Study of Cellulosic Fiber Degradation by Four Common Fungi: Chromatic Alterations and SEM Examination

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[Abstract]

In the present study, papers manufactured from cotton cellulose were used. White paper (without inks), paper with cinnabar ink (red ink and HgS), and paper with iron gall ink (black) were used to facilitate colonization by *Trichoderma viride, Penicillium roqueforti, Eurotium chevalievi,* and *Aspergillus flavus.* The identification of cellulose, and the inks used and binder material (Arabic gum) was achieved using FTIR spectra. Degradation of cellulosic fiber was performed by measuring the hyphal growth of the studied fungi using an environmental scanning electron microscope (ESEM). Color changes in the inoculated papers with each of the four fungi tested was determined by assessing the surface of the studied cellulosic fibers treated with tea tree oil (0.25%) and thyme oil (0.5%). The color change values (Δ E) refer to fungal growth. The least fungal growth (*A. flavus*) was found on the white and red papers treated with tea tree oil, with a Δ E value of 1.95, whereas the highest fungal growth (Δ E 39.17) was exhibited by *T. viride* on red paper treated with thyme oil. The greater value of Δ E between the control and inoculated samples of the same species in the same type of paper presented the highest fungal growth on the paper. From both the ESEM examination and the chromatic alteration of the inoculated papers with four fungi, *T. viride* was observed to be the most destructive fungus for the tested papers, whereas *A. flavus* was the least destructive.

1 Introduction

Documents in libraries consist of several organic materials such as papers, manuscripts, leather, and woods; these are all susceptible to natural biodegradation processes (Harvey, 1992), which can destroy historical records, resulting in the loss of valuable information (Cappitelli and Sorlini, 2005). A high fungal diversity has been found on all types of bibliographic documents and support materials, including parchment, laid paper, and wood-pulp paper (Mesquita et al., 2009).

Various chemical and physical factors can affect the biodeterioration of papers, and fungi appear to play an important role in the biodeterioration of paper materials (Zotti et al. 2008). Papers manufactured from cellulosic materials are susceptible to decay and disintegration over time by fungi and other biological agents (Devanathan, 2012). Degradation is initially caused by the presence of spores or vegetative cells on

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the surface of documents, and cellulolytic fungi are considered to be serious degrading agents of bibliographic documents (Fabbri et al., 1997). Fungi have extensive enzymatic activity, which facilitates colonization and the decay of paintings, textiles, paper, parchment, leather, oil, casein, glue, and other materials used for creating historical art objects (Sterflinger, 2010).

Yeasts and filamentous fungi of *Aspergillus* and *Penicillium* species were documented on the wooden substratum and estimated to be the main cause of biodeterioration in photograph collections from the Photographic Library of the National Archive of the Republic of Cuba (Borrego et al., 2010). Furthermore, *Fusarium* species have been isolated from the atmosphere of the examined rooms containing photographs (Ljaljević-Grbić et al., 2013). *Aspergillus fumigatus, Penicillium canescens,* and *A. versicolor* were found in a wood pulp sample and *Alternaria alternata* and *Toxicocladosporium* has been found in laid paper (Zyska, 1997; Szczepanowska and Cavaliere, 2000; Mesquita et al., 2009).

Changes in documents because of fungal colonization are either through discoloration by weak acids produced by fungi or by the accumulation of pigments (foxing) on the cellulose (Arai, 2000). This occurs because of the metabolic activity of microorganisms and results in metal or ink oxidation (Meynell and Newsam, 1978; Arai, 2000). The mechanism of cellulose degradation can be either physicochemical (Carter, 1996; Bogaard and Whitmore, 2002) or through biotic agents (Zang and Lynd, 2004). In a study by Zotti et al. (2008), it was noticed that some fungal species are not specifically cellulolytic, but they can be considered broad spectrum biodeteriogens. It is possible to consider these fungi as facultative for cellulose, but they are able to degrade it in the absence of other, more specific, growing substrates.

In the present study, papers manufactured from cotton cellulose were used. White paper (without inks), paper with cinnabar ink (red ink and HgS), and paper with Iron Gall Ink (black) were used to assess colonization by *Trichoderma viride, Penicillium roqueforti, Eurotium chevalievi,* and *A. flavus.* The degradation of cellulosic fiber was performed by measuring the hyphal growth of the studied fungi using an environmental scanning electron microscope (ESEM). Color changes in the inoculated papers with each of the four fungi tested were determined on the surface of the studied cellulosic fibers treated with tea tree oil (0.25%) and thyme oil (0.5%).

2 Materials and Methods

2.1 Paper Composition

In the present study, papers manufactured from cotton cellulose were used. White paper (without inks), paper with cinnabar ink (red ink and HgS), and paper with iron gall ink were used to determine colonization by four fungi. Arabic gum was used as a binding medium (Newman, 2000) for the pigments (Mazzeo, 2008).

2.2 Fourier Transform Infrared Spectroscopy with Attenuation Total Reflection

Inks (red and black), cotton cellulose, and the binder used (Arabic gum) were identified using Fourier transform infrared spectroscopy with attenuation total reflection (FTIR-ATR) spectra. Figs. 1 and 2 show the

FTIR-ATR spectra of the Arabic gum and iron gall ink under the test, which were recorded using a Nicolet 380 Spectrometer using a zinc selenid crystal, at wavelength range 650-4,000 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. FTIR absorbance frequencies for the treated samples were recorded with an average of 128 scans using a resolution of 4 cm^{-1}. The analysis of several samples of ink from the manuscript was undertaken by the Analytical Research Laboratory using FTIR. Furthermore, Fig. 3 presents the XRD spectra of cellulose fiber with cinnabar ink.

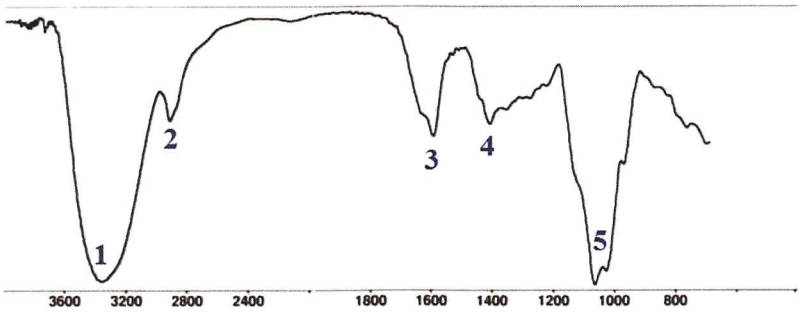

Fig. 1 FTIR spectra analysis of Arabic gum

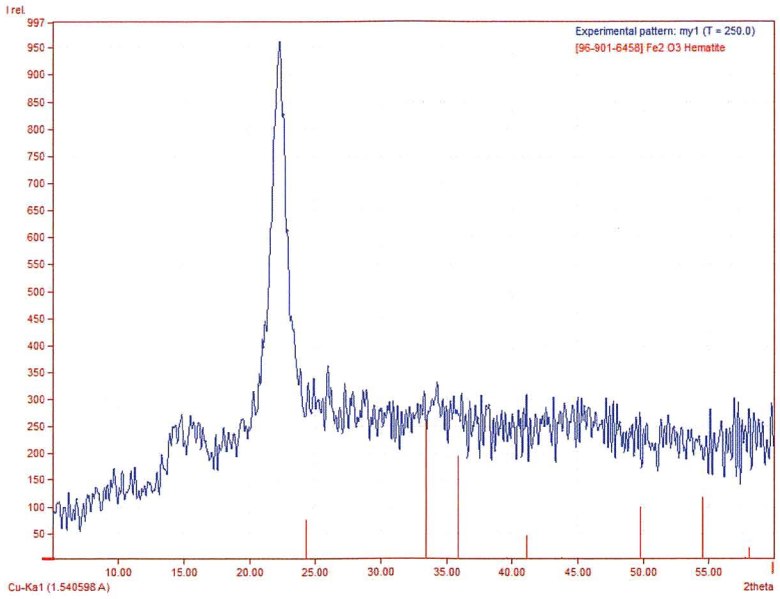

Fig. 2 FTIR spectra analysis of Iron Gall Ink

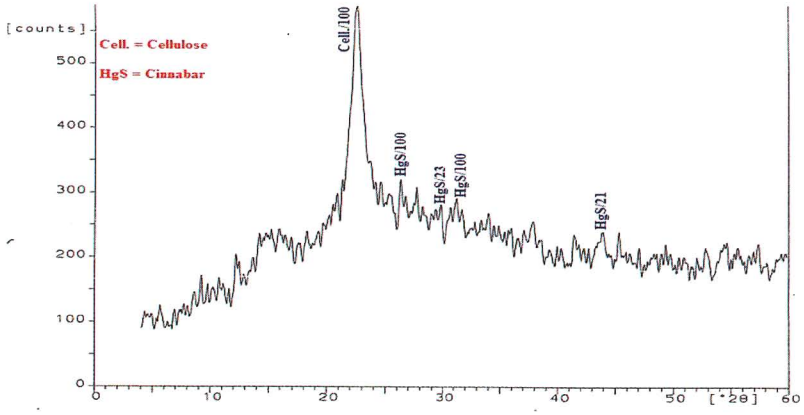

Fig. 3 XRD spectra analysis of cellulose fiber with Cinnabar ink

2.3 Colonization Test

The paper samples were cut into small pieces (20 × 20 mm) using a scalpel for the colonization test. Paper samples were sterilized using UV light exposure for 48 h, after autoclaving at 121°C for 6 h and drying in an oven at 105°C for 24 h (Miller et al., 2009). For the preparation of spore suspensions, 10 ml of sterilized distilled water was added to culture plates containing potato dextrose agar (7-days old) of *T. viride, P. roqueforti, E. chevalievi,* and *A. flavus.* The spores were gently freed using a camel brush. Paper samples were deliberately inoculated with fungi to study the color changes of paper samples before and after infection. Colonization was evaluated after a period of 5 years from inoculation.

2.4 Environmental Scanning Electron Microscope Examination

We observed the degradation of cellulosic fibers by measuring the hyphal growth of the studied fungi using an ESEM. After incubation, the microbial growth was examined using ESEM (SEM model-a FEI Quanta 200 ESEM FEG scanning electron microscope). The employed energy of the acceleration beam was 20 KV (Mansour and Salem, 2015).

2.5 Colorimetric Measurement

Color changes were observed on the surface of the studied cellulosic fibers. First, tea tree and thyme oils were used at concentrations of 0.25% and 0.5%, respectively, using micropipettes. Further, 1 ml of Tween-80 detergent was added to each oil concentration. A known amount of oil concentration was added to the cover of the Petri dish containing colonized paper with *T. viride, P. roqueforti, E. chevalievi,* and *A. flavus,* and the plates were covered for 48 h after fumigation with the essential oils.

Color difference measurement of restructured meat samples was conducted using a color difference apparatus (Handy Colorimeter NR-3000, Nippon Denshoku, Tokyo, Japan), calibrated with the standard whiteboard (D65/10, X = 82.43, Y = 87.40, Z = 89.77) to measure its L, a, and b value. The mean of three replicates was calculated. The delta values (ΔL, Δa, and Δb) indicate how much a standard and sample differ from one another in L, a, and b, and are often used for quality control or formula adjustment.

Tolerances may be set for the delta values. Delta values that are out of tolerance indicate that there is great difference between the standard and sample.

L scale: Light vs. dark, where a low number (0-50) indicates dark and a high number (51-100) indicates light.
a scale: Red vs. green, where a positive number indicates red and a negative number indicates green.
b scale: Yellow vs. blue, where a positive number indicates yellow and a negative number indicates blue.

The overall change in color indices because of aging was expressed as E according to the following formula (George, 1995):

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

Where $\Delta L^* = L^*_{sample} - L^*_{standard}$

3 Results and Discussion

3.1 Cellulosic Fiber Deterioration by Fungi

Fig. 4 shows the biodeterioration of cellulosic fibers caused by *E. chevalievi,* which has the ability to grow on cellulose fibers and produce enzymes that can degrade cellulosic fibers. Fig. 4A shows the growth of *E. chevalievi* over the fiber structure. Fig. 4B shows the occurrence of degraded cellulose fibers caused by the fungi and demonstrates spores inside the fibers, whereas hyphae cover the surface. Fig. 4C shows the deterioration of fibers with a high number of spores inside the degraded fibers, and the presence of spores inside the degraded fibers.

Fig. 5 shows biodeterioration of the cellulosic fibers caused by *P. roqueforti,* which has the ability to grow on cellulose fibers and produce enzymes that can degrade the cellulosic fibers. Fig. 5A shows the growth of *P. roqueforti* around and over the fiber structures. Fig. 5B shows the occurrence of cavity holes in the fiber caused by the fungal hyphae and the evidence of hyphae inside the fiber. Fig. 5C shows a clear increase in fiber damage and the presence of hyphae and spores inside the degraded fibers.

Fig. 6 shows biodeterioration of cellulosic fibers caused by *T. viride,* which has the ability to grow on cellulose fibers and produce enzymes that can degrade the cellulosic fibers. Fig. 6A shows the growth of *T. viride* covering the fiber structure. Fig. 6B shows the clear increase in fiber damage and the presence of hyphae and spores on the fiber. Fig. 6C shows the occurrence of cavity holes in the fibers caused by the fungal hyphae and obvious mycelia, which cover and penetrate the fibers.

Fig. 7 shows the biodeterioration of cellulosic fibers caused by *A. flavus,* which has the ability to grow on cellulose fibers and produce enzymes that can degrade the cellulosic fibers. Fig. 7A shows the growth of *A. flavus* on the fiber structure. Fig. 7B shows the occurrence of an opening structure in the fiber caused by the fungal hyphae and evidence of hyphae around the fiber. Fig. 7C shows the clear increase in fiber damage and the presence of hyphae around the degraded fibers and a swelling-like appearance in the fibers.

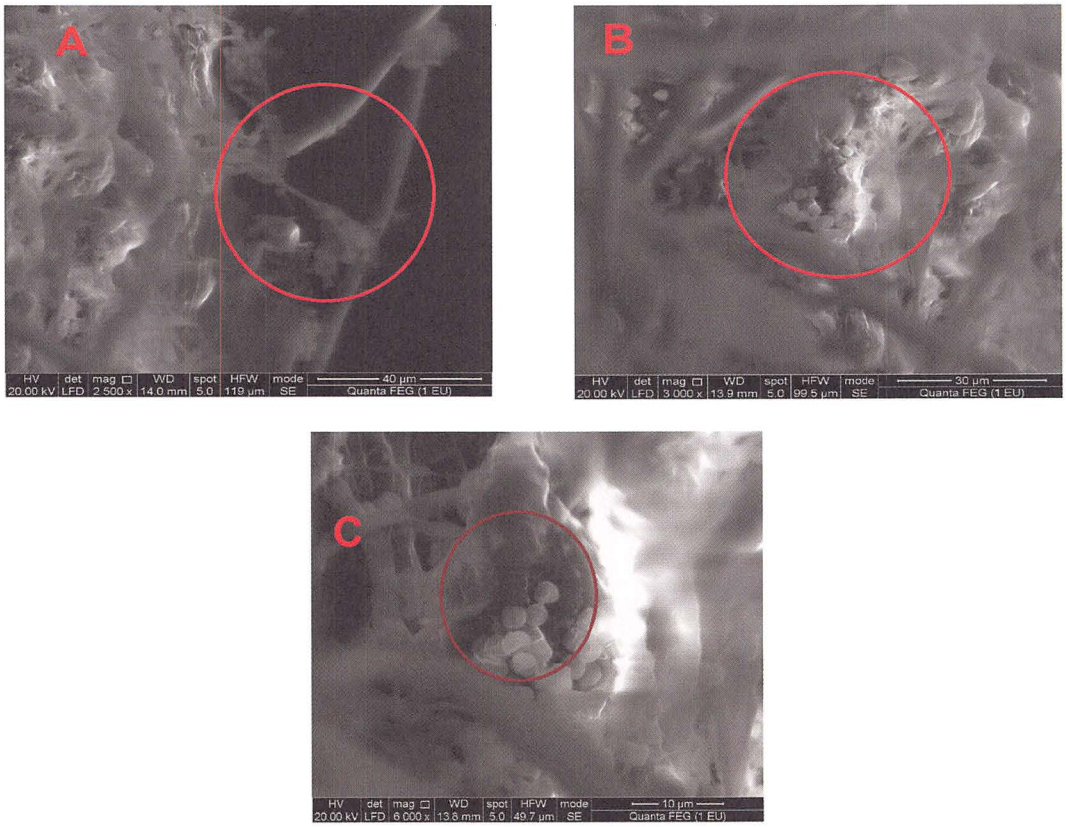

Fig. 4 Deterioration of cellulose fibers by *Eurotium chevalievi*

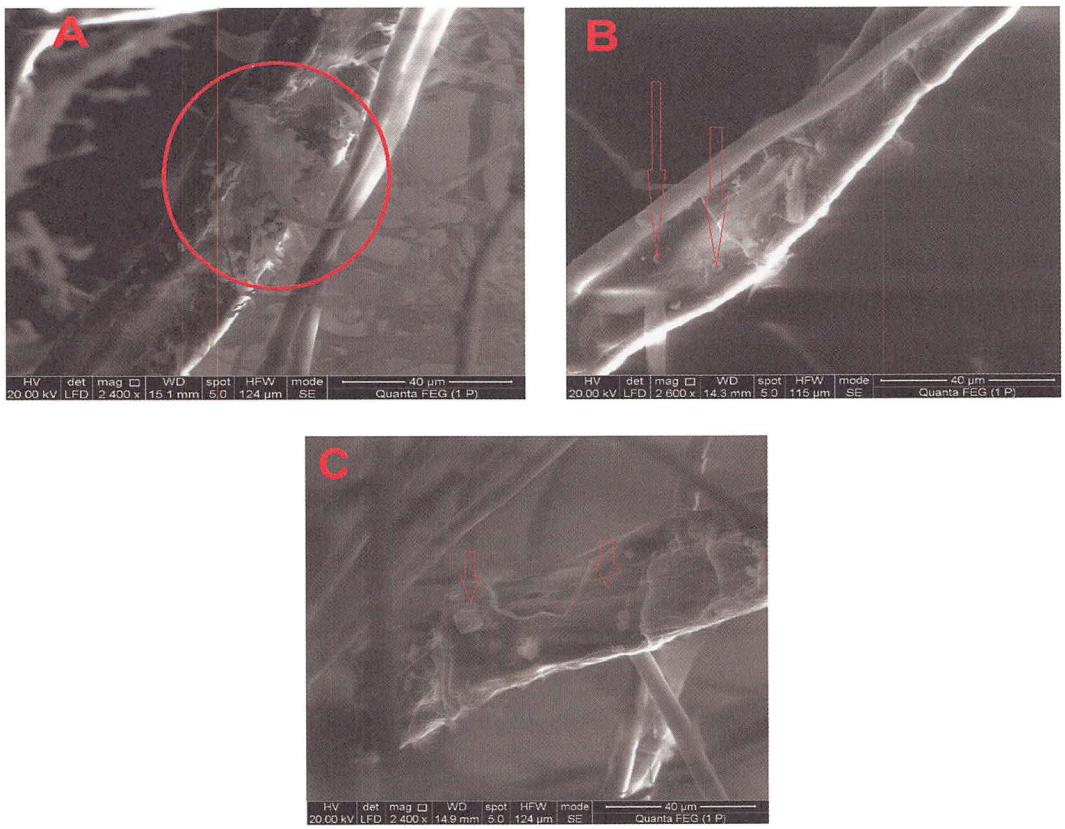

Fig. 5 Deterioration of cellulose fibers by *Penicillium roqueforti*

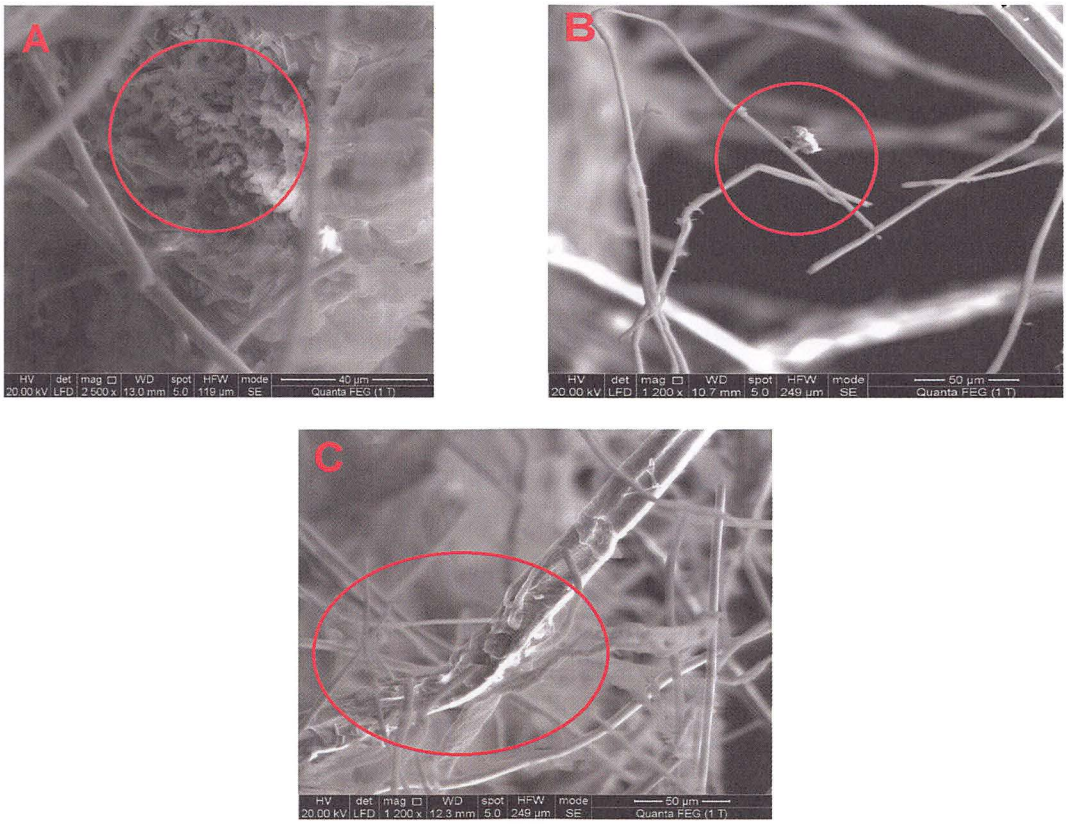

Fig. 6 Deterioration of cellulose fibers by *Trichoderma viride*

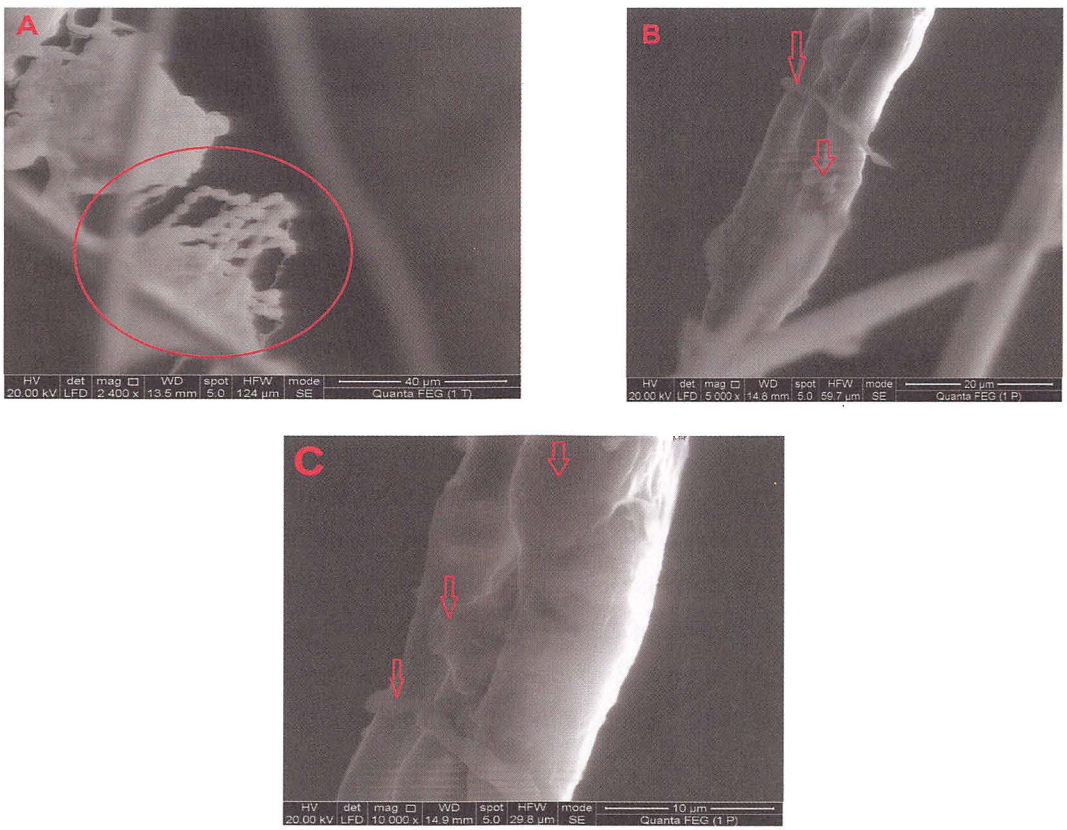

Fig. 7 Deterioration of cellulose fibers by *Aspergillus flavus*

The fungal species tested here showed different growth patterns on the paper samples. Cavity formation inside wood cell walls was probably because of enzymatic attack beneath the hyphae (Anagnost et al., 1994; Schwarze and Fink, 1998). Reis-Menezes et al. (2011) showed the entry of *Cladosporium* sp. hyphae through one of the pores in a calcium carbonate layer over the cellulose of some types of paper, suggesting that they are searching for cellulose as a nutrient.

Fungi produce mycelia, conidia and ascospores to utilize the substrate efficiently, when the paper type is favorable (Pinzari et al., 2006). *Trichoderma* sp. are classified as mesophilic organisms with a low xerotolerance (Lupo et al., 2002; Kredics et al., 2003). *Trichoderma* sp. can grow rapidly on natural and artificial substrates resulting in the breakdown of polysaccharides and destruction of cell wall integrity (Van-Wyk and Mohulatsi, 2003). *Penicillium* sp. are known for their ability to produce extracellular enzymes including cellulase (Krogh et al., 2004). In addition, some *Penicillium* sp. can degrade pectin, whereas a greater number of species can degrade xylan (Yoon et al., 2007). The production of xylanase enzymes by *Aspergillus* spp. has been studied by de Vries and Visser (2001); hydrolytic enzymes include endo-1, 4-β-glucanases; exo-1, 4-β-glucanase, and 1, 4-β-glucosidases for cellulose hydrolysis; and endo-1, 4-β-xylanases and β-xylosidases for hemicellulose hydrolysis (Biely and Tenkanen, 1998).

A. flavus has already been isolated from paper materials, and can colonize paper (Nyuksha, 1994a, b; Zyska, 1997), which is often present on other biodeteriorated objects of different materials such as handmade textiles (Montegut et al., 1991).

3.2 Color Change

Table 1 presents the changes in color of the test papers (white, red, and black) treated with tea tree and thyme oils as, colonized by four common fungi (*T. viride, P. roqueforti, E. chevalievi,* and *A. flavus*).

In treated papers (white, red, and black) with tea tree oil and inoculated with each of the four fungi (Table 1), the results showed that the lowest ΔE value for *A. flavus* was 1.95, which was found on the white and red papers, followed by black paper inoculated with *A. flavus* (ΔE 2.25). The largest color change (ΔE 18.35) was observed for the red paper inoculated with *P. roqueforti,* and white paper (ΔE 18.33) followed by black paper inoculated with *T. viride* (ΔE 12.69). Furthermore, the white papers inoculated with *E. chevalievi* and *T. viride* showed changes in colors with ΔE values of 7.70, and 7.40, respectively, whereas red papers had ΔE values of 7.79, and 7.57, respectively.

In the treated papers (white, red, and black) with thyme oil and inoculated with *T. viride, P. roqueforti, E. chevalievi,* and *A. flavus* (Table 1), *E. chevalievi* grew least on the black paper with an ΔE value of 2.18 followed by *T. viride,* where the ΔE was 2.90. The results obtained from the calculated ΔE initially showed that *T. viride* was the fungus that caused the greatest color change in the papers (red color treated with thyme oil), with an ΔE value of 39.17, which is expected because it is a dematiaceous mold. Moreover, the white papers inoculated with *P. roqueforti* and *E. chevalievi* showed changes in colors with ΔE values of 7.76, and 6.83, respectively, whereas the red paper had an ΔE value of 7.02 when inoculated with *E. chevalievi.*

Treatment	ΔL	Δa	Δb	ΔE^*
Control white Tea tree oil	0.0	0.0	0.0	0.0
Trichoderma viride	−5.66	−4.46	−2.97	7.40
Penicillium roqueforti	0.60	−13.48	−12.44	18.33
Eurotium chevalievi	−0.16	−5.40	−5.45	7.70
Aspergillus flavus	1.27	−0.97	−1.13	1.95
Control Red Tea tree oil	0.0	0.0	0.0	0.0
Trichoderma viride	−5.42	−4.47	−2.53	7.57
Penicillium roqueforti	0.60	−13.48	−12.44	18.35
Eurotium chevalievi	−0.16	−5.40	−5.48	7.79
Aspergillus flavus	1.27	−0.97	−1.13	1.95
Control Black Tea tree oil	0.0	0.0	0.0	0.0
Trichoderma viride	−12.61	1.03	1.01	12.69
Penicillium roqueforti	−4.00	0.92	3.50	5.39
Eurotium chevalievi	−5.66	1.14	0.48	5.79
Aspergillus flavus	−1.35	0.48	1.73	2.25
Control white Thyme oil	0.0	0.0	0.0	0.0
Trichoderma viride	−2.66	0.87	−3.73	4.63
Penicillium roqueforti	−6.09	0.27	−4.76	7.76
Eurotium chevalievi	−6.15	1.30	−2.68	6.83
Aspergillus flavus	−3.36	0.44	−4.80	5.88
Control Red Thyme oil	0.0	0.0	0.0	0.0
Trichoderma viride	−8.83	−32.16	−20.55	39.17
Penicillium roqueforti	2.61	−2.63	−2.82	4.67
Eurotium chevalievi	1.33	−4.61	−5.12	7.02
Aspergillus flavus	1.22	−2.74	−2.67	4.01
Control Black Thyme oil	0.0	0.0	0.0	0.0
Trichoderma viride	−1.42	0.33	2.51	2.90
Penicillium roqueforti	−6.15	0.96	1.48	6.86
Eurotium chevalievi	−0.56	0.88	1.91	2.18
Aspergillus flavus	−3.82	0.17	2.08	4.45

*ΔE: total color difference

Table 1 The chromatic alteration of papers treated with tea tree and thyme oils after colonization with four common fungi

Overall, the values of ΔE shown in Table 1 refer to the fungal growth. The smallest fungal growth (*A. flavus*) was found on the white and red papers treated with tea tree oil, with a ΔE value of 1.95, while the highest value (ΔE 39.17) occurred by *T. viride* on red paper treated with thyme oil. The greater value in color changes (ΔE) between the control and inoculated samples of the same species in the same type of paper presented the highest fungal growth on the paper (Reis-Menezes et al., 2011).

From the ESEM examination and chromatic alteration of the inoculated papers with four fungi, *T. viride* appears to be the most destructive fungus for the tested papers and *A. flavus* the least destructive.

Paper texture can also be affected by degradation of cellulose fibers (Reis-Menezes et al., 2011). Saprophytic soil fungi (such as *Aspergillus*) and those that continue the biodeterioration process, which are specialized in degrading cellulose are the group most likely to start the deterioration process in books (Szczepanowska, 1986). In contrast, *Trichoderma* species are cellulolytic fungi and are responsible for a substantial part of damage caused to paper items (Szczepanowska, 1986).

The paper surface can be affected by a range of pigments produced and excreted by these fungi, which cause changes in the colors. Pigments formed during fungal metabolism, are composed of complex chemical substances which are present in the spores and mycelia, and are secreted by fungal cells (Szczepanowska and Lovett, 1992).

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