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Prevalence of Fungal Contamination in Some of Biology Department's Laboratories

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A B S T R A C T

Fungi are ubiquitous in distribution and are a serious threat to public health in indoor environments. The study was conducted to evaluate surface sampling in conjunction with air sampling for the detection of fungal contamination in Biology Department's laboratories and determine the best detergent which treats with it.

The study was carried out at the Biology Department, College of Science, Al-Mustansiriyah University, and Baghdad. It was includes (i) isolation fungal samples on Potato Dextrose Agar (PDA) and identification these isolates by using morphological and microscopical characteristics and (ii) evaluation of three detergents on the most prevalent species of isolated fungi by using disk diffusion method and measuring the zone of inhibition against the tested isolate around each detergent. The results identified ten fungal species isolated from these laboratories, *Alterneria sp.*, *Aspergillusniger*, *Aspergillusflavus*, *Aspergillusterreus*, *Cladosporium sp.*, *Penicillium sp.*, *Rhizopus sp.*, *Fusarium sp.*, *Geotrichum sp.* And *Oxysporum sp.*. Among all, *Aspergillusniger* was the more prevalent in all scanned laboratories. On the other hand, formalin when tested on *A. niger* was more effective than other detergents. We concluded that all tested laboratories had fungal contaminants due to numerous contamination sources. *Formaldehyde fumigation will be very necessary to sterilize these laboratories.*

Keywords: Fungal Contaminations, Indoor, Disinfectants

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INTRODUCTION

All around the world, life style changes have resulted in a shift from open air environments to air tight, energy efficient environments at home and work places, where people spend a substantial portion of their time[1,2]. In these environments, improper maintenance, poor building design or occupant activities often result in a condition called as ''Sick Building Syndrome'' (SBS), where occupants experience adverse health effects that appear to link with the time spent in a building[3,4] . The complaints may be localized to a particular room or widespread throughout a building and relief usually occurs soon after leaving the building[5]. Headaches, pressure on the head and throbbing, and feelings of tiredness are the most common signs of SBS. Microbial contamination in laboratories and hospitals is becoming serious problem worldwide and characterization of such contaminants offer hope for treating some laboratory acquired infections (LAI). It is important to determine the nature of micro-organisms which are as result of such accidents in order to devise mitigation techniques before outbreaks [6]. To exploit the benefits associated with cell culture procedures, there are laboratory quality and bio safety practices that are overlooked, that lead to microbial contamination in large numbers in the cell cultures and in laboratory environment which may lead to (LAI) to people[7]. The contamination risks pose danger to laboratory personnel as well increase the costs of in-house cell culture procedures [8].Numerous building materials and furnishings can be colonized and

damaged by fungi, especially under humid or wet conditions. Some fungi can produce toxins that can cause health effects upon direct contact with skin, inhalation or ingestion [6]. However, the presence of surface-associated fungi in indoor environments may go undetected if traditional air sampling methods with culture analysis are the only monitoring methods used. The detection of airborne and surface-associated fungal contaminants in indoor environments is necessary for risk assessment and to determine the extent of remediation for contaminated environments. In this study, air and surface sampling was conducted in Biology Department's laboratories to isolate and identify the fungal contaminants; and determine the best detergent which treats with it.

MATERIAL AND METHODS

Preparation of culture media (Potato Dextrose Agar - PDA-)

It was prepared according to the manufacturer recommendation by dissolving 39grams of (PDA) powderin1000ml of distilled water and adjusted the pH to 5.5andsterilized the medium in autoclave(121ºC under 15lbs/In^{2}pressure for 20minutes), thenadded1ml of antibiotic (chloramphenicol) in 1L of sterilized culture medium to eliminate the bacteria. The medium was used for isolation and identification of fungi.

Isolation and identification of fungi

Fungi were isolated from the air of 6 laboratories of department of biology, College of Science, Mustansiriyah University, through the exposing three petri-dishes containing sterilized medium (PDA) by opening them in laboratory for ten minutes then closed, also swabs was wiped on the surface of laboratory benches and inoculated on PDA media. All petri-dishes incubated at 28ºC for 5-7 days.

Growing of fungi colonies on PDA were sub-cultured by transferring a small mycelia plugs from the colony margins. Pure culture was obtained by sub-culturing many times then identified on the basis of their morphological characters by observing colony feature (colony and texture) and microscopically by staining with lactophenol cotton blue and observe under microscope for the conidia, conidiophores and arrangement of conidia. Fungi were identified and classified as depended on taxonomic keys [10.11]. The percentage of occurrence and frequency of isolation to each isolated fungal genus and species were calculated according to the following formula[12]:-

$$\% \text{ occurrence of Genus} = \frac{\text{Colonies number of genus}}{\text{total number of genera colonies}} \text{ X } 100$$

$$\% \text{ occurrence of species} = \frac{\text{Colonies number of species}}{\text{total number of species colonies}} \times 100$$

Preparation of different detergents

Different detergents were used in the current study. Formaline was obtained from the storage of department of biology –college of science and two industrial detergents hypochloride sodium the common name (fas) ,contains of 6% hypocholide sodium and flash were purchased from the market of Baghdad , contains of 10% hydrochloride acid HCl and Prepared two dilutions 1/2 , 1/4 for each detergent using by sterile distilled water .

Preparation of fungal spore suspension

Mycelial fragments is prepared simply by adding 20 ml of sterile distilled water to a culture plate PDA containing the fungus growth *Aspergillus niger* in age colony 7 day. Then transfer spores in to sterile vial by sterile syringe[13].

Effect of different detergents on growth of fungal isolate

Aspergillus niger was the predominent isolate in all samples in this study, therefor was tested its ability to resist of these detergent hypocholide sodium (fas) , formalin, and flash at two dilutions 1/2 , 1/4 the depending upon the agar diffusion disk . Filters sterilize from whatmman No.1 in diameter 35mm were soaked in petri-dishes contained solutions of each detergent for two dilutions for ten minutes then put it in the middle of PDA plates after adding 1 ml of spore suspension of *Aspergillus niger* and spreading it on whole plate by streaking method, then incubated all plates in incubator at 28ºC for 4-7 days with triplicate for each treatments. The diameters of the inhibition zones were measured by ruler (cm)[14].

RESULTS AND DISCUSSION

Isolation and identification of fungi

A total of ten fungal isolates were obtained and identified from different laboratories. They included *Alterneria sp.*, *Aspergillusniger*, *Aspergillusflavus*, *Aspergillusterreus*, *Cladosporium sp.*, *Penicillium sp.*, *Rhizopus sp.*, *Fusarium sp*., *Geotrichum sp.* And *Fusariumoxysporum.* Fungal species isolated and their frequencies are shown in table.1 and table.2. The frequency of occurrence shows that *Aspergillusniger* was the most common fungal species in which it occurred in nine out of the twelve isolates tested. Results showed that "the Parasitology & Histology Lab." was the less contaminated laboratory by fungi, where only one species (*Cladosporium sp.*) was isolated from its air; and two species (*Rhizopus sp.* and *Aspergillusniger*) was isolated from its dust (see table.1).

Table 1: Occurrence of fungi in some of undergraduate laboratories in department of Biology

The place	Source of Isolate			
	Air	Frequency (%)	Dust	Frequency (%)
Parasitology &Histology Lab.	*Cladosporium sp.*	100	*Rhizopus sp.* *Aspergillusniger*	62.1 37.9
Food Processing and Soil Microbiology Lab.	*Aspergillusniger* *Aspergillusflavus* *Penicillium sp.* *Alternaria sp.* *f.oxysporum*	17.14 20 22.85 14.28 25.71	*Aspergillusniger* *Rhizopus sp.*	45.45 54.55

Table 2: Occurrence of fungi in some of postgraduate laboratories in department of Biology

The place	Source of Isolate			
	Air	Frequency (%)	Dust	Frequency (%)
Mycology & Phycology Lab.	*Aspergillusniger* *Rhizopus sp.* *Cladosporium sp.* *Aspergillusterreus* *Geotrichum sp.*	40.9 40.9 4.5 4.5 4.5	*Aspergillusniger* *Rhizopus sp.* *Fusarium sp.* *Geotrichum sp.*	50 37.5 4.1 8.3
Microbiology Lab.	*Aspergillusniger* *Rhizopus sp.* *Geotrichum sp.* *Fusarium sp.*	35.48 58.06 6.45 3.22	*Cladosporium sp.* *Fusarium sp.* *Oxysporum sp.* *Geotrichum sp.*	50 25 25
Plant Tissue Culture Lab.	*Aspergillusflavus* *Geotrichum sp.*	50 50	*Aspergillusniger* *Aspergillusflavus* *Penicillium sp.* *Aspergillusterreus* *Alternaria sp.*	66.66 12.5 8.33 4.16 4.16
Molecular biology lab.	*Rhizopus sp.* *Aspergillusniger* *Geotrichum sp.* *Fusarium sp.* *Aspergillusflavus*	52.94 17.64 11.76 11.76 5.88	*Aspergillusniger* *Rhizopus sp.* *Aspergillusflavus* *Penicillium sp.* *Alternaria sp.*	31.03 31.03 24.13 3.44 3.44

Results showed that the fungal contamination is high in the biology department's laboratories. This might be attributed to the fact that more people enter these laboratories. There are many reports on fungi isolated from indoor environments[15,16]. Fungi are able to grow on almost all natural and synthetic materials, especially if they are hygroscopic or wet. Inorganic materials get frequently colonized as they absorb dust and serve as good growth substrates for *Aspergillus fumigates* and *Aspergillusversicolor*[17].Mitsukoand others[18] discovered that presence of microbes in a room indicates the presence of people and their levels may get high when the building is heavily populated. Fungal contaminants were also found associated with the dust of benches. Typically, fungi make up two-thirds of all of airborne, living organisms [19]. Miller and others[20] had isolated *Cladosporium*, *Penicillium*, *Aspergillus*and *Alternaria* as the most common fungi in biosafety cabinets. Regularly used furniture has been reported as a major source of fungal spores [21]. Shade around the house has also been reported to increase indoor fungi counts fivefold [22]. Fungi grow anywhere indoor, where there is moisture and a food source. Many building materials consist of cellulose materials that are particularly suitable for fungi growth when they are wet. Other materials that also support fungi growth include dust and paints.

Effect of different detergents on growth of fungal isolate

In the next step of our study was the indication of commercial detergents which can be most effective on laboratories contaminating fungi. Because of *Aspergillus niger* was the prevalent isolate in all samples in this study, we tested effect of three detergents on this species. Antifungal tests were carried out by disk diffusion method and evaluated by measuring the zone of inhibition against the tested isolate around each detergent dilution.

Inhibition zones shown in table.3 for two dilutions of each detergent tested against *A. niger*. Formalin in both dilutions was more effective than other detergents (see Figure.1), while flash never showed any inhibition in both tested dilutions. Hypocholide sodium (Fas) gave moderate effect in dilution 1/2, reaching 6 cm as zone of inhibition, while in other dilution (1/4) didn't shown any effect see (Figure.1).

Table 3: Inhibition zone (cm) for two dilutions of some detergents against *Aspergillusniger*.

Detergents	Formalin		Fas		Flash	
Concentration	1/2	1/4	1/2	1/4	1/2	1/4
Inhibition zone (cm)	9	9	6	0	0	0

The inhibitory effect of detergents may attribute to the toxic effect of some ingredients that elongate the fungal lag phase, inhibit normal cell elongation, and spore germination. Detergents as surface-active agents have detectable influences in permeability of the cell walls to different materials and metals Al-Garniand others[23]and Khan and Karuppayil[24].Control of fungi in the indoor environments has traditionally focused on identifying the source of contamination control, use of filters, cleaning etc. Glutaraldehyde shows high toxicity and its vapors irritate eyes, nose and throat. Formaldehyde stimulates irritation of mucosa and is also reported as a carcinogen. Cresol is less toxic but extensive use may be harmful [25].Only a few studies have specifically focused on the effects of germicidal UV light. Currently various manufacturers are marketing germicidal UV lamps for controlling contamination, including fungal contamination in indoor environments, as well as Air Handling Units (AHU's) and ducts [26].

CONCLUSIONS

Based upon the research results we conclude:

- All tested sites had fungal contaminants. The contaminated areas were in walls, tables, doors and air.
- Each site contained more than one fungal contaminant.
- Aspergillus nigeris the prevalent fungal species inBiology Department's laboratories.

Formalin is the best detergent can be used to sterilize these laboratories

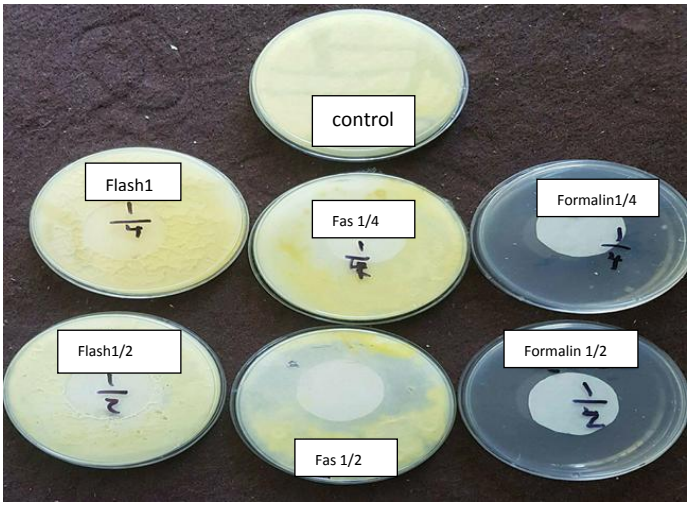

Figure 1: Inhibition zone for two dilutions of some detergents against *Aspergillusniger*.

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