



Original article

MICROBIAL DEGRADATION OF ANCIENT TEXTILES HOUSED IN THE EGYPTIAN TEXTILE MUSEUM AND METHODS OF ITS CONTROL

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Abstract

All ancient textile materials have a simple chemical composition, mainly cellulose and protein. This organic component increases the susceptibility of textiles to soaking up and retaining wet from the unfavorable conditions of high humidity and temperature, causing microbial deterioration. Microbial deterioration of archaeological textile was studied as a state from the Egyptian Textile Museum; isolation, purification, and identification of the causative microorganisms were occurring, where the most common microorganisms isolated from archaeological textiles were molds. Biological activities of the isolated microorganisms were studied and disinfection of archaeological textile was applied using different methods. The characteristics of test methods and disinfection include their application to historical objects. Historic textiles were analyzed from different perspectives: Stereo microscopes, SEM with EDX, FTIR, as well as fiber structure and fiber chemical composition. The results illustrated that the best concentrations of a specific mic-robicide for the bio-treatment of infected textile materials is Di-chloroxylenol at (1000 ppm). It is sufficient to inhibit all isolated microorganisms, followed by p-chloro-m-cresol at (1000 ppm) concentration, and Sodium azide at (2000 ppm) concentration.

Keywords: Textiles, Microbial, Antimicrobial agents, Enzymes, Biocides treatments

1. Introduction

The deterioration process includes any undesirable change in the properties of the material caused by the vital activities of organisms [1]. It mainly depends on the type and origin of fabric, contact with microorganisms or insects, and storage conditions (temperature, humidity, light, oxygen, dust, and pollution). Antique textile objects are an important part of the cultural heritage that needs to be maintained. However, preserving them for a long time is a real challenge not only for conservators and museum staff, but for microbiologists, chemists, textile scientists, and other experts, as well. Recently, it's generally agreed

that fungi and bacteria not only cause serious aesthetic destruction of paintings, costumes, ceramics, mummies, books, and manuscripts, but they inhabit and penetrate into the materials, leading to material loss owing to acid corrosion, enzymatic degradation, and physical alteration. Moreover, the decontamination of the infected artifacts, exhibition rooms, and depots could lead to high expenditure for museums [1-3]. Fungi and bacteria cause serious damage to historic materials as a result of secreting organic acids and specific enzymes such as cellulose, protease and ligninases [4]. During the

deterioration of textiles, microorganisms produce extracellular cellulolytic and proteolytic enzymes as well as secreting pigments and acids. The deterioration of textiles leads to odors, staining, discoloration, a decrease in strength, and a change in pH. [1,5]. Stains appear due to the action of exo-pigments, which are secreted by microbial cells and diffuse into the fabric. The ability of textiles to absorb and retain moisture from the surrounding environment in the museums, coupled with their organic components, makes them highly susceptible to fungal deterioration. Advanced changes to the properties of textile materials are commonly done by the natural ageing that causes historical textiles to become more susceptible to microbial deterioration [6-8]. The present study isolated the fungi deteriorating historical

textiles at the Egyptian and the Coptic Museums in Cairo and found out that the most dominant fungi species belong to *Alternaria*, *Aspergillus*, *Chaetomium*, *Penicillium* and *Trichoderma*. The storage conditions and contact of textiles with microorganisms are also important to be investigated. The examples reported in literatures indicate that archaeological textiles that have permanent contact with water and soil (historical textile objects, e.g. mummies, textiles in the graves, tombs, crypts, sunken ships, and soldier's uniforms) are more susceptible to deterioration. Such susceptibility also depends on the degree of polymerization and crystallinity of the polymer fibers; the fabric thickness and the type of weave [6].

2. Material and Methods

2.1. Sampling

Microbial swabs were taken from six showcases (14, 28, 30, 31, 133 & 144) at the Egyptian Textile Museum. This process was done through scratching the surface of the infected materials by sterilized cotton swabs and transferred directly into four prepared agar media (Cellulose agar, Gelatin agar, Dox agar,

2.2. Media

The media used in this study can be summarized, as follows: List of media used for growth, preservation or identification of microbial isolates obtained from

2.2.1. Productive medium

According to Ammar, et al., [11], the medium listed in tab. (1) was used for the production of cellulose and protease enzymes. The main source of carbon

and Nutrient agar). Plates were incubated at 28-30°C for 1-7 days depending on the microorganism. At the same time, air samples of showcases were taken from the surface of the same media to be compared to the results of the swabs.

the samples: Dox agar medium, Gelatin agar medium, Cellulose agar medium and Nutrient agar medium. Media used to enzymes productivity [9,10].

(sucrose) was replaced with 10g of cellulose and 10g of gelatin. It is composed of the following ingredients:

Table (1) Productive medium used for the production of cellulose and protease enzymes

Ingredient	g/L
NaNO ₃	2.0
KH ₂ PO ₄	1.0
MgSO ₄ .7H ₂ O	0.5
KCl	0.5
FeSO ₄ .7H ₂ O	0.01
Sucrose	20
Distilled water	1L
pH	6.5-7

2.2.2. Detection medium

As have been mentioned by Ammar, et al., [12] the detection medium was used for the selection of cellulolytic and

proteolysis isolates. It is composed mainly of the ingredients listed in tab. (2):

Table (2) Detection medium used for the selection of cellulolytic and proteolysis isolates

Ingredient	gL ⁻¹
Arabic gum	2.0
Agar	15.0
Cellulose powder (for cellulose activity)	10.0
Gelatin powder (for protease activity)	10.0
Distilled water	1000 ml
pH	7.0

2.3. Identification of microbial isolates

The identification of all microbial isolates was carried out at the Lab. of Microbiology, Conservation Center, Grand Egyptian Museum. Colonies grown in the media (Cellulose agar, Gelatin agar, Dox agar, and Nutrient agar). Plate's media were purified on the same medium. Each

single colony was picked for identification according to [13-15] by determining the morphological characteristics using *Carl Zies* light microscope attached to an analysis unit and a digital camera, then they were compared with the references standard.

2.4. Determination of cellulose and protease activity of the isolated microorganisms

2.4.1. Enzyme production

Production was carried out in 250 ml. conical flasks. Each one contains 100 ml. of the production medium (for the production of cellulose and protease enzymes. The main source of carbon (sucrose) was replaced with 10g of cellulose and 10g of gelatin, respectively). Flasks were sterilized at 121 °C for 15min. After cooling, they were inoculated with 2 ml. of standard

2.4.2. Enzyme assay

Cup plate clearing zone technique (CCZ) was used for assaying the activities of cellulase and protease enzymes. It was carried out by pouring 20 ml. aliquots of the detection medium [11] into a sterile Petri dish and allowed to solidify. A sterile corn borer (15mm diameter) was used to make three cups in each plate and 0.1 ml of the supernatant (cell-free enzyme) of each isolate was placed into the three

inoculum of each isolate. The inoculated flasks were incubated at 28 -30 °C for proper time. At the end of the incubation period, the liquid cultures were centrifuged at 3000 RPM for 15 minutes. The supernatant was taken for the determination of enzyme (cellulases and protease) activity, as described below.

cups. The plates were incubated at 30°C for 24 h. Then, they were flooded with a Lugol's iodine solution to assay cellulose and an acid mercuric chloride solution to assay protease. Enzyme activities were compared based on the diameter (mm) of the clear zone. Isolates showing the highest activity for each enzyme were used in the subsequent experiments.

2.5. Determination of minimal inhibitory concentration (MIC) of antimicrobial agents against the isolated microorganisms

Three commercially available microcides were purchased from Aldrich Company (Germany) and tested against the isolated microorganisms to determine their MICs. They were Di-chloroxylenol, Sodium acid, and p-chloro-m-cresol. A stock solution of each microcide was prepared by

dissolving 1g/L ethyl alcohol (95%). Gradient concentrations of each microcode (from 900 to 3000 p.m.) were prepared by diluting the stock solution with alcohol. 1 ml of spore suspension was spread on Dox agar plate. The plates were allowed to dry, then a cork purer was used to

make three pores in each plate. On one plate, 100 μ l of each concentration (from 900 to 3000 p.m.) of the tested microcides were placed in each pore. The plates were incubated at 30 °C for 1-3 days compared with control plates (ethyl alcohol instead of microcides). The MIC was determined by measuring the

2.6. Effect of microcides on infected textile

In this experiment, the modern pieces of textile (modern linen samples; measured 10cm×10cm, weighted 3g., colored beige and collected from the Textile Company in Mahalla, Egypt) were infected with the isolated microorganisms, incubated for two months at room temperature. After the end of the incubation

inhibition zone according to Brantner, et al., [16]. MIC test is the gold standards for deciding the susceptibility of organisms to a specific antimicrobial substance so accustomed decide the performance of all different strategies of susceptibility testing [17].

3. Results

3.1. Microbial isolates

According to Ammar, et al. [12] and Gilman [13] the resulted microbial colonies subjected to preliminary characterization depending on the type of organism and their morphology proved that there are three main genera were identified: *Aspergillus*, *Cladosporium* and *Pacillomyces*, as listed in tab. (3) & shown in fig. (1). In this regard, it can be seen that the genus *Pacillomyces carneus* was dominant in the six showcases of the total fungal isolates, followed by *Aspergillus*

period, the pieces of textile were treated with the best microcides. Then the effect of microcides on textile was determined using Binocular dissecting stereomicroscope, FTIR (Fourier Transform Infrared), in addition to environmental scanning electron microscope (ESEM).

and *Cladosporium*. In addition, the species of *Pacillomyces* isolated from all showcases indicate that the spores of its microorganisms were present in all showcases and caused the infection to the pieces of textile. In addition, the environmental condition inside and outside the showcases were suitable for microbial growth. They were recorded inside the showcases as 26 °C and 74% for temperature and relative humidity, respectively.

Table (3) The isolated microorganisms on different media (*Cellulose agar*, *Gelatin agar*, *Dox agar*, and *Nutrient agar*)

Swabs site	Media used			
	<i>Cellulose agar</i>	<i>Gelatin agar</i>	<i>Dox s agar</i>	<i>Nutrient agar</i>
Air of showcases	- <i>Asp. Niger</i>	<i>Asp. flavus</i>	<i>Asp. niger</i>	-
Showcase 14	- <i>Pacillomyces carneus</i>	-	<i>Pacillomyces carneus</i>	-
Showcase 28		-	<i>Pacillomyces carneus</i>	-
Showcase 30		-		-
Showcase 31		-		-
Showcase 133		-		-
Showcase 144	- <i>Clado. herbarum</i> - <i>Pacillomyces carneus</i>	-		-

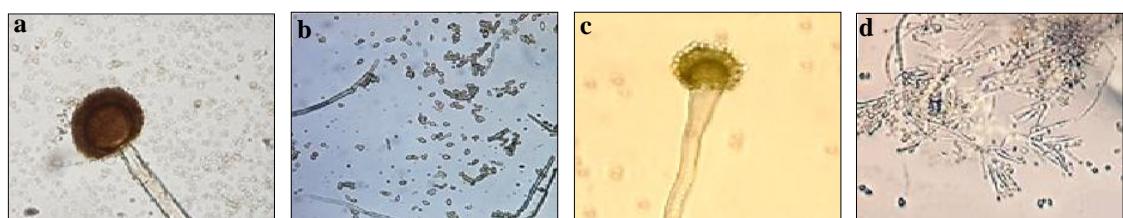


Figure (1). Shows isolated microorganisms **a.** *Asp. niger*, **b.** *Asp. flavus*, **c.** *Clado. Herbarum* and **d.** *Pacillomyces carneus* showing conidia and conidiophores (400X).

3.2. Determination of celluloses and protease produced by the isolated microorganisms using cup plate technique

The resulted data, tab. (4) of the tested microorganisms proved that they varied in the ability to produce cellulase and protease enzymes. Thus, they vary in

the degree of decomposing protein and cellulose. The tabulated data show that *Paecilomyces carneus* achieved the highest cellulytic and proteolytic activities.

Table (4) The ability of the isolated microorganisms to produce cellulases and proteases enzymes

Microbial isolates	Diameters of clearing zone (mm)	
	Celluloses	Proteases
<i>Asp. niger</i>	30	28
<i>Asp. flavus</i>	27	25
<i>Paecilomyces carneus</i>	33	30
<i>Clado. herbarum</i>	26	24

3.3. Determination of MIC of antimicrobial agents against the isolated microorganisms

In this part of the study, the prepared microcides applied to the isolated fungi to determine the MIC that inhibited each fungal species proved that in the case of Dichloro-xylenol, good response could be detected at all concentration except for 900 ppm that gave no response in all isolates. Therefore, it could be reported that using Di-chloroxylenol at (1000 ppm) gave the diameter of a clear zone ranged (19-22 mm). In the case of sodium azide, no response could be detected at 900 and 1000 ppm concentrations of all isolated microorganisms except for *Asp. niger* and *Asp. Flavus* that gave responses at (1000 ppm) with diameters of 19 and 18 mm, respectively. It could be reported that using Sodium azide at (2000 ppm) gave the diameter of a clear zone ranged (20-

24 mm). P-chloro-m-cresol showed good response at all concentration except for 900 ppm that gave no response in all isolates. Thus, it could be reported that using p-chloro-m-cresol at (1000 ppm) gave the diameter of a clear zone that ranged (18-21 mm). These data can be used to recommend the best concentrations of a specific microbicide for the bio-treatment of infected textile materials by any of the tested fungi. For instance, Di-chloroxylenol at (1000 ppm) is sufficient to inhibit all isolated microorganisms, followed by p-chloro-m-cresol at (1000ppm) and Sodium azide at (2000 ppm). These results are listed in table (5) & shown in fig. (2).

Table (5) Determination of inhibition zone (mm) of fungal species grown on Czapek agar as affected by three microcides; Di-chloroxylenol, Sodium azide and p-chloro-m-cresol (from 900 to 3000 ppm).

Fungal isolates	Mean diameter of inhibition zone (mm) at different concentrations (ppm)											
	Di-chloroxylenol				Sodium azide				p-chloro-m-cresol			
	900	1000	2000	3000	900	1000	2000	3000	900	1000	2000	3000
<i>Asp. niger</i>	0	22	30	35	0	19	24	28	0	20	25	31
<i>Asp. flavus</i>	0	21	25	28	0	18	22	27	0	19	24	28
<i>Paecillomyces carneus</i>	0	20	24	28	0	0	20	25	0	21	27	32
<i>Clado. herbarum</i>	0	19	22	27	0	0	21	24	0	18	23	29



Asp. Flavus



Asp. niger



Pas. Carneus



Clado. herbarum

Figure (2) Shows determination of inhibition zone (mm) of fungal isolates grown on Czapek agar

3.4. Method of treatment

The above results illustrated that Di-chloroxylenol can be used as the best antimicrobial at (1000 ppm) concentration to control the deterioration of textile through three methods of application. The first method (direct method) is used to control the infection by a specific brush for cleaning and treatment, the second method (injection method) uses a specific syringe to apply the antimicrobial only on the infected area. In the third method (indirect

3.5. Microcides and their effects on infected textile

In this experiment, the pieces of infected textile were treated with the best microcides, i.e. Di-chloroxylenol to control their deterioration. The effects of microcides on modern infected pieces of textile before and after the application were determined using Binocular dissecting stereo microscope, FTIR, and ESEM. The results of morphological data obtained by Binocular (26-x) show the penetration of fungal threads into the textile sample, and revealing weakness with individual filaments and dark brown spots. They also proved that, Di-chloroxylenol is the best antim-

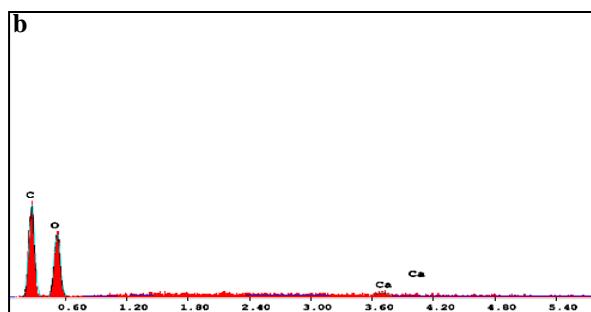
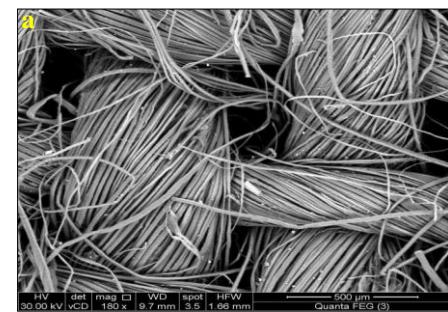
method), the treatment is applied on the object by fumigation method without contacting the object. Therefore, the third method can be used because it is always safe. In this method, the best antimicrobial is used by dissolving the best MIC of each substance in ethyl alcohol (95%) and the volume used according to the volume of the showcase containing the pieces of textile.



Figure (3) Shows binocular stereo microscope of **a.** modern linen textile sample before infection (26-x), **b.** modern linen textile sample (26-x) affected by fungal growth, weakness and fragmentation of fibers after infection for 60 days at room temperature, and treatment with 1000 ppm Di-chloroxylenol.

Table (6) Elemental analysis of modern linen textile sample before and after infection for 2 months.

Wt %	Analytical Results									
	C	O	Na	Mg	Al	P	S	Cl	Ca	Total
Before	51.24	48.15	-	-	-	-	-	-	0.61	100.0
After	58.15	33.86	1.81	0.79	0.42	0.41	0.67	1.63	2.27	100.0



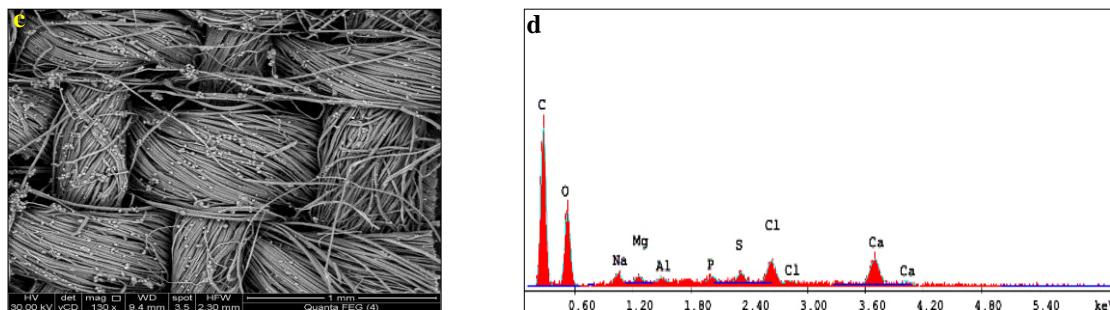


Figure (4) Shows **a.** SEM photomicrograph of a modern linen textile sample before infection (500-x), **b.** EDX pattern of major elements of a modern linen textile sample before infection, **c.** SEM photomicrograph of a modern infected linen textile sample affected by fungal growth on the surface (500-x), **d.** EDX pattern of major elements of a modern linen textile sample after infection

Through determining the chemical properties of linen by FTIR equipment, it could be noted that there is a net change in dipole moment during the vibration of the molecule or the functional group (specific groups of atoms or bonds among molecules, indicating the characteristic chemical reactions of these molecules) for infrared activity leading to the absorption of infrared radiation. Figure (5) illustrates that the chemical changes within the linen structure are due to microbial infection resulted in the degradation of organic compounds into tiny compounds which have other chemical specific groups. In addition, specific groups of atoms or bonds disappeared within the molecules that are responsible for the characteristic chemical reactions of those molecules.

Furthermore, it indicates that chemical changes inside the linen are due to microbial infection resulted in the degradation of organic compounds into small compounds (which have another chemical functional groups). Also, it is attributed to the disappearance of other chemical groups and producing substances with (H^+) and (OH^-) groups which changes pH values of textile samples. In considering the different functional groups in (control) sample without infection against infected sample, the following bands were obtained: hydroxyl group, hydrogen-bonded O-H stretching at $\nu \approx 3300-3500\text{ cm}^{-1}$, the C-H stretching at $\nu \approx 2800-3000\text{ cm}^{-1}$, the C-O vibration and stretch linkage at $\nu \approx 1020-1100\text{ cm}^{-1}$.

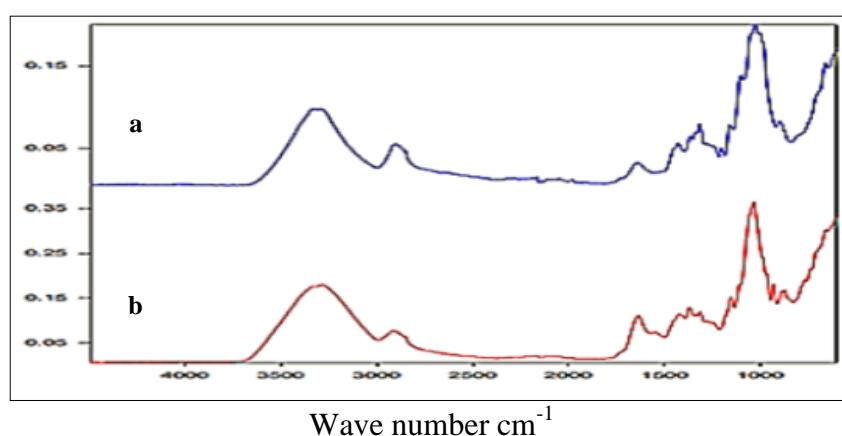


Figure (5) Shows FTIR chart of linen textile sample **a.** before & **b.** after infection by *Asp. flavus*

4. Discussion

The present study aims to highlight the effect of microbial deterioration on the ancient textile and the best methods

to resist it. In addition, some analyses were done too textile samples before and after infection using different perspectives:

(Stereo microscopes, SEM with EDXS, FTIR, fiber structure, and fiber chemical composition. The microorganisms of six showcases (14, 28, 30, 31, 133 & 144) in the Egyptian Textile Museum; air sample were isolated onto four prepared agar media (Cellulose agar, Gelatin agar, Dox agar, and Nutrient agar), three genera were identified: *Aspergillus*, *Cladosporium* and *Paecilomyces*. Our results illustrate that the genus *Paecilomyces carneus* was the dominant genus in the six showcases of the total fungal isolates. Also, they indicate the presence of various species of *Aspergillus* and *ALT*, which agree with those obtained by Abed-EL Hameed, (1999) [18]. In addition the same species “*Aspergillus*, as well as *Alternaria*, *Cladosporium*, *Acremonium sp.*, *Epicoccum sp.*, and *Fusarium solani*” were isolated from wooden coffins at the Egyptian Museum [19]. The isolated microorganisms were tested to produce the cellulase and protease enzymes. The tabulated data show the highest catalytic and proteolytic activities were observed by *Paecilomyces canoes*. These data match those obtained by Pekhtasheva, et al., 2012 [20] proving that during the bio-deterioration of ancient textiles, microorganisms produce extracellular cellulolytic and proteolysis enzymes, as well as secreting pigments and acids. Furthermore, assimilation-micro-organisms use fibers as a nutrient source; and/or degradation-fabrics are damaged due to the growth of microorganisms and secreted metabolites. Additionally, it could be argued that *Cheatomium*, *Myrothecium*, *Stachbotrys*, *Verticillium*, *Alternaria*, *Penicillium*, and *Aspergillus* as fungal genera are the most active microorganisms that involve in the degradation of cellulose fabrics, especially the two last genera [21]. The results of tested microcides, Di-chloroxylenol, Sodium azide and p-chloro-m-cresol (from 900 to 3000 ppm) show that Di-chloroxylenol reported good response at (1000 ppm) concentration giving the diameter of a clear zone range of (19-22 mm) in all isolates. In the case of Sodium azide, a good response was shown at (2000 ppm) concentration giving the diameter of a clear zone range of (20-24 mm) in all isolates. In case of p-chloro-m-crucial

good response was reported in concentration 1000 ppm gave the diameter of clear zone ranged from 18-21 mm in all isolates. All the previous data can be utilized to recommend the best concentrations of a specific microbicide for the bio-treatment of infected textile materials. Di-chloroxylenol at (1000 ppm) concentrations was sufficient to inhibit all isolated microorganisms, followed by p-chloro-m-cresol at (1000 ppm), and Sodium azide at (2000 ppm). In a similar study, it could be asserted that dichlorophen is sufficient for the treatment of the textile sample to completely prevent fungal growth and was the least harmful to the textile in the Golestan Museum of Iran [22]. Additionally, it could be attested that the third method “indirect method applied the treatment on the object by fumigation” is the best one to apply the antimicrobial by dissolving the best MIC of each substance in ethyl alcohol (95%) and the volume used according the volume of the showcase holding the pieces of textile. These results agree with those obtained by Kakoaei, et al., (2014) in similar case [23], where, they show that fumigation techniques are the suitable method for inactivation of *Bacillus anthracis* strain spores with methyl bromide (MB). Regarding the evaluation processes of microcides on modern infected textile pieces by different techniques; the morphological data obtained by binocular, fig. (3) show the penetration of fungal threads into textile sample revealing weakness with individual filaments and dark brown spots. The results of ESEM investigation, fig. (4-a, c) shows significant changes were detected in carbon's percentage varying from 51.24 to 58.15 % and calcium's percentage increased from 0.61 to 2.27%. In addition, appearing of the elements sodium (1.81), magnesium (0.79%), aluminum (0.42%), phosphorus (0.41%), sulfur (0.67%) and chloride (1.63%), tab. (6) & fig. (4-b,d) after infection is attributed essentially to the partially surface erosion of the object. Similar findings were previously reported and interpreted by Gadd (2004)

[24] and Abdel-Kareem, (2010) [25]. They proved that fungal abilities to penetrate into the material by hyphal growth and lead to mechanical deterioration of the object. In addition, they leads to biocorrosive activity due to excretion of organic acids or oxidation of mineral cations [26,27]. Also, it could be said that the fungal growth on the object's surface can also alter these surfaces due to their metabolism. This process finally lead to the generating of organic acids (oxalic acid and citric acid) that have chelating properties by weakening the metal oxygen bond, increasing the solubility of some metals, and forming complexes with the mineral cations present on the surface matrix [28-31]. Finally, it could be asserted that the chemical changes within the linen structure due to microbial infection lead to the degradation of organic compounds into

tiny compounds (which have other chemical specific groups). Also, disappearance of specific groups of atoms or bonds within molecules is responsible for the characteristic chemical reactions of those molecules. In considering the different functional groups in (control) sample without infection against the infected sample, fig. (5) the following bands were obtained: hydroxyl group, hydrogen-bonded O-H stretching at $\nu \approx 3300\text{-}3500\text{ cm}^{-1}$, the C-H stretching at $\nu \approx 2800\text{-}3000\text{ cm}^{-1}$, the C-O vibration and stretch linkage at $\nu \approx 1020\text{-}1100\text{ cm}$. as have been argued previously similar studies, attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was used to detect microbial metabolic products on carbonate mineral surfaces following Escherichia coli growth Heather, et al., (2008) [32] and Serre, et al., (2015) [33].

5. Conclusion

Ancient textile materials exposed to microbial deterioration. Under unfavorable conditions of high humidity and temperature. The most common microorganisms isolated from archaeological textiles are molds. The isolated microorganisms have the ability to produce cellulases and protease enzymes, which gave negative effects on the textile. Treatment of infected textile with Di-chloroxylenol as best antimicrobial at concentration 1000 ppm gave good results in controlling the infection and showed no changes in physical, morphological and mechanical properties of the infected textile.

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