Studying the effect of fungal spots with thermal aging on archaeological cotton textiles

Mahmoud Abo-Elmaaref
Conservation Dept., Faculty of Archaeology, Sohag University, Egypt

Mohammed Marouf, Harby E. Ahmed
Conservation Dept., Faculty of Archaeology, Cairo University, Egypt

Abstract:

Cotton textiles are rare and rarely found in museums. In the presence of such textiles in uncontrolled museums condition, they are more vulnerable to damage by heat, humidity, insects, microorganisms "bacteria, fungi", This study presents the results of study of one of the most dangerous factors damage to cellulosic textile "cotton", microorganisms "fungi" In the presence of accelerated artificial aging, The study dealt with the impact of fungal stains and thermal aging on the mechanical properties and chemical composition of cotton fibers, Where many tests and analysis were carried out scanning electron microscope (SEM), USB Microscope, stereo microscope, and Fourier transform infrared spectral analysis (FTIR), In addition to some mechanical and physical tests such as measuring tensile strength and color change of samples by CIE-Lab values.

Key words: Cotton, fungus, thermal aging, stains, deterioration.
1-Introduction

cellulosic textiles, such as cotton and linen, are highly sensitive and more vulnerable to damage if exposed to display or storage in uncontrolled museum condition. In this case it becomes susceptible to many damage factors such light, heat, insects and microorganisms (bacteria, fungi). Conservation intervention including cleaning, disinfection, control, reinforcement and museum display of such items are essential for maintaining and preserving the properties of textile objects from damage and deterioration \(^{(1,2,3)}\).

Many researchers studied the impact of artificial thermal aging on textile samples and evaluated the changes resulting from this process, which resulted in changes in polymerization degree, acid content, and color change. This is in addition to the strong impact of Tensile strength and elongation of the experimental samples as a result of this process \(^{(4,5)}\). Several studies have also identified the most important fungal strains on the archaeological collections. These studies concluded that the most important fungal strains dominated four strains, respectively (Alternaria, Aspergillus, Cladosporium, and Penicililum). Microorganisms also attack textiles easily, which means the rapid deterioration and damage of textile fibers, where microorganisms pose a threat to textile materials in all stages of its manufacture, from obtaining raw material from farms to storage and transport to the final product \(^{(6)}\). The attack of microorganisms is considered a malicious destructive damage to historical textiles, due to the fact that such organisms remain latent for very long periods within the textile materials. In that period, the microbes are working to reproduce and therefore microbial damage can occur for compounds that appear to be in good condition and under good control.
The microbial damage to archaeological textiles leads to chromatic changes, staining, odor and rot as a result of the release of these organisms a volatile compounds, in addition to enzymatic degradation and mechanical deterioration due to fungus growth activity\(^7\). Fungal deterioration of textiles causes changes in the properties of textiles such as loss of strength, general durability, discoloration, and appearance. In addition, many fungi contain coloured substances that can cause stains and spots on textile objects. These stains contain chemical substances which deteriorate a textile object even if the fungus is dead. For this reason it is important task to remove fungal stains from textile objects. The chemical changes occurring during fungal growth result in decreased fabric strength and lead to partial or total destruction of the material. Mould can be dangerous to people who work in museums and in some cases can pose major health hazards\(^8,9\). Textile materials are damageable by bacteria and microscopic fungi. Bacterial degradation of textile materials is more intensive than the fungal one. The damaging bacterium genera are: Cytophaga, Micrococcus, Bacterium, Bacillus, Cellulobacillus, Pseudomonas, and Sarcina. Among the fungi damaging textile materials in the air and in the soil the following are detected Aspergillus, Penicillium, Alternaria, Cladosporium, Fusarium, Trichoderma\(^10\). The most important fungal strains that dominated in ancient linen textiles found in the Coptic and the Egyptian Museum in Cairo. The monitoring showed that the most important fungal strains dominated Aspergillus, Penicillium, Chatomium, Alternaria, and Trichoderma virida\(^11\). Fungal damage to cotton textiles is also more damaging and causes many problems for cotton fiber such significant decrease of strength of the fibers, disturbance of technological process (the smallest particles of sticky mucus excreted by some species of bacteria and fungi leads to sticking of executive parts of machines, abruptness increase and waste volume increase). Damaging of cotton fibers, fabrics and textile products by microorganisms is primarily accompanied by occurrence of colored yellow, orange, red, violet spots and then by putrefactive odor, and, finally, the product loses strength and degrades\(^10\).
2-materials and methods:

2-1 **Samples and chemicals**
- Egyptian cotton obtained from the Egyptian Company for Textile Industries "Dentex"
- Sucrose, Sodium nitrate, Di-potassium phosphate, Magnesium sulphate, Potassium chloride, Iron sulphate, Agar were supplied by Fluka C
- **Methods**
  2-2-1 isolation, identification and cultivation of fungi on cotton fibers.
  Fungi were isolated from Historical Textiles from Museum of faculty of applied arts, Helwan University the following nutrient media (Czapek Solution Agar) were prepared with concentration: Saccharose 30.0 g, Sodium Nitrate 2.0 g, Di-potassium Phosphate 1.0 g, Magnesium Sulfate 0.5 g, Potassium Chloride 0.5 g, Ferrous Sulfate 0.01 g and Agar 15.0 g were Saccharose is the sole carbon source, and sodium nitrate is the sole nitrogen source in Czapek-Dox Broth and Czapek Solution Agar. Dipotassium phosphate is the buffering agent, and potassium chloride contains essential ions. Magnesium sulfate and ferrous sulfate are sources of cations. Agar is the solidifying agent in Czapek Solution Agar. (12) Samples of raw and thermally artificial cotton were cultivated on Petri dish containing PDA medium and incubated on 25-30 °C. Petri dishes were sterilized before cultivation process. After 7 days all the colonies were isolated, purified by using the hyphal tip and single spore techniques. Fungal species that identify are the following: Aspergillus flavus, Penicillium duclauxii, Trichoderma sp, Aspergillus niger, Unknown mycelium, Aspergillus chevalieri, Aspergillus fumigatus and Aspergillus niger. Figer 1,2 show the shape of fungal strains.
USB Digital Microscopy for isolated fungal strains were ‘a’ Aspergillus niger, ‘b’ Aspergillus fumigatus, ‘c’ Aspergillus chevalieri.

Fig 1

USB Digital Microscopy for isolated fungal strains were ‘a’ Aspergillus ‘flavus’, ‘b’ Penicillium duclauxii, ‘c’ Trichoderma sp, ‘d’ Unknown mycelium.

Fig 2

2–2–2 thermally artificial ageing

The ageing process is the effect of the material and its components on the surrounding environmental factors of temperature, humidity, biological damage, gases and other environmental influences over time periods for exposure to such factors. (12) Where the accelerated artificial aging technology was used for natural fabrics dyed with natural dyes in order to obtain samples similar in their conditions to the ancient textiles, and study the effect of ageing process on experimental samples and measure the extent of their degradation in mechanical properties of tensile strength and elongation or in terms of chromatic change due to the effect of light on the dyes and the color stability of those dyes. (13) The researchers’ opinions differed in the time periods and temperature used in the thermal aging process of textile samples, some of which dealt with accelerated thermal -aging on textile samples after applying some drawings at 140 ° C for a period of 72 Hour heat in a convection oven. This period of exposure is estimated to be 200 years ageing due to natural factors (14).
thermal aging of silk samples dyed with natural dyes in a convection oven under Temperature of 100 °C for time periods 3, 6, 10, 12, 15, 18 days, where stated that the ageing for 3 days under a temperature of 100 °C equals 25 years of normal ageing of textile samples \(^3\). However, these samples may not be representative of the chemical state of the ancient textiles, because of the different deterioration agents (such as heat, light, pollution and microorganisms), which affected the historical object\(^14\). The accelerated thermal aging of cotton samples under study at 200 °C for a period of 5 days in a convection oven was carried out at the restoration lab at Sohag University.

2–2–3 Examinations and analysis

*Morphological study*

The morphological surface of the raw samples and ageing samples stained with fungal strains were studied to illustrate the changes in the fibers due to the presence of fungal stains and thermal aging. The morphological surface of the fibers was used in the stereo microscopy and Scanning Electron Microscope (Quanta 200 ESEM FEG from FEI Scanning Electron Microscope).

*Color measurement*

Color changes of Cotton fabrics before and after ageing were examined by CIE lab system (Commission Internationale de l'Eclairage). The CIE-Lab values of the dyeing ware measured using a double beam Optimatch spectrophotometer (Data color international Spectra flash SF450-UK). colors are given in CIE Lab coordinates, L* corresponding coordinate to the brightness (100 = white, 0 = black), while a* to the red–green coordinate (positive sign = red, negative sign = green), and b* to the yellow–blue coordinate (positive sign = yellow, negative sign = blue). The total color difference \(\Delta E^*\) between two color stimuli \(\Delta E^* = \left\{ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right\}^{1/2}\) \(\text{\cite{16,17,18}}\).
**Tensile strength and elongation measurement**

Tensile and elongation measurement was conducted at the National Institute of Measurement and Calibration in Cairo according to the standard (H5KT / 130-5000N / [E139-34A.TSX-2.5] -EN ISO 13934-1,1999 Maximum Force & Elongation- Strip Method). The distance between the jaws of the machine 5 cm, measuring temperature 20 °C and relative humidity 65%, while the dimensions of the samples used in the measurement process was 3 × 15 of the sample where the average value was taken to three measurements as a minimum for each sample.

**Fourier Transform Infra-Red Spectroscopy (FTIR)**

FTIR is a physical chemical method based on measuring the vibrations of molecules raised by IR beam in a specific wavelength (19). There are three main methods to analyze solid samples with infrared spectroscopy, the first is the use of an alkaline halide tablet. The second method by milling, and the third method by films. The method of analysis depends on the nature of the sample. The method of using an alkaline halide tablet is based on mixing the solid sample with dry powder from a halide and grinding and pressing until a clearer and more transparent disc of the sample is formed with alkaline halide. The most common alkali halide is commonly used in this analysis Potassium bromide KBr (20). Through the use of infrared spectroscopy, it is possible to know the functional groups that are made up of cotton fiber and affected by thermal process and fungal stains. The spectral range (1500 cm⁻¹-1900cm⁻¹) is characteristic of the aging cellulose degradation products (21). Where the products of cellulose damage from the different carbonyl groups appear, the increased vibration in this range is attributed to the cellulose oxidation products (22). The spectral range (1730 cm⁻¹ and 1620 cm⁻¹) is attributed to the groups of carboxylic, aldehyde and Cnjugated carbonyl groups respectively, ranges (1740 cm⁻¹: and 1730 cm⁻¹: aldehyde – carboxyl), 1710 cm⁻¹: ketone / 1620 cm⁻¹: carbonyl conjugated (23) 1720 - 1690 cm⁻¹) Ester (1750 - 1730 cm⁻¹) Aldehyde (1740 - 1720 cm⁻¹) (24).
3- Results :

The results of the study revealed that there are seven fungal strains isolated and identify from a piece of historical cotton fabric preserved in the Museum of the Faculty of Applied Arts, Helwan University, in preparation for the cultivation of these strains on samples of raw cotton, where these strains Aspergillus flavus, Penicillium duclauxii, Trichoderma sp, Aspergillus niger, Unknown mycelium, Aspergillus chevalieri, and Aspergillus fumigatus, The microscopic examination of the thermally cotton samples of the previous fungal strains showed that the morphological surface of the aging cotton fibers and the fungal strains were affected as shown in Figure (3,4,5).

Fig (3) SEM microscopy where "a" represents a raw cotton sample, "b" ageing cotton sample showing severe damage," c" surface of the fiber stained with fungal colonies.

As shown in (Figures. 4 and 5) with the stereoscopic microscope, change the color of the cotton fibers and color them with fungal colonies.

Fig( 4) shows the fungal strains growing on ageing cotton samples (a) Aspergillus niger with a magnification of 10^30x, (b) Aspergillus flavus, (c) Aspergillus chevalieri with a magnification of 10 × 50x.
Fig( 5) shows the fungal strains growing on ageing cotton samples(a) Penicillium duclauxii ,(b) Aspergillus fumigatus , (c) Trichoderma sp.

Measuring of the mechanical properties of the ageing cotton samples and samples deteriorated with fungal strains showed that the tensile strength and elongation of the cotton samples was strongly affected, This is evidenced by the strong decrease in tensile strength and elongation of the samples after the ageing process and cultivation of the fungal strains on cotton samples , Figure( 5, 6) shows the effect of fungal strains and the heat-aging process on the tensile strength and elongation of raw cotton .

Figure (5) A diagram showing the results of tensile strength and elongation of non-ageing cotton samples.
Figure (6) A diagram showing the results of the tensile strength and elongation of ageing cotton samples.

The results of the total chromatic change values measured by CIE lab system for ageing and degraded cotton samples by fungal strains showed a significant change in total chromatic change values ($\Delta E$) as well as increased yellowing rate ($\Delta b^*$) samples after thermal aging. Table (1) shows the total color change values for cotton samples before and after the heat-aging process and growth of fungal strains.
Table (1) Show the color changing of aged cotton samples.

<table>
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<tr>
<th>n</th>
<th>name</th>
<th>ΔL*</th>
<th>Δa*</th>
<th>Δb*</th>
<th>ΔE</th>
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<td>-3.94</td>
<td>-3.01</td>
<td>20.47</td>
<td>21.07</td>
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<tr>
<td>2</td>
<td>Unknown mycelium non ageing</td>
<td>-16.21</td>
<td>-1.48</td>
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<td>20.49</td>
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<td>3</td>
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<td>0.01</td>
<td>21.53</td>
<td>30.34</td>
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<tr>
<td>4</td>
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<td>-18.95</td>
<td>-2.69</td>
<td>17.70</td>
<td>26.07</td>
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<tr>
<td>5</td>
<td>Trichoderma sp ageing</td>
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<td>-2.60</td>
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<td>-2.57</td>
<td>16.32</td>
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<td>7</td>
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<td>-3.27</td>
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<td>8</td>
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<td>-1.69</td>
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<td>9</td>
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<td>-2.84</td>
<td>16.36</td>
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<td>2.65</td>
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The results of use infrared spectroscopy (FTIR) for ageing and degraded cotton samples by fungal strains showed that the functional groups of the samples were influenced by the aging process and by the fungal growth. This was demonstrated by increasing the vibration intensity of the spectrum at 1900–1500 cm⁻¹, a characteristic spectrum of degradation products of cellulosic materials. Figure (7,8) shows the change in the FTIR spectrum of ageing cotton and cotton sample that stained with fungus Trichoderma sp.

Figure (7) shows the change in the FTIR spectrum of ageing cotton, where "b" represents the sample before aging "a" represents the sample cotton after aging.
4—Discussion:-

4-1 Effect of fungal spots and thermal aging on the morphological surface of cotton fiber :-
SEM microscope and the stereoscopy microscope use to study the effect of fungal stains and thermal aging on the morphological surface of cotton fibers, Figure 3 shows the effect of thermal aging and fungal spots on the surface of cotton fibers, It shows drying, crusting, severe deterioration and significant change in surface morphology of cotton fibers.

4-2 Effect of thermal aging and fungal spots on the mechanical properties of cotton fiber: -
It is clear from the results of the tensile strength and elongation of the cotton samples the positive relationship between the tensile strength and elongation, where it is clear that both ageing and non-ageing samples are affected by the stains, it shows a strong decrease in the tensile strength and elongation of cotton samples. In the standard cotton sample tensile strength samples were 24.8 kg / f and the elongation 7.07% before ageing process.
After thermal ageing samples were tensile strength 16.98 kg / f and its elongation 5.14%. The effect of the fungal strains on tensile strength and elongation of the samples prior to aging was very similar. After the ageing process the following fungal strains were the most effective strains in the tensile strength and elongation of cotton samples Unknown mycelium, Aspergillus chevalieri, Trichoderma sp, Aspergillus flavus, as shown in diagrams in figure 5, 6.

4-3 Effect of thermal aging and fungal stains on the color change values of cotton fibers:

The results in table.1 show the effect of thermal ageing and deterioration factors on cotton samples. These results can be explained as thermal ageing increases oxidation. On oxidation, the side groups of the polymers turn into chromophoric groups (probably quinones) causing the material to develop yellow, brown, grey or light pink color. It is noticed that all the cotton samples were more darkness in brightness value (L) after thermal ageing. This difference in varying darkness degrees depending on the type of damage associated with the process of thermal ageing. The total darkness of aged cotton was -3.94, One can see that the cotton samples damaged by fungi with thermal ageing has a sever darkness around -31.42. By noticed the b value to the yellow–blue coordinate (positive sign = yellow, negative sign = blue) of aged cotton samples. One can see that all the samples has yellowness around 11.65 to 24.45, the total color difference (ΔE*) of aged cotton samples has a sever change around 21.07 to 36.35.

4-4 Fourier transform infrared spectral analysis (FTIR):

Spectral analysis using an infrared spectrometer with FTIR transformer was used to study the change in chemical composition and functional groups of cotton fibers due to thermal ageing and stains, Figure 4 shows changes in the spectral range of samples The intensity of vibration at the spectral range from 1800-1500 cm -1 was very clearly demonstrated for both the ageing cotton samples and the ageing cotton sample stained with fungal strain Trichoderma sp, .
Increase intensity of the vibration in this range is due to the oxidation and hydrolysis of the cellulose molecule (-COOH, -CHO, -CO), where oxidation and hydrolysis processes increase the fiber content of the aldehyde groups as a result of the molecular oxidation of alcohol groups and their transformation into the aldehyde groups and increase the composition of the carboxyl groups \(^{(23,24)}\). The 2363 cm\(^{-1}\) range also appeared in both the ageing cotton and Trichoderma sp sample is a distinctive range that indicates the presence of carbon dioxide (CO2) from the atmosphere, indicating that ageing samples increase CO2 absorbed during aging as a result of increased sample surface activity during the aging process. The spectral range 2900 cm\(^{-1}\) in ageing cotton sample and range 2901.23 cm\(^{-1}\) with the Trichoderma sp sample, It is a spectrum of CH2 vibration associated with the appearance of the carbonyl range and indicates the presence of cellulose damage products.

5- Conclusions:

The effect of fungal spots on the mechanical and chemical properties of cotton fiber was investigated. The effect of fungal stains on the mechanical and chemical properties of raw cotton fiber was studied and compared with the other properties after the thermal ageing of the samples. The mechanical and chemical properties of the fibers were compared with the same samples prior to the thermally ageing process. The results showed the strong impact of the tensile strength and elongation of cotton samples after the ageing, as well as the sharp change in the color change values of the samples. The effects of the mechanical properties of the samples were affected by the chemical composition of the samples and spectral analysis of the infrared spectrometer with the FTIR transistor indicate this effect. The spectrum of ageing samples showed that the characteristic ranges of degradation products of cellulosic materials were highly affected due to fungal strains and thermal aging. The study show by using examination method such SEM and stereomicroscopy, the highly damage of the morphological surface of cotton samples due to ageing process and fungal spots.
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