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INDOOR FUNGAL AIRSPORA AND MICROORGANISMS COMMUNITIES ASSOCIATED WITH OLD MANUSCRIPTS OF GEBO OF EGYPT

Sahab, A.F.*; Fatma Tawfic; Sohair Sahaba*** and Sawsan Moustafa******

* Plant Path. Dept., NRC, Cairo, Egypt.

** Biol. Dept. Fac. of Sci., King Abd El-Aziz Univ., Saudi Arabia.

*** Microbiol. Lab., GEBO, Cairo, Egypt.

****Biol. Chem. Dept., Fac. of Sci., Cairo Univ., Egypt.

ABSTRACT

The indoor airspora in different locations and sites of the archive and library of the GEBO building have been investigated as one year's collection using the agar plate method. All the screened locations of the GEBO building were highly polluted air, and could be considered a main contamination source.

Among the air-borne fungi isolates collected around the year were 50.4% of *Aspergillus* , 18.4 % of *Alternaria*, 9.8 % of *Rhizopus*, 8.4 % of *Fusarium*, 5.5% of *Penicillium*, 4.2% of *Trichoderma*, 3.1% of *Mucor* and 1.8 % of unidentified fungi as could be arranged depending on their occurrence in the samples.

Also, About 70 representative isolates developed on agar media were isolated from old manuscripts samples. The fungal genera could be arranged on the basis of there frequent occurrence as follows: *Penicillium* (41.7 %), *Aspergillus* (29.5 %), *Alternaria* (11.8 %), *Fusarium* (9.8%) and *Trichoderma* (6.6 %) of the total fungal count.

The obtained results show that only 12 out off 56 fungal isolate had a high ability to decompose CMC in Czapek's agar medium. Other 21 and 19 isolates gave moderate and slight effect respectively, while 4 isolates showed no growth. The most active fungal isolates (12 isolates) comprised three isolates of *Aspergillus niger* , 3 isolates of *Fusarium oxysporum* , 2 of *Penicillium funiculosum* and 4 of *Trichoderma viride* .

Data also showed that isolate no.1, 2, 3 and 4 of *Trichoderma viride* and no. 2 and 3 of *F.oxysporum* showed higher cellulase activity (Cx) than other fungal isolates, these isolates caused 74.81%, 91.26 %, 91.26%, 71.01%, 70.08 and 70.08 loss in viscosity respectively.

Keywords: airspora moulds, indoor air, old documents, contamination, cllulolytic activity.

INTRODUCTION

Air is the most common medium for the dispersal of spora and hyphal fragments of fungi (Hisao, 1996). Moulds are ubiquitous group of saprophytes or parasite fungi which ecologically highly distributed in nature. Their spores have been found in soil, water, plant and other decayed organic materials and they pervade the outdoor and indoor air. The best known and most prevalent health effect on indoor fungi is their ability to degrade various cultural properties, including manuscripts, book and documents... ext. Moreover, some fungi have the ability to induce allergic respiratory disease in susceptible individuals. Other deleterious health effects of exposure to indoor fungi result from nonallergenic fungal metabolite mycotoxins.

Several studies on fungal airspora have been carried out in Taiwan as stated by Han *et al.*, 1981 and Chang *et al.*, 1983. Han and Chang (1981) have

investigated the Hualsin airspora by means of the gravity slide method, recording 12 genera of fungi. Hsiao (1996) reported that among the fungi isolated, the majority comprised species of *Cladosporium* (33.18%) , *Aspergillus* (11.06%), Yeast (7.14%), *Penicillium* (3.44%), *Fusarium* (1.74%) and *Alternaria* (1.02%). Cvetnic and Pepeljnjak (2001) studied the airborne mycoflora of indoor environment. The spores of the high incidence of *Penicillium* and *Cladosporium* had the ability to grow on deteriorated leather, paper and cotton or wool fabrics (Zyska, *et al.*, 1995)

Paper is primarily composed of cellulose and other substances, i.e., lignin, hemicelluloses, pectin, waxes, tannins, proteins and minerals (Roberts, 1996).

Many workers, later on, proved that many species of Deuteromycetes, i.e., *Alternaria, Aspergillus*, *Cheatomium, Humcola, Myrothecium, Penicillium, Stachybotrys, Stemphylium, Trichoderma*, and *Ulocladium* are frequently isolated from books, documents and prints (Kowalik, 1980 and Galo, 1985). Sahaba, (1988) reported that the most active cellulose decomposes associated with deteriorated manuscripts, documents and books of the GEBO, of Egypt are fungal strains.

The aim of this work is to screen and identify the indoor aeromycoflora during one year period and also to survey microorganisms polluting valuable old manuscripts and their activity in degradation of cellulose materials.

MATERIALS AND METHODS

Locations:

The study on the indoor aeromycoflora was conducted for one year period in 2001. Seven places (3 locations in the archive and 4 in the library) of theGeneral Egyptian book Organization (GEBO) of Cairo, Egypt were selected as indoor air sample sites.

Sampling:

Sampling of airspora was carried out using the culture plate technique based on gravity – setting method adopted by Madelin and linton (1972) . Two kinds of isolation media were used: Czapek's Dox agar medium, containing (g/L) : 20, sucrose; 2 $NaNO_3$; 0.5 Mg SO_4. $7H_2O$; 05, KCl, 0.01, $FeSO_4$ and 20 agar. The second was: Potato dextrose agar medium (PDA) , containing(g/L):200peeledpotato;10,dextrose and 20, agar. Rose bengal(1/3000w/v) + Penicillin and Streptomycin antibiotics were added as bacteriostatic agents. The plates were exposed for 15 minutes to the air, then sealed and send to laboratory for incubation and identification.

Also, five deteriorated old valuable mansucripts (El-Azony family) obtained from the store of GEBO were selected . The surface of PDA and CMC- Czapek's agar (sucrose was substituted with CMC, 10.0g) media poured into plates were wiped by sterilized cotton swabs. The Petri-dishes were incubated at 28°C for 7 days and 15 days for cellulolytic fungi.

Identification:

The identification of mould isolates were carried out on the basis of their macro and microscopically characteristic sporulation according to the keys of Thom and Raper(1945), Gilman(1957), Barnett and Hunter (1972)

and Nelson, *et al.* (1983). The frequency occurrence of each genus was expressed as the percentage of samples containing a given organism.

Inoculation and Cultivation:

For the screening of the cellulolytic activities, cultures of the isolated fungi grown on PDA (plus 5% avicel) slants for 7 days at 30°C were transferred to 250 ml flasks each containing 50 ml sterilized Mandels and Weber (1969). It contained (g/L): 1.4 $(NH_4)2\ SO_4$; 2.0 KH_2PO_4; 0.3 Urea; 0.3 $CaCl_2$; 0.3 $MgSO_4$. $7H_2O$; .005 $Fe_2\ (SO_4)_3$.0014 $ZnSO_4$; .0016 $MnSO_4$; .002 CO Cl_2; Avicel (1%); Protease peptone (1%) and Tween 80 (0.1%), with finai PH of 5.0. The flasks were incubated on a rotary shaker (180 rpm) at 28-30°C for 3 days.For solid cultivation , experimental cultures were made in plates each containing culture medium and inoculated centrally with a disk(5-mm in diameter) and incubated at 28°C and the diameter growth were daily measured (Sahaba, 1988).

Selection of cellulose degrading microorganisms:

The isolates which gave high growth were chosen to measure their cellulolytic activity by using CMC as substrate and measuring the loss in the medium growth viscosity caused by fungi according to Matta and Dimond (1963).

The following formula was used in calculation of the percentage loss in viscosity.

$$\text{Loss in viscosity \%} = To - Ti / To - Tw \times 100$$

Where:

To = time of flow in seconds for 10 ml of control mixture with boiled filtrate.
Ti = time of flow in seconds for 10 ml of reaction mixture with active filtrate.
Tw = time of flow in seconds for 10 ml D.W.

RESULTS AND DISCUSSIONS

Many locations in the building of the GEBO at Cairo as well as deteriorated manuscripts and documents were chosen for microbiological studies sample from the atmosphere of 3 sites of archive (book store, old manuscripts and old documents) as well as 4 sites of library (manuscripts, fine arts, periodical hall and reading hall, more over from deteriorated rages of old manuscripts and documents were subjected to microbiological analyses

1-a Indoor fungal airspora of manuscripts stores and library halls.

In pursuance of data represented in Table (1) concerning the atmospheric fungal contamination during 2001 of either manuscripts stores (archive and library halls)

The following conclusions can be drawn:

Without any exceptions all screened locations of the GEBO building were of a highly polluted air, which could be considered a main contamination source. The various sites in each location showed various total fungal counts, during the seasons of the year.

The falling rate of microorganisms on each square meter per minute ranged, generally from 157.1 to 1942.6 in the atmosphere of different sites of archive and from 78.6 to 746.7 in the atmosphere of library. The archive sites

Table (1): Monthly total colony on agar plates falling on each square meter per minute of different sites of the G.E.O.B.-Building along one year (Jan. to Dec.,2001).

Month / Location & Sites	Jan.	Feb.	Mar.	April	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Mean Count	% Occurrence
I -Archive														
1- Book store	117.8	196.4	314.2	432.1	586.2	707.0	8880.6	896.8	707.0	586.2	314.2	196.4	494.5	24.19
2- Old Manuscripts	0.0	0.0	39.3	78.6	78.6	1117.8	436.8	544.7	335.0	732.5	146.3	54.5	289.5	14.16
3- Old documents	39.3	49.3	49.3	78.6	78.6	117.8	228.7	343.6	78.6	39.3	39.8	29.4	97.7	4.78
Total isolates	157.1	245.7	402.8	589.3	743.4	1942.6	1456.1	1785.1	1121.5	1358.0	500.3	280.3	293.9	-----
II -Library														
1- Manuscripts	413.9	227.7	358.3	398.4	498.4	398.4	196.4	196.4	314.2	235.7	157.1	78.6	291.9	14.28
2- Fine arts	263.3	526.6	263.3	368.6	105.3	220.4	117.8	127.1	127.1	117.8	39.3	0.0	194.7	9.52
3- Periodical Hall	224.4	424.4	289.6	258.8	746.7	756.6	353.5	392.8	392.8	392.8	374.9	157.1	397.0	19.42
4- Reading Hall	244.5	424.4	289.6	258.8	746.7	405.0	196.4	196.4	196.4	196.4	117.8	78.6	279.1	13.65
Total coolates	1146.1	1633.1	1199.8	1284.6	2097.1	170.4	8641.1	942.7	1060.5	942.7	689.1	314.3	290.6	------

showed the lower total fungal count during winter months (December, January and February reached 280.3, 157.1 and 245.7 colonies/ m^2 respectively). August (summer season) possessed the eminent falling rate, while fluctuating value occurred in the other months of the year.

Data also showed that library sites recorded the lowest fungal count during December (314.3 colonies/ m^2)while, during July the library sites has the highest level one (8641.1 colonies/ m^2) .The continuous fall of microorganisms inside the library 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 national and international valuable articles. Many reports dealing with the seasonal variations of fungal levels in indoor and outdoor air as those reported by Miller (1992) , Pasanen *et al.* (1996) and Cvetnic and Pepeljnjak (2001).

1-b Fungi occurring seasonally through out the year period:

Table (2) comprises the dominant fungal genera that dropped from air in either archive stores or library halls of the GEBO building along a year . these genera are *Alternaria* , *Aspergillus* , *Fusarium* , *Mucor* , *Rhizopus* , *Trichoderma* , *penicillium* and others of unidentified fungal isolates were ranked as " unknown " . There frequent occurrence percent differs especially from one month to another. The frequent occurrences were found to be ranging from 4.2 % in either September or November to 56.9 % in February for *Alternaria* , from 20.5 % in October to 75.3 % in November for *Aspergillus* , from 1.4 % in January to 14.5 % in October for *Fusarium*. The values were recorded nil in January till June and December to 14.5 % in October for *Mucor* and from nil in January , February , October , November and December to 41.1 % in May for *Rhizopus* and from nil in both February and November to 16.9 % in June for *Trichoderma*. Data also showed that the frequent occurrence percent for *Penicillium* were fluctuated from nil in April till September to 25.8 % in January for and from nil in January , February , May , June , October and December to 5.8 % in august for other unidentified fungi . in pursuance of the distribution of fungi genera along a year it could be observed that both *Alternaria* and *Penicillium* proliferate in cold months and others such as *Rhizopus* , *Trichoderma* and *Aspergillus* prefer a moderate warm months while, *Fusarium* , *Mucor* and others dominate in hot seasons .

By mediating the average of fungal isolates dropped from air through out a year the fungal genera could be arranged on the basis of their frequent occurrence as follows:
Aspergillus with 50.4 %, *Alternaria* with 18.4 % , , *Rhizopus* with 9.8 % , *Fusarium* with 8.3 %, *Penicillium* with 5.5 % , *Trichoderma* with 4.2 % , *Mucor* with 3.1 % and unidentified other fungi with 1.8 % .

2-survey of fungi associated with old manuscripts.

About 70 representative isolates developed on agar media were isolated from old manuscripts samples (Table, 3). The fungal genera could be arranged on the basis of there frequent occurrence as follows: *Penicillium* (41.7 %), *Aspergillus* (29.5 %), *Alternaria* (11.8 %), *Fusarium* (9.8%) and *Trichoderma* (6.6 %) of the total fungal count.

Table (2): Frequent occurrence of different fugal genera in the atmosphere of the GEBO-Building along one year during 2001 calculated in percentage.

Fungal Genera	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	average
Alternaria	21.6	56.9	11.6	13.1	16.2	5.3	9.7	14.2	4.2	37.0	4.2	27.0	18.4
Aspergillus	48.5	27.7	49.1	71.2	31.0	52.6	53.9	55.2	65.5	20.5	75.3	54.8	50.4
Fusarium	1.4	10.8	8.7	3.6	7.3	6.6	8.3	10.0	3.1	14.5	5.1	20.2	8.3
Mucor	0.0	0.0	0.0	0.0	0.0	0.0	2.1	4.1	10.5	14.5	5.1	0.0	3.1
Rhizopus	0.0	0.0	25.2	5.8	41.1	18.6	12.6	6.6	7.3	0.0	0.0	0.0	9.8
oderma	2.7	0.0	1.8	2.1	4.4	16.9	10.5	4.1	4.2	0.8	0.0	2.9	4.2
Penicillium	25.8	4.6	0.9	0.0	0.0	0.0	0.0	0.0	0.0	12.7	8.8	13.1	5.5
Unknowns	0.0	0.0	1.8	4.2	0.0	0.0	2.9	5.8	5.2	0.0	1.5	0.0	1.8

- **G.E.B.O. General Egyptian Book Organization, Corniche El-Nil, Boulac , Cairo ARE.**
- **Enumerations were carried out using three replicas for each event.**
- **Plates, with 9 cm in diameter, charged with agar medium were exposed to air for 5 min. , then incubated 7 days at 30°c.**
- **Counts were calculated in percentage for a fungal genera falling on a square metre per minute.**

Table (3): Frequent occurrence of different genera of fungi isolated from old manuscripts collected from different sources.

Sample Names	El-Azawy Family Book (1820-1822)					Total	Frequency %
Sample No.	1	2	3	4	5		
Alternaria	-	4	2	1	-	7	11.8
Aspergillus	3	1	7	3	4	18	29.5
Fusarium	-	2	-	1	3	6	9.8
Penicillium	7	8	10	-	1	25	42.6
Trichderma	-	1	1	2	-	4	6.6
Total						60	

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 selection but also in composition of the population of dominated microflora (Tao, *et al.*, 1997).

Fourteen species which belong to five genera were identified and classified to:
Alternaria tenuis , Aspergillus candidus, A. flavus , A niger , A. paraziticus , A. tamarii Fusarium graminearum , F. moniliforme, F. oxysporum ; Penicillium chrysogynum P. decumbens, P. funiculosum ; Trichoderma harzianum and *T. viride* as shown in Table (4). The same fungi were also recorded by Mahmoud *et al.* (1980); Sahaba, (1988); Zyska, *et al.* (1995) and Wang *et al* (1999).

3- Selection of cellulose degrading microorganisms :

a- Growth on CMC – Czapek's agar solid medium:

Table (4) represents data on the cellulolytic activity of various representative fungal isolates. The degree of decomposition of CMC differed considerably between microorganisms. Data also show that only 12 out off 56 fungal isolates had a high ability to decompose CMC in Czapek's agar medium. Other 21 and 19 isolates gave moderate and slight effect

respectively, while 4 isolates showed no growth. The most active fungal isolates (12 isolates) comprised three isolates of *Aspergillus niger* , 3 isolates of *Fusarium oxysporum* , 2 isolates of *Penicillium funiculosum* and 4 isolates of *Trichoderma viride* .

Fungal isolates which showed high ability to use CMC as carbon source in the presence of inorganic nutrients were used. Table (4) shows that isolate no.1, 2, 3 and 4 of *Trichoderma viride* and no. 2 and 3 of *F.oxysporum* showed higher cellulase activity (Cx) than other fungal isolates, these isolates caused 74.81%, 91.26 %, 91.26%, 71.01%, 70.08 and 70.08 loss in viscosity respectively. These results are in agreement with that recorded by Tashpulator (1973), El-sayed (1980), Mahmoud, *et al* (1980), Sahaba (1988), and Caneva, *et al* (1991) they reported that the most active cellulolytic organisms belonged to fungi.

The present study revealed that most active cellulolytic microorganisms associated with deteriorated manuscripts, documents and books are fungal strains. This may be due to the high acidity of paper manufacture since 1850 as a result of the replacement of gelatin by alum-resin as stated by Venter, (1966) and Carlton (1970) or due to the great tolerance to environmental conditions, i.e. fungi can live with a lower water content than other microorganisms (Caneva *et al.,* 1991).Some species of these fungi belong to the opportunistic fungi , which may be agents of mycosis specially in patients with immune deficiency. *Penicillium* , *Aspergillus and Fusarium* spp . have other deleterious health effects , result from none allergic fungal metabolites mycotoxins . The mycotoxins produced to the substrate may be present in carcinogenic or immunosuppressive properties (Cvetnic and pepeljnjak, 2001).

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مدي تواجدالفطريات في هواء مبني الهيئة القومية لدار الكتب المصرية والمصاحبة لبعض المخطوطات القديمة

احمد فرحات سحاب – فاطمة حسن توفيق – سهير صحابة – سوسن درويش
المركز القومى للبحوث- جامعة الملك عبد العزيز- الهيئه العامه للكتاب- جامعة القاهره

تم دراسة مدي تواجد الفطريات الموجودة في هواء عدد من المواقع في الأرشيف والمكتبات المتواجدة في مبني الهيئة القومي لدار الكتب المصرية خلال (عام ٢٠٠١) بإستخدام بيئة الأجار حيث وجد أن جميع المواقع سواء في مخازن الأرشيف والمكتبات كانت عالية التلوث بالفطريات وقد أمكن ترتيب العزلات الفطرية المتواجدة في الهواء علي أساس النسبة المئوية لتواجدها في الهواء كالأتي:

الأسبرجلس ٥٠,٤% والألترناريا ١٨,٤% والريزوباس ٩,٨% والفيوزاريوم ٨,٣% والبنسليوم ٥.٥% التريكودرما ٤,٢% والميوكر ٣,١% وفطريات أخري غير معروفة بـ ١,٨% .

كما وجد أن ٧٠عزلة من الفطريات التي تم عزلها من المخطوطات القديمة أمكن ترتيبها علي اساس النسبة المئوية لتواجدها كالآتي : البنسليوم ٤١,٧% والأسبرجلس ٢٩,٥% والألترناريا ١١,٨% والفيوزاريوم ٩,٨% والتريكودريما ٦.٦%

كما أوضحت الدراسة ان من بين ٥٦عزلة فطرية تم إختبارها كان ١٢ فقط لها القدرة علي تحليل الكربوكسي ميثيل سليولوز الموجودة في بيئة تشابكس وأن ١٩,٢١عزلة فطرية كانت قدرتها متوسطة او قليلة علي الترتيب وأن اربع عزلات لم تتمكن من النمو.

أمكن تعريف الفطريات التي لها قدرة علي تحليل السليولوز كتالي :

- الأسبرجلس نيجر (٣عزلات) وفيوزاريوم اكسيسبورم (٢عزلة) والبنسليوم فيونكيولوزم (٢ عزلة) والتريكودرما فيريدي (٤عزلات) كما أوضحت الدراسة أن العزلات ارقام ١،٢،٣،٤ من التريكودريما فيريدي ، العزلة رقم ٣،٢ من الفيوزاريوم اكسيسبورم لهم القدرة علي تحليل الكربوكسي ميثيل سليلوز في بيئة تشابكس السائلة حيث بلغت نسبة الفقد في اللزوجة الي ٧٤,٨١% ، ٩١,٢٦% ، ٩١,٢٦% ، ٧٠,٠١% ، ٧٠,٠٨% ، ٧٠,٠٨% علي الترتيب .
- وقد خلصت الدراسة الي ضرورة الحد من أعداد الفطريات المتواجدة في هواء المخازن (الأرشيف) و المكتبات للحفاظ علي صحة المترددين والعاملين بها لوجود بعض الفطريات لها قدرة علي إفراز بعض السموم الفطرية ولاضرارها البالغة بالمخطوطات القديمة الهامة .