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Original article

STUDIES ON FUNGAL ISOLATES INVOLVED IN BIODETERIORATION OF ANCIENT MANUSCRIPTS OF THE GENERAL EGYPTIAN BOOK ORGANIZATION (GEBO)

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Abstract
Microbiological contamination with fungi and bacteria can pose a significant destroy to old manuscripts or health hazard to those working in archives or library. Among 520 selected old documents, 162 manuscripts (31.15%) macroscopically showed fungal growth or damage and number of 75 documents (14.42%) were positive for presence of fungi but by culture fungal contamination revealed in 30 manuscripts (5.77%). Also, about 199 representative fungal isolates developed on PDA medium were isolated from old manuscripts samples. Aspergillus, Fusarium and Penicillium spp. were the main contaminating mould genera of all tested manuscripts and account over two third of contaminations. The fungal genera could be arranged on the basis of their frequent occurrence as follows: Aspergillus (45.57%), Fusarium (33.1%), Penicillium (8.6%), Alternaria (6.5%), Trichoderma (3.0%), Stymphylium (1.5%) and Nigrospora (0.5%) of the total fungal count. The obtained data also showed that of the 53 fungal isolates screened for cellulolytic activity on carboxy methyl cellulose (CMC) only 31(58.49%) had the ability to grow. Moreover, only 8 out off 31fungal isolates had a high ability to decompose cellulose powder in Czepek's medium. The growth of A. flavus was completely inhibited (100% inhibition) at rhiozolex concentration of ≤ 200ppm and benlate concentration of ≤ 400ppm. While, the growth of A. niger was also completely inhibited at rhizolex concentration of ≤ 400ppm and benlate at≤ 200ppm. The growth of F. oxysporum was completely inhibited by rhizolex at 1600ppm and benlate at ≤ 100ppm. On the other hand, fumigation with Para formaldehyde tablet for 9 days completely inhibited A. niger growth.

Keywords: *GEBO, Old documents, Fungi, Bacteria, Bio-deterioration, Cellulolytic activity*

1. Introduction

Archives preserve documents written on paper, papyrus, parchment and electronic supports. These organic and synthetic materials are deteriorated by physical, chemical and biological agents [1]. Biodeterioration of archival and library materials is a worldwide problem, which is great damage to unique manuscripts and books [2][3]. In addition, to international causes of the deterioration of paper due to its acidity and external agents are a major threat [3][4]. In this study we intend to discuss one of the biological factors as a main external group of factors that influence old documents. Of interest here is the biological deterioration of old documents due to the activity of fungi and bacteria and their control. Fungi are

decomposing organisms that play the main role destroying and degrading carbon and residue to nitrogen such as wood and paper. The ability of fungi to produce extracellular enzymes is well established [5][6]. They can produce hydrolytic enzymes such as cellulases, xylenases, pectinases, etc., [7][8][9] [10]. Many workers proved that some species of *Alternaria, Aspergillus, Chaetomium, Fusarium, Humicola, Myrothecium, Penicillium, Stachybotrys, Stemphylium, Trichoderma* and *Ulocladium* are frequently isolated from deteriorated manuscripts [11][12]. Isaac, 1995 [13], demonstrated that the most common fungi found in old paper are species of *Chaetomium* and *Penicillium* as well as *Trichoderma viride, Cladosporium herbarum* and *Stachybotrys* sp. Chemical control measures have been tested and found effective in the control of fungal growth *in-vitro* against several fungi [14][15][16]. The aim of this work is to determine fungi polluting valuable old manuscripts and that would able to degrade cellulose materials and their control using fungicides and paraformadehyde exposure. The aim of this work is to determine fungi polluting valuable old manuscripts and that would able to degrade cellulose materials and their control using fungicides and paraformadehyde exposure.

2. Materials and Methods

2.1. *Document analyzed*

Ten selected old valuable manuscripts, documents and books were collected from different parts of the repositories of EGBO. At first, objects were visually inspected for mould growth or any damage. The pages were swabbed by sterilized cotton swabs and directly wiped on the surface of PDA plate medium containing chloramphenicole to isolate fungal contaminants. The Petri dishes were incubated at 27±°C for 7 days and subseq-uently colonies were counted.

2.2. *Fungal identification*

The identification of mould isolates were carried out on the basis of their macro and microscopically charac-teristic sporulation according to the keys of some scientists [17][18][19][20]. The frequency occurrence expressed as percentage relative distribution genera or species were calculated according to Smith (1980) [21], where: Relative distribution = $\frac{\text{Number of colonies of the genus or species X100}}{\text{Total number of colonies of all genera or species}}$

2.3. *Qualitative determination of cellulolytic activity*

Cellulolytic activity was determined using CMC and cellulose powder as a source of carbon in a Petri dishes. The fungal strains isolated were incubated on modified Czapek's medium (CMC or Cellulose powder instead of sucrose). Mycelium discs of 5mm size from seven days old culture was cut and one such disc was placed at the centre of each agar plate. Three replicates were used for each treatment. The inoculated plates were incubated at 27±2°C and the radial growth and its density were measured when fungus attained maximum growth in one treatment.

2.4. *Effect of rhizolex and benlate fungicides*

Rhizolex and benlate fungicides were evaluated for their efficacy on mycelia growth of *Aspergillus flavus, A. niger* and *F. oxysporum* (selected for their high cellulolytic activity) by method recomme-nded for laboratory tests with fungicides [22]. Appropriate quantity of each fungicide was separately dispensed in molten sterilized PDA medium to make four concentrations: 25ppm, 50ppm, 100ppm, 200ppm and 400ppm. A 5mm-diam. disc obtained from the edge of the fungus colony, to be tested, was asceptically transferred to the centre of plate. Incubation was at 27±2°C and linear growths were measured for 7 days.

2.5. *Effect of exposure to paraformadehyde gas*

The effect of fumigation by paraformaldehyde taplets on the linear growth of an isolate of *Aspergillus niger* was examined. PDA plates were inoculated with a 5mm-diam. disc at the centre and placed into glass jar (5 liter) containing 3 taplets of paraformaldehyde. Plates in a jar without paraformaldehyd were used as control. Jars including petri dishes were incubated at 27±2°C and the linear growth were measured for 7 days when the tested fungal growth in the control treatment had reached the edge of the PDA plate. This test was repeated four times.

3. Results

3.1. *Macroscopic and microscopic examination of selected old documents*

Frequency and percentage of manuscripts shown in tab. (1) revealed fungal contamination by macroscopic, microscopic examination and culture in EGBO storage during this study. Among 520 selected old documents, 162 manus-cripts (31.15%) macroscopically showed fungal growth or damage. By direct microscopic examination among all the manuscripts, 75 documents (14.42%) were positive for presence of fungi but by culture fungal contamination revealed in 30 manuscripts (5.77%).

Table (1) frequency and percentage of manuscripts revealed fungal contamination by macroscopic, microscopic examination and culture in Dar Al-kotob storage during 2012.

Total Number of Manuscripts	***Fungal contamination detected by***		
	Macroscopic Examination	*Direct Microscopic Examination*	*Culture*
520	162(31.15%)	75 (14.42 %)	30 (5.77 %)

3.2. *Fungal genera and species isolated from old manuscripts and documents*

Data presented in tab. (2) showed that about 199 representative fungal isolates developed on PDA medium were isolated from old manuscripts samples. *Aspergillus, Fusarium* and *Penicillium* spp. were the main contaminating mould genera of all tested manuscripts and account over two third of contaminations. The fungal genera could be arranged on the basis of their frequent occurrence as follows: *Aspergillus* (46.2%), *Fusarium* (33.1%), *Penicillium* (9.0%), *Alternaria* (6.5%), *Trichoderma* (3.0%), *Stymphylium* (1.5%) and *Nigrospora* (0.5%) of the total fungal count. *A. niger* and *A. paraziticus* were isolated from the great part of manuscripts reached 20.6 and 11.06% respectively. Moreover, the obtained results showed that the percentage of fungi isolated from manuscripts that were tested ranged from 0.5 in Sharh Al-Aakida manuscript and 33.6% in Sharh Al-Mohazab manuscript. Also, more than fungi have been isolated from Sharh Al-Mohazab including 9 isolates of *A. niger* (13.4%), one isolate of *A. paraziticus* (1.4%), 51 isolates of *Fusarium* (76.1%) and 6 isolates of the fungus *Penicillium* (8.9%). As regard to Sharh Amr Albrahin document also showed high percentage of fungi with total frequent occurrence of 20.6%, including 15 isolates of *A. paraziticus* (36.6%), 15 isolates of *F. oxysporum* (36.6%), 10 of *A. niger* (24.4%) and one isolate of *Alt. tenuis* (2.4%).

Table (2) fungal genera and species isolated from old manuscripts and documents and their frequency occurrence on PDA medium.

Name of old document	*Fungal genera and species*														*Total*	*Frequenct Occurrence %*
	Alternaria spp.	*A. flavus*	*A. niger*	*A. ochraceus*	*A. paraziticus*	*A. terreus*	*A. versicolor*	*F. oxysporum*	*N. sphaerica*	*P. frequentance*	*P. corylophilum*	*P. spp.*	*Stymphylium spp.*	*Trichoderma viride*		
Sobh El- Asha (V. 2) (Kalkashandi)	-	1	18	-	-	-	-	-	-	-	-	3	-	-	22	11.0
Sharh Al-Mohazab (V.3) (Nawawy)	-	-	9	-	1	-	-	51	-	-	6	-	-	-	67	33.6
Sharh Al-Alakidah (Sanusy)	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	0.5
Sharh-Amr Albrahin (Sanusy)	1	-	10	-	15	-	-	15	-	-	-	-	-	-	41	20.6
Al-Aziz in-Al-Wagiz (Al-Rafei)	2	-	-	-	-	-	-	-	-	-	-	-	-	3	5	2.5
Al-Ogala (Al-Goghraffia) (Ayetuna print)	-	-	1	-	-	-	-	-	1	-	-	-	-	3	5	2.5
Hashiat Al- (Mohamed Al-Enbaby)	-	2	-	-	7	-	-	-	-	-	-	-	3	-	12	6.0
Goghraphia Al Omamia. (Mohamed Ali) V. 11738	5	1	1	-	-	-	-	-	-	1	-	1	-	-	9	4.5
Al-Pesat Al- Alamia, (Mohamed Ali)	5	1	2	-	6	-	-	-	-	-	7	-	-	-	21	10.5
Al-Alam Al-Osmany,1897	-	4	-	1	-	-	11	-	-	-	-	-	-	-	16	8.0
Total	13	9	41	1	23	7	11	66	1	1	13	4	3	6	199	-
Frequency %	6.5	4.5	20.6	0.5	11.6	3.5	5.5	33.1	0.5	0.5	6.5	2.0	1.5	3.0	-	-

A. = *Aspergillus* **F.** = *Fusarium* **N.** = *Nigrospora* **P.** = *Penicillium*

3.3. *Selection of cellulose degrading fungi*

3.3.1. Growth on CMC czapek's agar medium

Of the 53 fungal isolates screened for cellulolytic activity on carboxy methyl cellulose (CMC) only 31 (58.49%) had the ability to grow, tab. (3). Moreover, only 8 out off 31(25.8%) fungal isolates had a high ability to decompose CMC in Czepek's medium, including 4 isolates of *F. oxysporum* with radial growth between 82 and 88 mm, 2 isolates of *F. avenaceum* (Ø between 64 and 72 mm), 4 isolates of *T. viride* (Ø between 60 and 82 mm) and 2 isolates of *F. moniliforme* (Ø between 60 and 61mm). Other 17 and 2 fungal isolates gave moderate growth (Ø between 25-54 mm) and slight effect (Ø between 12-15 mm) respectively.

3.3.2. Growth on cellulose-czapek's agar medium

Of the 28 fungal isolates screened for cellulolytic activity on cellulose-Czapek's medium, tab. (3) only 10 (35.71) isolates showed maximum growth including 4 isolates belonging to *F. oxysporum* and 4 isolates of *T. viride* recorded radial growth between 70-73 mm with very good mycelium growth. While, the two isolates of *Stymphylium* spp. recorded radial growth between 78-80 mm with also very good appearance mycelium growth. On the other hand, the six isolates which belonging to *A. terreus*, *F. avenaceum* and *F. moniliforme* recorded linear growth between 0.0-65 mm with weak and moderate mycelium growth. Moreover, the other fungal isolates showed slight growth with moderate and weak mycelium growth.

Table (3) *in-vitro* growth of fungal isolates selected from deteriorated old manuscripts and air of the GEBO building on modified Czapek's medium (CMC and Cellulose instead of sucrose).

Fungal species and genera	*Growth on CMC-Czapek's medium*										*Growth on Cellulose -Czapek's medium*									
			Isolate No. 1		*Isolate No. 2*		*Isolate No. 3*		*Isolate No. 4*				*Isolate Nu.1*		*Isolate Nu.2*		*Isolate Nu.3*		*Isolate Nu.4*	
	No. of isolate	*(+)ve growth*	*Ø m m*	*Density*	*Ø m m*	*Density*	*Ø m m*	*Density*	*Ø m m*	*Density*	*No. of isolate*	*(+) growth*	*Ø m m*	*Density*	*Ø m m*	*Density*	*Ø m m*	*Density*	*Ø m m*	*Density*
Alternaria spp.	4	2	38	+	37	+	-	-	-	-	2	2	22	+	23	+	-	-	-	-
A. flavus	4	2	33	+	32	+	-	-	-	-										
A.niger	4	3	43	2+	45	2+	40	+	-	-	1	1	22	+	--	-	-	-	-	-
A.paraziticus	4	2	45	2+	42	3+	-	-	-	-	2	2	37	2+	43	2+	-	-	-	-
A.versicolor	4	2	42	3+	40	2+	-	-	-	-	2	2	36	2+	38	2+	-	-	-	-
A. terreus	4	2	37	2+	34	2+	-	-	-	-	2	2	65	3+	59	3+	-	-	-	-
Cladosporium sp.	1	1	15	+	-	-	-	-	-	-	1	1	18	+	-	-	-	-	-	-
Fusarium avenaceum	4	2	64	3+	82	3+	-	-	-	-	2	2	56	2+	61	2+	-	-	-	-
F. moniliforme	4	2	60	2+	61	2+	-	-	-	-	2	2	50	3+	52	3+	-	-	-	-
F. oxysporum	4	3	88	4+	87	4+	87	3+	-	-	4	4	73	3+	72	3+	70	3+	70	3+
P. crysogenum	2	1	12	+	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
P. corylophylum	1	1	15	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. frequantance	1	1	15	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stemphylium spp.	4	2	54	2+	46	2+	-	-	-	-	2	2	80	3+	78	3+	-	-	-	-
Trichoderma viride	4	4	68	3+	66	3+	66	4+	82	3+	4	4	73	3+	72	3+	70	3+	70	3+
Total	49	30									28	26								

A. = *Aspergillus* **F.** = *Fusarium* **P.** = *Penicillium*

* Each figure represents average of three replicates incubated at 27 C for 7 days

-= No growth += weak growth 2+= moderate growth 3+= heavy growth 4+= vigorous growth

3.4. *Effect of fungicides on fungal growth*

3.4.1. Effect of rhizolex and benlate

It was revealed from the results, tab. (4) that rhizolex and benlate fungicides at different concentrations significantly inhibit the mycelia growth of A. *flavus*, *A. niger* and *F. oxysporum* and the activity was increased as the concentration of fungicides increased. Data also showed that the growth of *A. flavus* was completely inhibited (100% inhibition) at rhiozolex concentration of ≤ 200ppm and benlate concentration of ≤ 400ppm. While, the growth of *A. niger* was also completely inhibited at rhizolex concentration of ≤ 400ppm and benlate at≤ 200ppm. The growth of *F. oxysporum* was completely inhibited by rhizolex at 1600 ppm. and benlate at ≤ 100ppm.

Table (4) effect of rhizolex and benlate fungicides on percentage inhibition of mycelial growth of *Aspergillus flavus*, *A. niger* and *Fusarium. oxysporum* isolated from Hashiat Al-allm 1 (470)

Fungicide	***Rhizolex***				***Benlate***			
Fungi / *Conc.*	*A. flavus*	*A. niger*	*F. oxysporum*	*Mean*	*A. flavus*	*A. niger*	*F. oxysporum*	*Mean*
0 (control)	0.00 K	0.00 K	0.00 K	0.00E	0.00 F	0.00F	0.00 F	0.00 E
50 ppm	24.63 J	38.87 H	31.83 I	31.78 E	30.53 E	49.63 D	75.93 C	52.03 D
100 ppm	75.17 DE	64.70 F	53.30 G	64.39 D	52.53 D	81.87 B	100.0 A	78.13 C
200 ppm	100.0 A	79.29 CD	74.03 E	84.43 C	72.67 C	100.0A	100.0 A	90.89 B
400 ppm	100.0 A	100.0 A	82.20 C	94.07 B	100.0 A	100.0 A	100.0 A	100.0 A
800ppm	100.0 A	100.0 A	86.67 B	95.56 B	100.0 A	100.0 A	100.0 A	100.0 A
1600ppm	100.0 A	100.0 A	100.0 A	100.0 A	100.0 A	100.0 A	100.0 A	100.0 A
Mean	71.40 A	68.98 B	61.15 B	-	65.10 C	75.93 B	82.18 A	-

3.4.2. Effect of paraformadehyde gas

Vapor of paraformaldehyde passing overhead the PDA medium inoculation with *A. niger* caused inhibition of the fungal linear growth as demonstrated in tab. (5). Fumigation with the paraformaldehyde tablet for 9 days completely inhibited the fungal growth. Also, the activity of inhibition was increased as the times of exposure increased.

Table (5) effect exposure time of paraformaldehyde gas on the linear growth (mm) of *Aspergillus niger* isolated from Hashiat Al-allm 1(470).

Exposure time (day)	***Growth (mm) after 6 days incubation***	***% inhibition***
0	90.0	0.00
3	25.11	72.10
6	20.00	77.77
9	0.00	100.0
12	0.00	100.0
15	0.00	100.0

4. Discussion

Archives preserve documents written on paper, papyrus, parchment and electronic supports. These organic and synthetic materials are deteriorated by physical, chemical and biological agents [1]. Biodeterioration of archival and library materials is a worldwide problem, which is great damage to unique manuscripts and books [2] [3]. Among three methods used for fungal isolation, culture was more sensitive than direct microscopic examination and also was more reliable than macroscopic inspection. So, it could be recommended that culture should be used for routine inspection especially in libraries containing old and valuable archival materials *Aspergillus*, *Fusarium* and *Penicillium* spp. were the main contaminating mould genera of all tested manuscripts and account over two third of contaminations. In general, the quantitative and qualitative differences in frequent occurrence of fungal genera between a tested manuscripts to another, ascertain that the environmental conditions play a great roll not only in relation but also in composition of the population of dominated microflora [23]. The same fungi were also recorded by many researchers [14][24][25][26]. Also, many detected species particularly *Trichoderma*, *Fusarium* and *Penicillium* readily form huge numbers of spores, which easily dispersed and will act as sources of new

infection. Regarding the cellulolytic activity of the 53 fungal isolates screened for cellulolytic activity on carboxy methyl cellulose (CMC) agar medium, only 31 (58.49%) had the ability to grow. Moreover, only 8 out off 31fungal isolates had a high ability to decompose cellulose powder in Czepek's agar medium. It is well known that the majority of fungal isolates obtained from the air of archives, libraries and museums exhibited cellulolytic activity produce acid, excrete pigments on the paper and contribute to the formation of biofilms, which accelerate the deterioration of the different document substrates [27][25]. These results are in agreement with that recorded by others [28][14][29]. Applications of fungicides and fumigants have provided a good control of fungi. Results showed that the growth of *A. flavus* was completely inhibited (100% inhibition) at rhiozolex concentration of ≤ 200ppm and benlate concentration of ≤ 400ppm. While, the growth of *A. niger* was also completely inhibited at rhizolex concentration of ≤ 400ppm and benlate at≤ 200ppm. The growth of *F. oxysporum* was completely inhibited by rhizolex at 1600ppm and benlate at ≤ 100ppm. On the other hand, fumigation with paraformaldehyde tablets for 9 days completely inhibited A. *niger* growth. The fungicides benlate and rhizolex has been extensively used *in-vitro* against several fungi. [14][15][16].

5. Conclusion

To avoid bio-deterioration of old manuscripts, it is recommended to store the valuable documents in a suitable environment, ideally with a relative humidity of 44-55% and constant temperature below 20 °C without use of chemicals. This study will help preserve the deteriorated old manuscripts from biodeterioration caused by fungi by using rhizolex and benlate fungicides against paper decay fungi (A. flavus, A. niger and F. oxysporum) and for sterilization we can use fumigation by paraformaldehyde tablet for 9 days exposure.

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