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A comparative Study for The Effect of Laboratory Aging and Fungal Infection on Properties of Raw and Dyed Linen Textiles

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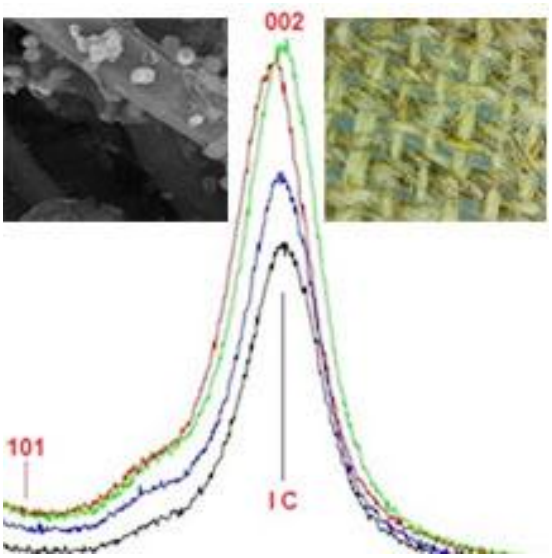
HIGHLIGHTS

- Comparison of the effect of exposure of linen textiles to laboratory aging processes and infection with fungal spots.
- Studying the effect of fungal strains and aging processes on the cellulose content of linen fibers.
- Clarification of the greatest effect resulting from the exposure of linen textiles to both fungal strains and laboratory aging processes.

GRAPHICAL ABSTRACT



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ABSTRACT

Archaeological textiles are among the most sensitive organic collectibles exposed to damage factors, whether environmental factors such as heat and light or biological factors such as infection with microorganisms. This research aims to compare the results of exposure of raw and dyed linen textiles to natural aging factors, that have been simulated in the laboratory, in addition to biological aging factors through the cultivation of some fungal strains on raw and dyed linen fabrics.

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The study used two types of linen fibers, the first type was raw linen fibers, and the second was linen fibers that were dyed with madder (*Rubia tinctorum*) which is considered one of the sources of red dye. Many methods of examination and analysis were used, such as scanning electron microscope SEM to examine the morphological surface of the fibers, measuring the tensile strength and elongation to test the mechanical properties. The measurement of color change was carried out using the CIE lab system, in addition to measuring the crystallinity index of the cellulose polymer. The main components of the fibers were detected using X-ray diffraction XRD. The results showed that the effect of fungal strains on the mechanical and chemical properties of raw linen textiles was greater than the effect of aging processes, while the tensile strength and elongation of linen dyed with madder were significantly decreased due to the light aging process.

1. Introduction

Archaeological textiles contain many natural fibers, whether these fibers are of plant origin such as cotton and linen, or animal origin such as wool and silk. These fibers contain natural polymers, that have physical and mechanical properties as well as chemical properties. These properties are changed from time to time by many environmental factors such as heat, humidity, light, and ozone as well as microbial colonies. The union of all these factors which were mentioned above works on the aging of these natural fibers and makes them more fragile[1]. Therefore, the early identification of the factors of damage that affect textiles and other organic collectibles is one of the most important requirements for preventive conservation measures, as well as understanding the biodegradation processes resulting from the microbial activity of microorganisms that most stimulate the biodegradation processes, whether ancient or modern [2]. Some of the structural properties of fibers and textiles also affect the occurrence of biodeterioration of archaeological textiles. The degree of polymerization of the fibers, the length of the chain in the cellulose molecule, the orientation and crystallization rates of the fibers, as well as the exposure of the fibers to mechanical, chemical, or light damage. All of these factors make the fibers and textiles vulnerable to the occurrence of biological damage. Due to microorganisms, loose textiles are less resistant to biological damage than tightly woven textiles and fabrics because they contain more dirt and biological pollutants between the fibers, which in turn creates a conducive climate for the growth of microorganisms and the occurrence of biological damage[3]. The degradation of cellulosic materials by microorganisms depends to a large extent on the degree of crystallization of cellulose, as amorphous cellulose is easily attacked by microorganisms, unlike crystallized cellulose, so the degree of orientation or angle at which the fibers are placed in the longitudinal axis also affects the processes of biodegradation, as cellulose fibers with a high degree of orientation are less susceptible to microbial attack than cellulosic fibers with a low degree of orientation [4]. It is known that all types of fibers are sensitive to the photo-oxidation processes that occur as a result of light rays, especially ultraviolet and infrared rays, as ultraviolet rays break down the cellulosic chains of cellulosic fibers, resulting in the decomposition of cellulose. Infrared rays also lead to an increase in the surface heating of the fibers, which results in physiochemical changes, and light increases the temperature and impurities of the atmosphere, in addition to accelerating the aging process. In such conditions, textiles and fabrics may become more susceptible to the growth of living organisms [5] Many researchers also showed that when linen fabrics were exposed to ultraviolet rays for 200 hours, these textiles became darker, and a decrease in the tensile and elongation rate of samples was observed by about 15%. The analysis of these samples using XRD showed a decrease in the crystal size of the fibers. In the longitudinal dimensions, the lateral dimensions and the crystal index remain unchanged [6]. The high relative humidity is one of the most important factors in the process of growth of microorganisms, as the fibers in the process of acquiring water depend on their hygroscopic and porosity prop-

erties, increasing the relative humidity in the fibers to more than 65%, which works to swell the fibers. It creates a favorable climate for the growth of microorganisms, especially mildew, as for the process of bacterial growth, it needs fabrics with a high degree of relative humidity, which may reach more than 95% [5]. Some other factors play an important role in the processes of stimulating biological damage to textiles by microorganisms, including:

- Fiber content: All fibers represent a permanent supporter for the growth of microorganisms, but natural fibers of cellulosic or protein origin are more affected by microorganisms than synthetic fibers, and natural fibers of cellulosic origins, such as cotton and linen, are more susceptible to damage resulting from the growth of microorganisms.
- Poor fiber surface; "cleanliness textile surfaces": Microorganisms often begin to grow on the surfaces of soiled textiles or textile surfaces containing additives such as bleaching materials, and the growth rate of microorganisms decreases on textiles with clean, non-soiled surfaces.
- Acidity & Alkalinity: The acidity and alkalinity of archaeological textiles affect the growth rates of microorganisms, as these organisms can coexist and grow under acidic and alkaline conditions under a pH ranging between pH4:9 [7]. The process of impacting microorganisms on archaeological textiles and their fibers takes place in two ways: the first is direct, in which microorganisms such as bacteria, fungi, and other microbes use the textile material as a food source for them. The second method is indirect, in which the tissue material is destroyed and deteriorates as a result of the metabolic processes of microorganisms [8, 9]. Natural polymers such as cellulose, starch, and protein are completely decomposed by enzymes secreted by microorganisms such as cellulase-degrading cellulose enzymes, starch-dissolving amylase, and protein-degrading protease enzymes. The decomposition of these polymers results in new materials that represent the vital component of the food of the microbial cell of microorganisms. Industrial polymers are almost very persistent in the environment and are not subject to such enzymatic degradation by microorganisms. The synthetic polymers with molecular weights greater than 10,000 that are degraded by the enzymatic activity catalyzed by microorganisms include PEG, polyester, and polyvinyl alcohol. PVA, where aliphatic polyester polymers are degraded by a group of lipase enzymes that break down fats, as for PEG, PVA polymers, are degraded by enzymes that decompose the real structure of polymer molecules [10].

2. Materials and Methods

2.1. Materials

- Linen textiles are produced by The Egyptian Textile Industries Company (Dintex) in Cairo. The number of threads per square centimeter of the linen fabric was 33 threads in the warp direction, and 30 threads in the weft direction
- Madder dye *(Rubia tinctorum)* was purchased from the Egyptian local market.

2.2. Methods

2.2.1. Linen sample dyeing stage

The Madder Roots Dye (*Rubia tinctorum*) were ground well into a powder form, soaked in water for 24 hours, then heated to 30-60 ^{o}C for two hours. The extract was left to cool and then filtered well until a clear and transparent color of dye was obtained. For the dyeing process, a percentage of the solution that was previously filtered from Madder Roots was used (a bath volume of 20 ml was used, per 1 gram of the dye), then the linen fibers that had been previously boiled well and rinsed to remove any sizing materials were dyed. After dyeing, the dye residue was removed by rinsing three times with cold water (5 min, at room temperature) [11, 12].

2.2.2. Laboratory aging and cultivation of fungal strains on experimental raw and dyed linen samples

Two types of accelerated aging were carried out, the first is thermal aging, and this aging was carried out in a convection oven

for 72 hours at a temperature 140 C^0, which is equivalent to nearly 200 years of natural aging that archaeological materials can be exposed to, especially textiles [13, 14]. The second type of aging is light aging using ultraviolet rays, The light aging process that was performed on the samples, was carried out at the National Institute for Standards (NIS) in Cairo at Metro Loggia Textile Laboratory, using ultraviolet light according to the International Standard ASTM D6544 - 12 Standard Practice for Preparation of Textiles before Ultraviolet (UV) Transmission Testing. The measurement method was carried out by measuring the radiation levels of the mercury ARC lamp using a radiometer for long ultra-violet rays (NIS 268 UVA), which has the maximum response range at 365 nm, as well as short UVC rays, UVC 268, which has a maximum response at 254 nm, where the distance between the center point on the bulb of the lamp and the detector was 20cm. The average reading for UVA was 5.5616 mW/cm^2 and UVC was 3.0782 mW/cm^2, and the exposure time of the samples was 5 hours at a relative humidity 36% ± 4% [11]. As for the stage of cultivating the fungal strains on the experimental raw and dyed linen samples, the samples were prepared for the cultivation of the fungal strains that were isolated and purified from the microbial study that was carried out on the display and storage of the Sohag National Museum, which concluded that three fungal strains dominate the display and storage atmosphere, namely: *Aspergillus flavus ، Trichoderma sp ، Penicillium duclauxii* [15]. Where the raw and dyed samples of linen were placed after sterilization inside a sterilization booth, the samples were wetted with sterile distilled water, the raw and dyed samples were injected using a sterile injection needle with the three fungal strains and then placed in Petri dishes inside an incubator for a while from 7-14 days under the temperature 25-28 ^oC [16].

2.2.3. Examination and analysis procedures

- **Microscopic examination**

The surface morphology of the linen textiles was investigated by SEM and stereo microscope, to show the changes and damage which happened to the fibers. Small samples were taken from different areas of the textiles. Samples were investigated under Quanta FEG250(NRC) Scanning Electron Microscope SEM and stereo microscope (OPTIKA microscopes ITALY) in Organic monuments Laboratory, Faculty of Archeology, Sohag University [15, 17, 18], the investigation by SEM aimed to monitor the changes in the surface morphology of the samples before and after artificial accelerated aging and infection with fungal species [19].

- **Measurement of mechanical properties (Elongation and maximum force)**

The measurement of tensile strength and elongation of experimental samples was conducted at the National Institute of Standards (NIS) in Giza. The measurement process was carried out using a US Tinius Olsen device with a strength of 5kN. Test conditions were under 25C° and 65% relative humidity, the distance between the jaws of the device was 5 cm, and the dimensions of the samples were 3 × 15 cm. The measurement was done according to international standards (ASTM D503506 (2008) standard test method for breaking force and elongation of textile fabrics (strip method) [15].

- **Measurement of physical properties (color change by CIE lab system)**

The measurement of values of the color change of linen samples infected with fungi and laboratory-aged, were measured using a portable colorimeter type (PCE-CSM7 S/N 330242 made in the UK) in Organic monuments Laboratory, Faculty of Archeology, Sohag University, Total color change (ΔE*) of linen samples Infected with fungi and laboratory aging was calculated according to the following equation [20, 21]:

$$\Delta E^* = \{(\Delta L^*)^2 + (\Delta b^*)^2 + (\Delta a^*)^2\}^{1/2}$$

- **Measurement of chemical properties (pH, crystallinity index XRD)**

The process of measuring the pH of aged linen samples infected with fungal strains isolated from Sohag National Museum was carried out using a device (pH meter(HACH HQ11d). The measurement was done ac-

cording to international standards (T529 om-04 TAppI 2004) [22]. The effect of laboratory aging and fungal infections on linen samples was also measured by measuring the crystallization index of the cellulose polymer, the main component of linen fibers were detected using XRD analysis. The analysis and measurement were carried out at the XRD unit, Faculty of Science, Sohag University, using a Bruker D8 Advance device at room temperature, and the scanning range of the 2θ angle was 10-80 [23].

3. Results and discussion

3.1. Effect of laboratory aging processes and fungal infection on the morphological surface of raw and dyed linen fibers

Through microscopic examinations using SM, and SEM of the aged raw and dyed linen fibers infected with fungal strains, darkness and yellowing of the laboratory-aged samples were detected in addition to staining of the sample´s surface with spores of fungal strains growing on them. All of these aspects resulted in disfigurement of the surface of the fibers, and the occurrence of clear color changes as shown in Figs. 1 and 2. SEM examination of the laboratory-aged samples infected with fungal spots showed severe dehydration for the thermally aged samples, and fiber breakage as a result of the laboratory light aging process by using ultraviolet rays, in addition to the deformation of the morphological surface of the fibers with spores of fungal strains growing on linen samples, whether raw or dyed as shown in Fig. 3.

3.2. Effect of laboratory aging processes and fungal infection on the mechanical properties of raw and dyed linen fibers

It is clear from the test of the mechanical properties of the samples "tensile and elongation forces" as shown in the graphs in Fig. 4 that there is a Positive relationship between the tensile strength and elongation of the fibers, this was observed in lower tensile rates, immediately followed by a decrease in the elongation of the samples. Where the samples of raw linen infected with fungi recorded a decrease in the tensile strength and elongation of the samples, where the tensile rate of the linen sample infected with *A. flavus* was 26.4005 kg/f, which represents a loss equivalent to 51.2% of the sample tensile strength and elongation of 1.076%, which represents a loss of 40.6 % of sample elongation compared to the uninfected raw sample . The raw linen sample infected with *A. flavus* showed the lower tensile strength and elongation compared to the uninfected raw linen sample, where the standard sample tensile value was 54.1469kg/f and its elongation was 1.813%, this indicates the strong effect of the fungus on the mechanical properties of cellulosic fibers, specifically linen fibers [15]. As for the madder-dyed linen samples, they also recorded a decrease in the tensile and elongation values, which is illustrated by the graph in Fig. 5. Considering the samples with the lowest tensile and elongation values, it was found that the sample of linen dyed with light aging madder, where the tensile strength of the sample after the aging process reached 28.032 kg/f, which showed a loss (41.1% of the sample tensile strength) and its elongation 3.288%, this represents a loss (42%) Compared with the standard sample [24]. Immediately followed by the madder-dyed linen sample infected with *Trichoderma sp*. The tensile strength of the sample infected with the fungus was 37.2196 kg/f and its elongation was 2.979%.

3.3. Effect of laboratory aging processes and fungal infection on the physical properties of raw and dyed linen fibers

Tables 1 and 2 show the color change values for thermally and lightly aged dyed and raw linen samples, as well as those infected with the fungal strains *A. flavus, Trichoderma, and P. duclauxii*, Where it shows the value of ΔL*, which refers to the rates of the darkness of the samples or their brightness (black-white), as it expresses the lowest value of the darkness of the samples, while the value from (0-100) refers to the brightness of the samples [25].

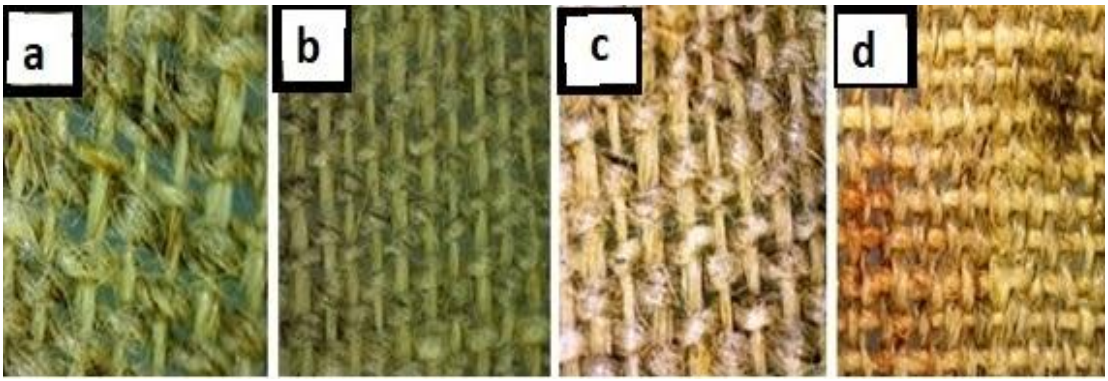

Fig. 1. Raw linen fibers, where (a) Raw linen which has a plain weave structure1/1, (b) A thermally aged sample showing the tendency of the sample to darkness, (c) Light aging linen showing yellowing of the sample, (d) Linen infected with fungi on which fungal spots appear.

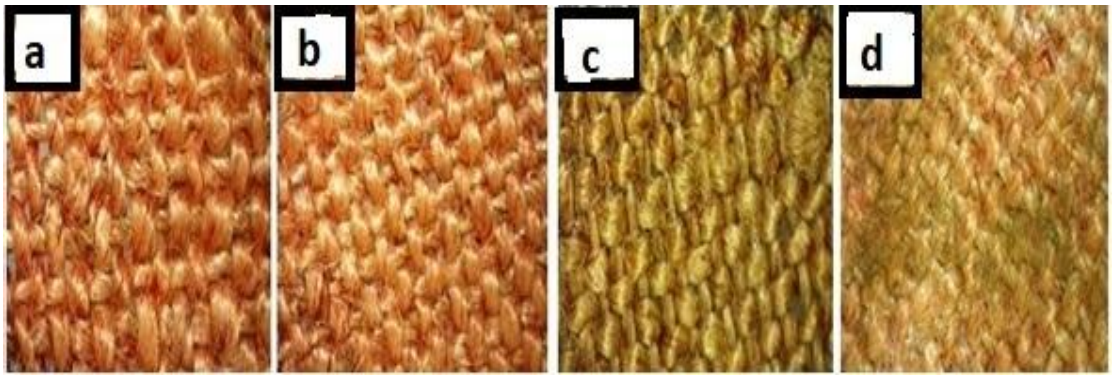

Fig. 2. (a) Linen fibers dyed with madder, (b) A thermally aged sample which shows the tendency of the sample to darkness, (c) Light aging linen showing fading of color sample, (d) Linen infected with fungi on which fungal spots appear

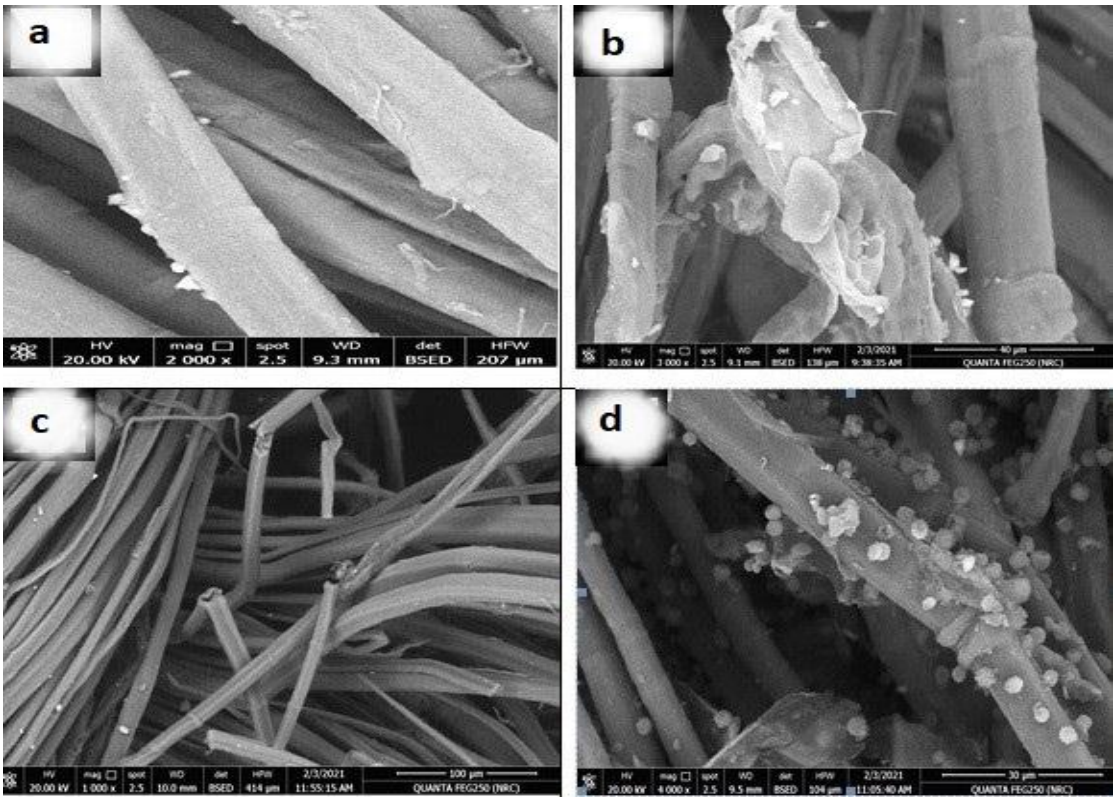

Fig. 3. SEM images where (a) Linen fiber (b) The severe damage to the fibers resulting from the thermal aging process (c) The effect of light aging processes on the fibers represented by the occurrence of breakage of the fibers, (d) Deformation of the morphological surface of linen fibers by fungal spores.

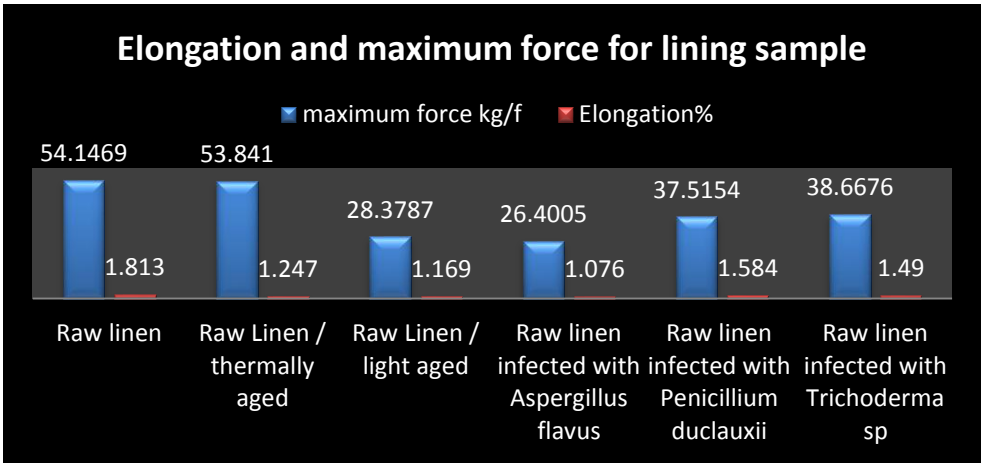

Fig. 4. Diagram showing the effect of laboratory aging processes and infection with fungal strains on the tensile strength and elongation of raw linen samples.

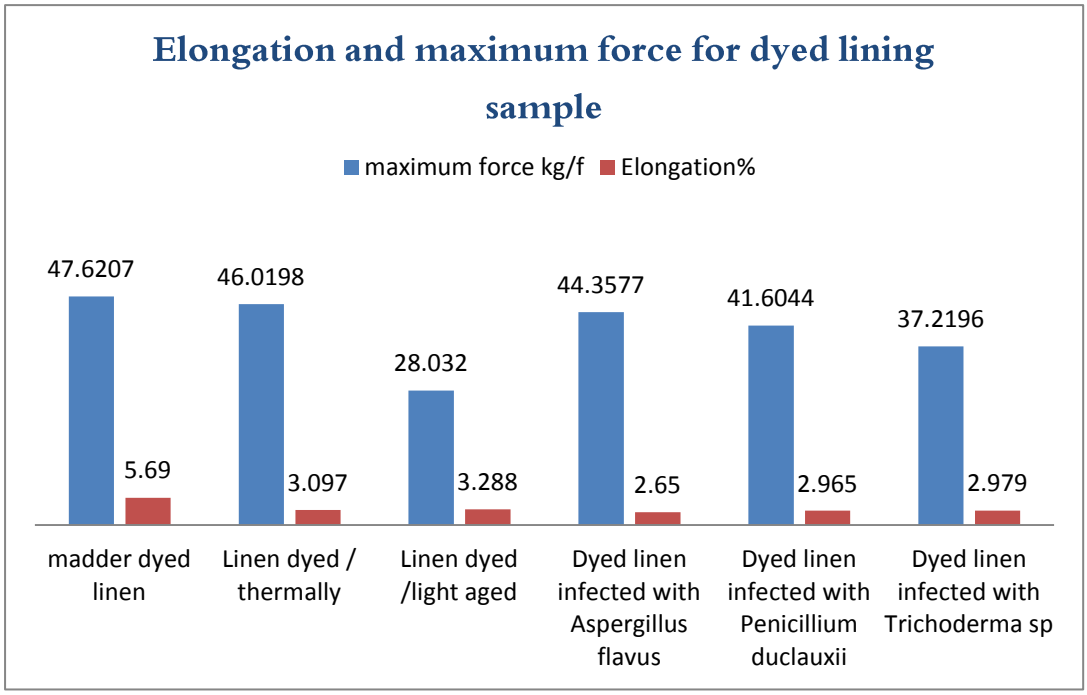

Fig. 5. The effect of laboratory aging processes and infection with fungal strains on the tensile strength and elongation of madder-dyed linen samples.

Table 1. The color change values for aged raw linen samples infected with fungal strains.

No.	Samples	L*	a*	b*	ΔL*	Δa*	Δb*	ΔE*
1	Raw linen	68.08	0.76	7.64				
2	Raw Linen / thermally aged	67.88	1.05	8.59	-0.20	0.29	0.95	1.01
3	Raw Linen/light aged	67.37	0.81	7.58	-0.17	0.05	-0.06	0.71
4	Raw linen infected with *A. flavus*	62.73	1.09	12	-5.35	0.33	4.36	6.91
5	Raw linen infected with *P. duclauxii*	65.05	0.96	9.06	-3.03	0.20	1.42	3.08
6	Raw linen infected with *Trichoderma sp*	67.06	0.88	8.81	-1.02	0.12	0.54	1.75

Table 2. The color change values for aged dyed linen samples infected with fungal strains.

No.	Samples	L*	a*	b*	ΔL*	Δa*	Δb*	ΔE*
1	Linen dyed with madder root (Rubia tinctorum	60.33	8.37	9.93				
2	Dyed Linen / thermally aged	59.87	8.01	11.01	-0.46	-0.36	1.08	1.23
3	Dyed Linen/light aged	59.22	8.12	9.69	-1.11	-0.25	-0.24	1.16
4	Dyed linen infected with *A. flavus*	61.12	6.05	12.95	0.79	-2.32	3.02	3.89
5	Dyed linen infected with *P. duclauxii*	58.97	7.96	10.49	-1.36	-0.41	0.56	1.53
6	Dyed linen infected with *Trichoderma sp*	63.04	5.72	8.91	2.71	-2.65	-1.02	3.72

Table 1 shows the increase in the total color change rate ΔE* for the linen samples infected with fungi, in particular the linen sample infected with *A. flavus*, where the value of the total color change for the sample infected with the fungus was 6.91, followed by the rest of the samples infected with fungi, then the lightly and thermally aged samples . Table 2 shows that the ΔL* values of madder-dyed linen samples varied between white and black, as we note that the linen sample infected with *Trichoderma* fungus tilted the ΔL* value of the white color, where the ΔL* value of the sample was 2.71, while the darkness rate of the linen sample increased in samples infected with *P. duclauxii*, where the ΔL* value of the sample reached -1.36, as well as the darkness rate of the light aging sample increased, as the ΔL* value of the sample reached -1.11. For the total color change values ΔE* for the samples, it was found that the samples infected with fungal spots achieved the highest total color change values for the linen dyed samples, where the madder sample infected with *A. flavus* achieved the highest total color change values for the samples. The total color change value for the sample was ΔE* (3.89),and by calculating the color change values between the raw and dyed linen samples, we found that the raw linen samples were more affected than the madder-dyed linen samples.

3.4. Effect of laboratory aging processes and fungal infection on the pH and crystalline index of raw and dyed linen fibers

As shown in Table 3 which represents the pH of thermally and lightly aging raw linen samples, as well as those infected with fungal spots., a beginning in the rise of acidity values of the studied samples compared to the standard sample was recorded. The pH value reached 5.52 in the linen sample infected with the fungal strain *A. flavus*,, while the pH value of the standard sample was 7.40. In Table 4 which shows the pH of linen samples dyed with thermally and lightly aged madder, and infected with fungal spots, a beginning of a rise in the acidity values of the samples compared to the standard sample was detected, in particular the linen sample infected with the fungal strain *P. duclauxii*, where the pH values reached pH 5.40 While the pH value of the standard sample was 6.95. The evaluation of the effect of aging processes as well as infection with fungal strains on the chemical properties of raw and dyed linen fibers depended on studying the degree of crystallization of the fibers. The evaluation process was carried out through X-ray diffraction. The evaluation process was carried out and the crystallization rates of the fibers were calculated according to the following equation: CI= IC-Iam ÷ IC ×100 [26], Where CI is the crystalline index for the crystalline region in the sample, Iam represents the amorphous region [27] in the sample. Through the analysis, it was found that there was a decrease in the indicator of crystallization of cellulose polymer, the main component of cellulose fibers, as a result of the aging processes and infection with fungal spots, as shown in the Tables 5 and 6, Through the indicators of crystallization of raw and dyed linen fibers, it was found that the largest decrease in the crystallization indicators of samples was the samples of linen infected with fungi, immediately followed by the light aging samples of linen, as shown in the XRD pattern, to calculate the crystallization index of the thermally and lightly aged linen samples infected with fungal spots in Figs. 6 and 7.

Table 3. pH of aged raw linen samples infected with fungal strains

No.	Samples	pH
1	Raw linen	7.40
2	Raw Linen / thermally aged	6.39
3	Raw Linen/light aged	6.01
4	Raw linen infected with *A. flavus*	5.52
5	Raw linen infected with P. duclauxii	6.38
6	Raw linen infected with *Trichoderma sp*	6.50

Table 4. pH of aged madder-dyed flax samples infected with fungal strains

No.	Samples	pH
1	Linen dyed with madder root (Rubia tinctorum)	6.95
2	Dyed Linen / thermally aged	6.51
3	Dyed Linen/light aged	5.61
4	Dyed linen infected with *A. flavus*	5.71
5	Dyed linen infected with *P. duclauxii*	5.40
6	Dyed linen infected with *Trichoderma sp*	5.50

Table (5) The calculation of crystallization indices for aged raw linen samples as well as those infected with fungi

No.	Samples	Crystalline area IC	Amorphous area Iam	crystallization index%
1	Raw linen	2θ 22.946 Y 13422	2θ 19.029 Y 3302	75.3%
2	Raw Linen / thermally aged	2θ 23.241 Y 10474	2θ 19.11 Y 2689	74.3%
3	Raw Linen/light aged	2θ 23.143 Y 9426	2θ 19.029 Y 2503	73.4%
4	Raw linen-infected fungus	2θ 23.634 Y 8522	2θ 19.339 Y 2866	66.3%

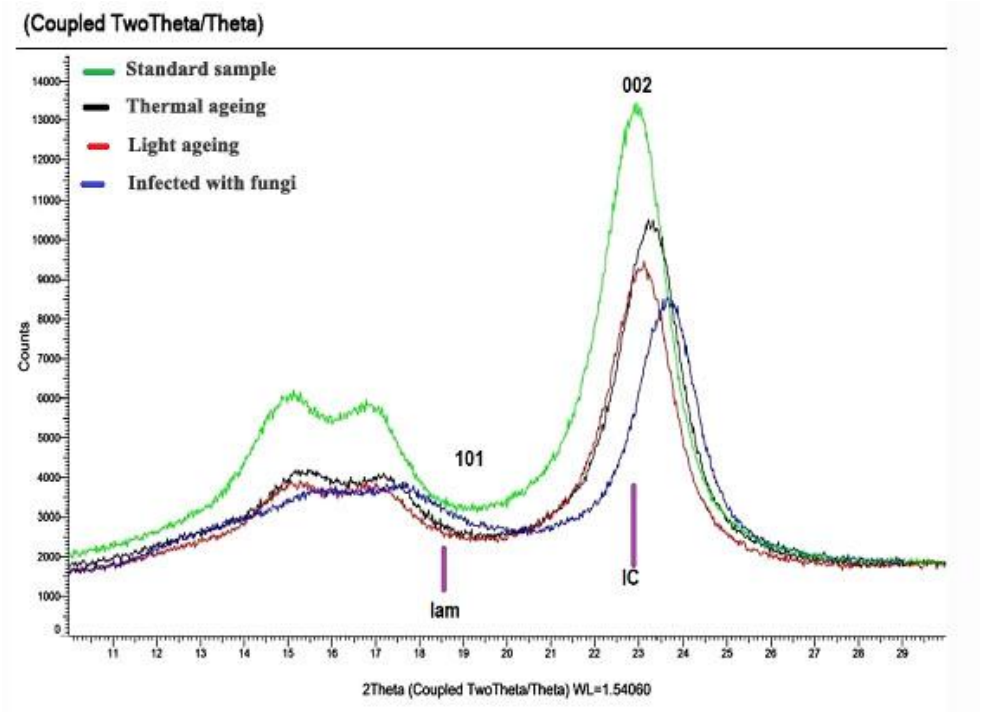

Fig. 6. The XRD patterns for calculating the crystallization index of raw aged linen fibers infected with fungi.

Table 6. The calculation of crystallization indices for aged dyed linen samples as well as those infected with fungi

No.	Samples	Crystalline area IC	Amorphous area Iam	crystallization index%
1	Linen dyed with madder root (Rubia tinctorum)	2θ 23.028 Y 13052	2θ 19.013 Y 2761	78.9%
2	Dyed Linen / thermally aged	2θ 23.044 Y 8748	2θ 19.029 Y 1994	77.2%
3	Dyed Linen/light aged	2θ 22.979 Y 12825	2θ 18.784 Y 2911	77.3%
4	Dyed linen infected with fungus	2θ 22.995 Y 10264	2θ 18.964 Y 2484	76.7%

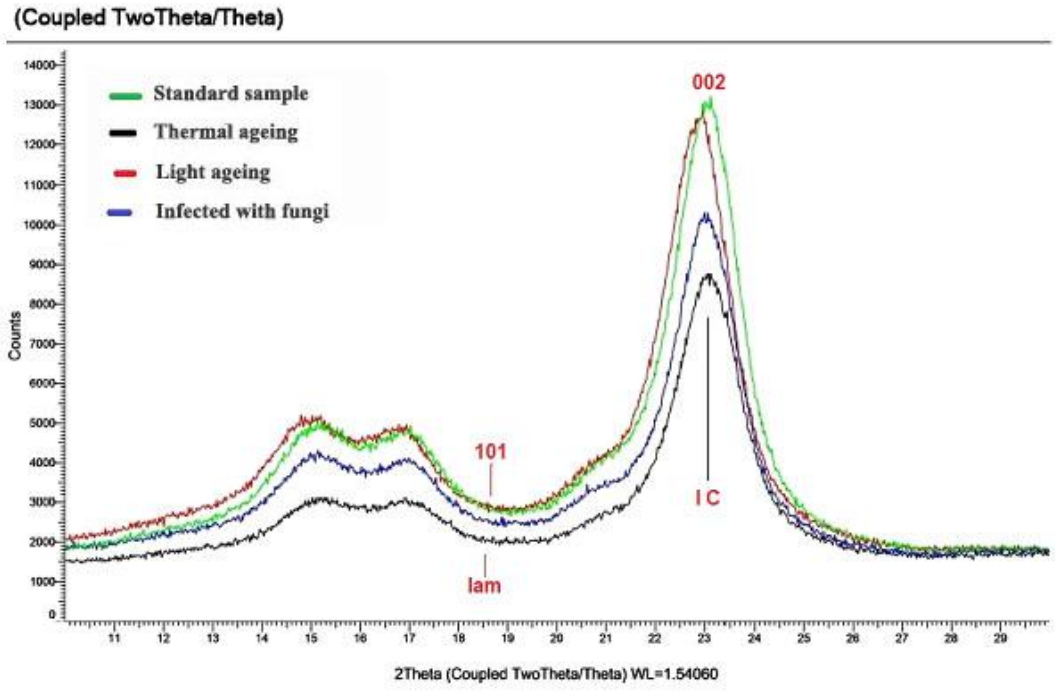

Fig. 7. The XRD patterns for calculating the crystallization index of dyed aged linen fibers infected with fungi.

4. Conclusion

Archaeological textiles are among the most sensitive organic collectibles exposed to damage factors, whether natural factors such as (heat - light) or biological factors as a result of infection with microorganisms. By comparing the effect of laboratory aging processes (thermal-light) for linen samples with samples infected with fungal strains, it was found that the effect of fungal strains on raw and dyed linen samples had the greatest impact on the mechanical, physical, and chemical properties of linen fibers. Where we note the severe decrease in tensile strength and elongation, the severe color changes of the raw and dyed linen samples, in addition to the severe decrease in the crystal content of the cellulose polymer, and the main component of the cellulosic fibers, as a result of its infection with fungal strains, from which it is

clear that the infection of the linen samples with fungal strains was more destructive to the fiber properties, than physical, Mechanical or chemical exposure of the fibers to laboratory aging conditions, whether thermal or light.

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