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Studies on Indoor Air Quality in the Repositories of the National Library and Archives of Egypt

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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. Quantitative analysis revealed air microbiological contamination with moulds ranged from 15.72-369.45 cfu x103 /m3 of air, while bacteria ranged from 94.35-660.48 cfu x103 /m3. The air sample analysis yielded 2917.08 fungal colonies classified into 7 genera of which Penicillium spp. (20.22%), Aspergillus flavus (20.76%), Trichoderma viride (12.94%), Alternaria tenuis (10.79%) and Aspergillus niger (10.51%) were the main contaminating mould of all tested repositories and they together constituted 54.42% of the total airspora. Aspergillus flavus and Penicillium spp. were the dominant component in the indoor air with a total concentration of 605.48 and 589.74 cfux102 /m3 with frequency occurrence of 20.76 and 20.22% respectively. Its highest concentration was recorded in August (259.49 and 314.53cfu x103 /m3) and the lowest during winter in January (141.54 and 125.81 cfux103 /m3) respectively. The obtained data showed that, of the 53 fungal isolates screened for cellulolytic activity on carboxymethyl cellulose (CMC) only 31(58.49%) had the ability to grow. Moreover, only 12 out off 31fungal isolates had a high ability to decompose CMC in Czepek's medium. All essential oil tested materials were found to highly effective and gave 100% reduction in the growth of F. oxysporum and T. viride fungi at the higher concentration of 0.4%. The Tea tree essential oil was most effective against F. oxysporum responsible for 65.3 mean % inhibition followed by Anise essential oil responsible for 63.2 mean% inhibition without significant difference. While, Rocket essential oil was the most effective against T. viride responsible for 70.4 mean % inhibition***

Keywords: GEBO, fungi, bacteria, bio-deterioration, cellulolytic activity, essential oil.

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 (Walker, 2003). Biodeterioration of archival and library materials is a worldwide problem, which is great damage to unique manuscripts and books (Zyska, 1993 and Shamsian et al., 2006). In addition, an international cause of the deterioration of paper due to its acidity and external agents is a major threat (Wessel, 1970 and Shamsian et al., 2006). As environmental microorganisms can deteriorate the different supports of heritage significance, as well as to affect human health, who inhale 10 m3 of the air every day and spend between 80-95% of their live indoor (Dacarro et al., 2003 and Kalwasinska et al., 2012).

Of interest here is the biological deterioration of old documents due to the activity of fungi and bacteria and their control. It is important to investigate the microbial concentration of indoor air at repositories (archives, documents and book storages) to preserve the cultural heritage. These microorganisms can be carried by dust particles into the indoor archive repositories by means of the people and the air ventilation systems (Nevalainen and Morawaska, 2009 and Borrego et al., 2012).

Several studies on fungal and bacterial airspora have been carried out by Gupta et al. (1993), Cvetnic and Pepelynak (2001), Sahab et al. (2003), Jothish and Nayar (2004), Hedayati et al. (2005), Deepak (2008). Jankiewicz et al. (2008) and Kalwasinska et al. (2012). Also, Abdel-Hameed et al. (2013) reported that, *Alternaria, Aspergillus, Cladosporium, Penicillium* and yeasts were the predominant genera in indoor and outdoor and the abundance of genera varied by season and region. Sahab et al. (2003) detected 50.4% of *Aspergillus*, 18.4% of *Alternaria*, 9.8% of *Rhizopus*, 8.4% of *Fusarium*, 5.5% of *Penicillium*, 4.2% of *Trichoderma* and 3.1% of Mucor from air of manuscripts storage during one year.

Now, researchers have focused for developing new methods for preserving old documents against microorganisms. The health impacts caused by moulds in buildings and library are major concern for libraries and achievers and indoor applications (Jankiewicz et al., 2008). The search for natural solutions that are user friendly and showing negligible to humans are increasingly sought. The plant extracts such as essential oils are well known for their antimicrobial properties (Sridhar et al., 2003, Wang et al., 2005, Yang and Clausen, 2008 and Zyani et al., 2011).

The aim of this work is to determine: first, screen and identify the common airborne fungi and bacteria in the indoor of Egyptian General Book Organization (GEBO). Second, to asses fungal activity on cellulose material degradation.Third, to study the antifungal activity of five essential oils against the most cellulolytic fungi.

2. Material and Methods

2.1 Characterization of the locations

Studies were conducted at eight floors (as indoor sampling sites) of the Egyptian General Book Organization (EGBO)

during one month from each of winter, spring and summer seasons of the year 2012. Samples were taken from various repositories containing ancient documents. The temperature and relative humidity were measured inside of repositories at each point of sampling at the moment when the microbiological sampling was performed using a digital thermohygrometer (SATO, Model, SKL-200, Japan).

2.2 Microbiological sampling of air

The determination was made in January, May and August representing seasons of winter, spring and summer, 2012 respectively. The sedimentation method suggested by Omeliansky (Bogomolova and Kirtsideli, 2009). Open Petri dishes at 1m from the floor were placed for 3 minutes in five different points of repositories. Culture media employed were potato dextrose agar+ chloramphenicol (0.1%) to isolate fungi and nutrient agar for bacterial growth. After exposure period the Petri dishes were sealed and subsequently incubated at 28±2°C for 72 hours for bacteria and 7 days for fungi. Once dishes incubated, fungal and bacterial colonies were counted. Colony forming units per cubic meter (cfu/m3) were determined, taking into account the following equation described by Omeliansky (1940):

Where:

$$No.of\ \ microbes\ (cfu/m3)\ =\ 5a.104\ (bt) - 1$$

a: number of colonies per Petri dish.,
b: dish surface, cm2,
t: exposure time (min).

Also, relative microbial distribution of genera or species was conducted according to Smith (1980), where:

$$Relative\ distribution\ = \frac{Number\ of\ colonies\ of\ the\ genus\ or\ species\ \ X100}{Total\ number\ of\ colonies\ of\ all\ genera\ or\ species}$$

2.3 Fungal identification

The identification of mould isolates were carried out on the basis of their macro and microscopically characteristic sporulation according to the keys of Gilman (1957), Barnett and Hunter (2000), Domsch et al., (2007) and Samson et al. (2010). The frequency occurrence expressed as percentage relative distribution of genera or species were calculated as mentioned before.

2.4 Qualitative determination of cellulolytic activity

Cellulolytic activity was determined using CMC and cellulose powder as a source of carbon in 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 center of each agar plate. Three replicates were used for each treatment. The inoculated plates were incubated at 28±2°C and the radial growth and its density were measured when fungus attained maximum growth in one treatment.

2.5 Effect of essential oils:

Essential oils of Star Anise, Fennel, Rosemary, Rocket and Tea tree oil obtained from National research center (NRC) of Egypt were tested against the most cellulolytic fungi. The inhibitory effect of the essential oil was calculated against the linear growth of Fusarium oxysporum and Trichoderma viride fungal isolates in-vitro. For each of the essential oil, five concentrations, i.e., 0, 0.05, 0.1, 0.2 and 0.4 % were prepared into sterilized molten and cooled PDA medium. Later 15 ml of the molten medium was poured into sterilized Petri plates and then inoculated with mycelium discs of 5 mm size and placed at the center. Three replications were used for each concentration. The plates were incubated at 28±2°C and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the essential oils was expressed as per cent inhibition of mycelial growth over control, calculated by using formula by Vincent (1947)

$$\text{Treatment Percent inhibition } = \frac{\text{Growth in control} - \text{growth in}}{Growth\ in\ control} \times 100$$

2.6 Statistical analysis

The collected data were statistically computed using the software Mstate-c for Windows. Results were expressed with the standard error of the treatment means for 95% confidence limits.

3. Results and Discussion

3.1 Total colonies of fungi and bacteria of different locations

Many locations in the building of the EGBO at Cairo, Egypt were chosen for microbiological studies.

Table 1: Total colonies of fungi and bacteria (cfu x 10^3/m3/min) falling in different sites of Egyptian Dar Alkotob building over a period of three different seasons of 2012

Floor No.	Name of Location	Total fungal count (cfu x 10^3/m3/min)				Total bacterial count (cfu x 10^3/m3/min)			
		Winter January	Spring May	Summer August	Total	Winter January	Spring May	Summer August	Total
1-	*Foreign periodical storage*	55.04	78.62	117.92	251.58	94.35	196.57	212.29	503.21
2-	*Arabic periodical*	86.99	117.92	172.99	377.40	204.43	283.06	377.42	864.91
3-	*Documents storage*	15.72	15.72	39.31	70.75	149.39	157.26	188.71	495.36
4-	*Old manuscripts*	47.18	70.76	86.49	204.43	172.98	298.74	298.79	723.38
5-	*Koran karim (ventilated)*	125.81	204.43	267.33	597.57	235.89	275.20	369.55	880.64
6-	*Papyrus and maps*	86.49	133.67	172.98	393.14	117.94	157.26	235.89	511.09
7-	*Documents (Non ventilated storage)*	94.35	125.80	133.68	353.83	243.75	369.55	377.42	990.72
8-	*Art documents (Non -ventilated)*	133.67	165.12	369.58	668.37	495.36	629.05	660.48	1784.87
Total		644.75	912.04	1360.28	2917.07	1714.09	2319.54	2720.55	6754.18

Table 2: Total number of fungal genera and species (cfu x 10^3/m^3/min) and their frequency occurrence percentage from the atmosphere of different location of the GEBO building during 3 seasons of the year 2012

Fungal genera and species	*Time of determination during year 2012 (Seasons and months)*						Total	%
	Winter (January)		*Spring (May)*		*Summer (August)*			
	Total	*%*	*Total*	*%*	*Total*	*%*		
Alternaria tenuis	62.90	9.76	102.23	11.21	149.41	10.98	314.54	10.79
Aspergillus chevalerie	15.78	2.44	-	-	23.59	1.73	39.32	1.35
Aspergillus flavus	141.54	21.95	204.45	22.41	259.49	19.08	605.48	20.76
Aspergillus humicola	31.45	4.88	23.57	2.59	-	-	55.04	1.89
Aspergillus niger	70.77	10.79	94.36	10.34	141.54	10.40	306.67	10.51
Aspergillus ochraceus	23.59	3.65	31.45	3.45	62.90	4.62	117.94	4.04
Aspergillus spp.	70.54	10.98	94.36	10.34	15.73	1.16	180.63	6.19
Cladosporium spp.	-	-	15.73	1.72	7.86	0.58	23.59	0.81
Fusarium avenaceum	7.86	1.22	31.45	3.45	23.59	1.73	62.90	2.16
Fusarium oxysporum	31.45	4.88	39.31	3.45	125.82	9.25	196.58	6.74
Mucor spp.	7.86	1.22	23.59	2.59	15.73	1.16	47.18	1.62
Penicillium spp.	125.81	19.51	149.40	16.38	314.53	23.12	589.74	20.22
Trichoderma viride	55.04	8.54	102.23	11.21	220.17	16.18	377.44	12.94
Total	644.59		912.13		1360.368		2917.08	

The concentrations of fungi and bacteria recorded a high variation in spite of having similar values of temperature and RH can be observed. Without any exceptions, all screened locations of the EGBO building were highly polluted (Table, 1). The same trend was also observed in the same locations by Sahab et al. (2003). The results indicated that, the total amount of fungi in the investigated repositories, which constituted indoor background, ranged from 15.72-369.45 cfu x103 /m3 of air, while the amount of bacteria at different sampling sites ranged from 94.35-660.48 cfu x103 /m3. The highest count was observed during summer month (August), 1360.28 and 2720.55 cfu x103 /m3 for fungi and bacteria, respectively and the lowest during winter month (January) detected 644.75 and 1714.09 cfu x103 /m3 for fungal and bacteria, respectively.

Data also showed that, the number of mould fungi was lower than the bacterial count at all detected time. The same result was also recorded by Reponen et al. (2001) and Borrego et al. (2012). The concentration of fungal and bacterial counts in the indoor of non- ventilated art document repositories (Table, 1) with a higher relative humidity and temperature were higher than those obtained in other locations at EGBO. In this repositories fungal concentration oscillated between 133.67 and 369.58 cfu x103 /m3 in January and August with total count of 668.37 cfu x103 /m3 , while bacterial concentration ranged between 495.36 and 660.48 cfu x103 /m3 during winter and august, respectively with total count of 1784.87 cfu x103 /m3 .

Moreover, the lowest total fungal and bacterial count (70.75 and 495.36 cfu x103 /m3), respectively were detected at the storage of the floor (3).The continuous fall of microorganisms inside the building and on the stored valuable manuscripts along the year indicated that, there are shortage in precautions and in the maintenance, storage and handling of such valuable documents .Numerous studies emphasis the fact that, rooms with efficient ventilation or air conditioning systems and guaranteed air tightness are less contaminated than rooms where air-conditioning was not installed (Gorny, 2004, Lugauskas and Krikstaponis, 2004 and Kalwasinska et al., 2012). The results obtained in our study are comparable to the findings of a study conducted by several investigators (Sahab et al., 2003, Jankiewicz et al., 2008, Kalwasinska et al., 2012 and Abdel-Hameed et al., 2013)

3.2 Fungal genera and species isolated from different locations

Data illustrated in table (2) indicated that, a total of 2917.08 fungal colonies belonging to 7 genera were identified from indoor of EGBO of Egypt building. *Penicillium spp*. (20.22%), *Aspergillus flavus* (20.76%), *Trichoderma viride* (12.94%), *Alternaria tenuis* (10.79%) and *Aspergillus niger* (10.51%) were the main contaminating mould of all tested repositories. They were present throughout the year and they together constituted 54.42% of the total airspora.

Aspergillus flavus and *Penicillium spp*. were the dominant component in the indoor air with a total concentration of 605.48 and 589.74 cfu x103 /m3 with frequency occurrence of 20.76 and 20.22%, respectively. Its highest concentration was recorded in August (259.49 and 314.53cfu x103 /m3) and the lowest during winter in January (141.54 and 125.81 cfux103 /m3), respectively. The same trend was also observed, as general for the other detected moulds, as the highest concentration was showed during summer in August and the lowest during winter in January. Many reports dealing with the microbial levels in indoor air as those reported by Sahab et al.(2003), Deepake (2008) and Jankewicz et al. (2008). Shamsian et al. (2006) studied the fungal contaminations in historical manuscripts at Astan Quds museum library. They detected that, fungal genera of *Aspergillus, Penicillium* and *Mucor* could damage paper of manuscripts.

4. Selection of Cellulose Degrading Fungi

4.1 Growth on CMC Czapek's agar medium

Of the 53 fungal isolates screened for Cellulolytic activity on carboxymethyl cellulose (CMC) only 31(58.49%) had the ability to grow (Table, 3). Moreover, only 12 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). Moreover, 17of total 31 fungal isolates gave moderate growth (Ø between 25-54 mm).But only 2of 31 gave slight effect (Ø between 12-15 mm), respectively. These results are in agreement with that, recorded by El-Sayed (1980), Sahaba (1988) and Sahab et al. (2003).

4.2 Growth on cellulose-Czapek's agar medium:

Of the 28 fungal isolates screened for cellulolytic activity on cellulose-Czapek's medium (Table, 3) only26 had the ability to grow. Only 10 (35.71%) fungal 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.

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

Fungal genera and species	Growth on CMC-Czapek's medium										Growth on Cellulose -Czapek's medium									
	No. of isolate	(+) ve growth	Isolate No.1		Isolate No.2		Isolate No.3		Isolate No.4		No. of isolate	(+) growth	Isolate No.1		Isolate No.2		Isolate No.3		Isolate No.4	
			Ø mm	Density	Ø mm	Density	Ø mm	Density	Ø m m	Density			Ø mm	Density	Ø m m	Density	Ø mm	Density	Ø mm	Density
Alternaria spp.	4	2	38	+	37	+	-	-	-	-	2	2	22	+	23	+	-	-	-	-
Aspergillus cheivaleri	1	1	25	+	-	-	-	-	-	-	1	1	18	+	--	-	-	-	-	-
Aspergillus flavus	4	2	33	+	32	+	-	-	-	-										
Aspergillus humicola	1	1	32	+	-	-	-	-	-	-	1	1	12	+	-	-	-	-	-	-
Aspergillus niger	4	3	43	2+	45	2+	40	+	-	-	1	1	22	+	--	-	-	-	-	-
Aspergillus paraziticus	4	2	45	2+	42	3+	-	-	-	-	2	2	37	2+	43	2+	-	-	-	-
Aspergillus versicolor	4	2	42	3+	40	2+	-	-	-	-	2	2	36	2+	38	2+	-	-	-	-
Aspergillus 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+	72	3+	-	-	-	-	2	2	56	2+	61	2+	-	-	-	-
Fusarium moniliforme	4	2	60	2+	61	2+	-	-	-	-	2	2	50	3+	52	3+	-	-	-	-
Fusarium oxysporum	4	4	88	4+	87	4+	80	3+	82	3+	4	4	73	3+	72	3+	70	3+	70	3+
Nigrospora sphaerita	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium crysogenum	2	1	12	+	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Penicillium corylophylum	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium frequantance	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium spp.	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stemphylium spp.	4	2	54	2+	46	2+	-	-	-	-	2	2	80	3+	78	3+	-	-	-	-
Trichoderma viride	4	4	66	3+	60	3+	62	4+	82	3+	4	4	73	3+	72	3+	70	3+	70	3+
Total	53	31									28	26								

**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

Table 4: Effect of essential oils on percent inhibition of mycelia growth of *Fusarium oxysporum and Trichoderma viride*

Tested	*Fusarium oxysporum*						*Trichoderma viride*					
Conc. %	fungi	0.05	0.1	0.2	0.4	Mean of inhibition%	0	0.05	0.1	0.2	0.4	Mean of inhibition%
Rosemary	0	36.9	48.4	52.5	100		0	50.6	41.1	74.3	100	
	h	g	fg	efg	a	**47.6**	f	bcda	de	abcd	a	**53.2**
Anise	0	57	75.9	83.3	100		0	16.3	52.5	90.6	100	
	h	defg	bcd	ab	a	**63.2**	f	cde	bcde	ab	a	**51.9**
Tea tree oil	0	71.9	74.4	80.4	100		0	47	81.4	69.9	100	
	h	bcde	bcde	abc	a	**65.3**	f	cde	abcd	abcd	a	**59.7**
Rocket	0	58.9	58.2	64.5	100		0	81.8	83.3	86.7	100	
	h	cdefg	defg	bcdef	a	**56.3**	f	abc	abc	abc	a	**70.4**
Fennel	0	52.3	53.7	64.1	100		0	40.8	64.4	82.2	100	
	h	efg	efg	dcdef	a	**54**	f	de	abcd	abc	a	**57.5**
Mean	0	55.4	62.1	68.95	100		0	47.31	64.55	80.56	100	

On the other hand, the six isolates which belonging to *A. terreus*, *F. avenaceum* and *F. moniliforme* recorded linear growth between 50-65 mm with moderate and heavy mycelium growth.

Moreover, the other 10 fungal isolates showed slight growth with moderate, and even no mycelium growth. 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 (Florian, 2004 and Borrego et al., 2012).

5. Antifungal activity of essential oils

The antifungal activities of five essential oils on percentage inhibition of mycelial growth of F. oxysporum and T. viride are shown in Table (4). These essential oils were tested by agar diffusion plate method caused significant reduction in the growth of above mentioned fungi. The rate of growth reduction was directly proportional to the concentration of tested material in the medium. Results showed that, all tested essential oil possess a remarkable antifungal activity against the two tested fungi compared to control.

All tested materials were found to highly effective and gave 100% reduction in the growth of the two fungi at the higher concentration of 0.4%. The Tea tree essential oil was most effective against *F. oxysporum* responsible for 65.3 mean % inhibition followed by Anise essential oil responsible for 63.2 mean% inhibition without significant difference. While, Rocket essential oil was the most effective against *T. viride* responsible for 70.4 mean % inhibition. On the other hand, Rosemary and Anise showed less effect on the growth of *F. oxysporum* and *T. viride* respectively, as the mean percentage inhibition were 47.6 and 51.9% respectively. The plant extracts such as essential oils are well known for their antimicrobial properties (Sridhar et al., 2003, Wang et al., 2005, Yang and Clausen, 2008, and Zyani et al., 2011).

Our data on the antifungal properties of oils suggested that, these oils should be examined and further evaluate its potential as a natural fungicide against deteriorated fungi.

6. 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 also demonstrated the in-vitro antifungal activity of essential oils against paper decay fungi and potential use oils preservative for the control of paper decay by many fungi. However, for the development essential oils as alternative of synthetic fungicides, further studies are required to evaluate toxicity and the effectiveness of treatment.

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