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Deterioration of different paper types by fungi isolated from Cairo University´s old library

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Abstract

Biodeterioration of library materials is a worldwide problem due to presence of huge numbers of libraries allover the world. The current research aimed to study the paper deterioration by fungiisolated from Cairo University´s old library, Egypt. The most ten frequent paper deteriorating species were used. They were *Aspergillusflavus*, *A. niger*, *A. parvisclerotigenus*,*A.oryzae, A. tubingensis, Cladosporiumcladosporioides*, *Penicillium commune*, *P. crustosum*, *P. digitatum* and *P. italicum*. Artificialinfestation of 4 paper types by the ten fungal species indicating variation in utilization ability depending on paper type (papyrus followed by newspaper, printer and then whatmanfilterpaper) and on fungal species. Determination of remaining cellulose value after paper infestation and fungal growth indicated that *A. flavus* was the highest cellulose utilizable in Whatman filter paper, *A. niger* for Papyrus paper, *A. oryzae* for newspaper and *P. commune* for printer papers. Atomic force microscope (AFM) was used in case of *P. commune* infesting the 4 paper types. The images indicated reduction in surface roughness of all paper types with Whatman paper being the most affected one. AFM indicated also disruption in the normal system of infested paper surface which appeared with many peaks compared to one peak in normal paper.

Key words: Archive Books; Paper Deterioration;Cellulytic Fung;, Atomic Force Microscope.

1. Introduction

The archive and libraries in Cairo University hold a vast of documents and historical books since its foundation in 1908. The past maintenance and management conditions of some of the library collections were not suitable and documents are now susceptible to biodeterioration by many microorganisms. Tropical countries like Egypt with high relative humidity and high temperature have environmental conditions that encourage the development of microorganisms in libraries and archives.

For several centuries, paper was the main material for storing cultural heritage in museums and libraries all over the world **[1]**. The conservation of paper-based artifacts from deterioration poses a serious problem for several archives, libraries and museums **[2]**.

The action of fungi on documents, books and maps leads to great cultural losses **[3].** Cellulose degradation and secondary metabolites production by fungi are the main reasonsfor paper deterioration **[1]**.The organic nature of the paper makes it suffer from biodeterioration by fungi which are daily contaminating the paper collection **[4]**. Infestation is caused by unsuitable storage and display conditions, which allow conidia and spores in aerosols to fall on damp and dirty papers **[5, 6].** Furthermore, the restoration of cultural objects was made of many types of organic materials and hygroscopic compounds which support mold growth such as egg yolk, casein, poppy seed, hemp seed oil, Chinese wood oil, resins and rabbit skin glue. Décors made of

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textile, straw, clay, natural feathers or hairs are also supportive to microbial growth **[7]**.

Paper is a discontinuous artificial system consists of cellulose fibers and additional components differ according to the papermaking process, fiber type and the historical period in which it was produced **[8]**. Cellulose is the most abundant natural polymer on earth. It is a linear polymers of β-D-glucose in the pyranose form linked together by 1,4-glucosidic bonds. The degradation of cellulose based paper is important especially in archives and museums. During the degradation two main reactions occur, hydrolysis of glucosidic bonds and oxidation of glucopyranose rings **[9]**.

Historical and modern libraries include collections of historical books, periodicals, maps, atlases, newspapers, photographic and painting pictures, in addition to audiovisual materials including audio and optical disks **[10, 11].** Each object is affected differently by a wide variety of microorganisms. Fungal deterioration of paper damages historical records resulting in the loss of valuable information. However, this biodegradation is very important as a recycling process **[12]**.

Some fungi involved in the deterioration of library books may be harmful to library professionals and users, due to the production of mycotoxins which cause several diseases **[3, 13, 14]**.

Compactus shelving is one of the systems utilized for the preservation of library materials because it permits for more effective use of space and protection against dust deposition **[15]**. However compactus shelves can be problematic when used in maintenance environments missing effective climate control systems **[16]**. Resistance to heat and humidity exchange between the micro-environment within the compactus units and the external environment can cause danger to kept objects, particularly when these objects are consist of hygroscopic materials. International Federation of Library Associations (IFLA) commends 50-60 % relative humidity and 18-20 ºC air temperature for efficient conservation of documents like books and periodicals in archives and libraries. In practice, it is hard to preserve a stable temperature and relative humidity level even with the use of air condition **[15]**.

The Atomic Force Microscope (AFM) has been used as a mean for measuring roughness for plant materials **[17]**. AFM provides surface topography of biomaterials through three-dimensional (3D) information regarding surface morphology and mechanical properties without the need for pretreatment**[18]**. AFM is important for studying surface roughness (Ra) or average deviation at the nanoscale, having resolution far exceeding that of other optical based methods **[19].**

The main objective of the current study was to study the paper deterioration by fungi isolated from Cairo University´s old library.

2. Materials and Methods

2.1. Artificial infestation of paper material

Ten fungal species, previously isolated from deteriorated books located in Cairo University old library, were used to inoculate different paper types and kept at controlled temperature (27 ºC) and humidity (100%), in order to verify the actual capability of these isolated species to grow and deteriorate paper material **[20]**.The fungal species were *Aspergillusflavus*, *A. niger*, *A. parvisclerotigenus*,*Aspergillusoryzae, A. tubingensis, Cladosporiumcladosporioides*, *Penicillium commune*, *P. crustosum*, *P. digitatum* and *P. italicum*.In this experiment, the accelerated mold growth test was carried out to detect the ability of the fungi to use the paper components as a single source of nutrients. The nutrient source for the mold growth was only the cellulose and additives contained in each type of paper **[21]**.

2.1.1. Tested Paper types

Four paper types with different composition were used in this study. The first type was filter paper Whatman No. 1 (made of pure cellulose). Whatman paper is considered as a standard model **[22]**. The second type was Papyrus paper used by ancient Egyptians in writing and drawings. The third type was Newspaper taken from the Egyptian Newspaper. The fourth was printer paper A4, 75 grams (a product from PT. Indah Kiat Pulp & Paper Tbk).

2.1.2. Preparation of samples

- **Paper samples**

The paper samples were cut into uniform squares of 4 cm^2. Then, they were sterilized in an autoclave to eliminate airborne fungal and bacterial cells from the surface prior to inoculation. After that, they were placed in humid sterilized chambers. The humid plates (Figure 1) used in these tests consisted of large sterile Petri dishes contain small sterile petri dishes filled with sterile distilled water to obtain relative humidity of 100%.

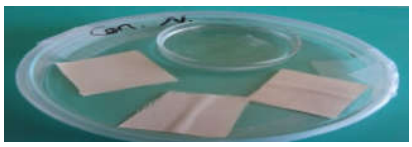

Figure (1): Humid plate

- **Inocula**

Spore suspensions were prepared from the ten tested fungal species by scraping the surface of 7-days-old cultures with a sterile loop in a 0.02% sterile Tween 80 and distilled water, then some washing and centrifugation steps were done to remove mycelia. A defined volume (2 ml) of each spore suspension was diluted with nutritive Sabouraud Broth (g l^{-1}: D-Glucose (dextrose) 40.0; Mycological Peptone10.0 and pH at 5.6 ± 0.2), in order to inoculate the paper squares. Each paper square was inoculated with 100 µl of spore suspension. For control samples, paper squares were inoculated with 100 µl of nutritive broth. The inocula were placed in the center of the paper samples using a micropipette. Three replicates for each treatment were used. Humid plates were kept in a thermostatically controlled incubator at 27ºC for 28 days. Inoculations were done within a laminar flow cabinet to apply aseptic conditions.

2.2. Examination of fungal growth on the paper samples

After 10 days in the humid plate, the fungal growth level on the paper samples (control and inoculated) was evaluated by simple visual examination and was indicated by plus or minus, ranging from ‘‘-ve’’ to ‘‘5+ve’’ according to the growth intensity; ‘‘5+ve’’ corresponding to a high sporulation and ‘‘-ve’’ to the absence of growth.

2.3. Quantitative determination of cellulose in paper samples

Updegraff [23] used simple and fast colorimetric method for determination of cellulose. It was based on sedimentation of cellulose by removing lignin, xylosans and hemicellulose with acetic acid/nitric acid reagent. Cellulose was then dissolved in 67 % H_2SO_4 and detected by the anthrone method of **Scott and Melvin [24]**. This method gives quantitative recovery of purified cellulose from paper. The acetic/nitric reagent was prepared by mixing 150 ml 80 % acetic acid and 15 ml concentrated nitric acid. The anthrone reagent was prepared by dissolving 0.2 gram anthrone in 100 ml concentrated H_2SO_4 which prepared fresh daily. Chill about two hours in refrigerator prior to use. To plot a standard curve, stock solution was prepared by dissolving 50 mg pure cellulose, dried for 6 hours at 105°C and cooled over anhydrous alumina, in 10 ml 67% H_2SO_4 (v/v) with gentle heat. This was diluted to 500 ml with distilled water to contain 100 µg cellulose/ml. Then, dilutions of stock solution (50, 100, 150, 200 and 250 µg cellulose/ml) were analyzedat wave length of 620 nm using anthrone reagent.After 28 days incubation of papers inoculated with different fungal spore suspensions, the remaining cellulose in all paper types (control and inoculated) were determined as follow: To each 50 mg paper sample, 3 ml acetic/nitric reagent was added gradually. One ml was added firstly and mixed well on vortex mixer, then adding the remaining 2 ml and remixing. Tubes were then placed in boiling water bath for 30 minutes with marble cover to reduce evaporation. Water bath level was maintained at same level as the liquid in the tubes. Centrifugation for 5 minutes at 5000 rpm was done then the supernatants were discarded. Distilled water (10 ml) was added gradually in a manner similar to acetic/nitric reagent. Again, centrifugation for 5 minutes at 5000 rpm was done and the supernatants were discarded. Similar to the manner of acetic/nitric reagent, 10 ml 67 % H_2SO_4 was added. Tubes were let stand for one hour then 1 ml from each tube was diluted to 50 ml by distilled water. After that, 1 ml of this dilution was placed in tube and 4 ml distilled water was added to obtain final volumes up to 5 ml. Tubes were placed in an ice bath to cool. To each tube, 10 ml of cold anthrone reagent were added by layering with pipet. Then, they were mixed well on vortex mixer – a parafilm cover was suitable to avoid splashing. Tubes were returned to ice bath until all tubes were mixed. Tubes were let stand at room temperature for 5-10 minutes. Optical densities were read on a spectrophotometer in glass cells at wave length of 620 nm against a reagent blank.

2.4. Topographic examination of cellulose in paper samples by atomic force microscope

Before being observed with AFM, artificially deteriorated samples were cleaned from spores and aerial fungal mycelium by a soft brush used for paper restoration under a biological hazard cabin to be able to document the paper’s surface following fungal spoilage. The atomic force microscope (AFM) had been used to detect the decomposition of cellulose fibers caused by *Penicilliumcommune*, as a model, in the four types of paper. Images gained from the analysis of artificially deteriorated samples had been used for a comparison with topographies obtained from control samples. AFM provides both qualitative and semi-quantitative information on paper deterioration and aging **[25]**.The model of AFM used in this study was: Wet - SPM (Scanning Probe microscope), Shimadzu, Japan, present inAtomic Force Lab., Micro Analytical Center, Faculty of Science, Cairo University, Egypt. The topographies were be visualized as “False colour scale” images. The whole experiment was carried out in non-contact mode. Paper samples were 1 cm × 1 cm. AFM images had been performed using 10 × 10 μm^2 frames. Quantification of degradation effects can be estimated through “surface roughness” parameter. Local surface roughness is defined, on each 10 × 10 μm^2 frame, as the root mean square deviation of the surface height, from its average value.

Sample surface roughness was here meant as the average, amongst all measured areas of the same sample, of the local roughness values.

2.5. Statistical analyses

The results were expressed as the mean ± standard deviations (mean±SD). Data were analyzed by using SPSS statistical program, version, 16. Statistical analysis of experiments was performed using Student's t test, where *p*-values ≤ 0.05 were considered significant.

Table (1): Visual examination of fungal growth levels on different paper types

Fungal species	Paper types			
	Whatman No.1	Newspaper	Papyrus	Printer
Control (not infested)	-	-	-	-
Aspergillus flavus	+	+	++	+++
Aspergillus niger	+++	+++	+++	+++
Aspergillus parvisclerotigenus	++	++	++	+++
Aspergillus oryzae	+++	+++	+++	+++
Aspergillus tubingensis	+++	++	+++	++
Cladosporiumcladosporioides	++++	++++	++++	++++
Penicillium commune	++	+++	+++	++
Penicilliumcrustosum	+++	++	+++	++
Penicilliumdigitatum	+++	+++++	++++	++++
Penicilliumitalicum	+++	+++	++++	++

Data were recorded after 10 days of inoculation.

(-) Absence of growth, (+) Very low growth, (++) Low growth, (+++) Moderate growth, (++++) Good growth and (+++++) High growth.

3. Results

3.1. Examination of fungal growth on the paper samples.

All fungal species can grow and utilize the cellulose in the four types of paper but with different levels (Table 1 & Figures 2-5). The most utilizable paper type with high fungal growth was Papyrus paper followed by newspaper and printer paper. Whatman filter paper was the least utilizable and supported poor fungal growth. The tested fungal species showed different growth capabilities on the paper samples. *Cladosporiumcladosporioides*and *Penicilliumdigitatum* grew with the highest level. *Aspergillus niger*, *Aspergillus oryzae*and *Penicilliumitalicum* came next in the growth level. Intermediate growth level was detected by *Aspergillus tubingensis*, *Penicillium commune* and *Penicilliumcrustosum* then *Aspergillus parvisclerotigenus*. The least growth level was observed for *Aspergillus flavus*.

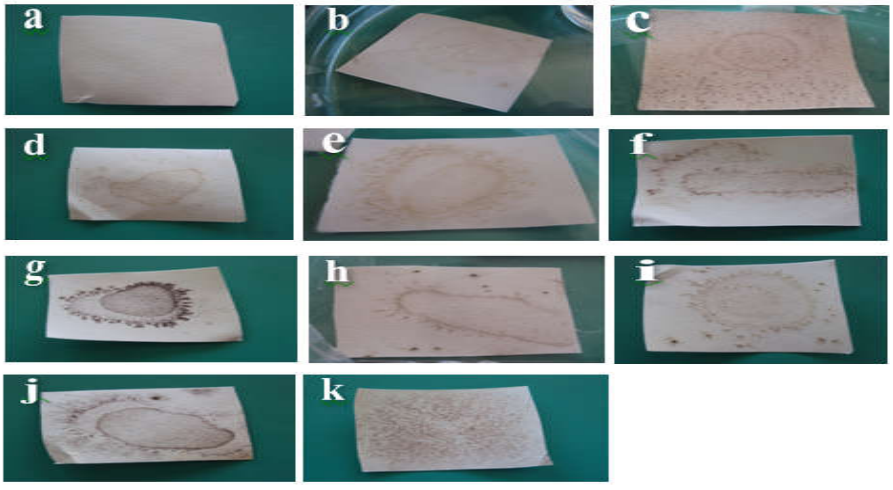

Figure (2): Fungal growth on infested Whatmanfilter paper. a) Control, b) *Aspergillus flavus*, c)*Aspergillus niger*, d) *Aspergillus parvisclerotigenus*, e) *Aspergillus oryzae*, f) *Aspergillus tubingensis*, g) *Cladosporiumcladosporioides*, h) *Penicillium commune*, i) *Penicilliumcrustosum*, j) *Penicilliumdigitatum* and k) *Penicilliumitalicum*.

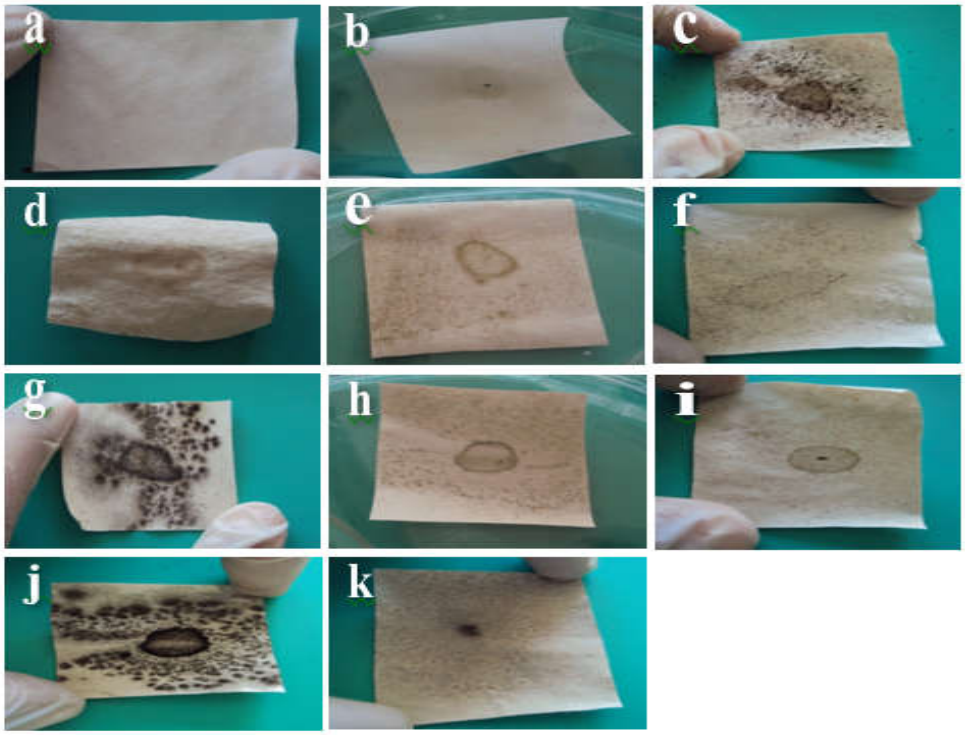

Figure (3): Fungal growth on infested newspaper. a) Control, b) *Aspergillus flavus*, c)*Aspergillus niger*, d) *Aspergillus parvisclerotigenus*, e) *Aspergillus oryzae*, f) *Aspergillus tubingensis*, g) *Cladosporiumcladosporioides*, h) *Penicillium commune*, i) *Penicilliumcrustosum*, j) *Penicilliumdigitatum* and k) *Penicilliumitalicum*.

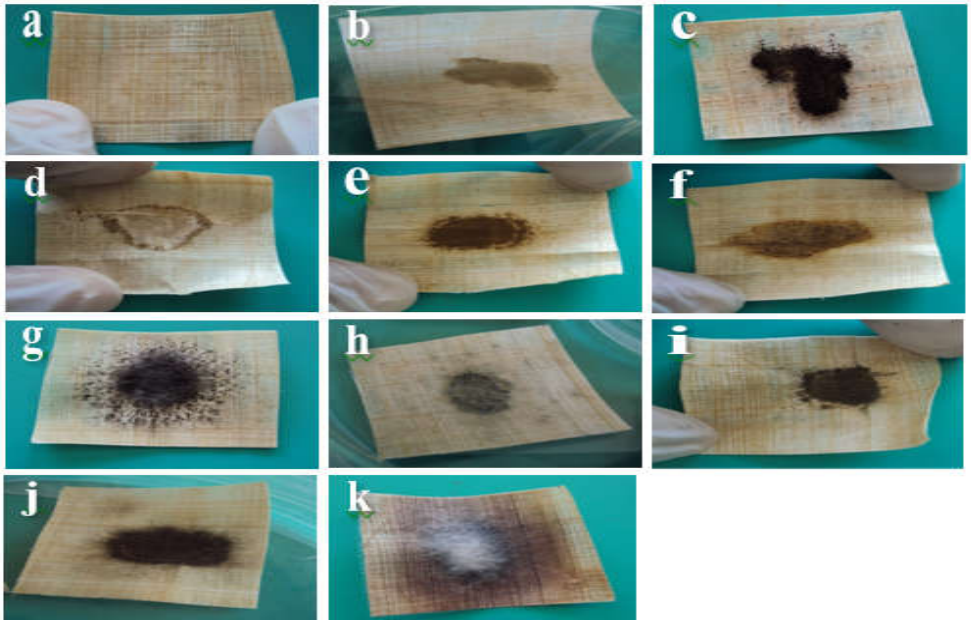

Figure (4): Fungal growth on infested Papyrus paper. a) Control, b) *Aspergillus flavus*, c)*Aspergillus niger*, d) *Aspergillus parvisclerotigenus*, e) *Aspergillus oryzae*, f) *Aspergillus tubingensis*, g) *Cladosporiumcladosporioides*, h) *Penicillium commune*, i) *Penicilliumcrustosum*, j) *Penicilliumdigitatum* and k) *Penicilliumitalicum*.

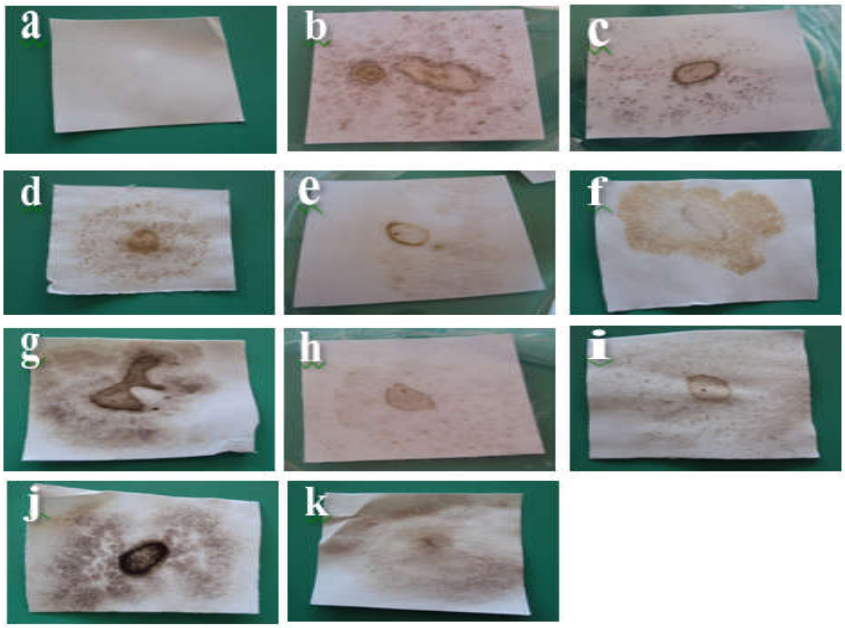

Figure (5): Fungal growth on infested printer paper. a) Control, b) *Aspergillus flavus*, c)*Aspergillus niger*, d) *Aspergillus parvisclerotigenus*, e) *Aspergillus oryzae*, f) *Aspergillus tubingensis*, g) *Cladosporiumcladosporioides*, h) *Penicillium commune*, i) *Penicilliumcrustosum*, j) *Penicilliumdigitatum* and k) *Penicilliumitalicum*.

Table (2): Remaining cellulose concentration and percent of hydrolysis in Whatman filter paper after artificial infestation by the ten fungal species

Fungal species	Remaining cellulose concentration (µg/ml) (Mean ± SD)	% of cellulose hydrolysis	Significance
Control (not infested)	58.26 ± 0.20	0.00	
A. flavus	4.58 ± 1.22	92.15	***
P. italicum	9.35 ± 2.14	83.94	***
A. oryzae	17.08 ± 0.53	70.68	***
P. commune	31.31 ± 2.03	46.25	**
C. cladosporioides	31.92 ± 3.13	45.20	**
A. tubingensis	32.53 ± 3.84	44.15	**
A. niger	34.47 ± 3.05	40.84	**
P. crustosum	40.46 ± 2.47	30.54	**
A. parvisclerotigenus	46.77 ± 0.18	19.72	*
P. digitatum	57.34 ± 3.05	1.57	ns

Data were recorded after 28 days of incubation. **ns:** non significance.

3.2. Quantitative determination of cellulose in paper samples.

It was found that the three fungal species *Aspergillus flavus*, *Aspergillus oryzae*and *Penicilliumitalicum*hydrolyzed cellulose from Whatman filter paper with high significant difference from control samples. The reduction in cellulose concentration of control recorded 92.15 %, 70.68 % and 83.94 %, respectively. The species *Aspergillus niger, Aspergillus tubingensis*,*Cladosporiumcladosporioides*,*Penicillium commune* and*Penicilliumcrustosum* came in the next rank in cellulose degradation fromWhatman filter paper with 40.84 %, 44.15 %, 45.26 %. 46.25 % and 30.54 % reduction in cellulose of control value. *Aspergillus parvisclerotigenus* hydrolysed cellulose of Whatman filter paper with low significant difference where 1.57 % reduction of control was detected. *Penicilliumdigitatum* showed non significant difference in decomposition of cellulose of Whatman filter paper (Table 2).

Aspergillus oryzae recorded the highest significant cellulose hydrolysis (76.47 % of control) after 28 days of incubation on newspaper. *Aspergillus niger*,*Penicillium commune* and *Penicilliumcrustosum* hydrolysed cellulose of newspaper with moderate rate, where the percentage decreases of control were 45.75 %, 50.33 % and 47.06 %, respectively. *Aspergillus flavus*,*Penicilliumdigitatum* and *Penicilliumitalicum* degraded the cellulose of the newspaper samples in lower rates, with 34.64 %, 28.11 % and 30.72 % decrease of control, respectively. The data also revealed non significant differences in the remaining cellulose concentration of newspaper samples after artificial infestation with species of *Aspergillus parvisclerotigenus*, *Aspergillus tubingensis* and *Cladosporiumcladosporioides* (Table 3).

Table (3): Remaining cellulose concentration and percent of hydrolysis in newspaper after artificial infestation by the ten fungal species

Fungal species	Remaining cellulose concentration (µg/ml)			% of cellulose hydrolysis	Significance
	Mean	±	SD		
Control (not infested)	15.56	±	0.30	0.00	
A. oryzae	3.66	±	0.31	76.47	***
P. commune	7.73	±	1.07	50.33	**
P. crustosum	8.24	±	0.61	47.06	**
A. niger	8.44	±	0.64	45.75	**
A. flavus	10.17	±	1.61	34.64	*
P. italicum	10.78	±	1.07	30.72	*
P. digitatum	11.18	±	1.07	28.11	*
C. cladosporioides	13.52	±	1.68	13.07	ns
A. parvisclerotigenus	15.35	±	1.38	1.30	ns
A. tubingensis	15.45	±	0.18	0.65	ns

Data were recorded after 28 days of incubation. **ns:** non significance.

The data revealed that *Aspergillus niger*,*Cladosporiumcladosporioides*,*Penicillium commune* and *Penicilliumitalicum*hydrolysed cellulose of Papyrus paper with high rate, where 73.67 %, 71.15 %, 70.59 % and 67.79 % reduction of control were recorded, respectively. *Aspergillus tubingensis* came in the next rank in cellulose hydrolysis of Papyrus paper (51.82 % reduction of control).

The growth of *Aspergillus flavus*, *Aspergillus parvisclerotigenus*, *Aspergillus oryzae*,*Penicilliumcrustosum* and *Penicilliumdigitatum*on Papyrus paper samples led to low significant cellulose hydrolysis ranged from 19.05 % to 36.13 % (Table 4).

Table (4): Remaining cellulose concentration and percent of hydrolysis in Papyrus paper after artificial infestation by the ten fungal species

Fungal species	Remaining cellulose concentration (µg/ml)			% of cellulose hydrolysis	Significance
	Mean	±	SD		
Control (not infested)	36.30	±	0.10	0.00	
A. niger	9.56	±	1.54	73.67	***
C. cladosporioides	10.47	±	0.92	71.15	***
P. commune	10.68	±	1.53	70.59	***
P. italicum	11.69	±	1.07	67.79	***
A. tubingensis	17.49	±	2.59	51.82	**
P. crustosum	23.18	±	4.43	36.13	*
A. flavus	23.89	±	2.46	34.17	*
A. parvisclerotigenus	24.10	±	2.61	33.61	*
P. digitatum	28.29	±	4.92	22.06	*
A. oryzae	29.38	±	1.54	19.05	*

Data were recorded after 28 days of incubation.

It was found that *Aspergillus oryzae*,*Penicillium commune* and*Penicilliumcrustosum*were accompanied with the highest significant cellulose hydrolysis (63.06 %, 66.07 % and 62.16 %, respectively) when they were grown on printer paper samples. Lower cellulose hydrolyses were accompanied with the growth of each of the following fungal species: *Aspergillus flavus* (31.53 %), *Aspergillus niger* (33.03 %), *Aspergillus parvisclerotigenus* (35.68 %) and*Cladosporiumcladosporioides*(32.34 %)on Printer paper samples. *Penicilliumitalicum* utilized cellulose of printer paper samples with low significant hydrolysis rate, where 18.32 % cellulose reduction of control was recorded. *Aspergillus tubingensis* and*Penicilliumdigitatum* showed non significant differences in the cellulose concentrations of control after artificial inoculation on printer paper samples with 5.71 % and 0.90 % cellulose hydrolysis of control, respectively (Table 5).

Table (5): Remaining cellulose concentration and percent of hydrolysis in printer paper after artificial infestation by the ten fungal species

Fungal species	Remaining cellulose concentration (µg/ml)			% of cellulose hydrolysis	Significance
	Mean	±	SD		
Control (not infested)	33.86	±	0.20	0.00	
P. commune	11.49	±	0.93	66.07	***
A. oryzae	12.51	±	0.61	63.06	***
P. crustosum	12.81	±	0.61	62.16	***
A. parvisclerotigenus	21.78	±	5.08	35.68	**
A. niger	22.67	±	1.38	33.03	**
C. cladosporioides	22.88	±	0.92	32.43	**
A. flavus	23.18	±	0.93	31.53	**
P. italicum	27.65	±	1.27	18.32	*
A. tubingensis	31.92	±	1.68	5.71	ns
P. digitatum	33.55	±	0.53	0.90	ns

Data were recorded after 28 days of incubation. **ns:** non significance.

3.3. Topographic examination of cellulose in papers infested with *P. commune* using atomic force microscope.

AFM had been used to detect the decomposition of cellulose fibres caused by *Penicillium commune* infested the four papers types. This fungal species was selected because it proved to be an efficient biodeteriorating agent for the tested papers. Ultra fine topographic images obtained from fungal deteriorated samples had been used for a comparison with topographies obtained from control (not infested paper samples).

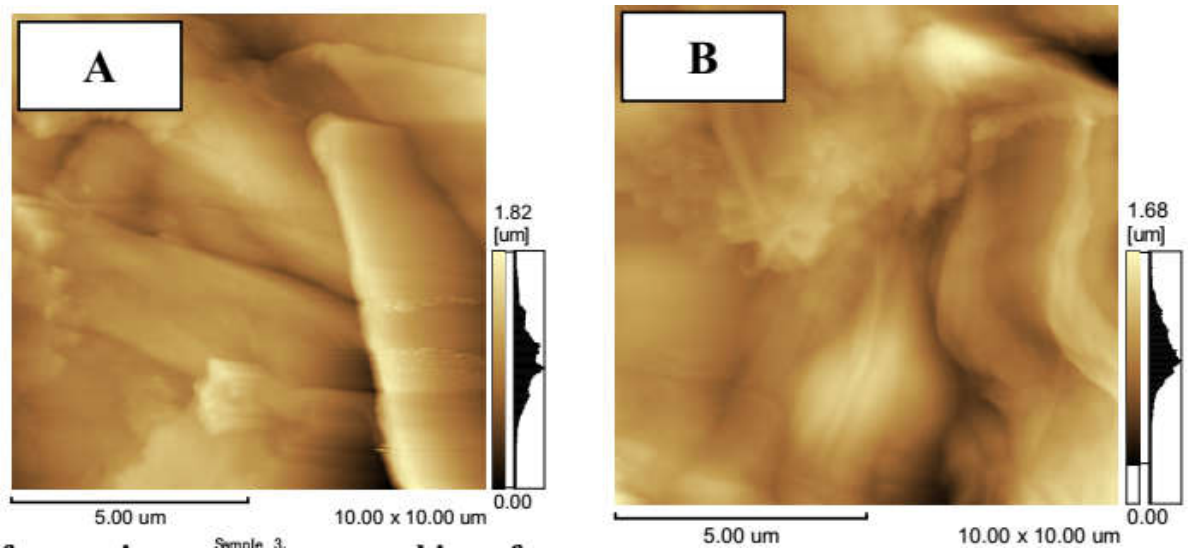

Figure (6): Atomic force microscope topographies of newspaper. A) Control. B) After artificial infestation by *Penicillium commune*.

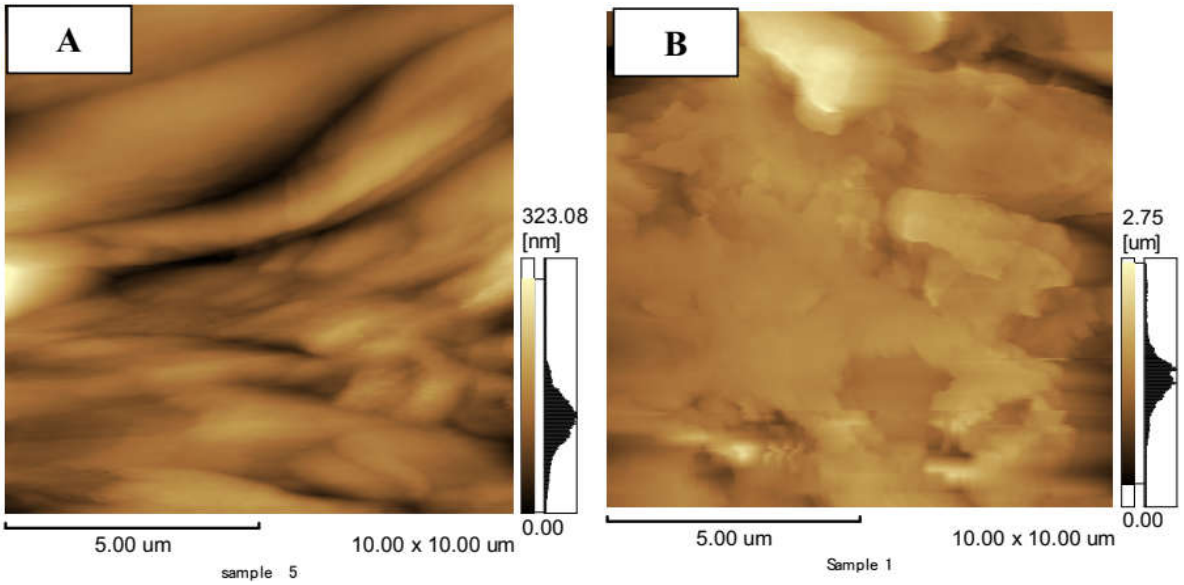

Figure (7): Atomic force microscope topographies of Papyrus paper. A) Control. B) After artificial infestation by *Penicillium commune*.

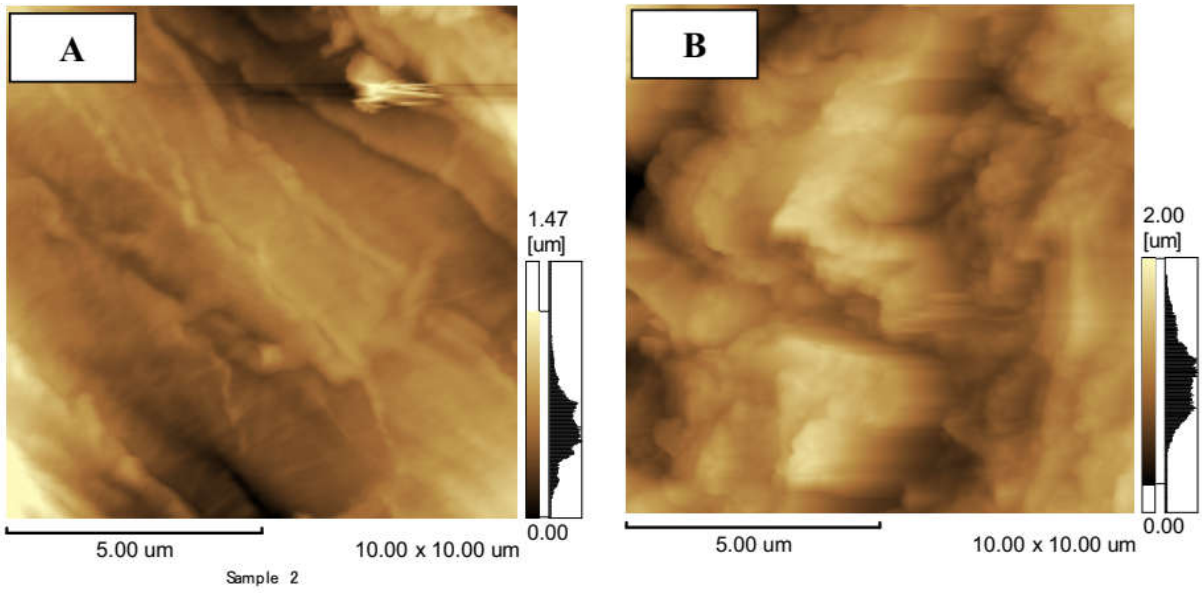

Figure (8): Atomic force microscope topographies of printer paper. A) Control. B) After artificial infestation by *Penicillium commune*.

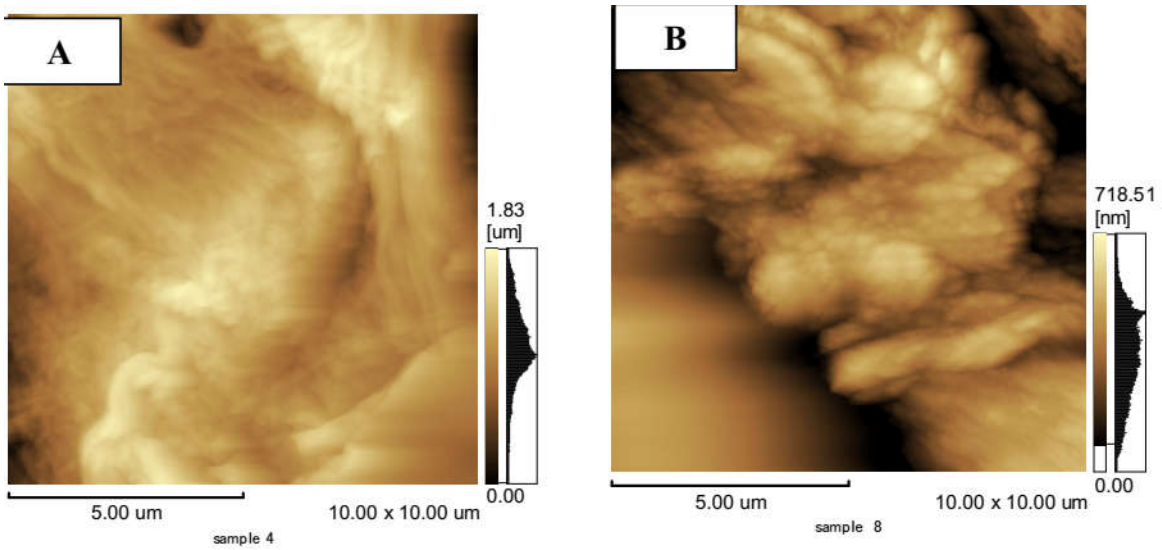

Figure (9): Atomic force microscope topographies of Whatman filter paper. A) Control. B) After artificial infestation by *Penicillium commune*.

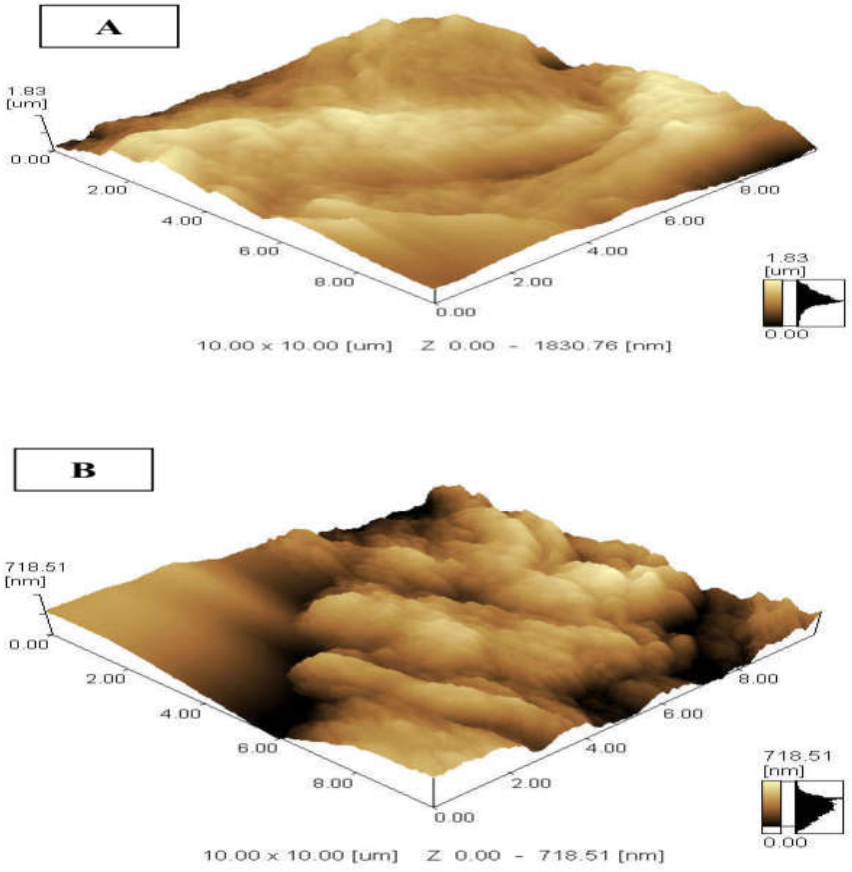

Figure (10): Atomic force microscope 3D topographical view of Whatman filter paper. A) Control. B) After artificial infestation by *Penicillium commune.*

Table (6): AFM quantitative estimation of surface roughness of the four paper types after artificial infestation by *P. commune* compared with control sample

Paper type	Surface roughness		Roughness decrease of control (%)
	Control	After deterioration by *P. commune*	
Whatman	2.21	1.02	53.85
Newspaper	2.32	1.78	23.28
Papyrus	2.09	1.78	14.83
Printer	1.82	1.63	10.44

The data of AFM topographies (Figures 6-9) showed distinct surface differences between the uninfested paper samples and the artificially deteriorated samples with *Penicillium commune*. These differences may be due to the cellulolytic activities or due to the acidic compounds produced by *P. commune* which affect greatly the cellulose fibers. Imaging of infested paper samples showed, generally, a non homogeneous decomposition of the fiber surface into fibril bunches.

The surface roughness is determined by the presence of one peak in the surface height distribution of the 3D topographies which is characteristic of paper samples. This system disrupted greatly in case of deteriorated paper of fungal infection and more than one peak appears in the 3D topographies (Figure 10 A, B).

Surface roughness is defined as the mean square (10 x 10 μm^2) deviation of the surface height, from its average value. Therefore, fibers in good conservation state will result in higher height deviation (high surface roughness) values than degraded fibers into fibril aggregates and smaller degradation compounds. As the surface roughness decreased, the cellulose biodeterioration increased.

The quantification of the cellulose degradation was evaluated by AFM by the surface roughness of the paper samples (Table 6). The data revealed a general reduction in the surface roughness of all paper types treated with *P. commune*. The highest reduction, however, was detected in Whatman paper with 53.85 % reduction of control. The least roughness reduction was observed in printer paper with 10.44 % reduction of control.

The data in table (7) and Figure (11, A, B) revealed high degradation in cellulose particles by *P. commune*. In the infested paper, the average of surface area (0.05 um^2) reduced by 96.73 % of control (1.53 um^2). The average mean radius of the cellulose particles also decreased from 0.62 um (control) to 0.09 um (infested paper) with percentage reduction of 85.48 %.

Table (7): Analysis of cellulose particle size of Whatman filter paper by AFM before and after artificial infestation by *Penicillium commune*

Parameters of particles	Control	After deterioration by *P. commune*	Reduction of control (%)
Average Surface roughness	2.21	1.02	53.85
Average Surface Area [um^2]	1.53	0.05	96.73
Average Mean Radius [um]	0.62	0.09	85.48

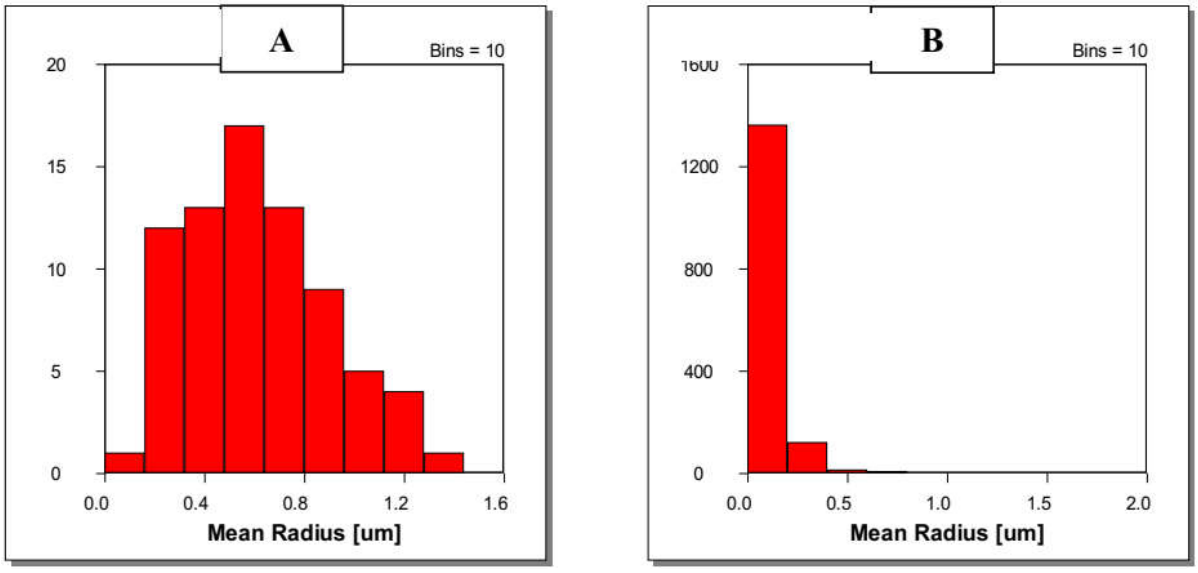

Figure (11): Mean radius of cellulose particles of Whatman filter paper, estimated by AFM. A) Control. B) After artificial infestation by *Penicillium commune*.

4. Discussion

Libraries and archives around the world suffer from biodeterioration of documents caused by microorganisms particularly fungi. By using conventional culture-dependent method, only a small amount of effectively colonizing organisms is identified. Preservation of cultural heritage is a probleme because of incomplete information of the deterioration agents **[22]**. Due to the fast reproduction and physiological activities of fungi, they are very adaptive to environmental conditions. Fungi are not only the main agents for

biodeterioration, they also are the main origin of allergic reactions, especially respiratory tract in susceptible users **[26]**. The study of degradation causes is of great significance to obtain an improved understanding of the mechanisms that leave objects of cultural heritage in a bad state of conservation.

Fungi cause damaging of important documents chemically, mechanically and aesthetically. They form hyphae, produce organic acids, secrete pigments leading to modification in the chemical and physical characters of the documents **[27].**

In the current work, all tested fungal species grew on the different paper types with various levels. The highest level was by*Cladosporiumcladosporioides*and *Penicilliumdigitatum* followed by *Aspergillus niger*, *Aspergillus oryzae*and *Penicilliumitalicum*, then by *Aspergillus tubingensis*, *Penicillium commune* and *Penicilliumcrustosum* then by *Aspergillus parvisclerotigenus*and finally by *Aspergillus flavus*. In close relation with current results, **Reis-Menezes*et al.*, [21]** infested 4 papers by different paper biodeteriorating fungi under 95% and 100% humidities without nutrients. The stereoscopic microscopy observation displayed that *Cladosporium* was the genus with highest capacity to grow on all papers studied followed by *Aspergillusniger*, *Trichodermaharzianum*and *Chaetomiumglobosum*. Relative humidity of 100% provided suitable condition for fungal growth. Growth slightly higher in the couché paper followed by Pólen (offset) and reference (cellulose). The recycled paper inhibited fungal growth because it has great variability in its make up. **Zotti*et al.*, [20]** infested whatman paper strips with 14 biodeteriorating fungi isolated from 18th century paper materials. All the 14 fungal species grow and developed mycelia. The time of growth and sporulation was variable, ranging from minimum (*A. flavus*, *P. restrictum*) to maximum with *Geomycespannorum*. **Pushalkar and Rao [28]** cultured fungal species *Aspergillusterreus*on a set of graded papers with known inorganic gradient (without any additive). The fungus grows and produces acidic metabolites and cellulolytic enzymes. It is also associated with library materials biodeterioration episodes **[29]**. The first reaction of paper deterioration is the hydrolytic degradation of cellulose molecules. Moisture plays an essential role with temperature and pH value. The second deteriorating process is the oxidative degradation of cellulose, mainly encouraged by the occurrence of oxygen in the environmental air. The third process is the thermal degradation of cellulose which concerns the breakage of chemical bonds. These processes depend on air pollution, biological attack and occurrence of bacteria and fungi in library rooms **[2, 12, 30, 31, 32].** Cellulolytic fungi decompose crystalline cellulose by producing combinations of cellulases that have different but complementary mode of action on the substrates **[33].**Cellobiohydrolase I (CBH I) is an exoglucanase that splits cellobiose units from nonreducing end of cellulose chain. Endoglucanase II (EG II) cleaves cellulose with chain, producing both cellobiose and glucose **[34]**. CBH I has been described to bind both crystalline and amorphous regions of cellulose, while endoglucanase (EG II) binds only the amorphous region **[35]**. Some paper deteriorating fungi exhibit strong cellulolytic activity such as *Alternaria*, *Botrytis*, *Chaetomium*, *Penicillium*, *Stemphylium*and*Trichoderma*; proteolytic activity such as *Aureobasidium*, *Chaetomium*, *Epicoccum, Mucor*, *Trichoderma*, *Verticillium*and lipolytic activity such as the above in addition to *Paecilomyces***[36]**. **Rojas *et al.*, [37]** found that 32 morphotypes filamentous fungi were isolated from industrial paper on different media at an advanced stage of biodeterioration. The isolates showed different degrees of amylolytic, cellulolytic and proteolytic activities on plate assays with *Eladiasacculum*the most active. Paper documents containing cellulose suffer from cellulose degradation which produces aliphatic acids such as acetic, formic, lactic and oxalic acids that lead to acidification **[38]**. The conservation procedures must include deacidification agents such as Ca-propionate **[39]** or aminosilanes **[40]**. Some filamentous fungi associated with paper deterioration are able otof dissolve cellulose fibers via the cellulolytic enzymes action or secrete pigments or organic acids which discolor paper and cause damage of cultural historical books **[41]**. Cellulose hydrolysis can pave the way for attacks by other non-cellulolytic saprophytic fungal species **[42]**. **Coronado-Ruiz *et al.*, [43]** isolated fungi responsible for biodegradation of a nineteenth-century art collection from the archive of the Universidad de Costa Rica and determine their cellulolytic activity. They reported that 95% of the species secrete extracellular enzymes that degrade cellulose into smaller oligosaccharides or monosaccharides.

In the current study, the remaining cellulose values after paper infestation by fungal species were determined. Values indicated that Whatman filter paperwas highely utilized by A. flavus, while Papyrus paper was utilized greatly by A. niger. A. oryzae was the highest cellulose utilizable in newspaper and P. commune for printer papers. in consistence with these results, Swapna and Lalchand [44] studied the cellulolytic activity of some fungi isolated from some books on book paper and newspaper. They determined the cellulolytic activity by the test of weight loss. The results showed that Aspergillus niger and Cryptocorynespiralis had the same loss of substrate percent (30%) after 10 days on book paper. In case of Newspaper, the weight loss after 10 days was about 29.70% caused by C. spiralis followed by A. niger (18.90%). The papers weight loss showed the amount of paper degraded by the fungus, thus reflecting its cellulolytic ability.

Atomic Force Microscope (AFM) permits three-dimensional (3D) imaging and measurement of unstained and uncoated structures in air or fluid permitting direct observation of native specimens and biological processes under native condition. In the present work, AFM was used in case of *P. commune* infesting the 4 paper types. The images showed a reduction in the surface roughness of all paper types with Whatman paper being the most affected one. AFM indicated also disruption in the normal system of infested paper surface which appeared with many peaks compared to one peak in normal paper. In this connection, **Piantanida*et al.*, [25]** reported that AFM determine the change in roughness due to deterioration of paper by presence of more than one distinguished peeks in the surface height distribution curve, whereas a single peak is characteristic to paper control samples. Surface roughness is defined, on each 10×10 μm^2 frame, as the root mean square deviation of the surface height, from its average value. Therefore, fibers in good conservation state will result in higher height deviation (i.e. surface roughness value) than fibers degraded into fibrils and other degradation products. They also reported that artificially deteriorated whatman paper sample and those naturally affected by biological agents displayed a distinct surface roughness differences when compared to control images. The biodegradation of cellulose fibers that appeared in AFM image can be attributed to activity of both cellulolytic enzymes and acidic compounds secreted by fungal cells. **Piantanida*et al.*, [45]** found non homogenous decomposition of fiber surface of whatman paper under accelerating ageing in climatic chamber when examined by AFM. Therefore, a comprehensive investigation of the degree of interaction between microbial growth and paper substrata is fundamental for deciding which kind of restoration should be applied. The set up of techniques, such as AFM, for a better understanding of biodeterioration of paper represent a step up towards a more conservation policy of historical libraries restoration.

5. Conclusion

The paper deterioration depends on the fungal species, the paper type, in addition to the environmental conditions. Whatman filter paper was highly affected by *A. flavus*, Papyrus paper by *A. niger*, newspaper by *A. oryzae* and printer paper by *P. commune*. Reduction in surface roughness of all paper types was oserved when using atomic force microscope (AFM) in case of *P. commune* infesting the 4 paper types. Whatman paper recorded the highest surface roughness reduction. AFM also showed disruption in the normal system of infested paper surface.

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الملخص العربي

تحلل الأنواع المختلفة من الأوراق بواسطة الفطريات المعزولة من المكتبة القديمة لجامعة القاهرة

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يعد التحلل البيولوجي لمواد المكتبات مشكلة عالمية بسبب وجود أعداد هائلة من المكتبات في جميع أنحاء العالم.يهدف البحث الحالي إلى دراسة تحلل الأنواع المختلفة من الأوراق بواسطة الفطريات المعزولة من المكتبة القديمة لجامعة القاهرة ، مصر. بعد عزل الفطريات تم استخدام أكثر عشرة أنواع شيوعًا في تدهور الورق. هم *Aspergillus flavus* و *A. niger* و *A. parvisclerotigenus* و *A.oryzae* و *A. tubingensis* و *Cladosporiumcladosporioides* و *Penicillium commune* و *P. crustosum* و *P. digitatum* و *P. italicum*. الإصابةالاصطناعية لأربعة أنواع من الورق من قبل الأنواع الفطرية العشرة أشارت إلى تباين في القدرة على الاستخدام اعتمادًا على نوع الورق (ورق البردى يليه ورق الجريدة ، ثم ورق الطابعة ثم ورق الترشيح واتمان) وعلى الأنواع الفطرية. أشار تحديد قيمة السليلوز المتبقية بعد إصابة الورق ونمو الفطريات إلى أن فطرة*A. flavus* كانت الأكثر استخداماللسليلوز في ورق الترشيح واتمان، و *A. niger* لأوراق البردي ، و *A. oryzae* لأوراق الجرائد ، و *P. commune* لأوراق الطباعة.تم استخدام مجهر القوة الذرية (AFM) في حالة إصابة الأنواع الورقية الأربعة بفطرة *P.commune*. أشارت الصور إلى انخفاض خشونة السطح لجميع أنواع الورق مع كون ورق واتمان هو الأكثر تأثراً. أشار استخدامAFM أيضًا إلى حدوث خلل في النظام الطبيعي لسطح الورق المصاب والذي ظهر مع العديد من القمم مقارنة بقمة واحدة في الورق العادي.