INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE

ISSN: 2067-533X Volume 10, Issue 2, April-June 2019: 307-316 www.ijcs.uaic.ro

ANTIFUNGAL ACTIVITY OF SOME SELECTED FUMIGANTS REGULARLY USED AGAINST FUNGI ISOLATED FROM REPOSITORY OF DAR- AL-KOTTOB OF EGYPT

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Abstract

The main goal of this work was to investigate the prevalence of fungi inside a repository of Dar al-Kutub during November 2017 and the biological fungicidal and fungistatic activities of some fumigants against isolated fungi. Indoor airborne fungi ranged from 10.482 to 83.857 cfu X10[2]. Which were seven species belonged to five genera of filamentous fungi were detected and identified. The percentage abundance of the species Aspergillus flavus (27.11%), Aspergillus niger (22.03%) and Penicillium chrysogenum (23.73%) were present in high values. On the hand, the other fungal genera could be arranged in descending order as follows: Fusarium spp. (7.63%), Trichoderma spp. (4.24%) and Alternaria spp. (3.39%). The antifungal effectiveness of three selected fumigants (phostoxin, dettol+isopropanol and paraformaldehyde) was determined. Complete reduction (100%) in the linear growth by phostoxin of all tested fungi, whereas a high growth reduction was also observed by paraformaldehyde. A potent effect was observed by dettol+isopropanol which can be arranged on descending order as follows: Penicillium chrysogenum (96.30%), Trichoderma harzianum (73.00%), A. niger (69.93%), and then F. oxysporum (55.97%). The most effective concentration was 20%, whereas the lowest levels of inhibition was observed against T. harzianum (18.87%), followed by F. oxysporum (30.30%) at conc. 5%. Dettol and isopropanol formulation as well as para-formaldehyde had a significant fungicidal activity on the growth of all tested fungi). As no growth was appeared (100% inhibition) from the fungal disc taken from plates of both fumigants against F. oxysporum, T. harzianum and P. chrysogenum. But, phostoxin showed fungistatic activity as different levels of inhibition ranged from 6.66 -73.33% were observed from discs of all fungi.

Keywords*: Fumigants; antifungal; Dettol; Para-formaldehyde; Phostoxin; Dar-elkottob.*

Introduction

Libraries are priceless nation's legacy that contains all sources of human knowledge and wisdom. The major constituents of the library materials including paper, leather, parchment, papyrus is organic in nature [1] and so they are susceptible to the deterioration by different factors biotic (biological) and abiotic (environmental) including the physical, chemical, accidental factors [2-4].

Among biological agents fungi constitute the biggest problem in biodeterioration process of materials stored in archives, library and repositories [5]. Because of the great ability of fungi to produce extracellular hydrolytic powerful enzymes, that cause serious damage to valuable

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documents mechanically, chemically, and aesthetically, in addition to production of pigments or weak acids this causes discolouration, disfigurement and staining of library materials [6, 7].

Absence of proper ventilation, darkness, high temperature and relative humidity (above 55%) provides favorable conditions for the growth encourage the spread of these fungi [8].

Also, dust that is air borne in nature represents a highly dangerous threaten to libraries and archives as it hygroscopic in nature and contain soil particles, metallic substances, fungal spores, so it can represent a microclimatic environment through it fungal spores can grow and flourish, besides that the accumulation of the dust plus high humidity transformed into dirt that sticks with the library materials and become difficult to remove [9].

Several lists of airborne fungi and their counterparts in artwork have been isolated from the indoor area like, *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus parasiticus, Aspergillus versicolor, Cladosporium cladosporoides, Cladosporium oxysporum, Curvularia lunata, Fusarium solani, Mucorhiemalis, Penicillium brevicompactum, Penicillium chrysogenum,* and *Rhizopus stolonifer* [10-12].

Several indoor microbial remediations were tested in order to eliminate the microorganisms from indoor environments. During the years many chemical substances were tested as liquid biocide or gases for fumigation [13, 14].

Paraformaldehyde could be used as a fumigant to control microbes mainly in closed systems as fungicide [15], in addition its use as general disinfection [16, 17] reported a series of articles on the use of paraformaldehyde in sterilization, and found that, formaldehyde gas is germicidal, yeasts, fungi and vegetative bacteria were easily killed by the gas and its effect on Its maximum activity were at closely systems at 80% RH.

Others, suggested that, paraformaldehyde exhibited fungicidal activity as a fumigant against *Aspergillus niger, Penicillium sp. Cladosporium sp.,* and *Emericella nidulans* [18].

The compositions of dettol include Chloroxylenol B.PC (4.8% w/v) [19]. Its antimicrobial action is due to its phenolic nature that makes disorders and changes the permeability of the microorganism's cell wall and stopping the enzymes function and finally led to the death of the cell [20-22].

A.R. Abbas and M.H. Ibtisam [23] reported that, dettol is more effective than formalin in their effect on fungus and less effective on bacteria. Chloroxylenol (dettol) at 10% was highly antifungal on *Aspergillus flavus.* According to *S.R. Mohammed and M.H. AL-Jibouri* [24] the most effective concentration of dettol was 10% for all isolated fungi. These finding agreed also with *K.M. Redal* [25] and differed with *S.M. Handel* [26] and *D.R. Smith* [27] who reported that formaldehyde was more effective than dettol.

Dettol at different concentrations showed a remarkable effect on fungi at all-time used, but 2.5% dettol solution was the most effective disinfectant against isolated fungi, in particular on *Aspergillus niger* and *Cunninghamella* [28].

Hydrogen phosphide, or phosphine (PH3), is a highly toxic gaseous compound used for protection of stored cereals and oilseeds against attack by insect and rodent pests [29]. According to previous reports, PH3 can have anti-fungal activity *J. Leitao et al* [30], although apparently it is only effective on actively growing organisms.

PH3, are very effective because of their high infusibility across physical and biological barriers, which gives them the capacity to very rapidly reach the susceptible targets that are hidden in the stored products, although the antifungal activity of phosphine against *A. paraziticus* has been noted [31].

The purpose to determine: firstly, to detect the prevalence of fungi inside repository of Dar al-Kutub in one month. Secondly, to evaluate the effects of some fumigants on growth by vapor or contact for fungicidal or fungistatic activity against fungi isolated from repository.

Materials and Methods

Description of the repository of old document

The Dar Al-kottob (National library & Archives is located at Cornish al-Nil, Cairo, Egypt. This building has 16 repositories located on 8 floors. One repository was selected for this research which characterized by their large size and their dimensions (Length 20m, width 6m and height 3.5m).This repository contain collections stored inside the repository of old books, manuscripts, albums, and maps.

Thermo-hydrometric sampling

Temperatures (T) and relative humidity (RH) are measured daily in the repository of the Dar al- kottob, since many years ago. The temperature and relative humidity were measured inside the repository at each point of sampling at the moment when the microbiological sampling was performed using a digital thermo hygrometer (SATO, Model, SKL-200, Japan).

Microbiological sampling of air

Sampling was done during November of 2017 (autumn) using sedimentation method suggested by *E. Bogomolova and I. Kirtsideli* [32]. Open Petri dishes at 1m from the floor were placed for 3 minutes in three different points of repository. Culture media employed were potato dextrose agar + chloramphenicol (0.1%) to isolate fungi. After exposure period the Petri dishes were sealed and subsequently incubated at 28±2°C for 7 days. Then, the colony count was performed and the necessary calculation of air were made in order to determine the fungal concentration expressed in colony forming unites per cubic meter (cfu/m^3), taking into account the following equation described by *V.L. Omeliansky* [33]:

$$\text{No. of fungal colony (cfu/m}^3\text{)} = 5a.10^4\ (bt)^{-1}$$

a: number of colonies per Petri dish., b: dish surface, cm^2, t: exposure time (min).

Also, relative density (RD) was conducted according to *G. Smith* [34]:

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

Fungal identification

The identification of mould isolates were carried out on the basis of their cultural, morphological of colonies and macro and microscopically characteristic sporulation according to different manuals of [35-38]. The frequency occurrence expressed as percentage relative distribution of genera or species were calculated as mentioned before.

Fumigant agents

Three agents of most common disinfectant were used in this study include dettol+ isopropanol (25:25mL/L), paraformaldehyde (20g/m^3) and phostoxin tablet (1g/m^3). All of these agents were used to evaluate the best activity as antifungal when used in fumigation of old documents and manuscripts repositories.

Effect of fumigants on fungal linear growth

The fungal isolates used in this study were *Fusarium oxysporum*, *Aspergillus niger*, *Trichoderma harzianum* and *Penicillium chrysogenum*. They were isolated from the air of old document repository as mentioned before these deteriorated fungi were routinely maintained on potato dextrose agar (PDA) at 28±2°C. PDA plates were prepared using Petri dishes (90mm) and inoculated with mycelia plug (5mm) from tested fungal isolates at the centre of the dish. The Petri dishes were placed without led, upside down and placed directly in three gars, the first contain dettol+isopropanol, the second contain paraformaldehyde and the third with phostoxin tablets at the same concentrations as mentioned before. After inoculation, jars and the plates were incubated at 28±2°C in the dark. The colony growth diameter was measured after the fungal growth in the control treatment had completely covered the Petri dishes. All treatments were replicated three times. Growth inhibition of treatment against control was calculated by percentage, using the formula:

$$\% \text{ inhibition} = C\text{-}T/T\ X100,$$

where: C is the hyphal extension (mm) in the control and T is the average of hyphal extension (mm) of plates treated with individual fumigant compound.

The fungistatic- fungicidal nature of the fumigants was tested by observing revival of growth of the complete inhibited mycelial disc following its transfer to non-treated PDA. A fungicidal effect was where there was no growth, whereas fungistatic effect was where temporary inhibition of microbial growth occurred.

Effect of different concentrations of dettol+ isopropanol

To check the antifungal activity of dettol + isopropanol on the linear growth against the above-mentioned fungi using poisoned food (PF) method **[39]**. In PF method the formulation was added to PDA immediately before it was emptied into the Petri dishes at a temperature of 45°C. The concentrations tested were 5, 10 and 20%. The control received the same quantity of sterile agar suspension (0.2%) mixed with PDA. The tested fungi were inoculated with 5mm mycelial plugs from 7 days old cultures and incubated at 28±2°C. Colony diameter was measured whenever respective colonies covered the medium surface in plates and growth inhibition of treatment against control was calculated.

Results and Discussions

Due to the high temperatures in the world during 2017 and hence, the relative humidity in the air of stores, especially where there is no air conditioning which will certainly lead to the proliferation of microorganisms, especially fungi that cause the deterioration of ancient manuscripts in repositories.

Regarding the temperature and RH values it is observed that, the mean temperature value (25.3°C) and RH value (63.8%) in the left side of the tested repository was approximately the same in the right side during the sampling time (Table 1).

Table 1. Environmental conditions (temperatures and relative humidity (RH) measured at left and right sides of repository during the sampling time.

Sampling	Temperatures (°C)			Relative humidity (RH) %		
	Min.	Max.	Average	Min.	Max.	average
Right	22.3	28.3	25.3	59.2	68.3	63.8
Left	22.4	28.1	25.3	58.8	68.7	63.8

There are strong correlation between poor conservation condition (Temp., RH, ventilation, light ext...) and biodeterioration of old manuscripts and archival papers [2- 4, 40]. During this study the temperature and RH average reached 25.3°C and 63.8% respectively. This environment is favored to some fungal species that are able to proliferate in the repositories.

Microbial air sampling

Quantitative analysis of air samples revealed heavily fungal contamination reaching 309.224 cfu $X10^2/m^3$ (Table 2). The high relative abundance of fungi probably indicates poor storage conditions in repository as well as higher temperature and relative humidity.

Table 2. Relative abundance (%) and colony forming units (cfu $x10^2/m^3$) in old document repository at the NLA

Fungi	Number of colonies	Relative abundance (%)	cfu $x10^2/m^3$.
Alternaria spp.	4	3.39	10.482
Aspergillus flavus	32	27.11	83.857
Aspergillus niger	26	22.03	68.134
Aspergillus sydowii	14	11.8	36.688
Fusarium oxysporum	9	7.63	23.585
Penicillium chrysogenum	28	23.73	73.375
Trichoderma harzianum	5	4.24	13.103
Total	**118**	**100**	**309.224**

Results in the present study were approximately similar to those previously found in National library and Archives of Egypt by *A.F. Sahab et al* [41] who detected fungal level at mean value of 15.72-369.45 cfu $X10^2/m^3$.

The presence of large numbers of fungal spores in the air of the repository may be due to the accumulation of dust and the lack of good storage conditions in the repository.

In this study, a total of seven species which belonged to five genera of filamentous fungi were detected and identified. The percentage abundance of the species *Aspergillus flavus* (27.11%), *Aspergillus niger* (22.03%) and *Penicillium chrysogenum* (23.73%) were present in high values and were the most isolated fungi, as the cfu$X10^2/m^3$ ranged from approximately 68.1 to 83.9. These data were in line with previous results available in literatures, which indicate these fungi were predominant in indoor environment [10-12, 14].

On the other hand, by mediating the average of other fungal isolates, the fungal genera could be arranged in descending order on the basis of their frequent occurrence as follows: *Fusarium spp.* (7.63%), *Trichoderma spp.* (4.24%) and *Alternaria spp.* (3.39%). The presence of large numbers of fungal spores in the air of the repository may be due to the accumulation of dust and the lack of good storage conditions in the repository.

Vapor effects of fumigant agents

The *in-vitro* evaluation of inhibitory vapour effect of dettol + isopropanol, paraformaldehyde and phostoxin on fungal growth was tested. The data presented in table 3 show that, the linear growth of the tested fungi was reduced by the vapour of the tested formulations in varying degrees.

Table 3. Vapour effect of dettol + isopropanol, paraformaldehyde and phostoxin on the percentage linear growth inhibition of some fungi isolated from old document repository

Fungi	linear growth inhibition %			
	Dettol + isopropanol (25,25ml/L)	**Para-formaldehyde**	**Phostoxin**	**Mean**
Fusarium oxysporum	55.97 DE	44.80 E	100.00 A	**66.92**
Aspergillus niger	69.93 B	100.00 A	100.00 A	**89.97 A**
Trichoderma harzianum	73.00 B	57.80 CD	100.00 A	**76.93 B**
Penicillium chrysogenum	96.30 A	68.87 BC	100.00 A	**88.39 A**

-Three replicates were used for each treatment.

- Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range tests. Means followed by the same letters are not significantly differed

Complete reduction (100%) in the linear growth of all tested fungi was observed when exposed to the vapour of phostoxin. On the other hand, a high growth reduction was also observed by dettol+isopropanol on the tested fungi which can be arranged on descending order as follows: *Penicillium chrysogenum* (96.30%), *Trichoderma harzianum* (73.00%), *A. niger* (69.93%), and then *F. oxysporum* (55.97%). Also, a potent effect was observed by paraformaldehyde against *A. niger* (100%), *Penicillium chrysogenum* (68.87%), *Trichoderma harzianum* (57.80%), then *F. oxysporum* (44.80%).

It could be observed from the present results that the fungal growth of all test fungi showed high sensitivity to fumigation by phostoxin followed by dettol+isopropanol then paraformaldehyde, and the most sensitive organisms were *Penicillium chrysogenum* and *A. niger* without significant difference between them, while the least sensitive organism was *F. oxysporum*. Phostoxin as a fumigant showed high antifungal activity. Similar data were observed by *J. Leitao et al* [30], although apparently it is only effective on actively growing organisms. This may be due to their high diffusibility across physical and biological barriers, which gives them the capacity to very rapidly reach the susceptible targets that are hidden in the stored products, although the antifungal activity of phosphine against *A. paraziticus* has been noted [31].

Effect of different concentrations of dettol + isopropanol on fungal growth

Using the PF technique, the results in table 4 and figure 1 indicated that, all the concentrations of dettol + isopropanol showed inhibitory effect on the linear growth of the tested organisms, besides increasing the concentrations of dettol+isopropanol inversely proportional with the linear growth of the tested isolates, in other words, as the concentration of dettol+isopropanol increase as the linear growth decrease. The most affective concentration was 20%, whereas the lowest levels of inhibition was observed against *T. harzianum* (18.87%), followed by *F. oxysporum* (30.30%) at conc. 5%.

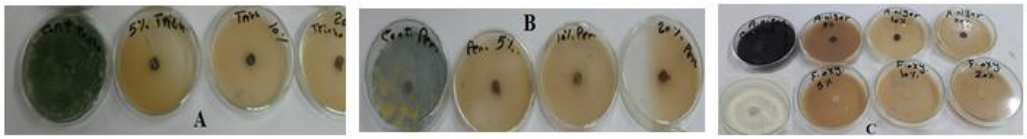

Fig. 1. Direct effect of different concentrations of dettol and isopropanol on mycelial growth (mm) of some fungi

Table 4. Vapour effect of different concentrations of dettol and isopropanol on linear growth inhibition (%) of some fungi

Fungi	Dettol + isopropanol concentration			
	5%	10%	20%	Mean
Fusarium oxysporum	30.30 DE	51.10 CD	57.57 C	**46.32 C**
Aspergillus niger	63.37 BC	83.00 AB	83.30 AB	**77.56 B**
Trichoderma harzianum	18.87 E	49.27 CD	64.80 BC	**44.31 C**
Penicillium chrysogenum	84.80 AB	94.43 A	100.00A	**93.08 A**
Mean	**49.34 CD**	**69.45 AB**	**76.42 A**	

-Three replicates were used for each treatment
-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range tests.
-Means followed by the same letters are not significantly differed

Data also showed that, *Penicillium chrysogenum* was the most sensitive fungi to the vapour of dettol+isopropanol. The inhibition rate reached (84.80 %) at conc. 5% and complete inhibition effect at (100%) at conc. 20%, whereas it displayed moderate antifungal activity against *A. niger* (83.30%) at conc. 20%, followed by *Trichoderma harzianum* (64.80%), and *F. oxysporum* came with (57.57%) reduction.

The antifungal activity of dettol+isopropanol as fumigant was variable between fungi, as against *Penicillium chrysogenum* was the most affective. The inhibition rate reached (84.80 %) at conc. 5% and complete inhibition effect at (100%) at conc. 20%. Whereas, it displayed moderate antifungal activity against *A. niger* (83.30%) at conc. 20%, followed by *Trichoderma harzianum* (64.80%), and *F. oxysporum* came with (57.57%) reduction. Similar results were reported by *C.R. Mahon et al* [20], *F. Tara* [21], *B.R. Kim et al* [22] and *H. Nowrozi et al* [28]. *A.R. Abbas and M. H. Ibtisam* [23] reported that, dettol is more effective than formalin in their effect on fungus and less effective on bacteria. These finding agreed also with *S. R. Mohammed and M. H. AL-Jibouri* [24] and *K.M. Redal* [25] and differed with *S.M. Handel* [26] and *D.R. Smith* [27], who reported that formaldehyde was more effective than dettol.

Fungicidal and fungistatic effect

Results of this experiment showed that, dettol + isopropanol formulation had a significant fungicidal activity on the growth of all tested fungi (Table 5). As no growth was appeared (100% inhibition) from the fungal disc taken from plates of dettol + isopropanol, also the same fungicidal activity was achieved against *F. oxysporum*, *T. harzianum* and *P. chrysogenum* taken from plates exposed to para-formaldehyde vapour. Only phostoxin showed fungistatic activity as different levels of inhibition (6.66-73.33%) were observed from discs of all fungi.

Table 5. Fungicidal and fungistatic effect of vapour effect of some fumigants (dettol + isopropanol, paraformaldehyde and Phostoxin) on the linear growth inhibition of some fungi

Fungi	linear growth inhibition %			
	Dettol + isopropanol (25:25:1000)	**Para-formaldehyde**	**Phostoxin**	**Mean**
Fusarium oxysporum	100.00A	100.00A	30.74D	**76.91B**
Aspergillus niger	100.00A	63.33C	71.85B	**78.39B**
Trichoderma harzianum	100.00A	100.00A	6.66E	**68.89C**
Penicillium chrysogenum	100.00A	100.00A	73.33B	**91.11A**
Mean	100.00A	90.83B	45.65C	

-Three replicates were used for each treatment
-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range tests. Means followed by the same letters are not significantly differed

Results of the present study detected the fungicidal activity of dettol + isopropanol formulation on all tested fungi, also the same fungicidal activity was achieved against *F. oxysporum*, *T. harzianum* and *P. chrysogenum* taken from plates exposed to para-formaldehyde vapour on the other hand, phostoxin showed fungistatic activity on all fungi. These results appear to be in partial agreement with previous reports [18, 24, 30]. Fumigants were thought to kill fungi by destruction either by coagulating the protein of the fungi by destroying its cell membrane or by removal of sulphonhydric group from the organisms [19, 42, 43].

Conclusion

It was revealed from the study that, fumigation with dettol+isopropanol, phostoxin and para-formaldehyde showed fungicidal and fungistatic activity against some fungi isolated from deteriorated old manuscripts and books.

The use of good fumigants should be encouraged to reduce cases of deterioration of old document caused by deteriorated fungi.

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Received: May 04, 2018
Accepted: May 12, 2019