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Essential Oil Nanoemulsion as Antifungal Agents in the Conservation of the Painted Limestone Stela

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Abstract

The objective of this work was to isolate and identify fungal strains colonizing painted limestone rectangular funerary Stela of Dedusobek (Cairo CG 20236 \ Sr 3-9714)–Egyptian Museum, and evaluate the resistance effects of isolated fungi to essentials oils nanoemulsion (cinnamon, tea tree, and lemon). Essential oils (EOs) have been well-known for their antimicrobial properties and EOs have also been applied to treat the biodeterioration of cultural heritages as powerful source in green conservation. The use of essentials oils nanoemulsion as an alternative to traditional biocides should be considered. In the present study, we used the tea, lemon, and cinnamon oil nanoemulsions as antimicrobial agents to treat Stela-born fungal deterioration. Our study showed that cinnamon oil nanoemulsion had strong antimicrobial activity in vitro assays, which has been successfully confirmed by application on the biofilm revealed in the painted limestone Stela. The applied essentials oil nanoemulsion were characterized using scanning electron microscopy (SEM) as well as color measurements by CIELAB system. The results revealed that the antimicrobial activity of cinnamon oil nanoemulsion solution was enough to kill the fungal contaminants.

Keywords: Biodeterioration; essential oil nanoemulsions; antifungal; cinnamon oil; cultural heritage; green conservation.

1. Introduction

Biodeterioration processes of mural paintings can cause different kinds of alterations such as discoloration of their own materials, the formation of crusts on surfaces as well as the loss of some of their constituting materials which leads to structural damage, and detachment of the paint layer from the support or it can cause aesthetic damages leading to pigment discoloration and formation stains. Microorganisms can cause severe problems due to the excretion of organic or inorganic acids [1][2]. The fungal hyphae can grow inside the paint layers causing mechanical destruction and by further growth, the smooth surface of the painting will be altered and the painting became rough [3]. Due to the damages that fungi can cause to mural paintings, their growth should be controlled. The conservation of mural paintings is based on the use of preventive and remedial methods. Preventive methods aim to inhibit biological attacks on stone works. Selection of suitable environmental conditions (temperature, light, humidity) that reduce the risk of microbial deterioration is only feasible indoors. Dirt and dust that had been deposited that had various organic substances as well as bird droppings were considered as potential sources of nutrition for microorganismsand to recover this periodical

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cleanings are needed [4][5]. Biocontrol appears to be a reliable alternative to chemical fungicides, because fungicides are highly toxic and impose environmental hazards for both the treated objected and conservators. So, these new trends used environmentally safe methods such as plant extracts as well as essential oils (i.e., thyme and its derivatives) to control fungal colonizing cultural heritage objects. These substances have high potentiality as antimicrobial agent with low toxicity for human and environment [6][7].

Essential oil is as aromatic product, of multifaceted composition, obtained from part of plant or the whole plant through several methods. It has specific taste and odor specific to the plant from which it was derived. This difference in composition could be due to plant variety, geographical locations, harvesting seasons, drying methods and extraction methods[8]**.** They are commonly used due to their pleasant or spicy smell. In addition, numerous recent studies reported the biological activities of essential oils such as antibacterial and fungicidal activities[9]. The small size of essential oil molecules allow them to easily penetrate through cell walls and affect various biochemical processes. Thus, the biological activity of essential oils depends on their composition, i.e. essential oils that contain substituted phenols (eugenol, thymol, carvacrol, and guaiacol) exhibit strong antibacterial and antioxidant effects [8]. Furthermore, essential oils are easily degraded by oxidation, volatilization, heating, and exposure to light and difficult to disperse in hydrophilic media [10]. The nanoemulsions have higher solubilization size in contrast to unstable emulsions and suspensions. The literature studies detailed that the essential oils, by nature, determine excellent antimicrobial properties [11].

In order to overcome these problems, nanoencapsulation techniques have been suggested leading to increase their dispersibility in aqueous preparations, increase chemical stability during storage, reduce organoleptic changes, and in some cases, even increase their antimicrobial action [12]. Nanoemulsions exhibit high kinetic stability and are thus stable for a long period of storage [13]. Essentials oils have eco-friendly and effective the control of biodeterioration on paintings [14]. EOs have applied in the field of cultural heritage preservation, to preserve papyrus, paper documents and wood [15][16]. Additional studies used an effective mixture of essential oils such as oregano, lemongrass, and peppermint against some fungi [17]. A previous work conducted by [18] proved cinnamon oil had the highest antifungal activities against the tested fungi *A. niger*, *A. flavus*, *A. ochraceopetaliformis*, *C. halotolerans*, and *N. goegapense.* Garlic oil had been confirmed as a antimicrobial materials against different fungal species *Aspergillus niger*, and tea tree, lavender and thyme oils shown result against much common fungi and bacteria infesting ancient documents[15][8] . Elsayedproved that clove and camphor essential oils are the most potent oils as antifungal of *A. alternata* and *A. niger* IN archaeological oil paintings [19].

The aim of this research is to estimate the ability of essentials oil nanoemulsions to inhibit the growth of fungal strains that inhabiting painted limestone Stela and examine the treatment impact on aesthetic and physical characteristics of the limestone Stela before and after microbial aging. Monitoring the variation in these characteristics through the SEM and color measurements was also undertaken.

2. Material and Methods

2.1. Samples

The painted limestone rectangular funerary Stela of Dedusobek(Cairo CG 20236 \ Sr 3-9714) was discovered in 1861 during the excavations by A. Mariette (director of the Egyptian Antiquities Service) in North Abydos. The Stela dated back to the dynasties 12 (Middle kingdom). The object's dimensions are 33 cm in height and 22 cm in width (Fig. 1). Sampling was carried out using sterile cotton swabs from the colored surface of the selected Stela under aseptic conditions and transferred in sterile tubes to the laboratory (Microbial Chemistry Department, National Research Centre, Dokki, Giza, Egypt) for fungal isolation and identification.

Figure 1: The Painted limestone rectangular with cornice of Dedusobek

2.2. Portable X-Ray fluorescence Spectrometer (pXRF)

Portable XRF was applied to point out the chemical composition of the pigment materials for Stela. Portable X-Glab ELIO spectrometer with excitation source was used to detect XRF spectra. This equipment is characterized by: Transmission x-ray generator 5-200 μA, 10-40 kv,rh anode "Ag,Au,MoW", 1 mm or 2mm collimator and filters set. Elio portable XRF analysis (PXRF) is designed for in-site analysis. ELIO is also known to have detection head large area silicon drift detector: 25 MM2 XRF detectors, 130 eV at MnKa with 10 kcpsinput photon rate (high resolution mode), 170 eV at MnKa with 200 Kcps input photon rate "fast mode".

2.3. Preparation of nanoemulsions based on Tea, Cinnamon, and Lemon

Three essential oils, namely (Lemon, tea tree and cinnamon) were purchased from National Research Centre, Dokki, Giza. Nanoemulsions were produced according to [20]. 1.5 ml tween 80 (that used as a Surfactant) was dissolved in 15 ml dis H_2O under magnetic stirring for 15 min, at the same time TEOS solution was prepared by dissolving 3 ml TEOS into 80 ml H_2O with continuous stirring. Afterwards, 5 ml of Tea oil was added dropsies into TEOS solution with continuous stirring for 20 min. the resultant emulsion ultrasonic for 10 min to obtain nanoemulsion. With similar manner, cinnamon and lemon nanoemulsion were prepared.

2.4. Fungal isolation

All samples were transmitted to the laboratory on the same day of collection and immediately processed. Each swab, wiped across fungal colonies on the Stela was immersed in a sterile glass vial containing 5ml of sterile distilled water and shaker for 2 hours on a reciprocal shaker. Then serial dilution from each sample has been performed up to 10^{-4}. 0.2 mL from dilutions 10^{-3} and 10^{-4} were used to inoculate Petri dishes (15cm diameter) each containing 100ml of solidified potato dextrose agar (PDA) medium (with and without) Rose Bengal [21]and neomycin, and the inoculated plates were incubated at 30 ± 2°C for 6–8 days. The growth was observed after 2days. The isolated fungi were purified by the streak method. The cultures were routinely transferred (every 6–8 days) onto fresh PDA agar plates by streaking. Before fungal cultures were used for inoculation of rice medium, the fungus was subjected to three transfers on PDA agar plates by the direct agar transfer method [22].

2.5. Molecular Identification of isolated fungal strain from Stela

DNA extraction, PCR and sequencing (18SrRNA) were established in the molecular identification of the isolated fungal strains. Identification had been performed by employing of the nuclear ribosomal internal transcribed spacer (ITS) region 1, 2, along with the short structural gene (5.8 S) as had been studied in the supporting information of preceding studies. The ITS region has been chosen to identify fungi as it has been lately documented as a common marker for fungal identification [23][24].Purification of the PCR products was carried out to eliminate separate PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore). Sequencing achieved by using Big Dyeterminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA).

2.6. Antifungal activities of oil nanoemulsions against the isolated fungal strains

The prepared oil nanoemulsions from tea tree, lemon and cinnamon oils were tested for their antifungal activity against three isolated fungal strains from painted limestone rectangular funerary Stela of Dedusobek (Cairo CG 20236 \ Sr 3-9714). The used medium was potato dextrose agar (PDA) placed in 10cm-diameter petri dishes. The plates were seed with spore suspension from each fungal isolate (10^6CFU). Using cork borer, three holes (1cm diameter) were initiated in each plate. 100microlitre from each oil nanoemulsion were placed in each hole. The inoculated plates were incubated at 30°C for 72h. The appearance of clear zone around any oil suspension containing-hole means that oil had antifungal activity against the test fungal strain.

2.7. Transmission Electron Microscope (TEM)

The TEM of the cinnamon nanoemulsions was achieved using JEM-1230 Model electron microscope set at 60kV (JEOL Ltd., Japan). The oil nanoemulsions were diluted in water at least ten times before testing. After that, a drop of the fully dispersed diluted specimen was deposited onto a copper mesh (200-grid and covered with carbon film) and dried at room temperature. This process was carried out at the National Research Centre, Egypt.

2.8. Gas chromatography/mass spectrometry (GC/MS) analysis of cinnamon oil

GC-MS investigation was carried out according to the reported procedures [25], using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS,TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness, was used GC/MS detection. The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison

of the irrelative retention time and mass spectra with those of the NIST, WILLY library data of the GC-MS system.

2.9. In vitro antimicrobial activity of essential oil nanoemulsions on experimental samples

The experimental samples were prepared according to the Case study in the Egyptian Museum- El Tahrir. Lime stone support of the following dimension (5 × 5 × 5 cm) was prepared and painted with a pigment layer and then left to dry for 15 days, before subjecting to microbial aging. Under aseptic conditions, experimental samples were divided into two groups: the first group was sprayed with cinnamon oil nanoemulsion and the other group was not treated with oil nanoemulsion. Both groups were contaminated by the three isolated fungal strains and incubated for 14 days at 30^{o}C. After incubation both treated and untreated experimental samples were subjected to stereo microscope, SEM and colorimetric investigation.

2.10. Scanning Electron Microscopy (SEM) examination

The extent of deterioration of cinnmon oil nanoemulsion-treated experimental samples and non-treated experimental samples after fungal attack were examined by scanning electron microscope (JEOL model 1200 EX) characterized by using SEM model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses). The equipment has accelerating voltage 30K.V. and magnification of 14x up to 1000000x.

2.11. Colorimetric Change

CIE lab color system "1976" with Spectro densitometer "Exact X-Rite, Switze land" is used to measure the different colors of experimental samples before and after antifungal treatments to identify any probable color changes that might be resulted after using the oils nanoemulsion treatments. The reflectance values were measured from 400 to 700 nm at 10 nm intervals with white standard #1633 [26][27]. The total color difference (ΔE) is calculated according to the following equation: $\Delta E = \{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\}^{½}$.

3. Results and Discussion

3.1. Portable XRF analysis

Based on the data obtained from the XRF; it has been concluded that the pigments were not applied directly into the surface of the limestone, but a thin layer of gypsum ($CaSO_4.2H_2O$) was applied first. The presence of calcium (Ca) is related to use of the limestone, which it is constituted by this material, it contained 68.01% of calcium (Ca), 31.69 % of sulpher, and 0.23 % of iron (Fe), Fe refer to harmful impurities in the compositions of the limestone (Fig. 2A). In the blue regions, the spectra (Fig. 2B) exhibited high intensities of silicon (Si 50%), copper (Cu 1.19%) and calcium (Ca 35%), characteristic elements of the Egyptian blue pigment (cuprorivaite). Therefore, the possibilities in this case are Egyptian blue ($CaCuSi_4O_{10}$) also known as calcium copper silicate or cuprorivaite), it produced in a sintering process. The coarse textured mass of polycrystalline blue was obtained from fusing copper, calcium and silica, and a few percent of a flux of soda by heating in crucibles in a furnace at about 850–1050 °C [28]. The XRF spectrum of the yellow pigment showed the presence of some elements such as: (Fe 9.46%, Ca 53.17%, and S 37.18%) which it confirmed to the presence of hydrated iron oxide, goethite ($FeO.(OH)_2$) with the presence of a percentage of sulfure, and this is due to the ground layer (gypsum) (Fig. 2C). The red pigment shows calcium (Ca 72%), iron (Fe 4.45%) and sulfur (S 22%). The presence of the iron (Fe) element confirmed that red iron oxides were used to make the red pigment (hematite Fe_2O_3). In ancient Egypt; this color has been used in a thick layer upon the gypsum white wash to have a good hiding powerbecause the metal is very hard to be fine grinded or to be good mixed with paint medium [29]as presented in (Fig. 2D). Ca 73.34% and S 21.29% presented the highest concentration in the white pigment. The possible pigment in this case is calcium sulfate $CaSO_4.H_2O$ [30](Fig. 2E). XRF analysis of green pigment that the elements that presented the highest concentrations were: (Ca 53%), (Cu 6.8%) and (S 28.29%). The presence of (Cu) and (Ca) suggest the possibility of copper carbonates green pigments (malachite $CuCO_3.Cu(OH)_2$). In addition, the presence of (Ca) and (S) might be due to the calcium sulphate $CaSO_4.2H_2O$ (Fig. 2F).

3.2. Isolation and identification of fungal strains from Stela

Three fungal strains (S1, S2 and S3) were isolated from swabs collecting from deteriorated Stela (Fig. 3 A). These fungal strains were molecularly identified using 18SrRNA protocol. BLAST search was applied for the produced sequence from DNA of the fungal isolates (S1, S2 and S3), it had been found that, the fungal isolates S1, S2 and S3 had the similarities of 100 % with *Aspergillus terreus* isolate JODI2 (accession no. OQ798892.1), 99% with *Aspergillus candidus* strain ZSF. (accession no.KT377249.1) and 99.42% with *Aspergillus flavus* strain azm015 (accession no. MH107055.1), respectively. The phylogenic trees of the fungal isolates were built as shown in (Fig.3 B, C &D).

According to the previous identification techniques, *Aspergillus terreus* isolate S1-EGY, *Aspergillus candidus* isolate S2-EGY and *Aspergillus flavus* isolate S3-EGY with GenBank accession numbers of OR591158.1, OR591160.1 and OR591163.1, respectively. The fungal isolates (S1, S2 and S3) cultures were placed in the Microbial Chemistry Department Collection of Microorganisms. Classical and traditional fungal identification protocols, depending on morphology and biochemical studies, have been frequently useful and numerous novel species currentely are identified according to this method which was known to be time consuming and not accurate [31].

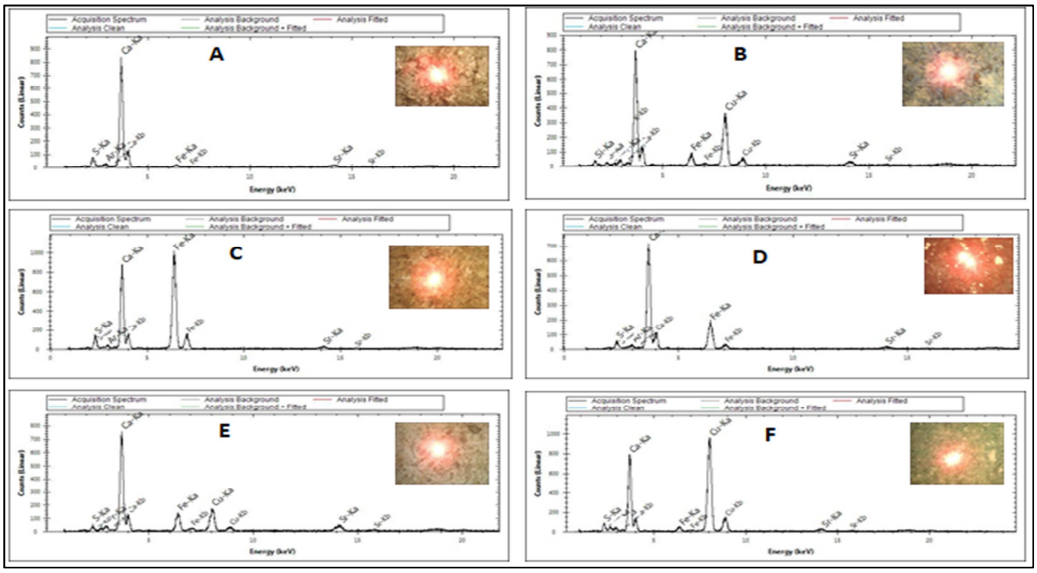
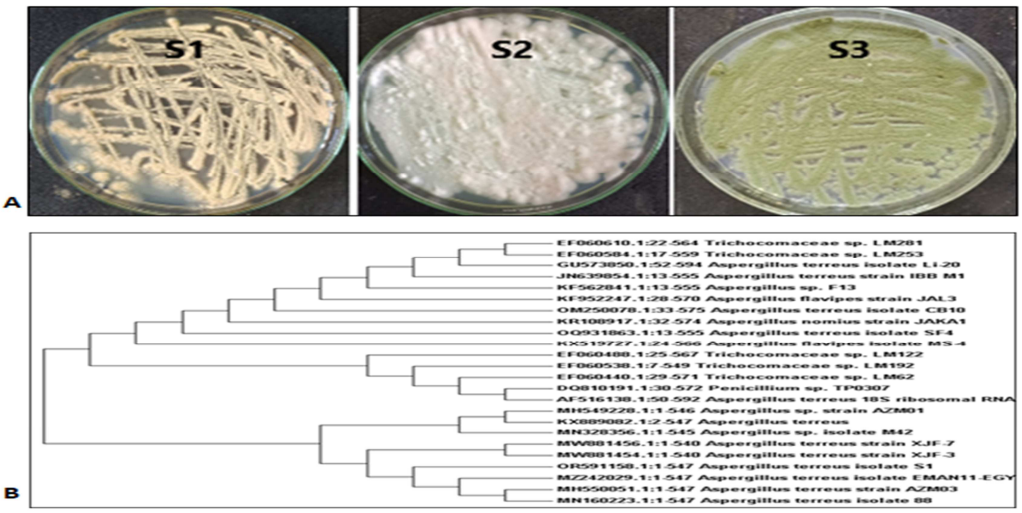


Figure 2: XRF spectrums of the stone (A).Blue pigment (B).Yellow pigment (C).Red pigment (D).White pigment (E).Green pigment (F).

3.3. Transmission Electron Microscope (TEM)

Three essential oils namely; Lemon, tea tree and cinnamon essential oil were prepared in nanoscale according to the method described above. Only cinnamon essential oil nanoemulsion was characterized as it was the only nanoemulsion that exhibited antifungal activities against the three isolated and identified fungal strains. (Fig. 4) shows the TEM images of cinnamon nanoemulsion. The droplet size of cinnamon nanoemulsions in the TEM images were approximately 6:10 nm. The droplet was oval and spherical shape as clarified through the TEM images.



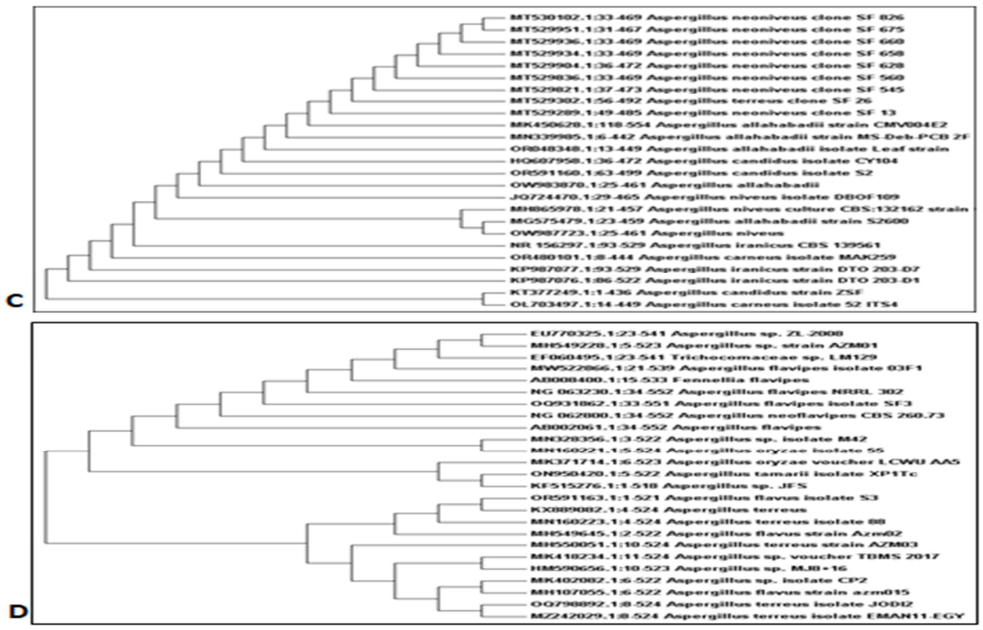


Figure 3: (A) The appearance of the isolated fungal strains on PDA medium**.** Phylogenetic trees of the *Aspergillus terreus* isolate S1-EGY (B), *Aspergillus candidus* isolate S2-EGY (C) and *Aspergillus flavus* isolate S3-EGY (D) isolates. The phylogenetic tree has been reconstructed using MEGA7 software

3.4. GC-MS investigation of cinnamon oil

GC-MS examination of the cinnamon oil comprises 10 compounds (Fig. 5). As is well known, the chemical analysis of cinnamon oil gives an impression about its ingredients that elucidate its bioactivity. The GC-MS of the total peak area of the identified ingredients constitutes 86.69%, the prospects of the chemical structures of the identified compounds are documented (Table 1): The maindetected compounds are 4-Amino-1,5-pentandioic acid, 1,3-Propanediol (3%), Methyl 2,2-Dicyanopropionate (10.25%), 3,3-Dimethoxy-2,2-di methylpropanol (15.29%), 2,2-Dimethylthiane-3,5-dione (6.78%), 2-Methoxyindan-1-one (6.02%), 4-(1-oxo-2-indanyl) butanoic acid (4.39%), Benzaldehyde,2-ethenyl (17.74%),1,2,3-Propanetriol, triacetate (8.97%) and 1-(3-Isopropenylphenyl) ethanone (19.28%). The identification was achieved via using computer search user-generated reference libraries, incorporating mass spectra.

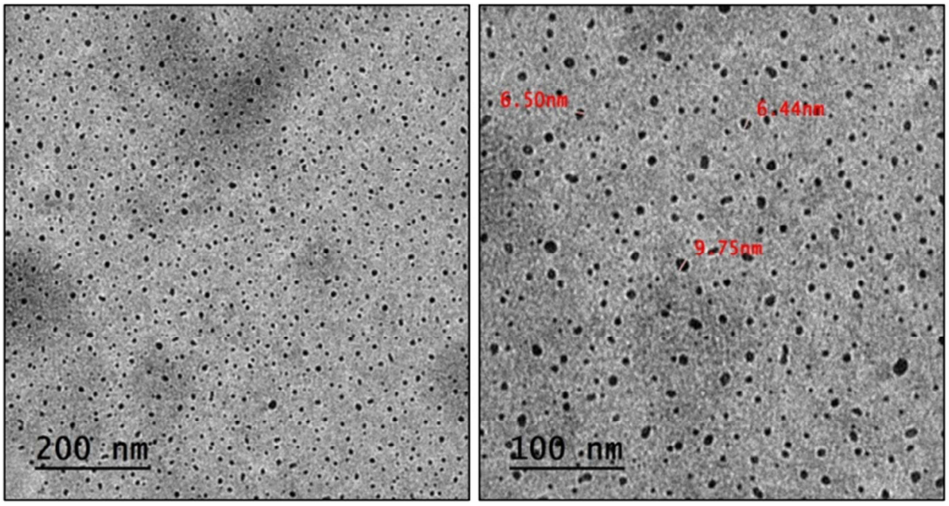


Figure 4: Transmission electron microscopy images of the cinnamon nanoemulsion: scale bar is 100 nm

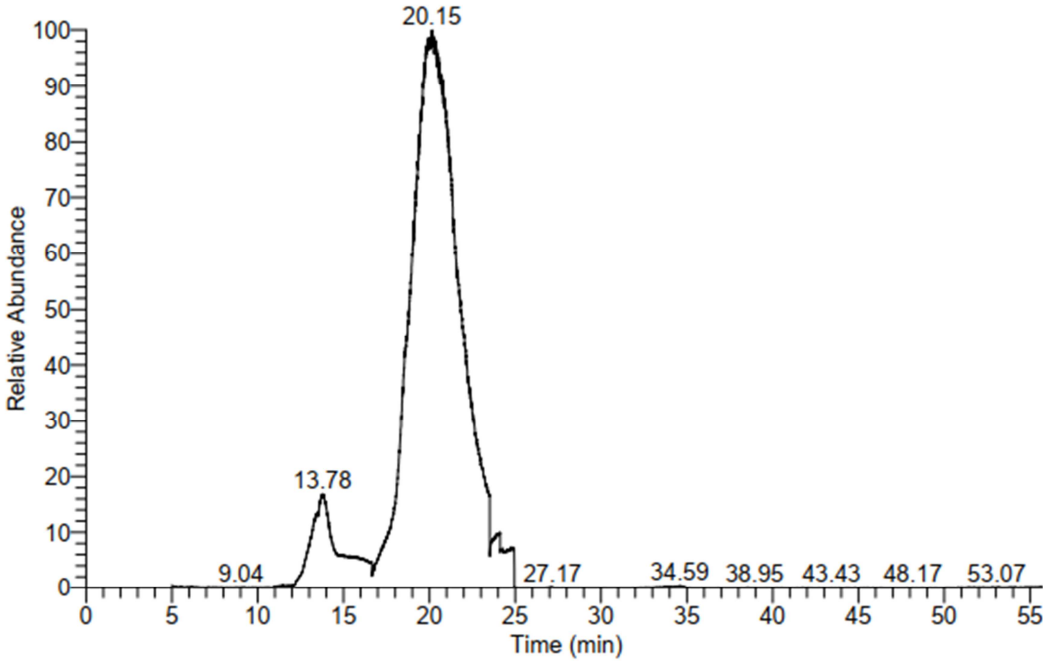


Figure 5: SEM-EDS chart shows the composition of the limestone support of the stela CG 20212

Table 1: Chemical compositions of cinnamon oil

No.	R_t	Area%[a]	M.W.	M.F.	Identified compounds
1	13.35	3	175	$C_7H_{13}NO_4$	4-Amino-1,5-pentandioic acid
1	13.77	10.25	76	$C_3H_8O_2$	1,3-Propanediol
3	13.85	15.29	138	$C_6H_6N_2O_2$	Methy 2,2-Dicyanopropionate
4	18.56	7.98	148	$C_7H_{16}O_3$	3,3-Dimethoxy-2,2-dimethylpropanol
5	19.78	6.78	160	$C_7H_{12}O_2S$	2,2-Dimethylthiane-3,5-dione
6	19.88	6.02	162	$C_{10}H_{10}O_2$	2-Methoxyindan-1-one
7	21.31	4.39	218	$C_{13}H_{14}O_3$	4-(1-oxo-2-indanyl)butanoic acid
8	23.50	17.74	132	C_9H_8O	Benzaldehyde,2-ethenyl-
9	24.10	8.97	218	$C_9H_{14}O_6$	1,2,3-Propanetriol, triacetate (CAS)
10	24.93	19.28	160	$C_{11}H_{12}O$	1-(3-Isopropenylphenyl) ethanone

3.5. Invitro antifungal activity of oil nanoemulsion against isolated fungi

Lemon, tea tree and cinnamon essential oil nanoemulsions were examined for their abilities to inhibit the growth of isolated microbial strains. The appearance of inhibition zones of the three isolated strains (S1, S2, and S3) around the tested oil nanoemulsion were considered as positive results. Results in (Fig. 6) revealed that the oil nanoemulsion from cinnamon oil exhibited antifungal activity against all isolated fungal strains *Aspergillus terreus* isolate S1-EGY, *Aspergillus candidus* isolate S2-EGY and *Aspergillus flavus* isolate S3-EGY. Many literatures were considered about the use of essential oils in preservation of historical cultural. For example, cinnamaldehyde, one of the maincomponents of the Cinnamon sp. Essential oils was used against fungal strains isolated from Cuban and Argentine documentary heritage. The antifungal activities of five essential oils against yeasts isolated from the royal tomb paintings at Tanis, Egypt, had been studied.The antifungal activities of Origanumvulgare essential oil was tested against seven *Aspergillus* species colonized different cultural heritage objects in Serbia [32][33].

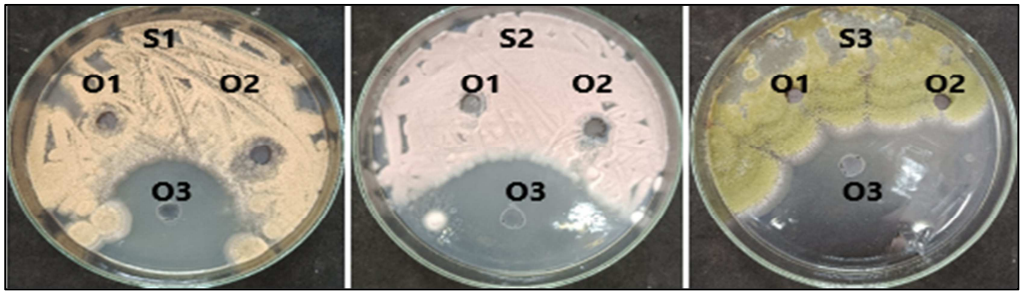


Figure 6: The antifungal activity of three prepared oil nanoemulsion against three isolated fungal strains from painted limestone rectangular funerary Stela. (01) tea oil, (02) lemon oil and (03) cinnamon oil

3.6. Assessment of the effect of cinnamon oil nanoemulsion on experimental samples

The effect of cinnamon oil nanoemulsion against the experimental samples infected with the isolated fungal strains (*Aspergillu sterreus* isolate S1-EGY, *Aspergillus candidus* isolate S2-EGY and *Aspergillus flavus* isolate S3-EGY) had been evaluated. A group of experimental samples were first sprayed with cinnamon oil nanoemulsion and infected with the three isolated fungal strains (separately) the other group was directly infected with the isolated fungal strain (separately). Both two groups were incubated at 30^{o}C and then evaluated using stereo microscope and SEM examinations. Visually a clear fungal growth was observed with non-sprayed experimental samples (Fig. 7 A, B&C, for fungal isolates S1, S2 &S3) whereas the cinnamon oil nanoemulsion-treated samples didn't exhibit any distinct fungal growth (Fig. 7 D, E &F) for fungal isolates S1, S2 &S3). SEM images of the untreated samples (Fig. 8 A, B & C) showed that the fungal hyphae and spores covered the surface and consequently degraded the samples. On the other hand, SEM images in (Fig. 8 D, E &F) showed no fungal growth and no deterioration with the three used fungal strains as they treated with cinnamon oil nanoemulsion. These results mean that cinnamon nanoemulsion prevents the growth of the inoculated microbial strains on experimental samples and protect them from microbial degradation. These results confirm that cinnamon nanoemulsion have no side effects on the samples, hence; cinnamon nanoemulsion is completely safe to be used in the treatment of painting limestone stela. [34] evaluated the biocidal activity of lemon and thyme essential oils against *Aspergillus niger*, *Fusarium solani*, *Penicillium Cyclopium*, and *Alcaligenes faecalis* inducing mural paintings deterioration, revealed that all the studied essential oils have inhibitory against fungal species. Some previous studies have mentioned that lemon essential oil has antifungal activity against many fungi isolated from cultural heritage[35].

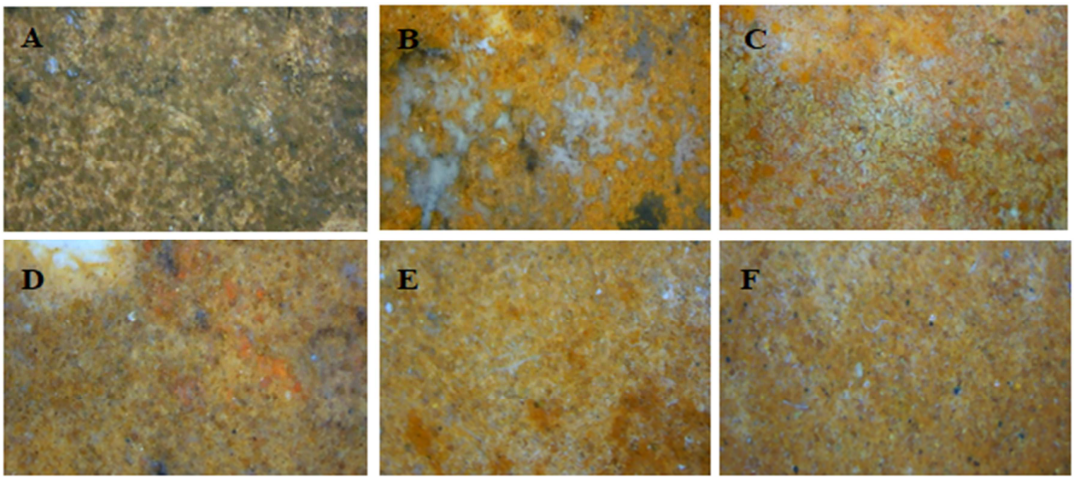


Figure 7: Stereo microscope images of samples untreated with cinnamon oil nanoemulsion (A, B &C) infected with (*Aspergillus terreus* isolate S1-EGY, *Aspergillus candidus* isolate S2-EGY and *Aspergillus flavus* isolate S3-EGY) compared to sample treated with cinnamon oil nanoemulsion prior to infection with *Aspergillus terreus* isolate S1-EGY, *Aspergillus candidus* isolate S2-EGY and *Aspergillus flavus* isolate S3-EGY (D, E & F)

3.7. Colorimetric measurements

Colorimetric studies have been established to evaluate the color change that had occurred to fungal infected samples and after treated with cinnamon nanoemlusion in comparison to native untreated samples. This is identified that the microbial covers the surface of the object and causes many physical changes, besides other chemical and mechanical changes [36]. The obtained results of the colorimetric measurements of the fungal aged control sample, and nanoemlusion sprayed samples aged with fungal contamination (Table 2) proved that no notable changes occurred due to the effect of cinnamon nanoemlusion with fungi. These results are easily recognized by naked eye, it is so clear that there is no notable difference among all treated and untreated samples.

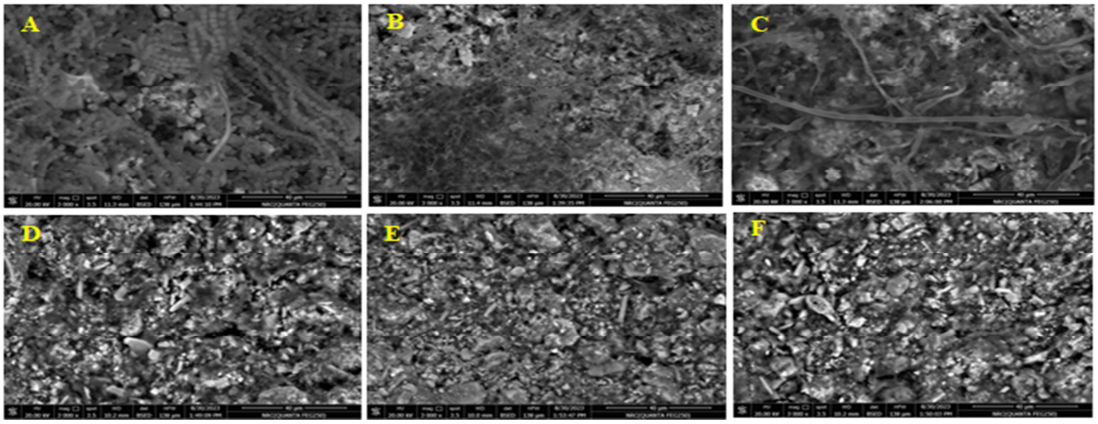


Figure 8: SEM images of samples untreated with cinnamon oil nanoemulsion (A, B &C) infected with (*Aspergillus terreus* isolate S1-EGY, *Aspergillus candidus* isolate S2-EGY and *Aspergillus flavus* isolate S3-EGY) compared to sample treated with cinnamon oil nanoemulsion prior to infection with *Aspergillus terreus* isolate S1-EGY, *Aspergillus candidus* isolate S2-EGY and *Aspergillus flavus* isolate S3-EGY (D, E & F).

Results (ΔE*) are calculated against the reference measurements (L*, a*, b*) of the yellow color area in the aged negative control and Positive control (samples spray with fungus and cinnamon nanoemlusion). ΔE for yellow color with *Aspergillus terreus*, *Aspergillus candidus* and *Aspergillus flavus* all results were calculated from the measurements L*, a*, b* for yellow color. ΔE was 1.99 (*Aspergillus terreus* + cinnamon nanoemulsion), 2.65 (*Aspergillus candidus* + cinnamon nanoemulsion) and 1.60 for (*Aspergillus flavus* + cinnamon nanoemulsion). Our results verified the usage cinnamon nanoemulsion in the disinfection of heritage buildings. It did not induce any adverse effect on the chemical characteristics of experimental samples either before or after artificial aging. The visual appearance of the surface of the conserved object should not affected by the treatment process. The total color difference (ΔE*) up to 3 units after a treatment application is generally considered unnoticeable to the human eye [37].

4. Practical Application for the Treatment of the Stela of Dedusobek

In this work, the results confirmed that cinnamon nanoemulsion has no side effects on the samples, hence; cinnamon nanoemulsion is completely safe to be used in the treatment of painting limestone stela. Thus, the spray method was used for the treatment of the spots spread all over the stela using the cinnamon nanoemulsion (Fig.9). We recommended the cinnamon nanoemulsion to be used as a great and safe alternative to the chemical compounds usually used on microbial treatments.

Table 2: Results of the colorimetric measurements

Sample name		Colour change			
		L^*	a^*	b^*	Δ E
Negative control (nanoemlusion only)	Cinnamon	50.32 50.9	24.11 26.24	33.66 34.5	------ 2.36
Positive control (fungi + cinnamon nanoemulsion)	*A.terreus*	49.38 48.38	21 21.35	31.33 29.65	------ 1.99
	A.candidus	47.43 49.26	22.96 22.07	31.76 30.07	----- 2.65
	A.flavus	49.49 48.52	21.95 20.8	33.52 32.98	------ 1.60

Figure 9: Treatment of the Stela by cinnamon nanoemulsion

5. Conclusion

This research gave vision to return back to green conservation by using natural materials in conservation of cultural heritage. The obtained results proved that cinnamon nanoemulsion was the most effective nanoemulsion among the three studied nanoemulsion as antifungal materials isolated fungal strains. Concerning the antifungal activity of cinnamon nanoemulsion on experimental samples, it is proved this nanoemulsion exhibited better results inhibiting the fungal growth. Cinnamon nanoemulsion is completely safe and has no side-effects on the experimental samples, and is recommended to be used as antifungal green conservation against fungal strains *A.terreus*, *A.candidus* and *A.flavus* infesting the painting limestone Stela. So, results ΔE were calculated from the measurements L*, a*, b* for yellow color. ΔE was 1.99 (*Aspergillus terreus* + cinnamon nanoemulsion), 2.65 (*Aspergillus candidus* + cinnamon nanoemulsion) and 1.60 for (*Aspergillus flavus* + cinnamon nanoemulsion). The total color difference (ΔE*) up to 3 units after a treatment application is generally considered unnoticeable to the human eye. The results of this study can help in preserving the cultural heritage based on the innovative results of these eco-friendly fungicides. The results proved that using EOs nanoemulsions in sterilization of cultural heritage based on painted stone is recommended.

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