and others free of these bodies. When the hyphae were treated with 60% camphor oil (Fig 9c); many changes occurred such as the presence of very granulated cytoplasm and the disappearance of electron-dense bodies from vacuoles. An undulated plasma membrane and cell wall which is composed of four layers were detected.

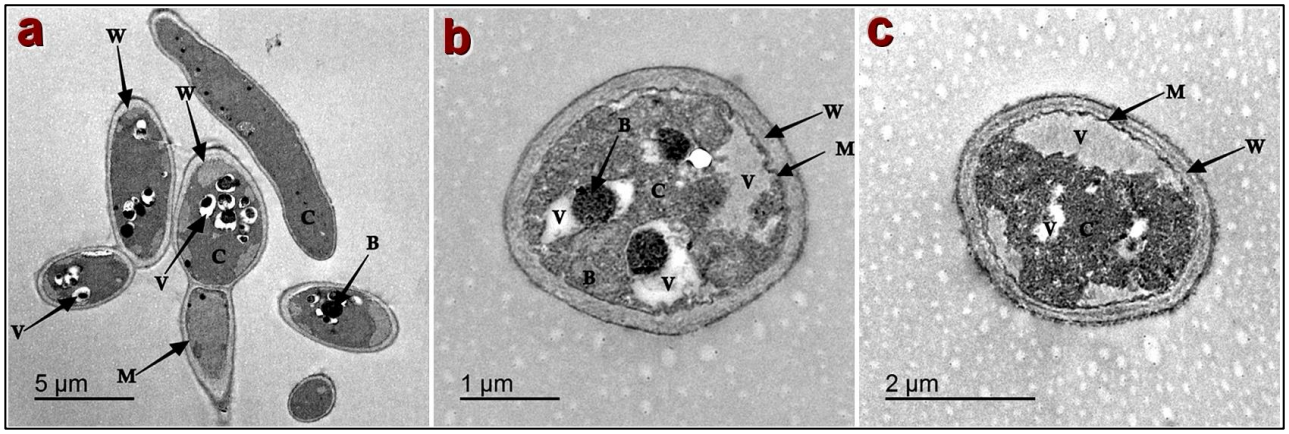


Figure 9. TEM micrographs show the A. niger, (a) 0% (untreated control) shows numerous vacuoles (V) containing electron-dense bodies (B), uniform and smooth cytoplasm (C), an electron-lucent cell wall (W) and an integrated cell plasma membrane (M), (b) treated hyphae with 40% camphor oil shows thick wall (W), undulated plasma membrane (M), vacuoles (V) containing electron-dense bodies (B) and others (V) free of these bodies and a granulated cytoplasm (C), (c) treated hyphae with 60% camphor oil shows a very granulated cytoplasm (C), the disappearance of electron-dense bodies from vacuoles (V), other types of vacuoles (V), an undulated plasma membrane (M) and cell wall (W) which is composed of four layers.

3.2. In vitro assessment for antifungal activity on SPMs

The results of antifungal activity of the four essential oils on *A. Alternata* and *A. niger* in agar plates had proven the optimum efficacy of only camphor oil and clove oil at concentration 60% (v/v), the maximum growth inhibition was 76% in case of clove oil against *A. Alternata*, so both oils were applied to SPMs infested by both fungi at concentration 60%, lower concentration (40%) and higher concentration (80%). The results of both oils at these different concentrations (Fig. 10) revealed that the more increase of oil concentration, the more efficacy in fungus inhibition, camphor oil is generally more efficient than clove, and it is more efficient against *A. alternata* than *A. niger*, camphor oil concentration (80%) achieved 98.% of *A. alternata* inhibition and 95.6 of *A. niger* inhibition, while clove oil concentration (80%) achieved 87.3% of *A. alternata* inhibition and 86% of *A. niger* inhibition.

Regarding the viability of spores; concentration 80% of both oils resulted in a higher number of dead spores in comparison to lower concentration, camphor oil killed 98.7% of the live *A. Alternata* spores (1650 killed, 22 lives), while clove oil killed 97.04% of the live *A. Alternata* spores (1250 killed, 38 lives) (Fig. 11). As for *A. niger*; camphor oil killed 97.4% of the live spores (150 killed, 4 lives), while clove oil killed 91.72% of the live spores (145 killed, 12 lives) (Fig. 12).

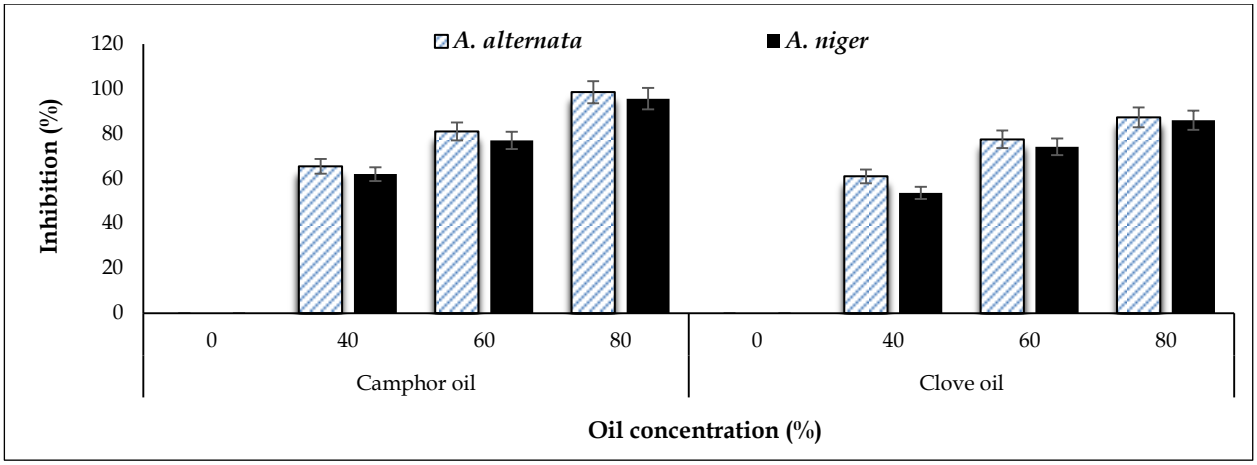


Figure 10. Effect of essential oils at different concentrations on the sporulation of two of heritage-infesting fungi, Alternaria alternata and Aspergillus niger on SPMs.

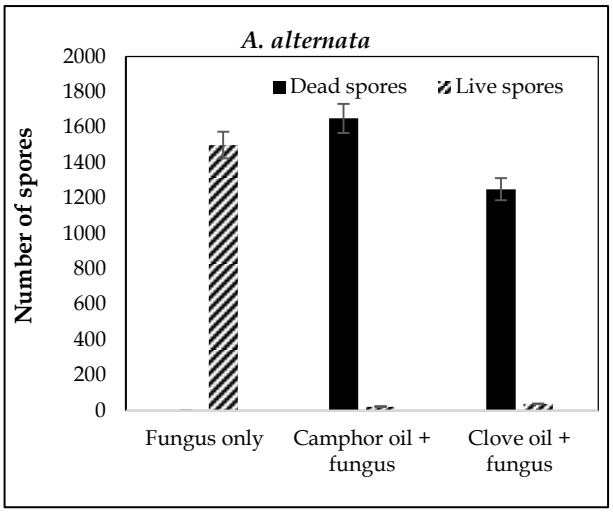


Figure 11. Effect of essential oils at 80% (v/v) concentration on the viability of spores of A. alternata on SPMs

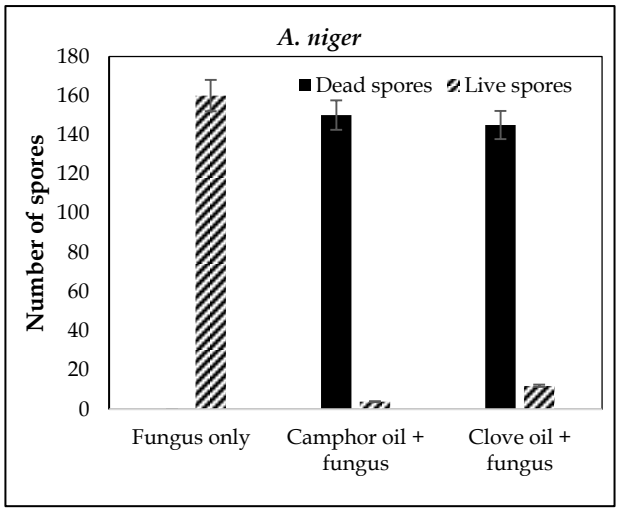


Figure 12. Effect of essential oils at 80% (v/v) concentration on the viability of spores of A. niger on SPMs

3.2.1. SEM

The SEM micrographs (Fig. 13) of SPMs aged control sample (no fungus and oils) and SPMs with no fungus but sprayed with oils concentration 100% clearly revealed that no notable morphological changes appeared in the SPMs due to oils concentration 100%. These results confirm that both oils in the highest concentration (100% v/v) have no side-effects on the SPMs properties, hence; both are completely safe to be used in treatment of oil paintings. Regarding the small macro-cracks appeared in the micrographs; this is the normal morphology of the surface modern and ancient oil paintings as well.

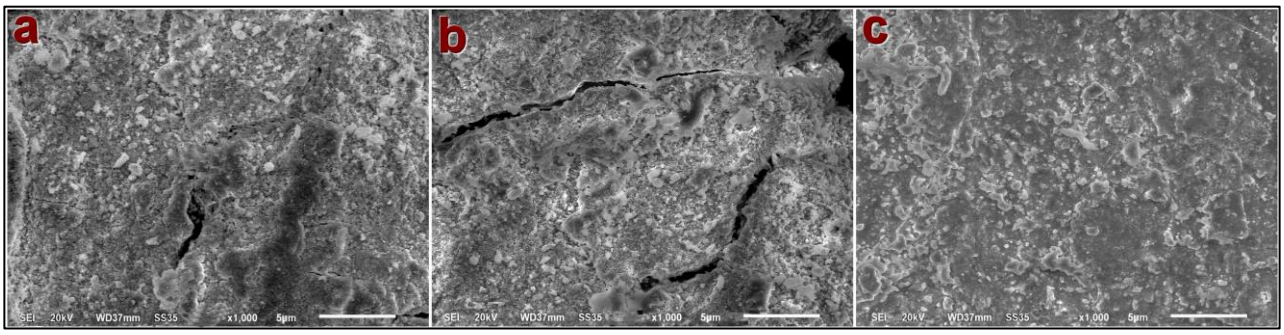


Figure 13. SEM micrographs of SPMs; (a) aged control samples with no fungus and oils, (b) SPM with no fungus but sprayed with camphor oil concentration 100%, (c) SPM with no fungus but sprayed with clove oil concentration 100%.

3.2.2. Stereo microscopy

The results of stereo microscopy (Fig. 14) of SPMs aged control sample (no fungus and oils), SPMs with no fungus but sprayed with oils concentration 100%, and SPM with *A. alternata* and *A. niger* treated with camphor and clove oils 80%, are clearly confirming that no notable changes appeared in all treated SPMs in comparison to the aged control untreated sample. These microscopy graphs clearly revealed the distribution of both fungi spores on the infested SPMs.

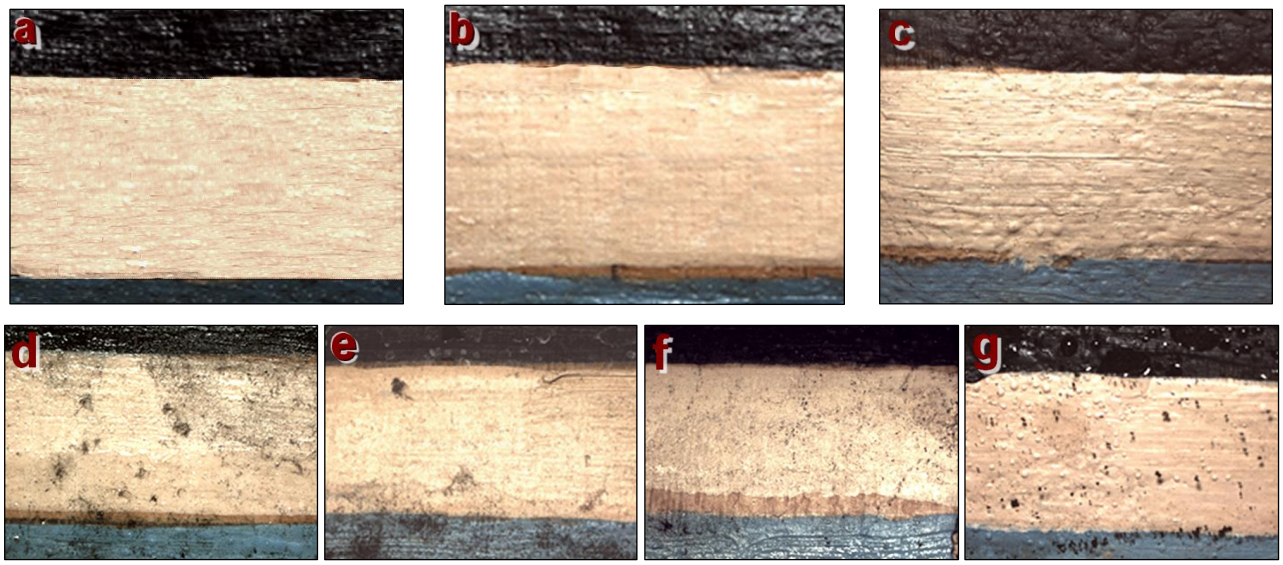


Figure 14. Micrographs of stereomicroscope for SPMs; (a) aged control with no fungus and oils, (b) SPM with no fungus but sprayed with camphor oil 100%, (c) SPM with no fungus but sprayed with clove oil 100%, (d) SPM with A. alternata and camphor oil 80%, (e) SPM with A. alternata and clove oil 80%, (f) SPM with A. niger and camphor oil 80%, (g) SPM with A. niger and clove oil 80%.

3.3. Colorimetric measurements

The obtained results of the colorimetric measurements of the aged control SPMs, 100% oil-sprayed control SPMs and 80% oil-sprayed SPMs with fungus (Table 2) proved that no notable changes occurred due to the effect of both oils either in concentration of 100% oil without fungus, or 80% oil with fungus, only very little decrease in ΔL* of white and yellow colors due to very fine deposits of fungal remains on the surface of the SPMs, these very little changes might be occurred also in other dark colors such as black, blue and red, but not identified because the tones of these color are originally dark. These results are easily recognized by naked eye, it is so clear that there is no notable difference among all treated and untreated SPMs. All these color results (ΔL*, Δa*, Δb*, ΔE*) are calculated against the reference measurements (L*, a*, b*) of the five colored area in the aged control SPMs with no fungus and no oil; Black; L*=2.1, a*=-6.6, b*=-12.5, White; L*=97.4, a*=-3.1, b*=16.2, Blue; L*=29.7, a*=-2.3, b*=-33.6, Red; L*=42.6, a*=24.8, b*=3.5, Yellow; L*=74.1 , a*=-4.2 , b*=53.6. A critical chromatic discussion is given elsewhere (Bratitsi et al., 2018).

Table 2. Results of colorimetric measurements of control and oil-treated SPMs

		Oil only					***A. alternata* + Oil**					***A. niger* + Oil**				
		Black	White	blue	Red	Yellow	Black	White	blue	Red	Yellow	Black	White	blue	Red	Yellow
Clove oil	ΔL*	-0.12	-0.33	-0.15	-0.12	-0.12	-0.23	-0.53	-0.37	-0.67	-0.34	-0.12	-0.23	-0.45	-0.42	-0.65
	Δa*	0.00	0.13	-0.17	0.14	0.13	0.00	0.22	-0.15	0.13	0.17	0.00	0.21	-0.1	0.11	0.17
	Δb*	0.00	0.15	-0.12	0.09	0.15	0.02	0.45	-0.32	0.29	0.43	0.02	0.25	-0.15	0.32	0.49
	ΔE*	0.12	0.38	0.25	0.20	0.23	0.23	0.72	0.51	0.74	0.57	0.12	0.39	0.48	0.53	0.83
Camphor oil	ΔL*	-0.19	-0.36	-0.17	-0.11	-0.16	-0.26	-0.48	-0.34	-0.43	-0.54	-0.14	-0.37	-0.52	-0.32	-0.34
	Δa*	0.00	0.12	0.15	0.12	0.15	0.04	0.13	-0.18	0.16	0.12	0.00	0.22	-0.09	0.21	0.13
	Δb*	0.00	0.11	0.13	0.06	0.13	0.07	0.27	-0.25	0.22	0.36	0.09	0.20	-0.22	0.35	0.31
	ΔE*	0.19	0.39	0.26	0.17	0.25	0.27	0.56	0.45	0.50	0.66	0.16	0.47	0.57	0.51	0.47

4. DISCUSSION

The obtained results of antifungal activity of the marjoram, camphor, clove, and basil studied EOs against *A. alternata* and *A. niger* on agar plates revealed that all used EOs have a variant antifungal activity against the selected fungi, all used EOs had proven an antifungal activity against much fungi and bacteria infesting heritage objects (Bakkali et al., 2008; Axinte et al., 2011). In spite of *A. alternata* and *A. niger* were not included among the microorganisms inhibited by clove oil (*Syzygium aromaticum*) (Akthar et al., 2014); but our obtained results proved that it is the most potent oil used, our obtained results are completely agreed with other article (Borrego et al., 2016) confirming that clove and garlic oils had been proven as a potent antimicrobial materials against different fungal species including *Aspergillus niger*, these incompatible results can be attributed to variant formula of used oils, variant inoculation, variant incubation period or variant antifungal application methods. Our results relevant to camphor oil proved that it is a very efficient inhibitive oil for both fungi, agreed with other articles that proved its efficacy not only in *A. alternata* and *A. niger*; but also in many other types (Al-Harbi and Uddin, 2005; Cabello, 2006; Chang et al., 2001).

In the light of the previous studies; the concentrations of the EOs used as antifungal agents were 2, 3, 4, 5% (Ghoneem et al., 2016), other camphor concentrations of 1, 3, 5% (v/v) were used as antifungal and antibacterial (Zainudin and Azim, 2015), other clove concentrations of 0.25-2.5%(v/v) were used as antifungal and antibacterial (Abed el Hamid et al., 2010). These variant concentrations are completely different and dis agree with our used oils' concentrations (we used 20, 40, 60, 80% for antifungal activity). This dilemma can be attributed to our source of obtained EOs, which might be diluted, as we purchased all extracted, prepared and ready for applications. At any rate; there is no side-effects appeared during our experiments of the four used EOs, either on agar plates during the first phase or on SPMs during the second phase. Also; no side-effects appeared in case of concentration 100% (v/v) which applied directly on SPMs without fungus inoculation.

5. CONCLUSION

The research gave insight to return back to green conservation by using natural materials in treatment and conservation of archaeological objects. The obtained results proved that clove and camphor essential oils are the most potent oils among the four studied essential oils as antifungal materials of *A. alternata* and *A. niger* in different rates, and the more oil concentration; the more efficacy growth inhibition of fungi. Regarding the antifungal activity of both oils on agar plates; clove oil concentration 60% proved a better results of *A. alternata* growth inhibition than camphor oil concentration 60%, which gave better results in *A. niger* growth inhibition than clove oil. Regarding the antifungal activity of both oils on SPMs; the higher concentration at 80% of both oils was more efficient in growth inhibition of both fungi than the lower concentrations at 40 and 60%. Camphor oil concentration 80% proved better results of growth inhibition of *A. alternata* and *A. niger* than clove oil at the same concentration. Both oils are completely safe and have no side-effects on the SPMs, and both are recommended to be used as antifungal green materials against of *A. alternata* and *A. niger* infesting the heritage painting objects. The research is urgently recommending more studies focusing on using different painting pigments and different painting oil media as objectives for both oils, more studies for the antifungal activity of both oils against other fungi and bacteria, and more studies for other suitable essential oils for their antifungal and antibacterial activity in heritage oil paintings. The results of this research can help in rescuing the oil painting cultural heritage not only in Egypt, but also in all heritage-owning countries, based on the innovative results of these eco-friendly fungicides.

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REFERENCES

Abdel-Fattah, G. M., Shabana, Y. M., Ismail, A. E. and Rashad, Y. M. (2007) Trichoderma harzianum: a biocontrol agent against Bipolaris oryzae. *Mycopathologia*, Vol. 164, pp. 81-89.

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*, Vol. 10, No. 2, pp. 85-96.

Abed el Hamid, H., Darwish, S. S. and Bahaa, A. (2010) Microbial studies to evaluate biodeterioration of oil painting and its prevention, *Journal of the General Union of Arab Archaeologists*, No. 11, pp. 102-128.

Akthar, M. S., Degaga, B. and Azam, T. (2014) Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review, *Issues in Biological Sciences and Pharmaceutical Research*, Vol. 2 (1), pp. 001-007.

Alberto, B., Zambonelli, A. and Zechini, A. (1997) Ultrastructural studies of the effects of Allium sativum on phytopathogenic fungi in vitro. *Plant Dis*., Vol. 81, pp. 1241–1246.

Al-Harbi, H. and Uddin, N. (2005) Bacterial diversity of tilapia (Oreochromis niloticus) cultured in brackish water in Saudi Arabia, *Aquaculture*, Vol. 250, pp. 566-572.

Ali, M., Ali, F. M., Darwish, S., Saker, U., Ciliberto, E., Greco, E. and Viscuzo, E. (2015) Investigation and conservation of El-Shenawy palace photographic collection in Mansoura, Egypt, *Mediterranean Archaeology and Archaeometry*, Vol. 15, No. 3, pp. 165-185.

Ashurst, J. (1990) *Cleaning Masonry Building, in Conservation of Building and Decorative Stone*, London.

Rushdy, A. M., Wahba W. N., Abd-Aziz, M. S., El-Samahy, M. and Kamel, S. (2017) A comparative study of consolidation materials for paper conservation, *International Journal of Conservation Science*, Vol. 8, Iss. 3, pp. 441-452.

Atodiresei, G. V., Sandu, I. G., Tulbure, E. A., Vasilache, V. and Butnaru, R. (2013) Chromatic characterization in CIELab system for natural dyed materials, prior activation in atmospheric plasma type DBD, *Revista de Chimie*, Vol. 64. Iss. 2, pp. 165-169.

Axinte, L., Cuzman, A. O., Feci, E., Palanti, S., Tiano, P. (2011) Cinnamaldehyde, a potential active agent for the conservation of wood and stone religious artefacts, *European Journal of Science and Theology*, Vol. 7, pp. 25–34.

Badawy, M. E.I. and Abdelgaleil, S. A. M. (2014) Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi, *Industrial Crops and Products*, Vol. 52, pp. 776-782.

Baka, Z. A. and Rashad, Y. M. (2016) Alternative control of early blight disease of tomato using the plant extracts of *Acacia nilotica, Achillea fragrantissima and Calotropis procera*, *Phytopathologia Mediterranea*, Vol. 55, pp. 121-129.

Baka, Z. A. (2014) Antifungal activity of extracts from five Egyptian wild medicinal plants against late blight disease of tomato. *Archiv. Phytopathol. Plant Protec*, Vol. 47, pp. 1988–2002.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008) Biological effects of essential oils –a review, *Food Chem. Toxicol*, Vol. 46, pp. 446–475.

Biswas, J., Sharma, K., Harris, K. K., Rajput, Y. (2013) Biodeterioration agents: Bacterial and fungal diversity dwelling in or on the pre-historic rock-paints of Kabra-pahad, India. *Iranian Journal of Microbiology*, Vol. 5, pp. 309-314.

Blazquez, A. B., Lorenzo, J., Flores, M. And Gomez Alarcon, G. (2000) Evaluation of the Effect of Some Biocides Against Organisms Isolated from Historic Monuments, *Aerobiologi*a, Vol. 16, pp. 423-328.

Błyskal, B. (2015) Fungal deterioration of a woollen textile dyed with cochineal, *Journal of Cultural Heritage* Vo. 16, pp. 32–39.

Booth, C. (1977) *Fusarium: Alaboratory guide to the identification of the major species*. CMI, Kew, England.

Borrego, S., Valdés, O., Vivar, I., Lavin, P., Guiamet, P., Battistoni, P., Gómez de Saravia, S. and Borges, P, (2012) Essential Oils of Plants as Biocides against Microorganisms Isolated from Cuban and Argentine Documentary Heritage, *International Scholarly Research Network ISRN Microbiology*, Vol. 2012, Article ID 826786.

Borrego, S., Gómez De Saravia, S., Valdés, O., Vivar, I., Battistoni, P., and Guiamet, P. (2016) Biocidal activity of two essential oils on Fungi that cause biodeterioration of paper documents, *International Journal of Conservation Science,* Vol. 7, Iss. 2, pp. 369-380.

Bratitsi, M., Liritzis, I., Vafiadou, A., Xanthopoulou, V., Palamara, E., Iliopoulos, I. , 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, Vol. 18, No 2, pp. 175-212.

Burt, S., (2004) Essential oils: their antibacterial properties and potential applications in foods – a review. *Int. J. Food Microbiol,* Vol. 94, pp. 223–253.

Cabello, F. (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing for human and animal health and for the environment, *Environment Microbial*, Vol. 8, pp. 1137-1144.

Carmo, E. S., Lima, E., Leite de Souza E. and Barbosa de Sousa, F. (2008) Effect of *cinnamomum zeylanicum* blume essential oil on the growth and morphogenesis of some potentially pathogenic *Aspergillus* species, *Brazilian Journal of Microbiology*, Vol. 39, pp. 91-97.

Chang, T., Chen, F. and Chang, C. (2001) Antibacterial activities of leaf essential oils and their constituents from Cinnamomum osmophleum, *Journal of Ethnopharmacolog*, Vol. 77, pp. 123-127.

Ciferri, O. (1999) Microbial degradation of paintings, *Applied and Environmental Microbiology*, Vol. 56, Iss. 3, pp. 879–885.

Cooke, M. (2002) European review of biocides, *Pharma. Chem*. Vol. 1, pp. 48–50.

Di Carlo, E., Chisesi, R., Barresi, G., Barbaro, S., Lombardo, G., Rotolo, V., Sebastianelli, M., Travagliato, G., and Palla, F. (2016) Fungi and bacteria in indoor Cultural Heritage environments: microbial-related risks for artworks and human health. *Environ. Ecol. Res*. Vol. 4, pp. 257–264.

Domenech-Carbo, M. T., Osete-Cortina, L., De La Cruz-Canizares, J., Bolivar-Galino, F., Romero-Noguera, J., Fernandez-Vivas, M. A. and Martin-Sanchez, I. (2006) Study of the microbiodegradation of terpenoid resin-based varnishes from easel painting using pyrolysis-gas chromatography-mass spectrometry and gas chromatography-mass spectrometry, *Analytical and Bioanalytical Chemistry*, Vol. 385, No. 7, pp. 1265-1280.

Doménech-Carbóa, M. T., Bitossia, G., la Cruz-Cañizaresa, J., Bolívar-Galiano, F., del MarLópez-Miras, M., Romero-Noguera, J. and Martín-Sánchez, I. (2009) Microbial deterioration of Mowilith DMC 2, Mowilith DM5 and Conrayt poly(vinyl acetate) emulsions used as binding media of paintings by pyrolysis-silylation-gas chromatography–mass spectrometry, *J. Anal. Appl. Pyrolysis*, Vol. 85, pp. 480–486.

Domsch, K. W., Gams, W., and Anderson, T. H. (1980) *Compendium of Soil Fungi*, Vol. 1, Academic Press, London, UK.

El-Sheikh, S. M, Ali, M. F, Salama, K. K (2017) Low cost pulps with microemulsions for cleaning of fresco painting surfaces. SCIENTIFIC CULTURE, Vol. 3, No 1, pp. 41-46 (DOI: 10.5281/zenodo.192843).

Ellis, M. B. (1971) *Dematiaceous Hyphomycetes*. CMI, Kew, England.

Essa, A. M. and Khallaf, M. K. (2016) Antimicrobial potential of consolidation polymers loaded with biological copper nanoparticles, *BMC Microbiology*, Vol. 16, pp. 16-144.

Ghoneem, K. M., Saber, W. I. A., El-Awady, A. A., Rashad, Y. M., Al-Askar, A. A. (2016), Alternative preservation method against *Sclerotiumtuber* rot of Jerusalem artichoke using natural essential oils, *Phytoparasitica*,Vol. 44, pp.341–352.

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*, Vol. 60, pp. 50–59.

Gottsegen, M. D. (1987) *A manual of painting materials and techniques*, Harper and Row, New York.

Hayat, M. A. (1989) *Principles and techniques of electron microscopy (Biological Applications),* Vol. 1, The Macmillan Press Ltd., London.

Helmi, F., Elmitwalli, H., Rizk, M., and Hagrassy, A. (2011) Antibiotic extraction as a recent biocontrol method for *Aspergillus Niger* and *Aspergillus Flavus* fungi in ancient Egyptian mural paintings, *Mediterranean Archaeology and Archaeometry*, Vol. 11, No. 2, pp. 1-7

Hussain, A. I., Anwar, F., Hussain Sherazi, S. T. and Przybylski, R. (2008) Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations, *Food Chem*. Vol. 108, pp. 986–995.

Ibrahim, N., Eid, B. and Abdel-Aziz, M. (2017) Effect of plasma superficial treatments on antibacterial functionalization and coloration of cellulosic fabrics, *Applied Surface Science*, Vol. 39, pp. 1126–1133.

Izzo, F. C., van den Berg, K. J., van Keulen, H., Ferriani, B., and Zendri, E. (2014) Modern oil paints – formulations, organic additives and degradation: Some Case Studies, In: *Issues in Contemporary Oil Paint*, van den *Berg* K. et al. (eds), Springer, Cham .

Karbowska-Berent, J., Gorniak, B. G., Czajkowska-Wagner, L., Rafalska, K., Jarmiłko, J. and Kozielec, T. (2018) The initial disinfection of paper-based historic items – Observations on some simple suggested methods, International Biodeterioration and Biodegradation, Vol. 131, pp. 60-66.

Katusim-Razem, B., Razem, D. and Braun, M. (2009) Irradiation treatment for the protection and conservation of cultural heritage artifacts in Croatia, *Radiation Physics and Chemistry*, Vol. 78, Iss. 7–8, July–August, pp. 729-731.

Keshari, N. and Adhikary, S. P. (2014) Diversity of cyanobacteria on stone monuments and building facades of India and their phylogenetic analysis, *Int. Biodeter. Biodegr*, Vol. 90, pp. 45-51.

Knut, N. (1999) *The Restoration of Paintings*, Kӧln, Kӧnemann; Princes Risborough, translated by Cambridge, UK.

Krumbein, W. E. (2003) Patina and cultural heritage: a geomicrobiologist's erspective. In: *Proceedings of the 5th European Commission Conference Cultural Heritage Research*, Kozlowski, R. (ed.), pp. 39-47. Cracow.

Kurowski, G., Vogt, O. and Ogonowski, J. (2017) Paint-degrading microorganisms, *Technical Transactions*, Vol. 12, pp. 81-92.

Lopez-Miras, M., Pinar, G., Romero-Noguera, J., Bolı´var-Galiano, F. C., Ettenauer, J., Sterflinger, K. and Martı´n-Sa´nchez, I. (2013) Microbial communities adhering to the obverse and reverse sides of an oil painting on canvas: identification and evaluation of their biodegradative potential. *Aerobiological*, Vol. 29, pp. 301–314 .

Lozano, C. J., Mendoza, M. V., Arango, M. C. and Monroy, E. F. (2009) Microbiological characterization and specific methanogenic activity of anaerobe sludges in urban solid waste treatment, *Waste Manag*, Vol. 29, pp. 704-711.

Ma, Y. T., Zhang, H., Du, Y., Tian, T., Xiang, T. and Liu, X. D. (2015) The community distribution of bacteria and fungi on ancient wall paintings of the Mogao Grottoes, *Scientific Reports*, Vol. 5, article no. 7752.

Mansour, M. M., and Salem, M. Z.(2015) Evaluation of wood treated with some natural extracts and Paraloid B-72 against the fungus *Trichoderma harzianum*: wood elemental composition, *in-vitro* and application evidence. *Int. Biodeterior. Biodegr*., Vol. 100, May, pp.62-69.

Manzano, E., Rodriguez-Simón, L. R., Navas, N., Checa-Moreno, R., Romero-Gámeza, M., and Capitan-Vallvey, L. F. (2011) Study of the GC–MS determination of the palmitic–stearic acid ratio for the characterisation of drying oil in painting: La Encarnaciónby Alonso Cano as a case study, *Talanta*, Vol. 84, pp.1148–1154.

Martin-Sanchez, P., Nováková, A., Bastian, F., Alabouvette, C., and Saiz-Jimenez, C. (2012) Use of biocides for the control of fungal outbreaks in subterranean environments: The case of the Lascaux cave in France, *Environmental Science and Technology,* Vol. 46, pp. 3762-3770.

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

Mayer, R. (1991) *The Artists Handbook of Materials and Techniques*, The Viking Press, New York.

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

Moghtader, M. (2013) In vitro antifungal effects of the essential oil of *Mentha piperita* L. and its comparison with synthetic menthol on *Aspergillus niger*, *African Journal of Plant Science*, Vol. 7, No. 11, pp. 521-527.

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,* Vol. 7, Iss. 1, pp. 41-56.

Ogbulie, J. N. and Obiajuru, I. O. (2004) Microbial deterioration of surface paint coatings, *Global Journal of Pure and Applied Sciences*, Vol. 10, No. 4, pp. 485-490.

Pangallo, D., Chovanova, K., Simonovicova, A., Ferianc, P. (2009) Investigation of microbial community isolated from indoor artworks and their environment: identification, biodegradative abilities, and DNA typing, *Canadian Journal of Microbiology*, Vol. 55, pp. 277–287.Phillips, C. A., Laird, K. and Allen, S. C. (2012) The use of Citri-V™®—An antimicrobial citrus essential oil vapour for the control of *Penicillium chrysogenum, Aspergillus niger* and *Alternaria alternata* in vitro and on food, *Food Research International*, Vol. 47, pp. 310–314.

Romero-Noguera, J., Martin-Sanchez, I., Ramos-Lopez, J. M. and Bolivar-Galiano, F. (2010) Biodeterioration patterns found in dammar resin used as art material, *Electronic Journal of Biotechnology*, Vol. 13, No. 3, pp. 1-9.

Romero-Noguera, J., Bolívar-Galiano, F. C., Ramos-López, J. M., Fernández-Vivas, M. A. and Martín-Sánchez, I. (2008) Study of biodeterioration of diterpenic varnishes used in art painting: Colophony and Venetian turpentine, *International Biodeterioration and Biodegradation*, Vol. 62, No. 4, pp. 427-433.

Rajput, Y., Rai, V., and Biswas, J. (2012) Screening of Bacterial isolates from various microhabitat sediments of Kotumsar cave: a cogitation on their respective benefits and expected threats for complete biosphere and tourists. *Res. J. Environ. Toxicol*, Vol. 6, pp. 13-24.

Raper, K. E. and Fennel, D. I. (1965) The Genus Aspergillus, Baltimore, Williams & Wilkins Co. USA.

Raut, J. S., and Karuppayil, S. M.(2014) A status review on the medicinal properties of essential oils, *Industrial Crops and Products*, Vol. 62 , pp. 250–264.

Rios, J. L. and Recio, M. C. (2005) Medicinal plants and antimicrobial activity, Journal of Ethnopharmacology, Vol. 100, pp. 80-84.Salem, N., Zidan, Y. E., Mansour, M. M. A., El Hadidi, N. M. N. and Abo Elgat, W. A. A. (2016) Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi, *International Biodeterioration and Biodegradation*, Vol. 106, pp. 88-96.

Sakr, A., Ghaly, M., Helal, G. and Abdel Haliem M. (2018) Effect of Thymol against Fungi Deteriorating Mural Paintings at Tell Basta Tombs, Lower Egypt, *International Journal of Research Studies in Biosciences*, Vol. 6, Iss. 2, pp. 8-23.

Salama, K. K, Ali, M. F, Moussa, A. M. (2016a) Experimental study of the retouching materials applied on mural painting in el Sakakeny Palace. *SCIENTIFIC CULTURE*, Vol. 2, No. 3, pp. 1-4 (DOI: 10.5281/zenodo.47545).

Salama, K. K, Ali, M. F, Abubakr M. Moussa (2016b) Deterioration factors facing mural paintings in El Sakakeny Palace (problems and solutions). *SCIENTIFIC CULTURE*, Vol. 2, No 3, pp. 5-9 (DOI: 10.5281/zenodo.44897)

Salama, K. K., Ali M. F. and El-Sheikh, S. M. (2018) The different influence of nano materials on pigments, *SCIENTIFIC CULTURE*, Vol. 4, No. 3, pp. 1-7. (DOI: 10.5281/zenodo.1409798).

Schanda, J. (2007) *Colorimetry: Understanding the CIE System*, New Jersey, Wiley Interscience, John Wiley & Sons, Inc, Hoboken.

Seves, A. M., Sora, S. and Ciferri, O. (1996) The microbial colonization of oil paintings: A laboratory investigation, *International Biodcteriorulion and Biodqqadation*, Vol. 37, Iss. 3-4, pp. 215-224.

Seves, A. M., Sora, S., Scicolone, G., Testa, G., Bonfatti, A. M., Rossi, E. and Seves, A. (2000) Effect of thermal accelerated ageing on the properties of model canvas paintings, *Journal of Cultural Heritage*, Vol. 1, pp. 315-322.

Shabana, Y. M., Elwakil, M. A., Charudattan, R. (2000) Effect of media, light and pH on growth and spore production by *Alternaria eichhorniae*, a mycoherbicide agent for waterhyacinth, *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, Vol. 107, pp. 617-626.

Sterflinger, K. (2010) Fungi: Their role in deterioration of cultural heritage, *Fung Biol. Rev*, Vol. 24, pp. 47-55.

Stuart, B. H. (2004) *Infrared spectroscopy; Fundamentals and applications*, John Wiley & Son's, Chichester.

Stupar, M., Grbić, M. L. j., Dzamic, A., Uukovic, J., 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,* Vol. 93, pp. 118–124.

Stupar, M., Grbić, M. L., Simić, G. S., Jelikić, A., Vukojević, J., and Sabovljević, M. (2014) A sub-aerials biofilms investigation and new approach in biocide application in cultural heritage conservation: Holly Virgin Church (Gradac Monastery, Serbia), *Indoor and Built Environment*, Vol. 23, pp. 584-593.

Summerell, B. A., Salleh, B. and Leslie, J. F. (2003) A utilitarian approach to *Fusarium* identification, *Plant Disease*, Vol. 87, pp. 117-128.

Teixeira, B., Marques, A., Ramos, C., Neng, N. R., Nogueira, J. M., Saraiva, J. A., Nunes, M. L. (2013) Chemical composition and antibacterial and antioxidant properties of commercial essential oils, *Ind. Crops Prod.*, Vol. 43, pp. 587–595.

Valentin, N., Lidstrom, M. and Preusse, F. (1990) Microbial control by low oxygen and low relative humidity environment, *Studies in Conservation*, Vol. 35, No. 4 , pp. 222-230.

Veneranda, M., Blanco-Zubiaguirre, L., Roselli, G., Di Girolami, G., Castro, K. and Madariaga, J. M. (2018) Evaluating the exploitability of several essential oils constituents as a novel biological treatment against cultural heritage biocolonization, *Microchemical Journal*, Vol. 138, pp. 1–6.

Weisburg, W. G., Barns, S. M., Pelletie, D. A., Lane, D. J. (1991) 16S ribosomal DNA amplification for phylogenetic study, *J. Bacteriol.*, Vol. 173, pp. 697-703.

Wiszniewska, M., Walusiak-Skorupa, J., Pannenko, I., Draniak, M., Palczynski, C. (2009) Occupational exposure and sensitization to fungi among museum workers, *Occup. Med.*, Vol. 59, pp. 237-242.

Zainudin, Z., Azim and M. K. M. (2015), Study on the antibacterial and antifungal effects of camphor oil; *Cinnamomum camphora* with emphasis on the control of overpopulation of tilapia, *International Journal of Fisheries and Aquatic Studies,* Vol. 2, (5), pp. 337-340.