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THE MYCOBIOTA ASSOCIATED WITH 10 OLD MANUSCRIPTS OF EGYPT'S NATIONAL LIBRARY ARCHIVES AND THEIR BIODEGRADATION CHARACTERISTICS

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Abstract

In libraries and archives, fungi are the most common biodegrading agents. The goal of this study is to examine and identify the fungi associated with 10 old manuscripts from Egypt's National Library Archives, as well as to determine the most harmful isolates for old manuscripts to be cautious of in the future. 115 isolates were qualitatively tested for cellulase activity; the most potent fourteen isolates were subjected to quantitative estimation of cellulase as well as other biodegradative attributes, such as degradation of starch, gelatin, and acid and pigment production. Aspergillus spp. were the predominant genera in all of the samples, and without exception, all of the tested isolates have cellulolytic activity, with 14 isolates having the highest cellulolytic activity. Each of the fourteen fungal isolates has three biodegradative attributes, but three A. niger isolates (no. 2, 3, and 5), Chaetomium atrobrunneum, and Ulocladium atrum) reacted positively for all biodegradative qualities.

Keywords*: Old manuscripts; Cellulolytic ability; Biodegradation attributes; Egypt's National Library Archives.*

Introduction

Egypt, as the East's Lighthouse, is home to a number of rare manuscript libraries, including the Manuscripts Library at the Ministry of Endowments in the Mosque of Sayeda Zeinab, the Institute of Arabic Manuscripts, the Al-Azhar Library in the Sheikhdom Palace, the American University Library of Rare Books, and the Library of Alexandria. The Egyptian National Library Archives (NLA) surpasses all of these places by containing the largest number of rare paper collections, including rare manuscripts, documents, papyri, and other items dating back thousands of years [1].

It is known that the older and rarer the contents of a library, the higher their value. Manuscripts are the priceless history of peoples and a summary of knowledge in different eras, and preserving them has always been a priority for advanced nations [2]. Paper is a multi-component material mainly composed of: fibrous matter (cotton, linen, and hemp, which are mainly composed of cellulose materials; wood contains cellulose besides other components like hemicellulose and lignin); sizing agents (whether natural, such as starch, gelatin, and alum; or synthetic, like AKD, ASA, colourants, and other substances); mineral particles (talc, kaolin,

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calcium carbonate, etc.); the quality of the paper is determined by the nature, chemical structure, and characteristics of its components, as well as their interactions 3–5]. Cleanliness, human activities, and ventilation, as well as the libraries' environmental conditions such as temperature and relative humidity, play a significant role in the growth and survival of bio-degradative and bio-deteriorative agents. Microorganisms, specifically fungi, have a deleterious effect on both library materials and librarians and are responsible for the biodeterioration of cultural heritage inside libraries and archives [6–8].

Fungi are considered major threats to papers in libraries and archives and well-known agents for the decomposition of organic materials, especially cellulosic substrates, which are the major constituents of library materials [9, 10]. The ability of fungi to produce extracellular enzymes is widely known. They can make hydrolytic enzymes including cellulase, xylanase, and pectinase, among others [11]. They also cause mechanical, chemical, and aesthetic damage to precious papers by forming hyphae, producing and excreting pigments (colours), as well as organic acids [12]. *M.K. Zonouz* [2] and *J.E. Smith & D.R. Berry* [13] have mentioned the production of gluconic acid, lactic acid, and citric acid by fungi like *Aspergillus, Rhizopus,* and *Penicillium species*. Fungi associated with library materials are *Aspergillus spp.* including *Aspergillus niger, Aspergillus clavatus, A. flavus, A. glaucus, A. terreus, A. repens, A. ruber, A. fumigatus, A. ochraceus, A. nidulans, Penicillium spp. including P. chrysogenum, P. funinculosum, P. pupurogenum, P. rubrum, P. variabile, P. spinulosum, P. fellutatum, P. frequetans, P. citrinum,, Cladosporium spp., Geotrichum spp.,* and *Alternaria spp., Curvularia spp., Chaetomium atrobrunneum, C. elatum, C. indicum, Fusarium sp., Mucor sp., Paecilomyces variotii, Rhizopus oryzae, Stachybotrys chartarum, Trichoderma harzianum, T. viride, Stemphyllium sp.,* and *Ulocladium sp*. as have been mentioned by previous studies [14-18]. The deterioration of archival materials by fungi has been stated before by many authors [12, 19–24]. The authors stated that fungi have the ability to decompose non-cellulosic materials and produce enzymes to decompose them, as they have the ability to produce amylase [25], caseinase, and gelatinase. Several investigators [13, 26–28] have also mentioned protease enzyme production by fungi.

The aim of this work is to identify the fungal load of 10 infested manuscripts in the NLA manuscript store in Egypt. The goal can be achieved by examining the relationship between environmental factors and the fungal load, as well as determining their cellulolytic activities qualitatively and quantitatively. Likewise, to determine their biodegradation potential using some qualitative assays and determining the relative frequency of the isolated genera and the predominant genus, based on these results, the most potent isolates will be chosen for further research.

Materials and Methods

Description of the sampling site

The entire fourth floor has been designated as the manuscript floor and is divided into three sites consisting of three departments: Papyrus Department, Manuscripts Department and Coins department. There is no provision for air conditioning or climate control in the store. As a result of these, there is an imbalance in air circulation within the store, as the temperature in the summer and spring months consistently reaches 30°C and in some cases exceeds 30°C.

Selection of heritage manuscripts sample

The samples were macroscopically examined and selected for further analysis based on macroscopic patterns of biodeterioration such as microbial stains with different colours, discoloration, structural damage, wetted paper, and musty odour; only the suspected manuscripts were selected (Fig. 1). A total of 10 manuscript samples suffering from deterioration symptoms were taken from the fourth floor containing the manuscript store (Fig. 1). The temperature and relative humidity were measured inside the repositories at the time of

sampling, when the microbiological sampling was performed using a digital thermo hygrometer (SATO, Model SKL-200, Japan).

Fig. 1. Photos of some deteriorated manuscripts present at NLA stores:
(A) 92 faqih hanafi trky; (B) hadith sahih muslim "bab alhuthi fi al'iinfaq, wakirahat al'iihsa';
(C) eulim alkalam talaeat 285; (D) adb trky 364 (kitab adab aldunya waldiyn)

Isolation of fungi from paper manuscripts

Sample collections were performed on a 1 cm^2 surface of each suspected manuscript by using sterile cotton swabs. The surfaces were wiped across fungal spots, then transferred to the laboratory in sterile tubes. Then, the swabs were directly streaked on the surface of PDA (Difco) and plates supplemented with chloramphenicol (100mg/L). Until a colony formed, the Petri plates were incubated at 28±2°C for 7 days. According to [29], fungus concentration was expressed in CFU/cm^2.

Identification of isolated fungi

Fertile moulds were purified by spreading a few spores on the surface of PDA plates and incubated at 28±2°C for 7 days. A single colony was aseptically subcultured on a slant of PDA. The isolates were tested for purity and stored on slant agar at 4ºC for further research. The identification of the obtained isolates was carried out on the basis of their macro- and microscopically characteristic sporulation according to the keys in [30–33]. The identification of fungal isolates was carried out in the plant Pathology Department of the NRC, Giza, Egypt.

Occurrence and frequency of the fungal isolates from selected manuscript

The relative distribution of fungal genera isolated from selected manuscripts was conducted, and the frequency of occurrence expressed as percentage relative distribution (RD) of genera or species was calculated according to [34], where:

$$\mathbf{RD} = \frac{\textbf{Number of colonies of the genus or species}}{\textbf{Total number of colonies of all genera or species}} \mathbf{X\,100\,\%} \quad (1)$$

Determination of the relative frequency (RF) of the fungal genera and their ecological categories

The relative frequency (RF) determination was determined according to [35] using the following formula:

$$\mathbf{RF} = \frac{\text{Times a genus is detected}}{\text{Total number of sampling realized}} \mathbf{X\,100} \qquad (2)$$

Biodegradation potential determination

Cellulolytic activity

Qualitative determination of the cellulolytic activity

All the pure fungal isolates were screened individually for their ability to hydrolyze the cellulose by using the plate screening method, which involved modified Czapek's agar plates with the following ingredients: 20g/L of agar, 2.0g/L of $NaNO_3$, 1.0g/L of K_2HPO_4, 0.5g/L of KCl, 0.5g/L of $MgSO_4{\bullet}7H_2O$ and 0.1g/L of $FeSO_4{\bullet}7H_2O$ and at pH = 5.5, without carbon source, and1% carboxymethyl cellulose (CMC) or 1% Avicel was added as a sole carbon source [36]. Then incubate all the plates at 25ºC for 7 days. For observations, petri dishes were stained using 1% Congo red dye (30min) followed by destaining with 1M NaCl solution for 20min. Clear zones could be observed only around colonies of the active fungal strains [37, 38]. The experiment was carried out in triplicate. The active cellulolytic fungal species were selected based on the diameter of the hydrolysis zones surrounding the colonies (14 isolates). The selected fungal isolates were tested for qualitative determination on filter paper as well as pigment secretion, according to [18, 39, 40].

Quantitative determination of the cellulolytic activity

Experimental cultures with CMC or FP: 10.0g/L (1%) as a carbon source were created in 250mL flasks each containing 50mL of Mandels and Weber's media [41] at an initial pH of 5 and inoculated with 10% of the spore suspension (1×10^6) of the tested fungi. The inoculated flasks were incubated at 30ºC in a static condition for different intervals (3, 5, 7, 9, 11, 13, and 15 days). The fungal mycelia were removed from the culture through filtration by FP (no. 1), and the filtrate was centrifuged at 14000rpm for 15 minutes at 4ºC. The fungal dry weight was also measured. The culture supernatant was used for the determination of the cellulase activities of FPase and CMCase. This was achieved according to the method of [41], where the resulting sugars were determined by a 3,5-Dinitrosalicylic acid (DNS) reagent according to [42], using glucose as a standard.

Filter Paperase assay (FPase Assay)

The IUPAC method was performed essentially according to the procedure prepared by *T.K. Ghose* [43]. At first, 1mL of 0.05M Citrate buffer, pH = 4.8, and 1×6 cm of filter paper was added to a test tube. Then, 0.5mL of cell-free culture supernatant was added to the tube. The tubes were incubated at 50°C for exactly 60min. At the end of the incubation, each tube was removed from the 50°C bath, and the cellulase reactions were stopped immediately by the addition of 2.5mL of the 3, 5-Dinitrosalicylic acid (DNS) reagent, according to [42]. Every tube was boiled for exactly 5.0min in a vigorously boiling water bath. Finally, the absorbance was measured at 540nm.

Carboxymethyl cellulase assay (CMCase Assay)

The determination of CMCase was performed essentially according to the procedure prepared by *T.K. Ghose* [43] as described before, with a modification of using 0.5mL of 1% (w/v) CMC as a substrate instead of filter paper strips. Enzyme activity was determined in terms of the International Unit (IU), which is defined as an amount of enzyme that liberates 1μmol of glucose equivalents per minute under the assay conditions [44].

Secretion of extracellular pigment

Tested isolates were inoculated on Oat Meal Agar Medium (OA) made in accordance with [36] in order to qualitatively assess extracellular pigment synthesis. Extracellular pigment

production was detected as a change in hue of the tested OA medium during the cultures' 7-day incubation at 25±1°C.

Acid or alkaline production

Quantitative determination was performed in Czapek's liquid media (10g glucose instead of sucrose), and pH was adjusted to 7 after the inoculation. Incubate the cultures for 3 days at 25±1°C for 3 days on a platform shaker with a 300rpm rotation according to [39].

Proteolytic activities

Gelatin hydrolysis assays were used to measure the qualitative level of proteolytic activity, according to [45]. Frazier's reagent was applied to each plate after 7 days of incubation at 30°C, and the presence of a translucent halo around the growth indicated a successful hydrolysis of gelatin.

Amylolytic activity

According to [39], the qualitative determination of amylolytic activity was performed using the techniques presented by [18], and each culture petri plate received 5mL of Lugol's reagent after incubation at 30°C for 7 days. The presence of a colourless zone around the colony was interpreted as a sign that the hydrolysis was successful.

Results and Discussion

Meteorological characters

During this study (February 2019–January 2020), temperature and relative humidity were recorded using the portable thermo hygrometer. The average temperature inside the store ranged between 22 and 34°C, and the RH ranged between 31.5 and 57.5%, as shown in figure 2.

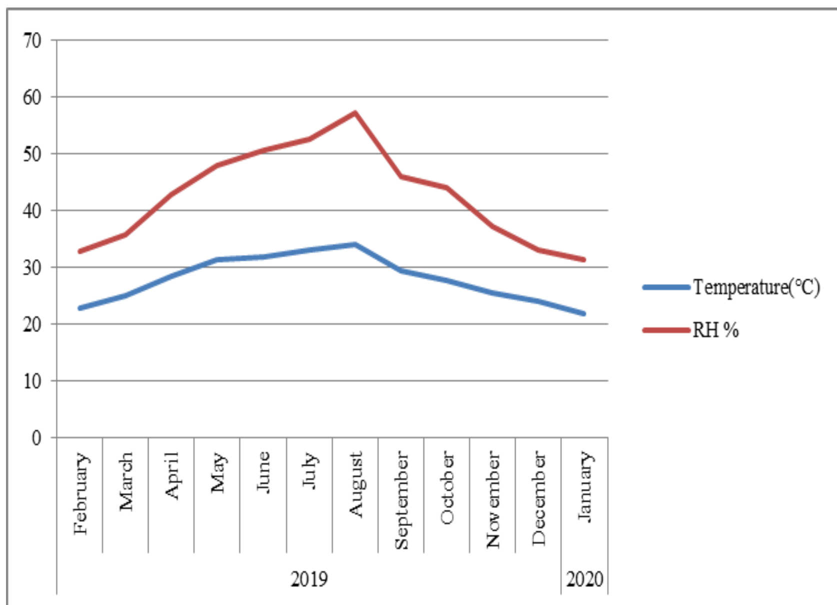

Fig. 2. Temperatures and RH of the manuscripts store for one year

It was observed that the conditions inside the National Library and Archives building are suitable for the spreading of different microorganisms and the biodeterioration of old documents. Based on these results, it was noticed that temperature and RH levels in libraries have risen above the acceptable limits as recommended by [46, 47], who cleared that a

temperature of 20±2°C and relative air humidity of 50±3% are recommended for collection storage in the studied institution types. Although many authors disagree on optimum temperatures of 21.1°C and 30–50% RH [48], with RH below 65% and a temperature of 0–25°C, *J. Singh* [49] reported optimum temperatures of 18.3°C and 35% RH. Similarly, *J. Skóra et al.* [50] found a correlation between microclimatic conditions and the number of microorganisms in the air in the tested working environments. In general, the higher the temperature and the more favourable the nutrition at a given relative humidity, the less time is required for spore germination [51]. Therefore, the main solution in preservation is climatic control, and other ways like fumigation and cleaning are accessories solution. The application of an electrical vacuum cleaner and controlling the temperature and relative humidity (RH) by installing air conditioning all over the stores and adjusting the temperature to not exceed 22°C and the RH to 50%. The results obtained in our study are comparable to the findings of a study conducted by several investigators [52-55].

Survey of fungal isolates

A total of 293 fungal isolates were isolated from 10 old manuscripts of the NLA of Egypt. All deteriorated manuscripts showed various forms of degradation symptoms, microbial stains with different colours, dirt accumulation, ripples, dissolving inks, and other features (Table 1 and Figure 1).

Table 1. Selected manuscripts for isolation and bio-deterioration symptoms

No.	Manuscripts title	Manifestation of deterioration													No. of isolates	Fungal conc. (CFU/cm^2)	No. of tested isolates
		Acidity	Fading colors	Tears	Fungal stains (Molds)	Blots	Skinning	Previous repairs	Ripples or moisture	Water damage	Dissolving inks	Grease	Accumulation of dust	Insect infection			
1	62hadaya			*	*	*			*	*		*	*		38	19	17
2	16 eurud tymur	*			*					*	*	*			20	10	10
3	660 tarikh tymwr						*		*	*		*			32	16	11
4	13 tasawaf talaeat		*		*					*	*	*	*		19	10	8
5	fqh shafaeaa talaeat 198	*			*			*	*	*		*	*	*	13	7	5
6	adb trky 364	*	*		*			*	*			*	*		18	9	8
7	tarikh 2800	*	*	*	*				*	*	*		*	*	63	32	22
8	eulim alkalam talaeat 285	*	*		*				*	*		*	*		23	12	9
9	71 faqh shiei			*	*		*		*	*	*		*		40	20	14
10	92 faqih hanafi trky	*	*		*		*	*		*	*		*	*	27	14	11
	Total														293		115

The fungal concentration for each old manuscript was shown in Table 1, which greatly differs from one manuscript to another based on the degree of deterioration, the chemical composition, the nature of the material itself, the fungal isolate physiology and surrounding environment, the exposure of the object, the method and frequency of surface cleaning, and store housekeeping [19]. The fungal concentration for each old manuscript varies from one manuscript to another, ranging from 7-32CFU/cm^2, where the Manuscript No. 7 was the most contaminated of the tested items and Manuscript No. 5 was the least contaminated with 5CFU/cm^2.

The microorganisms isolated from each manuscript have been counted; each microorganism isolated has been purified, identified, and numbered. Figure 3 illustrates some photos taken under a microscope for different isolates.

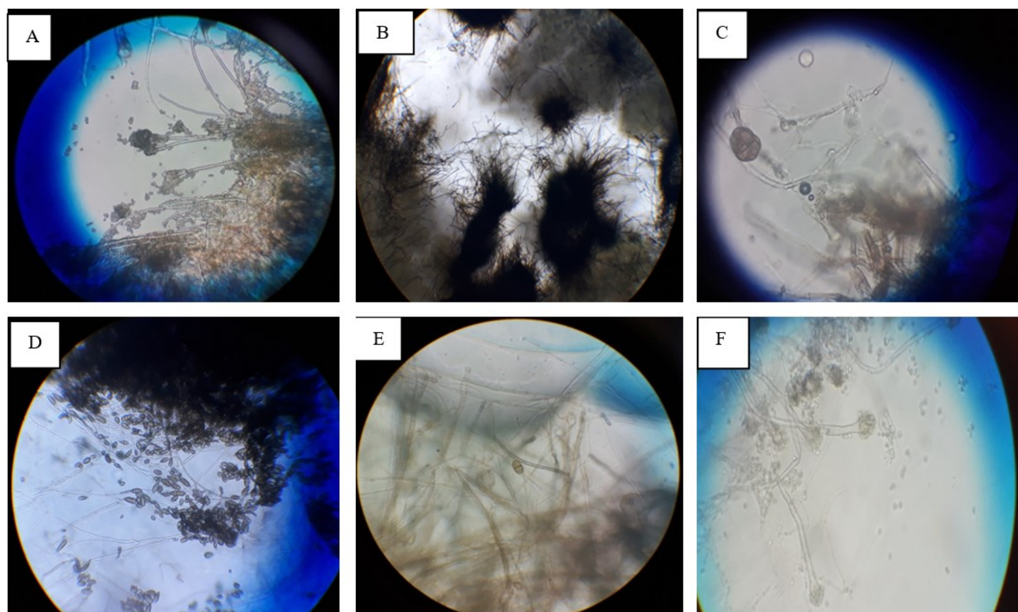

Fig. 3. Microscopic photos for some species of isolated fungi from the manuscriptes of the NLA's of Egypt:
A - *Gliocladium fimbriatum*; B - *Cheatomium atrobrunneum*; C - *Ulocladium atrum*;
D - *Alternaria tenus;* E - *Stemphyllium sp.*; F - *Aspergillus humicola*

The fungal concentration differs greatly from one manuscript to another based on the degree of deterioration, the chemical composition, and the nature of the material itself. Fungal isolates physiology and the surrounding environment, the exposure of the object, the method and frequency of surface cleaning, and store housekeeping [19].

Generally, manuscripts no. 7 (tarikh 2800), no. 1 (62 hadayaa), and no. 9 (71 faqh shiei) showed a higher number of fungi (22, 17, and 14), with a relative distribution of 19.1, 14.8, and 12.1%, respectively (Table 2). On the other hand, manuscript no. 5 (fqh shafaeaa talaeat 198) had the lowest fungal count, reaching 5 isolates with a frequency of 4.3%. These results are compatible with [56], who isolated *Aspergillus*, *Paecilomyces, Chrysosporium, Penicillium,* and *Cladosporium* from paper-based materials stored in compactus-type shelving units. In addition, [57, 58] found the fungal genera *Aspergillus, Cheatomium*, *Eurotium, Penicillium,* and *Trichoderma* among the most commonly found species on paper substrates. Similarly, the same fungal genera and species are often present on deteriorated manuscripts [17, 59, 60]. Data also revealed that each manuscript assessed is a microbial ecosystem made up of a fungal community consisting of one or more fungus species, and several genera could be found in the same manuscript.

Data also showed that fungal diversity in a particular material does not depend on the concentration of the fungi present but depends on several other factors, such as the nature of the substrate and the percentage of easily degradable sources of carbon like sugars and starch, the water availability of the substrate, and the presence of other accessory sources of food on the substrate, like starch, albumin, or even metals from the industry or from inks [61]. All these factors promote fungal presence and growth [62]. Frequency occurrences of fungal genera isolated from 10 contaminated manuscripts of the NLA of Egypt revealed that the most dominant genera were.

Table 2. Relative Distribution (RD) (%) and Relative Frequency (RF) of isolated fungal genera and their Ecological Category (EC) of fungi, isolated from 10 old manuscripts

Manuscripts / Fungal genera	62 Hadayaa	16 Eurud tymur	660 Tarikh tymwr	13 Tasawaf talaeat	FQH shafaeaa talaeat198	ADB trky 364	Tarikh 2800	Eulim alkalam talaeat 285	71 FAQH shiei	92 FAQIH hanafi trky	Total isolates	RD (%)	RF (%)	EC
Alternaria		1	-	-	-	-	-	-	-	-	**1**	**0.87**	**10**	**R**
Aspergillus	11	8	8	6	4	6	16	7	12	5	**83**	**72.17**	**100**	**A**
Chaetomium	1	-	-	-	-	-	-	-	1	-	**2**	**1.73**	**20**	**R**
Cladosporium	-	-		-	-	-	-	-	1	-	**1**	**0.87**	**10**	**R**
Fusarium	-	-	1	-	-	-	-	-	-	-	**1**	**0.87**	**10**	**R**
Gliocladium	-	-	-	-	-	-	1	1	-	-	**2**	**1.73**	**20**	**R**
Hormodendrum	2	-	-	-	-	-	-		-	-	**2**	**1.73**	**10**	**R**
Mucor	-	-	-	-	-	-	-	1	-	-	**1**	**0.87**	**10**	**R**
Mycelia sterillia	-	-	-	-	-	1	-	-	-	-	**1**	**0.87**	**10**	**R**
Penicillium	1	1	-	1	-	1	2	-	-	5	**11**	**9.57**	**60**	**C**
Paecilomyces	2	-	1	-	-	-	2	-	-	-	**5**	**4.35**	**30**	**F**
Phycomycetes	-	-	-	-	1	-	-	-	-	-	**1**	**0.87**	**10**	**R**
Spondylocladium	-	-	-	-	-	-	-	-	-	1	**1**	**0.87**	**10**	**R**
Ulocladium	-	-	-	-	-	-	1	-	-	-	**1**	**0.87**	**10**	**R**
Unknown	-	-	1	1	-	-	-	-	-	-	**2**	**1.73**	**20**	**R**
Total	**17**	**10**	**11**	**8**	**5**	**8**	**22**	**9**	**14**	**11**	**115**	**100**		

RF between: 100- 81% the genus is considered ecologically as Abundant (A) 80–61%; as Common (C) 60-41%; as Frequent (F) 40- 21%; as Occasional (O) 20- 0.01% and as Rare (R) according to *P. Esquivel et al*. [35]

Aspergillus, which accounted for the bulk of the recovered mycodiversity in all the tested manuscripts, had the maximum abundance of 72.17%, followed in decreasing order by *Penicillium* (9.57%), then *Paecilomyce*s (4.35%), as illustrated in Table 2 and Figure 4.

While the minor fungal genera account includes *Alternaria*, *Cheatomium*, *Cladosporium*, *Fusarium*, *Gliocladium, Hormodendrum, Mucor, Mycelia sterillia, Paecilomyces, Phycomycetes, Spondylocladium, Stemphyllium, Ulocladium*, and *Unknown* (<5%), these results are in line with those of other researchers [1, 60, 62–64], similar to those reported by [16, 59, 65].

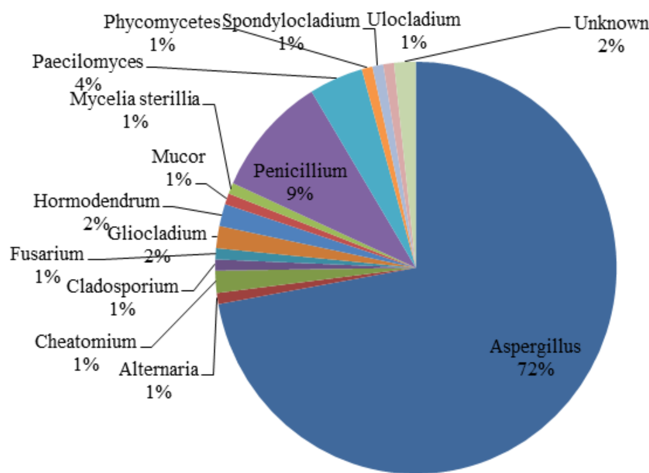

Fig. 4. Relative distribution of isolated fungal genera from 10 old manuscripts

Cellulolytic activity of fungal isolates

According to qualitative studies of some physiological characteristics of the isolated fungi, all the tested isolates were capable of growing and utilising CMC and Filter paper (FP) as a sole carbon source to varying degrees (Table 3). 115 fungal isolates were selected and quantitatively examined for their cellulolytic activity from a total of 239 fungal isolates recovered from 10 antique manuscripts from various eras at the NLA of Egypt.

As a qualitative method, the hydrolysis of CMC can then be detected using the Congo red dye test. Using the Congo red test, as previously stated, fungal species were chosen based on the diameter of the hydrolysis zone around the colonies.

Results showed that the activity of cellulose degradation varied from highly effective to feeble effective and was classified as highly effective (71–90mm), moderate (51–70mm), and feeble (11-50mm) (data not shown). The data revealed that 44 fungal isolates were recorded as highly effective (38.3%), 47 fungal isolates were recorded as moderately effective (40.9%), and finally 24 fungal isolates were recorded as feebley effective (20.9%).

14 fungal isolates, which have the most common species and most distinct zones of hydrolysis, were screened for their abilities to produce CMC_{ase} and Filter $Paper_{ase}$. Furthermore, for the quantitative determination of cellulose production, CMCase ranged from 0.093 to 0.21U/mL, while FPase ranged from 0.014 to 0.0313U/mL. *Cheatomium atrobrunneum* and *Aspergillus niger* (3) showed the highest CMCase and FPase production, detecting 0.21 and 0.031U/mL respectively, as shown in Table 3 and Fig. 5.

Table 3. Different biodegradation abilities of fungal strains isolated from 10 old manuscripts of NLA library of Egypt

No.	Tested fungal isolates	Cellulolytic activity		Amylolytic activity	Proteolytic Activity	Pigment Production (OA medium)	Acid or Alkaline production (pH)
		CMC degradation	Filter paper degradation	Starch degradation	Gelatin degradation		
1	*Aspergillus flavus* (1')	++++	++	-	+	-	**6.69**
2	*Aspergillus flavus* (5')	++++	++	+	+	-	**7.52**
3	*Aspergillus flavus* (6')	++++	++	+	+	-	**6.43**
4	*Aspergillus flavus* (10')	++++	+	-	+	-	**6.68**
5	*Aspergillus flavus* (12')	++++	++	+	+	-	**6.94**
6	*Aspergillus humicola*	++	+	-	+	-	**7.89**
7	*Aspergillus niger* (1)	++++	+++	-	+	-	**5.48**
8	*Aspergillus niger* (2)	++++	++	+	+	+	**2.70**
9	*Aspergillus niger* (3)	++++	+++	+	+	+	**2.89**
10	*Aspergillus niger* (4)	++++	++	-	+	-	**4.90**
11	*Aspergillus niger* (5)	++++	+++	+	+	+	**3.23**
12	*Chaetomium atrobrunneum*	+++	++++	++	+	+++	**7.38**
13	*Paecilomyces variotii*	++	±	+++	-	-	**5.77**
14	*Ulocladium atrum*	+++	+++	+++	+	+++	**6.15**

(+++) refers to excellent growth, activity and pigment production, (+++) refers to good growth, activity and pigment production, (++) refers to moderate growth, activity and pigment production, (+) refers to poor growth, activity and pigment production, (±) refers to very poor growth, activity and pigment production, (-) refers to no growth, no activity and no pigment production

It is worthy to note that every isolate has its own behaviour, and the production of cellulases (CMCase and FPase) differs from one isolate to another depending on many factors, viz., metabolic activity, type of substrate, incubation time, and the genetic background of the species [66]. Major tested isolates belonged to the *Aspergillus* genera (*A. niger,* 5 isolates), *A. flavus,* 5 isolates, and *A. humicola,* as they are the major dominant genera from the selected items. The cellulolytic activity of *Aspergillus* genera with their different species has been observed before by many authors [67-72]. The other tested isolates (*C. atrobrunneum, Ulocladium atrum,* and *Paecilomyces variotii*) were also detected as cellulase (CMCase, FPase) producers by many researchers [68, 69, 73-80].

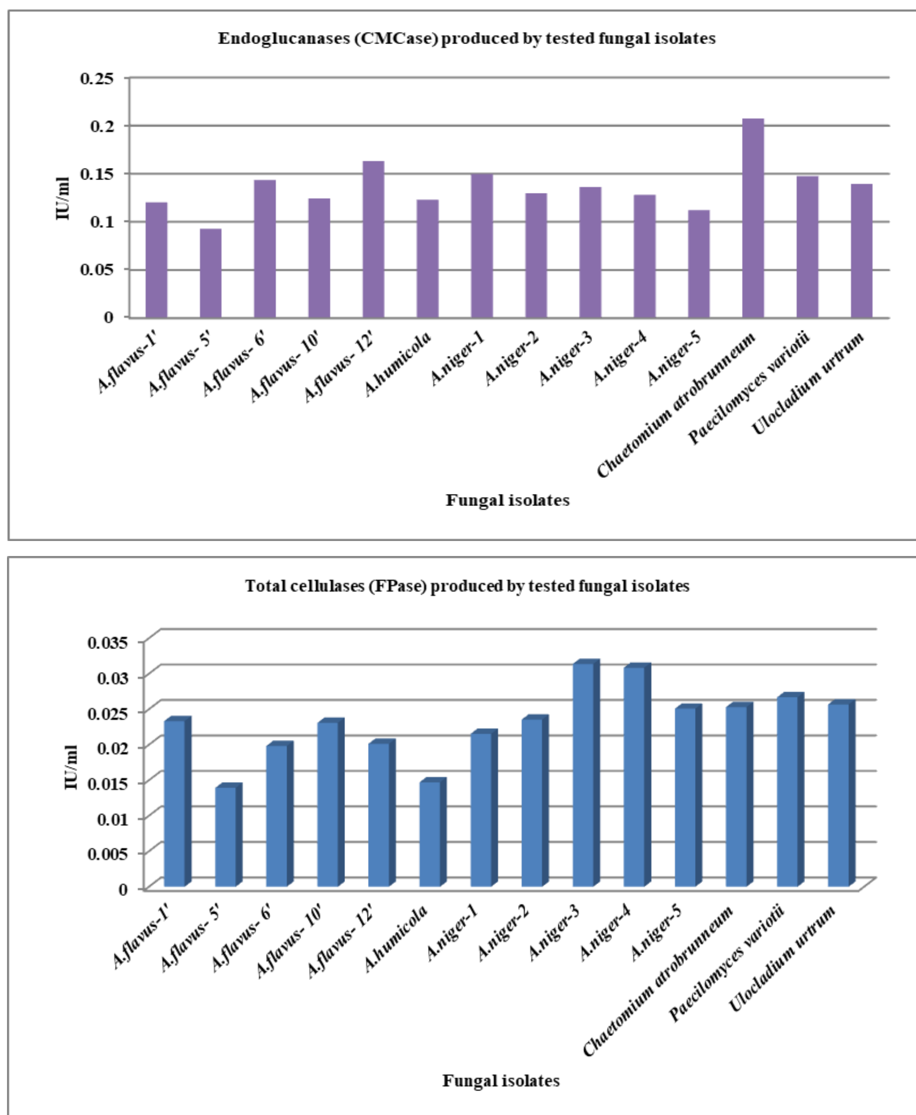

Fig. 5. CMCase and FPase of 14 tested fungal isolates obtained from NLA's manuscripts of Egypt

Besides cellulolytic ability, other different biodegradation abilities for the selected isolates have been tested by means of different assays, and the results have been summarised in Table 3.

Amylolytic activity of fungal isolates

Although starch is the third most common raw material in the paper mill industry, after cellulose fibre and mineral filler [81], only 9 isolates (64.29%) could degrade starch and produce amylase enzymes, while 4 tested isolates (28.57%) could not degrade starch. The most pronouncing activity was obtained by *Chaetomium atrobrunneum*, *Ulocladium atrum,* and *Paecilomyces variotii*, whereas all the other isolates showed moderate activity. In contemporary to present results, *C. Lucas et al.* [82] and *R. Bellaouchi et al.* [83] recognised *A. niger* as a high amylase producer. Likewise, many researchers proved that Chaetomium species

possess high amylolytic activity [84, 85]. *A.M. Abdel-Azeem et al.* [78] proved that 10 isolates of *Chaetomium globosum* have proteolytic, amylolytic, and moderate cellulolytic activity. Previous investigators also observed the same trend, as Ulocladium species were able to produce enzymes that decomposed amylose and cellulose [78], as shown in Table 3.

Proteolytic activity of fungal isolates

The results of proteolytic activity screening based on the clear zone diminution around the hydrolyzed gelatin showed that about 98.8% of the tested isolates could degrade gelatin and produce gelatinases. The clear zones were very clear and consistent for all isolates; they appeared rapidly after three days, and all the positive isolates were regarded as having highly proteolytic activity. On the other hand, only one isolate (*Paecilomyces variotii*) has no significant activity towards gelatin; this result is in harmony with *M.P. Rodarte et al.* [86], who stated that no proteolytic activity was observed for *Paecilomyces spp.* Several workers reported most of the tested fungal isolates as proteolytic enzyme producers with variable capabilities [83, 87-89] in Table 3.

Extracellular pigment production

Pigment production was demonstrated for the fourteen tested isolates on OA medium, the most suitable medium for pigment production by fungi. Data in Table 7 showed that only five isolates (35.71%) exhibited pigment production; this production of the pigments differed depending on media and fungal species. The present results showed that *Aspergillus niger* isolates no. 2, 3, and 5 secreted light black, dark black, and yellowish black pigments, respectively. While *Chaetomium atrobrunneum* can produce orange pigment on OA medium, also *Ulocladium atrum* can also produce olive brown or black pigment. Pigments produced by fungi differ in their colours and chemical composition [90, 91]. In the same direction, pigment production by A. niger was achieved by several investigators [29, 92-94]. *H. Szczepanowska and A.R. Cavaliere* [95] have also found *Chaetomium spp.* staining and deteriorating paper, implying that pigments can be released into the substrate and spread far from the origin source. Other studies reported similar results [96, 97]. In the same direction, *Ulocladium spp.* were reported to produce black pigment [98-101], as clarified in Table 3.

Acid or alkaline production

The production of acids and alkalines by the 14 selected isolates was detected in PDA broth medium with an initial pH value of 7, and the results are presented in Table 7. It can be seen from the table that most of the isolates produce acid or alkali to a different degree. The highest acid production was observed by *Aspergillus niger* isolates, ranging from a pH value of 2.70 to 5.48. Conversely, the three tested isolates (21.4%) showed a slight increase in pH values towards alkalinity; *A. humicola*, *A. flavus* isolate (no. 5'), and *Chaetomium atrobrunneum* have alkaline production with pH values of 7.89, 7.52, and 7.38, respectively. On the other hand, all the rest of the tested isolates (78.6%) represent 11 isolates that can produce acids with different values. The production of acids by fungi on cellulose substrates causes acid hydrolysis phenomena, which is the main cause of paper degradation, according to [3-5].

In general, the data illustrated in Table 3 revealed that all 14 of the selected isolates have the ability to biodegrade, and this ability differs from one strain to another. In this paper, there are only five isolates (*A. niger,* isolates 2, 3, and 5), *Chaetomium atrobrunneum,* and *Ulocladium atrum*), representing 36% of the total isolates, that are capable of degrading all the tested substrates besides the production of pigments. The other nine isolates, on the other hand, have varying degrees of degrading ability, viz., three isolates (21%), and six isolates (43%), with four and three biodegradation characteristics, respectively, as shown in figure 6.

The higher the biodegradative ability of the isolates, the greater their danger. As previously stated by [102, 103], isolates exhibiting numerous biodegradation characteristics are considered high-risk fungi and will be prioritised in future control measures because, given suitable conditions, their destructive effect will be greater than the others.

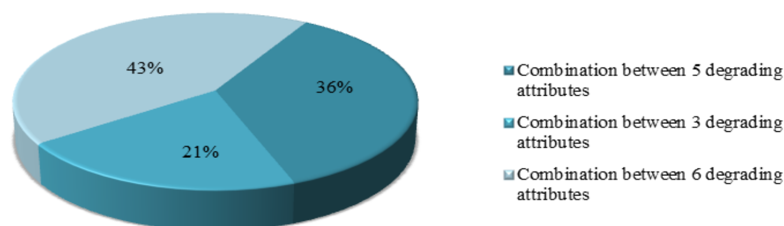

Fig. 6. Combination of biodegradation attributes of the tested fungi

Based on the biodegradation characteristics, notably the cellulolytic activity, *A. niger* (isolate no. 3) and *Chaetomium atrobrunneum* are designated highly-risked agents at Egypt's NLA library's manuscripts store and might cause significant damage if suitable circumstances are allowed, as they should be given special attention in the future.

Conclusions

The present study was conducted on 10 manuscripts from the National Library archives of Egypt. Environmental conditions were studied and it was found that temperatures and humidity ranged between 22-34°C and 31.5-57.5 %, respectively, which are the main factors of the increment in fungal growth as illustrated in the results.

From the obtained results, we could conclude that all of the samples were contaminated with fungi and that all of the tested isolates possessed cellulolytic activity.

The fungal concentration ranged from 7-32CFU/cm^2, *Aspergillus spp.* were the predominant species in all of the investigated samples, with a frequency occurrence of 72%.

A. niger (isolate 3) and *Chaetomium atrobrunneum* are the most potent organisms with the highest FPase (0.031U/mL) and CMCase (0.21U/mL) activities, as well as having good amylolytic and proteolytic activity and the ability to produce acids and pigments, suggesting that they are potentially hazardous to old manuscripts and could cause considerable damage if appropriate conditions are met, and that they should be given extra care in the future and also will have priority in future control.

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