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AN EXPERIMENTAL STUDY TO EVALUATE THE EFFECTIVENESS OF SOME ESSENTIAL OILS FOR INHIBITION THE MOST COMMEN TYPES OF FUNGUS INFECTED AND PERFORMANCE OF THE SAFE AND OPTIMAL SCIENTIFIC METHOD IN APPLICATION

BY

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Abstract

[EN] This Study aims to do an initial sterilization process in the excavation site after extraction of some organic artifacts from the burial environment immediately, using natural materials, volatile aromatic oils, without using any chemical pesticides that may react adversely with the materials and can be toxic to the public and harmful to the environment. This is summarized in performing an industrial laboratory aging (heat and humidity) for different samples (acacia wood and linen), then infecting them with common fungal infection, then completely inhibiting fungal growth using some aromatic oils (cinnamon and thyme) at a concentration of 1% using the evaporation method as the best technique in application which has proven effective in inhibiting fungi without causing any color change compared to spraying method.

KEYWORDS: Aging process; fungi infection; a green conservation; essential oils; spraying or evaporation technique, Colorimetric measurements.

تقييم فعالية بعض الزيوت العطرية في تثبيط أكثر أنواع الفطريات المصابة شيوعًا وأداء الطريقة العلمية الآمنة والمثلى فى التطبيق [AR]

تهدف هذه الدراسة إلى إجراء عملية تعقيم أولية ستجرى فى دراسة تطبيقية في موقع الحفر بعد استخراج بعض القطع الأثرية العضوية من بيئة الدفن مباشرة، باستخدام مواد طبيعية وزيوت عطرية متطايرة، دون استخدام أي مبيدات كيميائية قد تتفاعل سلباً مع المواد وقد تكون سامة للعامة ومضرة بالبيئة، ويتلخص ذلك في إجراء دراسة تجريبية حيث تم عمل تقادم صناعى معملي (حرارة ورطوبة) لعينات مختلفة تضاهى مادة الاثر(خشب سنط وكتان)، ثم إصابتها باصبات فطرية شائعة، ثم تثبيط نمو الفطريات تماماً باستخدام بعض الزيوت العطرية (القرفة والزعتر) بتركيز ١% باستخدام طريقة التبخر كأفضل تقنية في التطبيق والتي أثبتت فاعليتها في تثبيط الفطريات دون إحداث أي تغيير في اللون مقارنة بطريقة الرش.

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1. INTRODUCTION

At the first, the Organic excavation artifacts (such as wood, textiles, bones, ivory, horn, papyrus, mummies, etc.) are very susceptible to various deterioration agents like biological deterioration that is one of the most dangerous factors, especially with highly sensitive organic effects [1]

Fungi, specially can cause the physical deterioration of the organic artifacts by the growth of hyphal networks through the pore space system; The process involves the filamentous structures of the fungi penetrating through painting layers, the penetration also possibly being favored by turgor pressure inside the hyphae, lead to cracking, fissures, paint blisters and detachment of the paint layer [2] also ,Chemical deterioration occurs due to microbial metabolic products (including organic and in organic chemical deterioration occurs due to microbial metabolic products (including organic and inorganic acids and mycotoxins) and extracellular enzymes, which react chemically with variety components of organic artifacts leading to mineral dissolution such as pigments layers " Metal oxides" and structural changes in the other components [3] Moreover, theses fungi can release spores, hypha fragments, metabolites, toxins and allergens into the aerosol of the indoor environment of the cultural heritage, affecting human health at workplaces and causing serious respiratory infections, such as bronchial irritation and allergies [4]

And due to the long-term toxicity of chemical insecticides, we need to select a new highly environmentally friendly pesticide as a green conservation. Natural products are an excellent alternative to synthetic pesticides as a mean to reduce negative impacts to human health and the environment[5] . Volatile oils have attracted attention in recent years as a pest control agent due to their insecticidal effect

[1] G. Abd–Maksoud, E.A. Al-Shazly, A. El-Amin, Damage caused by insects during the mummification process: an experimental study, Archaeological and Anthropological Sciences, 3(3), 2011, p. 291–308.

[2] Di Carlo E., "Fungi and Bacteria in Indoor Cultural Heritage Environments: Microbial-related Risks for Artworks and Human Health," Environ. Ecol. Res., vol. 4, no. 5,(2016), p. 257–264.

[3] Hayet S., Sujan K. M., Mustari A., Miah M. A., "Hemato-biochemical profile of turkey birds selected from Sherpur district of Bangladesh," Int. J. Adv. Res. Biol. Sci, vol. 8, no. 6, (2021), p. 1-5.

[4] Elsayed Y., Shabana Y., "The effect of some essential oils on Aspergillus niger and Alternaria alternata infestation in archaeological oil paintings," Mediterr. Archaeol. Archaeom., vol. 18, no. 3, (2018), p. 71-87.

[5] Rodríguez-Gonzàlez, Á. , Garcia, S.Á. , López, Ó.G. , Silva, F.D. and Casquero, P.A. Insecticidal properties o ocimum basilicum and cymbopogon winterianus against Acanthoscelides obtectus, Insect pest of the common bean (phaseolusgaris, L.) (2019) , p. 1-14.

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Therefore, it is necessary to rapid intervention to sterilize some of these organic materials extracted at the excavation site before transferring it to museums with the least costs and available methods using natural materials in specific concentration 1% that have been proven in effective in inhibiting common fungi in extracted artifacts due to the relative humidity factor, which ranges between 30-40% for Fungi grow in such conditions with relatively good ventilation[6] Taking into consideration that the evaporation method has not been proven to cause any color change compared to the spraying method[7]

2.Materials and Methods:

2.1. Wooden and Linen samples

2.1.1Wood sample: A piece of acacia wood measuring 50 cm x 28 cm, showing the three parts of the wood (heartwood, sapwood, and park layer) as in(figure 1).

Fig.1, Shows the three parts of wood (heart wood - sap wood - park layer)

The wood species was identified by examination with a ZEISS optical microscope, and By examining the fibers, tracheids and vessels and comparing them with standard samples, it was identified and confirmed that they were made of acacia wood, which is a hard wood as in(figure 2).

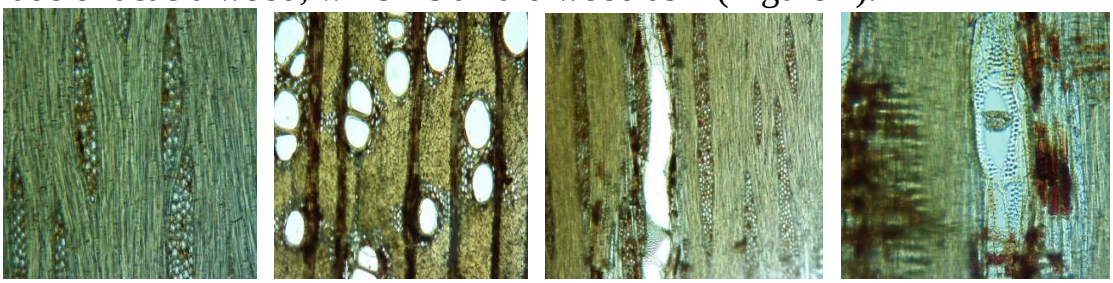

Fig. 2. examining the fibers, tracheids and vessels to identify the type of wood

As for the standard specifications for aging: Since there is no standard specification for the thermal aging of wood, the known standard specifications for paper and boxes made from wood pulp have been proposed due to the

[6] Plenderleith, H.G.and Werner, A.E.: "The conservation of antiquities and works of art: treatment, repair, and restoration" , Oxford, University press, London, (1971), p.12.

[7] Abd El-Aziz, M.F. and El-Sayed, Y.A. Toxicity and biochemical efficacy of six essential oils against Tribolium confusum (du val) (Coleoptera: Tenebrionidae), Egypt. Acad. J. biolog. Sci. (2009), p: 1 – 11.

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similarity in the chemical compounds between them (temperature 80°C and relative humidity 65%)[8]

The aging process was carried out for 72 continuous hours day and night (3 days), which is equivalent to 25 years under normal conditions. It was exposed to a temperature of 100°C and a relative humidity of 80% [9] , Then the internal moisture content was measured and compared to the internal moisture content after thermal and humidity aging.

After that the internal moisture content was measured before the thermal and moisture laboratory aging process.as in table 1

Sample	Wood sample before aging	Wood sample after aging
Wood Sample No.1	13%	11.3%
Wood Sample No.2	14%	12.6%
Wood Sample No.3	13.5%	11.6%
Wood Sample No.4	12.3%	11.0%
Wood Sample No.5	12%	10%
Wood Sample No.6	13.4%	10.7%

Table 1: show measure of moisture content of wood shambles before and after aging .

2.1.2. The linen sample: It is a piece of linen It was cut into 12 samples, as shown in the figure 3 . each sample is 5 cm * 5 cm. The internal moisture content was measured before the thermal and moisture laboratory aging process.as in table 2

The pieces were exposed to a temperature of 140°C and a relative humidity of 90% inside a monitoring oven for 72 hours, which is estimated to be about 200 years of aging under natural conditions, according to what was indicated by some previous studies on industrial aging processes [10].

Fig.3, Samples of linen pieces under experimental study before the artificial aging process

Samples	Moisture content	Moisture content

[8] BS6388:1996 – ISO 5630-3:1996, Paper and board – Accelerated Ageing – Part 3: Moist heat treatment at 80 degree and 65% relative humidity Paperback – http://www.iso.org – 29 Nov2022 -7:55 am.

[9] J. Evenson, P. Cox, M. Crews, " Effects of Accelerated Heat and Light Aging on Textiles Marked with Fabric Marking Pens, Textiles Specially Groub Post-Prints, (2004), p.23,32.

[10] O. Abdel-Kareem, Y. Zidan, N. Lokma, H, Ahmed," Conservation of A Rare painted Ancient Egyptian Textile Object from The Egyptian Museum in Cairo", (2008), p.9-16.

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	before aging	after aging
Linen sample No.1	11.4%	14.6%
Linen sample No. 2	11.1%	14.2%
Linen sample No. 4	10.5%	12.8%
inen sample No. 5	9.5%	13.1%
Linen sample No. 6	9.4%	13.6%
Linen sample No. 7	10.0%	13.9%

Table 2: show measure of moisture content of linen shambles before and after aging

2.1.3. Cinnamon oil: Cinnamon oil was extracted by steam distillation, where the leaves of the plant were collected and left to dry completely for several days, then placed in a steam distillation device for 4 hours. Second.

2.1.4. Thyme oil: It was also extracted from the thyme plant and was extracted by steam distillation, where the leaves of the plant were collected and left to dry completely for several days, then these ground leaves were taken and also placed in the distillation device, then the oils were stored in the refrigerator until they were used by steam , The two selected oils were extracted by the company supplying the microbiological laboratories of the Projects Sector - Ministry of Tourism and Antiquities (Delta Aromatic International Company) – in el Mohandessin - and they specialize in extracting aromatic, medicinal and food oils

2.2. Method

2.2.1. fungi infection

Microbial swabs taken from a mummy and a wooden coffin were received by the Microbiology Laboratory - Projects Sector of the Ministry of Tourism and Antiquities. They were taken by the researcher as in(figure 4) in order to determine the types of microbial load on them, and then to complete work on the isolated fungi from them to determine the possibility of using the essential oils under experimental study (cinnamon and thyme) as antifungals and the most appropriate concentration to completely inhibit the growth of these fungi, and to reach the optimal method for applying these oils to prevent any color change in the material of the artifact later

Fig.4, show the places where swabs were taken from a mummy immediately after its discovery and a coffin in the restoration laboratory of the Museum of Civilization to identify different types of fungi

2.2.2 identification of fungi

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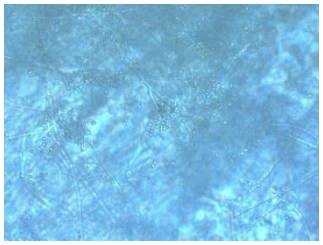
After the incubation period was over, the growths that appeared in the dishes were taken and a purification process was carried out for them to complete the laboratory experiments. The purified organisms were identified, as they were grown on nutritional media as in(Figure 5)

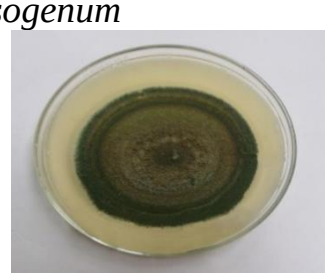
Fig.5, Isolated fungi are shown after purification in Petri dishes.

specifically for identification, and microbial slides were made from them to know the morphological characteristics and compare them with the standard morphological characteristics found in books and scientific references [11] specialized in identifying microorganisms as in (Figure 6). The results were as follows:

1	*Penecillium chrysogenum* *Penecillium regulosum*



Penecillium chrysogenum



Penecillium regulosum

Fig.6. show Microorganisms and their identification after cultivation in food environments

[11] Domsch KH., Gams , W. and Anderson T.H. Compendium of soil fungi. Academic press. Landon , Vols1-2, 1980.
Gilman L.C. A manual for soil fungi Indian edition arrangement with the original American publishers Iowa state uni. Press U.S.A, 1969.

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2.2.3 Dilution of the two selected essential oils under experimental study

First, it is difficult to mix these oils with water because they are poorly soluble in water, so it was necessary to use organic solvents to dilute them. Opinions differed among researchers about the best organic solvents, whether ethanol, alcohol, toluene, or dimethylformamide [12]

The effectiveness of 70% ethyl alcohol, toluene and dimethylformamide was tested and the following was observed: When using dimethylformamide, the diluted oil changed to a dark color, while toluene separated from the oil and did not merge. However, when using 70% ethyl alcohol, it was observed that it did not affect the color of cinnamon, thyme or lemon oil and merged completely. Based on the results of the experiment, it was decided to use 70% ethyl alcohol [13]

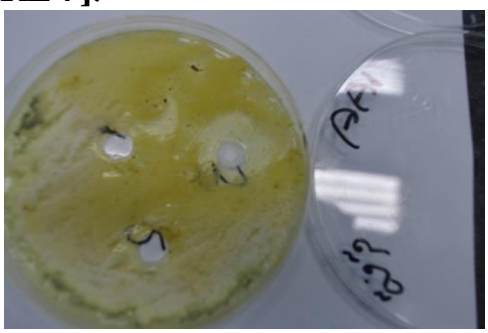
2.3.4 Laboratory tests to determine the appropriate inhibitory concentration for isolated fungi with the lowest oil concentration

Different concentrations were performed and tested on previously isolated fungi and the results were as follows as in table:-

1- cinnamon oil :

	10%	**1%**	**0.1%**
Penicillium chrysogenum	-	-	+
Penecillium regulosum	-	-	+

The results show that a 1% concentration of cinnamon oil mixed with ethyl alcohol is effective against the isolated fungi, as it is clear that a 1% concentration appeared to be more extensive in terms of the diameter of the area of fungal growth absence, which caused complete inhibition of fungal growth **[FIGURE 7]**.



Penecillium regulosum

Penecillum chrysogenum

2- thyme oil:

[12] Goudjil, M.B. & et.al " Biological Activities of Essential Oils Extracted from Thymus Capitatus " (Lamia cease), South Africa Journal of Botany Vol .128, p.5-12, 2020.

[13] Maisa Dehoum, "A Study of the Use of Natural Oils in the Treatment of Fungal Infestation of Murals, Applied to a Selected Model," PhD Thesis, Department of Restoration, Faculty of Archaeology, Fayoum University, p.113,114, 2021.

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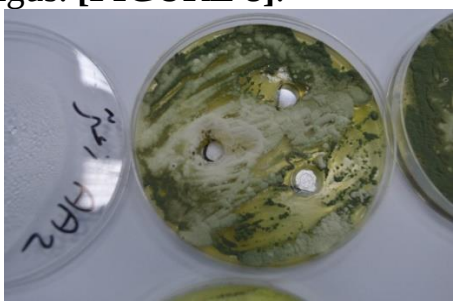
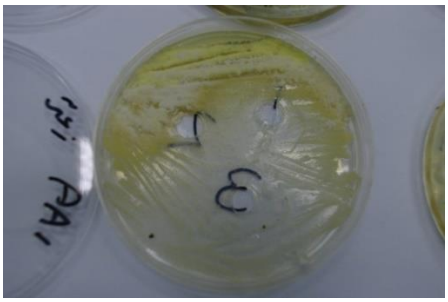
	10%	**1%**	**0.1%**
Penicillium chrysogenum	+	+	-
Penecillium regulosum	-	-	-

The results showed that a concentration of 1% of thyme oil mixed with ethyl alcohol is effective against the isolated fungi, while the other concentrations did not have any noticeable effect on the growth of the fungus, as it is noted that the average diameter of the inhibition growth zone is less than the diameter of the other oils, as shown in Figure, which caused complete inhibition of the growth of the fungus. **[FIGURE 8]**.



Penecillum chrysogenum



Penecillium regulosum

2.4. Infection stage for the selected samples under study (linen and wood)

After completing the laboratory study of essential oils in petri dishes and evaluating them to reach the best effective concentrations in resisting known fungi

The same fungi used in the previous experiment were used, which are the most widespread in organic discoveries or in the swabs taken from the extracted traces, namely Penicillium

chrysogenum and Penecillium regulosum

A suspension of the fungal spores under test was made using the nutritional medium of yeast extract (1 liter of water + 10 grams of yeast extract) and sterilized in the sterilization device (Autoclave) and then left to cool

Then those models or selected samples were inoculated with the suspension of fungal spores under study without treating them with the natural oils under study and inoculated with spores as shown in **[FIGURE 9]**.

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[FIGURE 9]: shows the stage of infection with fungal spots as a result of infection of the selected samples after inoculation with fungal spores

2.5. Exposure The fungal-infected samples to different concentrations of the selected oils and determination the optimum concentration for complete inhibition

The most effective concentration was used based on the laboratory experiment in Petri dishes, which is the 1% concentration of the two selected oils under study (cinnamon oil and thyme oil), based on the results of the microbiology laboratory affiliated with the Center for Research and Conservation of Antiquities.

All samples were preserved inside a polyethylene tent at room temperature 28 + -2 C for seven days [14], with the samples being monitored periodically during this period of time, during which the two selected oils were able to prevent the growth of the tested fungi using the spraying method as shown in [FIGURE 10]. Also these selected volatiles were applied on cotton pieces then tightly closed with something like

a polyethylene tent to prevent any leakage as shown in the picture [FIGURE 11].

[FIGURE 11] :The method of applying the essential oil

[14] Pandey, D. □., Tripathi, N. N., Tripathi, □. D. & Dixit, S. N. Fungitoxic and phytotoxic properties of the essential oil of Hypti s suaveolens. J. Plant. Dis. Protect. (1982).p. 344–349

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[FIGURE 10]: Shows method of applying the (using the fumigation technique) essential oils using the spray technique

3. Colorimetric Measurements

The aim of the color change scale test is to determine the percentage of change in color before and after treatment with the selected oils under study to evaluate the efficiency of the oils in terms of color change degrees after treatment and also to know the best method of application, whether by spraying or evaporation, so as not to produce any discolored spots on the artifacts later.

The color change was measured using the CLE system (L,a,b), which is an international system for measuring color change. The degree of brightness is symbolized on the device by the symbol L, as in [**figure 12**]

[**figure 12**]: The equipment used to measure the color change of experimental samples

Its value ranges from zero to one hundred (0:100), where 100 indicates pure white, zero indicates deep black, while the symbol a represents the colors red and green, and the symbol b represents the colors yellow and blue. The color difference between two samples is distinguished by the symbol Delta ▲, and the total color difference is symbolized by the symbol E▲

Four readings were recorded: -

Two readings related to cinnamon oil and thyme oil (by evaporation) as the results shown in [TABLE 1]

And two readings related to cinnamon oil and thyme oil (by spraying) as the results shown in [TABLE 2]

	symbol	L*	A*	b*	E▲
Readings before treatment with cinnamon oil	Linen sample	65.11	2.83	19.4	**0 . 61**
Readings after treatment Cinnamon oil	Linen sample	**64.53**	**2.89**	**18.73**	

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Readings before treatment with cinnamon oil	Wood sample	38,7	6,35	16,4	**0. 38**
Readings after treatment with cinnamon oil	Wood sample	**38.81**	**6.63**	**16.96**	
Readings before treatment with thyme oil	Linen sample	67	3,24	18,81	**0.77**
Readings after treatment with thyme oil	Linen sample	**66.10**	**3.37**	**19.22**	
Readings before treatment with thyme oil	Wood Sample	37,96	8,18	17,01	**1.23**
Readings after treatment with thyme oil	Wood Sample	39.20	8.84	17.55	

[TABLE 1] :shows the average readings before and after treatment with the two selected oils at a concentration of 1% (evaporation method)

	symbol	L*	A*	b*	E▲
Readings before treatment with cinnamon oil	Linen sample	63.83	2.45	18.30	**2.50**
Readings after treatment Cinnamon oil	Linen sample	**66.88**	**2.42**	**18.20**	
Readings before treatment with cinnamon oil	Wood sample	34.56	6,29	14.46	**2. 07**
Readings after treatment Cinnamon oil	Wood sample	**36.04**	**7.28**	**13.10**	
Readings before treatment with thyme oil	Linen sample	65.66	2.49	16.52	**3.94**
Readings after	Linen sample	**70.46**	**1.98**	**15.29**	

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treatment with thyme oil					
Readings after treatment with thyme oil	Wood sample	39.47	8,37	17,05	**2.85**
Readings after treatment with thyme oil	Wood sample	**36.48**	**7.81**	**14.95**	

[TABLE 2] :shows the average readings before and after treatment with the two selected oils at a concentration of 1% (spray method)

From the following results in the color change values ▲E, it is clear that the evaporation method used in applying the selected oils is the optimal and better method than application by spraying, whereas the results shown in the color change values for the evaporation method are less and did not exceed 1.23, while the results of the spraying method showed higher values than that, reaching 3.94

So ,in the researcher's opinion represents a slight color change, even if it is not apparent to the naked eye, especially on the trace material.

Therefore, the evaporation method used in treatment with aromatic oils is the optimal and better method than the application by the spraying method.

The following table **[TABLE 3]** shows the images that illustrate the amount of color difference through one of the programs dedicated to processing color change, Color Math, on the selected samples before and after treatment with cinnamon and thyme oil at a concentration of 1% (Evaporation technique), whereas the color change is very slight and completely invisible to the eye

Color difference of samples before and after treatment with the two selected oils (evaporation method)	**Samples**
	linen sample Cinnamon oil)evaporation method(Linen sample Thyme oil)evaporation method(

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Color difference of samples before and after treatment with the two selected oils (evaporation method)	**Samples**
	Wood sample Cinnamon oil)evaporation method(
	Wood sample Thyme oil)evaporation method(

The following table **[TABLE 3]** shows the images that illustrate the amount of color difference through one of the color change processing programs Color Math on the selected samples before and after treatment with cinnamon and thyme oil at a concentration of 1% (by spraying method), where the relative color change is shown.

Color difference of samples before and after treatment with the two selected oils under study (spray method)	**Samples**

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 	Linen sample Cinnamon oil (spray method) linen sample Thyme oil (Spray method)

Color difference of samples before and after treatment with the two selected oils (spray method)	**Samples**
 	Wood sample Cinnamon oil (spray method) Wood sample Thyme oil (spray method)

4. CONCLUSION

Based on the previous results, the following was confirmed:

1 -4.1. The efficiency of the two selected oils under experimental study (cinnamon and thyme) was determined at a concentration of 1% in completely inhibiting the fungus and preventing the appearance of any other fungal growth. A lower concentration was recorded after completing the periodic examination

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for a full week according to the reports of the microbiological laboratories of the Research and Conservation Center of Antiquities in the Projects Sector of the Ministry of Tourism and Antiquities.

4.2. Through visual examination and color change value test conducted by the restoration laboratories of the Faculty of Archaeology, Cairo University, it is clear that the method of applying the vaporization technique is better and safer than the Brazilian application of the spraying technique, after following up on the selected samples under experimental study and treating them with the two essential oils at a concentration of 1% under study (cinnamon and thyme) and recording the results. The highest color change percentage E▲ was recorded by Brazilian vaporization at 1.23, while the results of the spraying method showed a higher value than that, reaching 3.94, which represents in the researcher's opinion a slight color change, even if it is not apparent to the naked eye, especially on the material of the artifact, although all the results shown did not exceed five $E \leq 5$▲, which is consistent so far in the color change tests.

ACKNOWLEDGMENT: The authors of this paper are thankful to the Microbiology laboratory following the Research and Conservation Center of Antiquities in the Projects Sector of the Ministry of Tourism and Antiquities for helping and providing necessary and benefits information for this research work.

NOTES: There is another research talking about Initial sterilization of some of the mostly organic artifacts at the excavation site immediately after extraction using one of the essential oils by following the fumigation method inside tightly sealed tents before transporting them to museums or stores as a case study.

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