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AN INVESTIGATION OF THE BIOLOGICAL FUNGICIDAL ACTIVITY OF SOME ESSENTIAL OILS USED AS PRESERVATIVES FOR A 19TH CENTURY EGYPTIAN COPTIC CELLULOSIC MANUSCRIPT

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Abstract

The main goal of this work was to investigate the biological fungicidal activity of some commercial essential oils of tea tree, lavender and thyme, which were to be applied as alternative preservatives for ancient manuscripts. To achieve our goal, model samples of cellulosic paper were made to mimic the original manuscript, which was a Coptic manuscript known as Pascha (the sacred week), dated 1812. Twenty-three microorganism strains were isolated representing twelve fungal taxa and one bacterial taxa which were identified in all collected and analyzed in samples, which included Trichoderma viride, Penicillium roqueforti, Eurotium chevalieri, Aspergillus flavus and Bacillus subtilis. A scanning electron microscope (SEM) was used to investigate the growth of the associated microorganisms and their effect on the sample paper structure. Different concentrations of the aforementioned essential oils were applied on the mimic samples, which were then subjected to accelerated ageing corresponding to 25 or 50 years of a natural one. To characterize the applied oils on the samples, we made records by using FTIR-ATR, color measurements according to CIELAB system, and analyzed the mechanical properties of the tested samples. The results revealed that the samples treated with either tea tree oil or lavender oil, had ΔE values that decreased as the oil concentration increased. However, when samples were treated with thyme oil the reverse was obtained. For the treated samples exposed to 25 years of light ageing, we noticed that the higher obtained tensile strength and % elongation of treated samples followed the ranking order: thyme > lavender > tea tree oils. For the treated samples that were exposed to 50 years of natural light ageing, we observed that almost all tensile strength and elongation values of the treated samples were higher than that of the untreated ones. Moreover, we noticed that the inhibition of growth of the microorganisms was obtained at a low concentration of tea tree oil (0.25% v/v). This treatment was esthetically acceptable for archaeological objects, because it was colorless, transparent and safe. Based on the results we obtained, the optimized essential oil, which is the oil with an appropriate concentration, was selected to be added to the cellulosic pulp used for the leaf casting. Moreover, the same optimized essential oil was applied on the paper samples to be used as separators between the ancient manuscript pages. After dismantling, cleaning, leaf casting and rebinding of the damaged parts, the manuscript is then preserved.

Keywords: *Essential oil; fungicidal; Coptic manuscript; leaf casting; binding; conservation.*

Introduction

The contents of libraries, museums, archives and other documentation centers are the priceless heritage of mankind. Not only in the context of ancient lore, but also in the context of

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the medieval and modern age, manuscripts are considered as the most important source of authenticity. Manuscripts constitute our most precious national and cultural heritage. Thus, the preservation of manuscripts is a serious problem for custodians throughout the world and this is why every possible effort must be taken to save these treasures for the future generation.

Being organic in nature, cellulosic manuscripts are susceptible to decay and disintegration over time. Normally, chemical treatments are performed by using fumigation chambers, to protect them from the attacks of bacteria, fungi and any other insects [1]. Insecticides and pesticides are useless, as the pests develop immunity over time.

Researches in conservation provide essential information to conservators and to others who care for our cultural heritage, on the causes of deterioration, on revising conservation solutions, on options for appropriate methods of treatment and on the assessment of treatment performance and they also help in understanding the composition of materials and the techniques used to create works of art. In addition, also they resolve the issues related to dating and authenticating art objects.

Essential oils are widely used as components of drugs, biologically active additives and dietary supplements, as well as in aromatherapy, the food industry and cosmetics [2]. They are so commonly used largely due to their pleasant or spicy smell. In addition, numerous recent studies reported a biological activity of essential oils: they exhibit antibacterial, fungicidal, antioxidant and antiradical properties [3]. Essential oils consist primarily of low-molecular-weight mono- and sesquiterpene hydrocarbons, their oxygen analogues and phenol derivatives [4]. The small sizes of the molecules of essential oils 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 [5-7].

This applied study deals with the process of treatment and conservation of one of the biggest and most important Coptic manuscripts, dated 1812, known as Pascha (the sacred week), which consists of 206 pages. The manuscript was in poor condition as a result of its storage in a damp basement for a long time, which caused dirt accumulation, degradation and the binding of most of the pages, alongside with the fading of the ink from the printed pages. Thus, it was our target to investigate the possibility of using the commercial essential oils of tea tree, lavender and thyme as alternative preservatives in dealing with this bad condition of the precious manuscript and to prepare it to be exhibited in a proper manner.

Materials and Methods

Isolation, Culturing and Identification

The Coptic manuscript known as Pascha (the sacred week), dated 1812 was processed for fungal and bacterial isolation by using three different types of media, i.e. M40Y, potato dextrose agar (PDA) and Czapek's media, respectively. The conventional methods of swabbing and streaking were used [8]. The surfaces of the media plates were gently streaked with cotton swabs. The Petri dishes were incubated at 25°C±2 for fungi and at 37^{o}C ± 2 for bacteria, for 7 days. The resultant cultures were purified by using the hyphal tip and/or a single spore technique, as described by Mandhar et al. [9]. Macroscopic and microscopic characteristics of the obtained isolates, as well as color, size and morphology of the vegetative and reproductive structures were examined. Fungal isolates that speculated were identified by using the taxonomic keys of Ellis and Ellis [10] and Samson et al. [11]. Moreover, representative bacterial strains were analyzed by PCR primers and 16s RNA sequencing. The identification of bacteria taxa were carried out by Sigma Company.

Colonization test

To investigate the effects of using essential oils on the growth of the isolated fungal and bacterial taxa, mimic paper samples made of rag pulp (cotton linter and unbleached linen) were

cut (20 x 20 mm) by using a scalpel, then sterilized by using UV light exposure for 48 hours, after autoclaving at 121°C for 6 hours and drying in an oven at 105°C for 24 hours [12]. For the preparation of spore suspensions, 10 ml of sterilized distilled water was added to culture plates containing PDA (7-days old) and then spores were freed with the aid of a camel brush. Paper samples were deliberately inoculated with fungal and bacterial species to study the paper samples before and after infection. The colonization was evaluated after a three months period. Degradation and color change occurred were investigated.

Essential oils application: Effects of essential oils on the growth of microorganisms

Tea tree, lavender and thyme oils were applied on the surfaces of the mimic paper samples at different oils concentrations, i.e. 0.062, 0.031, 0.75, 0.50, 0.25, 0.12 and 1%. Few drops of Tween -80 detergent were added to each oil concentration. A known oil concentration was added to the autoclaved PDA medium immediately before solidification and poured into the plates. Plates were inoculated each with a 7 mm diameter disc taken from fresh (7-10 days old) cultures of a known fungus. The inhibitory effect of the tested treatments on linear growth of four microorganisms (*Trichoderma*, *Penicillium*, *Eurotium*, *Aspergillus flavus*, and *Bacillus subtilis*) was estimated.

Scanning Electron Microscope (SEM)

The SEM model wasa FEI Quanta 200 SEM FEG, which was used to study changes in surface morphology and colonization by microorganisms of both the inoculated and non-inoculated paper samples [13].

Yellowness Index Determination

The yellowness index for all different examined rag pulp paper samples treated with different concentrations of the essential oils, were measured and evaluated according to ASTM, D 1925 by using a Color Eye 3100 Spectrophotometer SDL, England. The mean value of three measurements was recorded for each sample.

Mechanical Measurements

Tensile strength and percentage elongation at break for all the samples under test were measured and evaluated by using a Shimadzu Universal Tester of type S-500, made in Japan. The measurements were carried out according to the ASTM-Standard Method 2000, D3822-96.

Fourier Transform Infra Red Spectroscopy with Attenuation Total Reflection

FTIR-ATR spectra of the samples under test were recorded by means of a Nicolet 380 Spectrometer using a zinc selenid crystal, in the wavelength range 650- 4000 cm^{-1}. To ensure reproducible contact between the crystal face and the fabric, a pressure of approximately 18 Kpa was applied to the crystal holder. The FTIR absorbance frequencies for the treated samples were recorded with an average of 128 scans by using a resolution of 4 cm^{-1}.

Results and Discussions

Isolation of microorganisms from the Coptic Manuscript

During the present investigation, a total of twenty-three microorganism strains were isolated from different parts of the ancient manuscript, i.e. unprinted, red ink prints and black ink prints. The isolated species represent twelve fungal and one bacterial taxa in all collected samples [14-16]. The microorganism species isolated from paper substrata are listed in table 1, their SEM identification images are shown in figures 1, 2 and 3.

Colonization of microorganisms on paper

The colonization of microorganism on either the unprinted paper samples or on that printed with red or black ink after two months displayed a severe fungal infection, including *Trichoderma viride. Penicillium roqueforti, Eurotium chevalieri, Bacillus subtilis and Aspergillus flavus*. Fungal growth on the surface of the ancient Coptic manuscript led to decomposition, randomly spread all over the investigated paper samples, resulting from the damage of cellulose and hemicellulose. Tests showed microorganisms growth on both sides of

the paper samples and between the fibers. That was generally observed only after an extended period of incubation and could be detected by scanning electron microscope (SEM). Each species had hyphal characteristics, either myofibrils or bacterial lytic holes. The species with myofibrils were detected on the top of the ink text, while the hyphae with bacterial lytic holes was observed beneath the ink, between paper fibers [17].

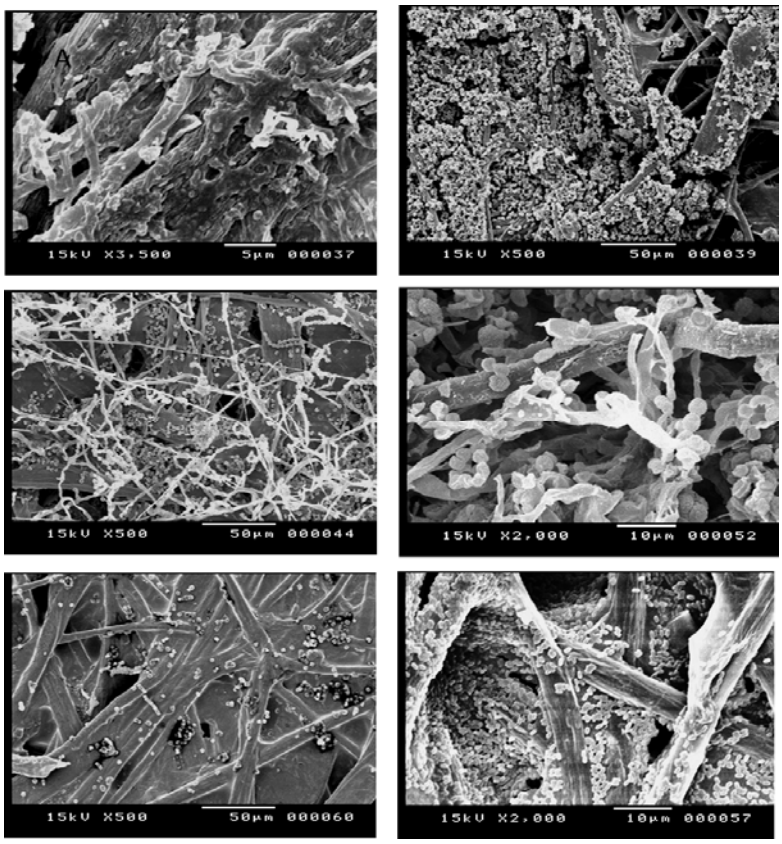

Fig. 1. The identified fungal taxa from the ruined paper of the ancient artifact: (A) Control (no treatment), (B) *Trichoderma viride*. (C) *Penicillium roqueforti*, (D) *Eurotium chevalieri*,(E) -*Bacillus subtilis* (F) *Aspergillus flavus*

Table 1. The isolated and identified fungal and bacteria strains from the ancient Coptic manuscript.

Microorganism location in the manuscript	The different media used in isolation		
	M40Y	**PDA**	**Czapek's**
Unprinted paper	Penicillium roquefortii Aspergillus candidus	*Bacillus subtilis* *Byssochlamys nivea* *Eurotium amstelodami* *Aspergillus humicola* *Aspergillus tamarii*	*Penicillium roquefortii* *Bacillus subtilis* *Aspergillus flavus*
Black ink on the paper	Eurotium amstelodami Aspergillus niger	*Penicillium roqueforti* *Bacillus subtilis* *Alternaria* sp. *Eurotium chevalieri* *Aspergillus flavus* *Trichoderma viride*	*Bacillus subtilis* *Aspergillus flavus* *Penicillium roqueforti*
Red ink on the paper	Penicillium roqueforti Eurotium amstelodami	*Trichoderma viride* *Bacillus subtilis* *Aspergillus fumigatus* *Aspergillus flavus*	*Bacillus subtilis* *Aspergillus fumigatus* *Aspergillus flavus*

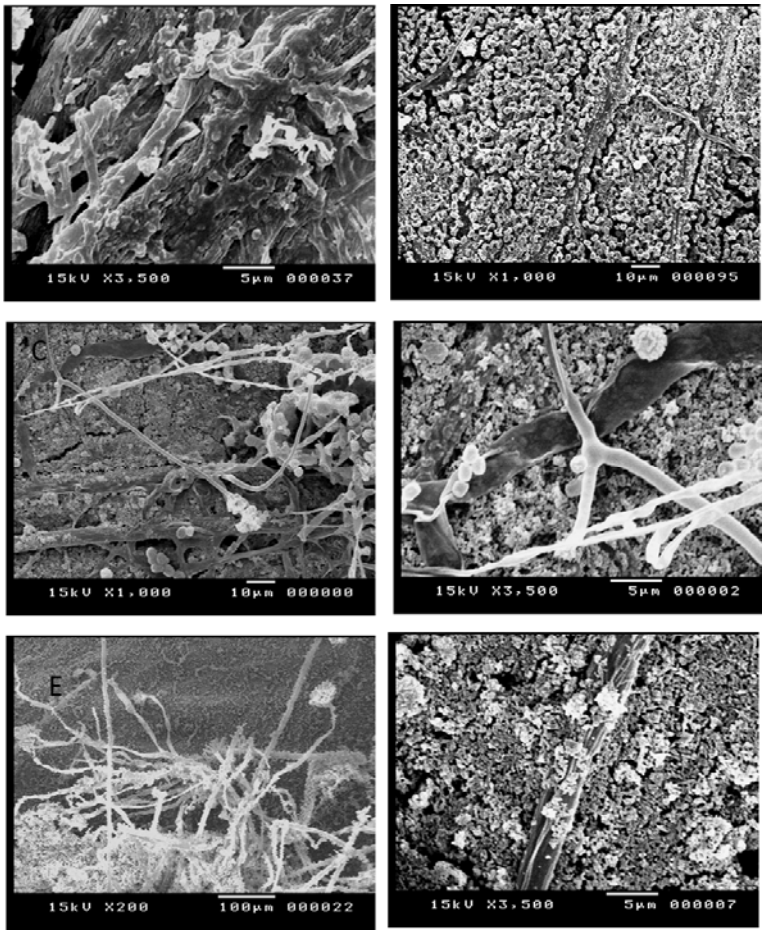

Fig. 2. The identified fungal taxa from the remains of the black ink printed pages of the ancient book: (A) Control (no treatment), (B) *Trichoderma viride*, (C) *Penicillium roqueforti*, (D) *Eurotium chevalieri*,(E) -*Bacillus subtilis* (F) *Aspergillus flavus*

The microorganism species on the paper artifact were identified by using both optical microscopy and SEM, based on the distinctive sizes and shapes of fungal spores and hyphae through the stain gram for bacteria. P. variotii was obtained following inoculation of sterile slabs with individual strains (Fig. 1A). It was observed that, the growth of Trichoderma viride covered the whole surface and was between the fibers of all tested samples, i.e. unprinted parts, red or black ink printed areas of the paper samples. Moreover, the conidiophores and conidia, from top to bottom, were bearing dense and vertically arranged branches carrying phialides. It was noted that Chlamydospores were present in single or in chains. *C. lunata* (Fig 1B), was in matured colonies which appeared dark brown to black. Also, Conidiophores arising from surface or aerial hyphae, septate, straight or flexuous conidia, that are relatively short, curved and smooth branching patterns intermingled with spore-forming structures. Regarding Fig 1C, it is clear that Conidiophores and spores were present. C. cladosporioides showed small sized simple or branched loose chained spores with lemon-shape. Conidiophore formed many aerial hyphae (Fig 1D), Eurotium chevalievi Conidiophores; vesicles globose to subglobose, conidial heads radiated. Conidiogenous cells arised directly from the vesicles. Also, the SEM images showed that, Conidia Ovate was broadly elliptical with ends often flattened, lobate-reticulate, produced in persistent chains. Bacillus subtilis (Fig 1E) a virgate, Gram-positive endospore

formed into the strep to bacillus form, in a rod shape. Aspergillus flavus (Fig 1F) showed conidial heads that were typically radiate, later splitting, to form loose columns. Conidiophores were hyaline and coarse. Conidia were globose to subglobose, pale green and conspicuously echinulate.

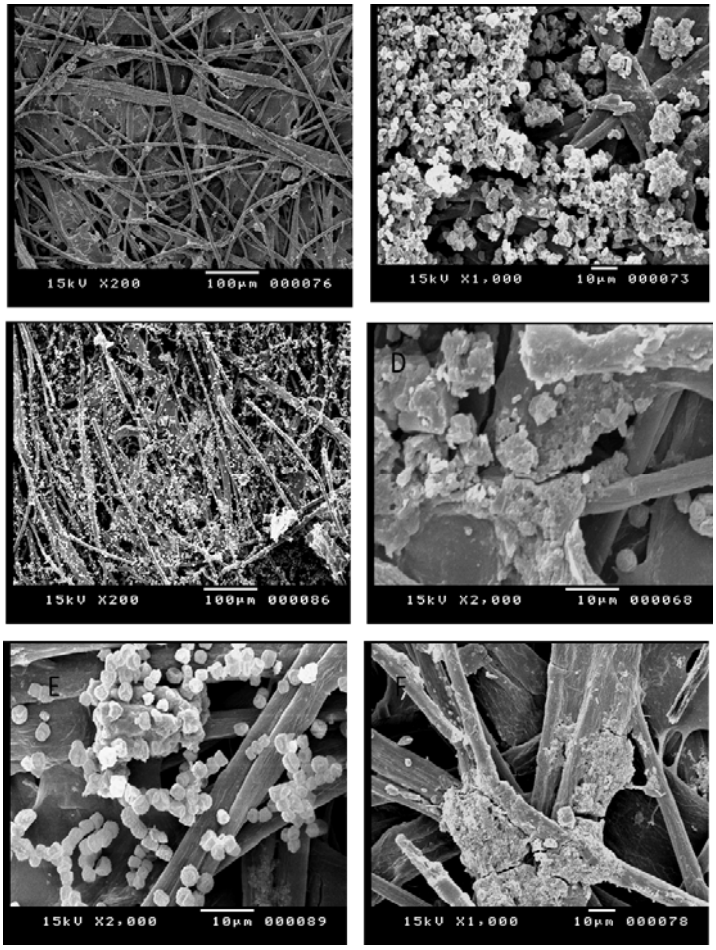

Fig. 3. The identified fungal taxa from the remains of the red ink printed pages of the ancient book: (A) Control (no treatment), (B) *Trichoderma viride*, (C) *Penicillium roqueforti*, (D) *Eurotium chevalieri*, (E) -*Bacillus subtilis* (F) *Aspergillus flavus*

Using essential oil on the fungal linear growth

Essential oils were also evaluated in the laboratory for their inhibition of mold growth on isolated microorganisms by one method. The analysis of the obtained results revealed that, Tea tree oil, at low concentration (0.25%v/v) was the most effective in inhibiting the growth of all fungal and bacterial isolates, followed by Lavender oil, which inhibited the growth of *Trichoderma viride* and Eurotium chevalieri at low concentration (0.25%v/v)and also *penicillium roqueforti* and *Aspergillus flavus Bacillus subtilis* at a concentration of 0.5%v/v. Finally , the least effective was *Thymus* oil, which inhibited the growth of *Trichoderma viride , Eurotium chevalieri, penicillium roqueforti*and and *Aspergillus flavus* at a concentration of 0.5%v/vand that of *Bacillus subtilis* at a concentration of0.1%v/v.

Table 2. The effect of different concentrations of the studied essential oils on the linear growth of the tested microorganisms

Conc. (%)	Tea tree oil						Lavender oil						Thymus oil					
	Aspergillus flavus	*Paeciomyces lacinus*	*Eurotium chevalieri*	*Trichoderma viride*	*Bacillus subtilis*		*Aspergillus flavus*	*Paeciomyces lilacinus*	*Eurotium chevalieri*	*Trichoderma viride*	*Bacillus subtilis*		*Aspergillus flavus*	*Aspergillus lilacinus*	*Paeciomyces chevalieri*	*Eurotium viride*	*Trichoderma subtilis*	*Bacillus*
Control	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90
0.031	41	36	20	90	***	48	29	23	90	***	52	45	67	90	***	41	36	20
0.063	34	32	18	87	**	36	23	14	76	***	40	31	32	82	***	34	32	18
0.125	26	20	14	42	*	22	18	11	32	**	32	22	14	40	**	26	20	14
0.25	0	0	0	0	0	18	12	0	0	*	25	17	9	10	**	0	0	0
0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*= slight growth; **= moderate growth; ***= good growth and ****= dense growth

The effect of the oil concentrations on the performance properties of the paper samples

Color parameters

The change in the yellowness and whiteness index can be considered as a sensitive indication of surface modification for the natural fabrics under test, during the different treatments [18]. Table (3) shows the effects of essential oils concentration on the color components L*, a* and b* values, the total color difference (**ΔE**), the yellowness index (**Y**) and whiteness index (**W**) of untreated and treated paper samples. The color difference values were calculated as the difference between the untreated samples (blank) and the treated ones. As shown in the results listed in table 3, adding EO into the tested paper sheets significantly affected the **L*** (lightness/darkness), ***a**** (redness/greenness) and ***b**** (yellowness/blueness) values of the samples surface. On adding tea tree and lavender oils to the paper, we found that the samples became lighter (higher L values) while the **a*** and **b*** components decreased on increasing the oil concentration. On the other hand, the whiteness index **W** obtained with both of the oils increased, while the yellowness index **Y** decreased, but still these parameters were lower than those of the untreated samples. This means that the whiteness index is biased in the blue-yellow dimension, such that higher whiteness index values are obtained if the treated material is lighter or slightly bluer than the untreated one. In the case of treating samples with thyme oil, it was clear from the results that the samples showed a remarkable decrease in the lightness L and whiteness **W** values, accompanied by an increase in the yellowness **Y**, **a** and **b** values.

In regard to the color difference parameter, it was noted that in case of samples treated with either tea tree oil or lavender oil, the **ΔE** values decreased as the oil concentration was raised, while in the case of using thyme oil the reverse was obtained, i.e. increasing **ΔE** with an increase in oil concentration.

Table 3. The color parameters of the untreated and treated samples

Essential Oil (EO)	*E.O. Conc.*	*L**	*a**	*b**	*ΔE*	*W*	*Y*
	Blank	93.00	-0.08	3.39	--------	67.25	6.90
Tea tree	0.125	91.04	0.12	7.92	4.79	42.25	15.78
	0.25	91.24	0.07	7.38	4.39	44.27	15.03
	0.5	92.45	0.04	6.49	3.25	50.43	13.00
Lavender	0.125	92.09	-0.35	5.66	2.46	54.43	11.09
	0.25	93.30	-0.17	5.59	2.30	57.47	10.75
	0.5	93.58	-0.04	4.80	1.44	61.45	9.50
Thyme	0.125	89.41	0.74	11.38	8.93	19.81	23.14
	0.25	89.17	0.81	11.88	9.25	18.17	23.88
	0.5	88.12	0.95	12.26	10.15	13.07	25.03

Mechanical properties

Tensile strength is indicative of fiber strength, fiber bonding and fiber length. Fiber length and coarseness also influence the tensile strength of paper [19, 20]. The tensile strength of paper is the maximum force per unit width that a paper strip can resist before tearing when applying the load in the direction parallel to the length of a strip.

The change in the mechanical properties of the treated paper samples using three different EO with different concentrations reflects not only the changes in the chemical structure of the polymer system but also in its morphology [21]. The data in table (4) clarify the effect of the different concentrations of tea tree, lavender and thyme oils on the mechanical properties of the paper samples aged by artificial light, corresponding to 25 and 50 years storage.

For the treated and untreated samples exposed to 25 years of natural light ageing, we noticed that almost all the treated samples showed an increase in their tensile strength and elongation than their corresponding untreated samples. Moreover, increasing the oil concentration leads to gradual decrease in both tensile strength and elongation of all tested samples, but still higher than that of the untreated ones. The higher obtained tensile strength and elongation of treated samples followed the ranking order: thyme > lavender > tee tree oils.

The prevalent deterioration reaction responsible for the natural and accelerated ageing of paper and cellulose is the acid hydrolysis of the glycosidic bonds between the glucose moieties of the cellulose macromolecules [22]. Their results indicated that the loss of strength and the developed brittleness were due to the loss of fiber strength, resulting from the acid-catalyzed hydrolysis of cellulose and not bond strength loss.

Regarding to the untreated and treated samples exposed to 50 years of natural light ageing, it is obvious that, samples treated with either tea tree oil or thyme oil showed gradual increase in both tensile strength and elongation on increasing the oil concentration. While those samples treated with lavender oil suffered from decrease in tensile strength and gradual increase in the elongation on increasing the lavender oil concentration. Also, it is clear that almost all tensile strength and elongation values of the treated samples are higher than that of the untreated ones.

Many scientists have correlated the resulting alteration in mechanical properties of cellulose with the lignin, a remnant of the raw plant material in paper, which was also believed to considerably contribute to the deterioration of paper, was proved to exert a protective influence on cellulose due to its antioxidant properties.

Table 4. Tensile strength and elongation values of the untreated and treated samples after 25 and 50 years exposure.

	Essential oil	E.O. Conc. (v/v%)	Tensile strength (N)	Elongation (%)
25 years ageing		Blank	10.04	0.934
	Tea tree	0.125	11.29	1.68
		0.250	10.84	1.36
		0.50	9.98	0.90
	Lavender	0.125	13.89	1.93
		0.250	11.08	1.88
		0.50	9.13	1.66
	Thyme	0.125	19.34	2.60
		0.250	16.87	2.13
		0.50	11.79	2.03
50 years ageing		Blank	10.16	1.72
	Tea tree	0.125	6.20	1.13
		0.250	11.70	1.35
		0.50	11.96	1.60
	Lavender	0.125	14.30	1.75
		0.250	12.86	1.88
		0.50	10.79	2.20
	Thyme	0.125	13.55	1.27
		0.250	16.08	2.01
		0.50	16.41	2.12

FTIR

Fourier-Transform Infrared Spectroscopy with attenuated total reflection (FTIR-ATR) can give highlight on the chemical changes that occurred in the paper samples as a result of the different treatments using different essential oils, by evaluating the variation in peak intensity values of structural functional groups. FTIR spectra of untreated paper samples as well as paper after treating with different concentrations of three different types of essential oils: tea tree, lavender and thyme oils are illustrated in figure 4.

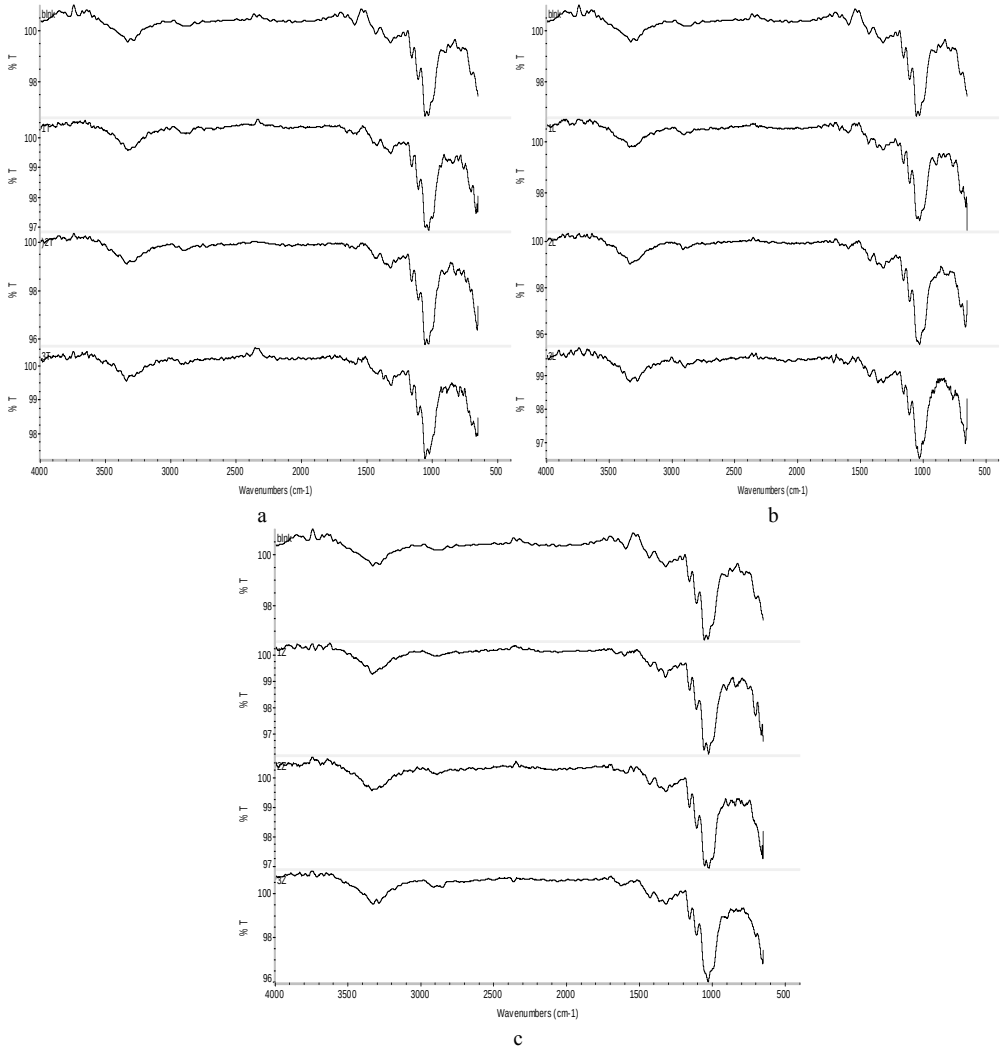

Fig. 4. FTIR spectra of untreated and treated paper samples using different concentrations of a - Tea tree oil, b - Lavender oil and c - Thyme oil

In considering the different functional groups in untreated paper sheets, the following bands were obtained : hydrogen-bonded O-H stretching at $\nu \approx 3700$ cm^{-1}, the C-H stretching at $\nu \approx 2864$ cm^{-1} , the C-H2 rocking vibration at $\nu \approx 1318$ cm^{-1} , peak around 1157 cm^{-1} related to CO-C and the OH bending at $\nu \approx 700$ cm^{-1} [23, 24].

FTIR analysis demonstrated that tea tree oil treated samples (Fig. 4a) showed certain peaks varying in their intensities due to the variation in the oil concentration; however, the peaks could be seen in the spectra of the treated samples are: aromatic ring structure C=C stretch around 1520–1515 cm^{-1}, which may be arising from certain aromatic components of each essential oil, vibrational peaks specific to methylene groups at ≈ 2850 and 2925 cm^{-1} and aromatic C–H bond at 700–750 cm^{-1} were also seen in the spectra [25].

The main composition of Lavender essential oil are linalyl acetate, linalool, lavandulol, leaf alcohol isobutyrate, and so on, which consists of molecule groups of -COOR, C=O etc. Their characteristic absorption peaks can be identified at 1665-1700 cm^{-1} due to stretching vibration absorption. The FTIR spectra of the lavender oil treated samples are shown in Figure 4b.

IR spectrum for thyme oil shown in Figure 4c, exhibits a broad band appearing at 3300 cm^{-1} assigned to the stretching vibration of (OH) group, 2860-2900 cm^{-1}due to stretching of CH3 group, band around 1430 cm^{-1}due to the C-C ring stretching. In the region 1380-1340 cm^{-1}due to O-H in-plane bending vibration, 1286 cm^{-1}due to isopropyl group region $CH(CH_3)_2$ str., a strong band 1244 cm^{-1} due to C-O stretching in phenol produce region, 800 cm^{-1} out-of-plane due to the aromatic C-H bending [26, 27].

Scientific recording, documentation and diagnostic of the manuscript

The manuscript under investigation is one of the biggest and most important Coptic manuscripts, it measured 55x35cm the text 23x33cm. Throughout the manuscript there were numerous pages with drawings and decorative designs in a variety of colored inks. The script's name was found on the latest page of the folio.

The manuscript was in poor condition as a result of storage in a damp basement for a long time which caused water staining and hydrolysis of paper also degradation due to fluctuating temperatures and humidity levels occurred while the books remained in storage. Severely mold damage of the leather cover and the folios was also observed (Fig. 5).

Fig. 5. Mold damage of the leather cover and the folios of the manuscript

Moreover, the glue sizing has penetrated throughout the stacks of papers thus, acting as binding agent between the pages. The pages were stuck together in lumps form and had the appearance of separate hard, solid mass resembling blocks as seen in Figure 6.

The paper was exceptionally dirty, with water stains and in-ground surface dirt particularly around the edges where the book was handled. The pages had prominent water stains around the edges.

In many cases, the ink had become faint or had been washed away entirely. The pages were badly torn and damaged, especially where they were handled at the fore edge and in the lower right corner. The leather cover had completely separated from the text block and the

boards were warped, misshapen and soft. The leather cover had stains that resulted from prolong exposure to water. The leather had completely deteriorated.

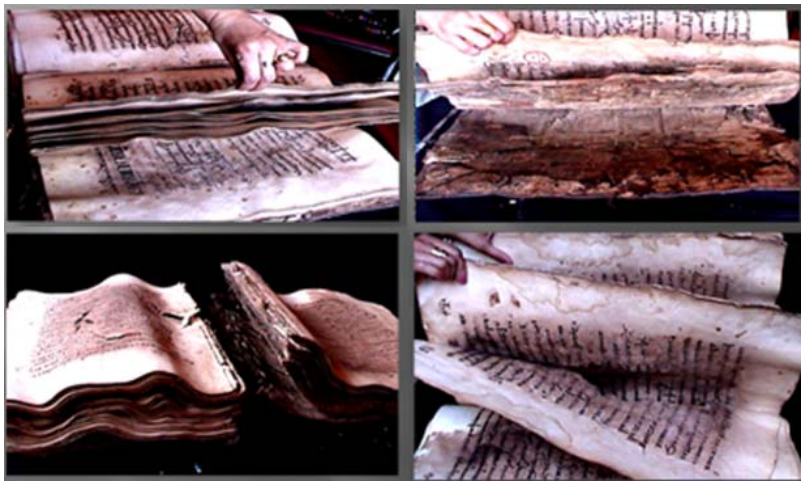

Fig. 6. The manuscript pages stuck into hard, solid mass and had the appearance of separate blocks

Dismantling the text block

Dismantling of the book could not proceed without knowing the correct sequence of the pages. At this stage, we tried to determine the correct location and order of the detached leaves of the manuscript as possible. Each section was removed from the text block by opening the section in the center and cutting the sewing thread. To keep the book in order, a small pencil number was placed on the top right hand corner of each page [28].

Surface cleaning

It was difficult to clean each page because they were stuck together in many cases, so we used the soft brushes and eraser minimalistic (a narrow range) to remove loose dirt. Care had to be taken when erasing, as the paper was very soft and easily damaged.

Separation process

Before the separation process, pH measurements were taken on a number of pages randomly. Spot tests revealed that the inks were stable enough to allow the pages to be washed. Each separate page or folio was sandwiched between Reemay® and washed for one hour in water. Then, the page or folio was lifted frequently from the washing water to release any soluble impurities from the paper. Finally, the washed pages were removed and placed onto drying racks to air dry [29]**.**

To release the adhered layers of the papers, the use of enzymes were proposed. The lump of paper was placed on a sheet of Melinex (Polyester sheet). These was immersed in a basin containing solution of 2 ml enzyme (novozymes, stainzyme plus) in 100 ml of distilled water at room temperature [30]. But the experimental and practice study proved better to raise the temperature to 37 ^{o}C this made easier for the solution to reach the core of the solid lump of papers and it is the ideal activity of the enzyme. After the lump being treated, it was thoroughly soaked and taken from the solution, still supported by the Melinex sheet and the dried firmly on the top of the lump with blotting paper until moisture no longer seeped from it. To separate the first page of manuscript a dry plotting paper was pressed firmly on the top of the lump this made the upper surface of block adhere to plotting papers then separated from the rest of the lump. Sometimes, the pages were detached from the lump with the help of thin knife and

various stainless steel “palette knives” used by dentists. During separation process, it was not possible to detach the pages regularly at a time because two or three together had more cohesion.

After 206 pages separation, the papers seem to be of very good quality and the ink has mostly remained black clearly visible. To conserve and prevent further damage and loss, it was decided to place all papers between blotting paper. After completely dried, some pages needed to repair torn using tissue paper wetted with methyl cellulose.

Leaf casting

After the folios separated, here came the next challenge which was to find a suitable method to fill in the large missing areas surrounded with fragile damaged parts. There were 30 folios suffering from severe loss at the central part. It was decided that leaf casting would be the most appropriate method to fill in the loss of the paper. This method would work on depositing pulp where there was a complete loss and also where the edges and the corners were thin and pulpy.

Rag pulp (cotton linter and unbleached linen) and pre-dyed chemical wood pulp (long fiber) was chosen for the new fiber. This leaf casting process was carried out at the laboratory of The National Library and Archives of Egypt.

After determining the area of the loss through placing the folios over a grid and counting the number of grid squares exposed by holes or uneven edges, gave the area of paper to be replaced. The paper thickness was measured with a micrometer. Also, the proper tint was determined through comparing it with the standard sheet.

The missing area of each folio had been calculated, dried pulp was weighed. It consists of 50% cotton, linen linter and dyed alkaline chemical wood pulp. Before beating, the pulp was disintegrated into slurry in blender, and then poured into a Hollander beater for 45 min. For superiority of tea tree oil (as proved from the previously discussed results) it was selected to be applied in the leaf casting stage.

In this stage, tea tree oil was added with 0.25% v/v concentration and we overcame the foaming effect through adding the Tween agent. The measured amount of pulp was then beaten into the blinder with 0.5% concentration. It was poured into the upper chamber of the leaf casting machine where the folio was placed on the Reemay after cutting a window in the plastic sheet compatible to the folio area [31]. After casting, each folio was removed from the upper chamber on its Reemay support and pressed between blotting paper in the hydraulic press (Fig. 7).

Rebinding

Due to the extreme deterioration of the original cover, the leather was almost decomposed. It was decided that it could not be used and to carry out the process of rebinding the manuscript with a new cover.

Firstly, the text block was hand sewn with link stitch, without wood sewing frame, according to the traditional Coptic binding. These bindings had chain stitch sewing those laces through the cardboard. The sewing was done through pairs of sewing stations with a needle on each end of the thread. The spine of the volume was flat .After all sections were attached, a strip of goat skin was cut to form the core of the headband which was sewed onto the headband the tail of the text block sewing with linen thread. The headband consists simply of a chain with each link catching up the previous one. Secondly reddish brown goat skin was used to make the new full leather binding typically to the original cover .The fastening type used was entirely made of leather and comprise five thongs with their toggles that are fastened at the front edge and one each at the head and tail of the upper cover with five long loops in the corresponding position on the lower cover, to hold the toggles in place and keep the book closed [32]. The loops are made of twisted braids as illustrated in Figure 8a. After the above stages were fulfilled, the manuscript now is ready for conservation as seen from Figure 9.

Fig. 7. The folios after leaf casting

Fig. 8. Where a - The leather braids made from strips,
b - The toggles from leather strips, and c - is the Coptic manuscript after rebinding

Fig. 9. The manuscript after treatment ready for exhibition

Conclusion

The main goal of this work was to investigate the fungicidal activity of the commercial essential oils of tea tree, thyme and lavender used as alternative preservatives for an ancient Egyptian Coptic cellulosic manuscript.

Our study revealed that tea tree oil was the most effective in inhibiting the growth of all fungal and bacterial isolates at a low concentration (0.25% v/v), followed by lavender oil and thymus oil, which was onlyeffective in inhibiting the growth of isolated microorganisms at higher concentrations (0.5% and 1.0% v/v).

Regarding to color parameters, we noted that the mimic paper samples fumigated with tea tree oil displayed the lowest color difference (ΔE) and the most adequate color parameters and yellowness index values, as compared to the corresponding results of the samples treated with either lavender or thyme oils.

All the obtained results were assured through the mechanical properties of the fumigated aged paper samples and by studying the change in the function groups of the FTIR charts.

As a result of the above mentioned data, the paper samples to be used as folio separators in the Coptic manuscript were fumigated with the optimized tea tree oil concentration before use.

Moreover, the same oil concentration was added to the pulp in the leaf casting machine, to fill the missing parts in the original manuscript.

Before preserving the Coptic manuscript under investigation, it was first essential to dismantle, clean and separate the folios of it. Moreover, leaf casting to fill in the large missing areas surrounded with fragile damaged parts was done by using rag pulp (cotton linter and unbleached linen) and pre-dyed chemical wood pulp (long fiber) treated with tea tree oil . Finally, rebinding the manuscript was done by using a new leather cover, due to the extreme deterioration of the original one, by following the documented Coptic binding techniques.

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