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Omnya A. El-Batrawy, Amany F. Hasballah

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Monitoring of toxinogenic producing fungi in air conditioning system dust at some clinics in Damietta Governorate

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Environmental Sciences Department, Faculty of Science, Damietta University, New Damietta-34517, Egypt

REVIEW ARTICLE

A total of 1,260 microbial and fungal isolates were obtained from 84 samples collected from filter dust, wing dust, indoor air without air conditioning, and indoor air with air conditioning (14 samples for each category) from May to August 2021 from 14 clinics in Damietta Governorate, North-East of Egypt. The Aeromycobiota was isolated using open plate and filtration methods, while the Filter and Wing Dust microbiota were isolated using the dilution plate method on Czapek's Yeast Extract Agar, Potato Dextrose Agar, and Dichloran-Glycerol-18 Agar. During this survey, eight species from five genera, *Aspergillus*, *Mucor*, *Fusarium*, *Penicillium*, and *Alternaria,* were isolated from dust. The results revealed that dust accumulated fungi (DAF) calculated in wing and filter dust ranged from 120 to 1240 and 60 to 480 CFU/cm^2, respectively. Clinics' indoor air before and after turning on the air conditioning system collected by the open plate technique is contaminated with eleven fungal species from six genera. While that was collected by filtration technique is contaminated with ten fungal species from six genera. Results revealed that some isolated fungi could produce aflatoxins as *A. flavus* & *A. fumigatus* and ochratoxin A as *A. niger* & *A.ochraceus*.

Keywords: Indoor air quality, Mycotoxins, Medical facilities, Aeromycobiota, Damietta Governorate

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CORRESPONDENCE TO
Amany F. Hasballah,
Environmental Sciences Department,
Faculty of Science, Damietta University,
New Damietta-34517, Egypt
Email: amany_ah@du.edu.eg
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INTRODUCTION

Few studies have addressed indoor air quality (IAQ) due to a variety of indoor microenvironments and technology restrictions on indoor air monitoring (Saraga et al. 2024). As people spend most of their time indoors, the quality of indoor air affects on their physical and mental health (El-Batrawy et al. 2024); therefore, there is a great interest in studying the quality of indoor air. The pollution of the outdoor environment affects on indoor indoor air quality (Hachimi et al. 2020 Chawla et al. 2023). Environmental factors such as weather conditions, moisture, air conditioning systems (ACS), patient density, and the presence of pathogens, and human activities, may affect indoor air in clinics (Yousefzadeh et al. 2022). Microorganisms in medical facilities include bacteria, viruses, and fungi affecting indoor air quality (Khan et al. 2009; Chawla et al. 2023; Carrazana et al. 2023 and Saraga et al. 2024). Most fungi can grow on moist materials and inorganics, which are preferred for forming colonies as they soak dust, causing good growth substrates for many species of fungi, such as *Aspergillus* (Samet and Spengler 2003). Clinics also include wooden materials, which fungi can grow on, particularly on surfaces that have been kiln-dried, such as *Cladosporium* and *Penicillium*(Sailer et al. 2010). Other genera of fungi, such as *Aspergillus*, *Alternaria, Cladosporium,* and *Penicillium,* are common in hospitals, homes and offices(Najjar, 2024). These fungi can grow on other materials inside clinics, such as Gypsum, Paper, glue, fiberglass insulation, books, and painted surfaces. Air filters and ACS are good surfaces for fungi colonies (Khan and Karuppayil 2012; Chawla et al. 2023). ACS uses air as a heat-transmission medium, which contains particles from industrial dust, motor vehicle emissions, and other pollutants, such as viruses and bacteria, that cause damage to human life (Elsaid and Ahmed 2021). A controlled microclimate must be maintained for sterilizing purposes in medical environments because air conditioning systems are required. However, if these systems are poorly planned, operated, or maintained, they may unintentionally become sources of indoor air contamination (Nascimento et al. 2023 El-Batrawy et al. 2024). These fungi are inhaled and are inducers of rhinitis, allergic asthma episodes, and bronchial irritation (Khan and Karuppayil 2012). The Damietta Governorate is situated in the northern part of the Delta, close to the coast at the mouth of the Nile River. Damietta may be exposed to air pollution as it is an urban area with numerous industrial and economic activities. Many industries include furniture and wood manufacturing, clothes, food products, metallic products, and shoe making. The wood and furniture industries represent the largest percentage of Damietta's industrial production (Hasballah and El-Henawy 2019). Air pollution with fungi was studied in Damietta governorate (El-Kuttan 2005; El-Morsy 2006; Abdel-Azeem and Rashad 2013; Hasballah and El-Henawy 2019; El-Batrawy and Hasballah 2020 and El-Batrawy et al. 2024) and from medical places and air conditioning system around the world (Oliveira et al. 2012; Khan and Karuppayil 2012; Pavan and Manjunath 2014; Ibietela and Robinson 2019; Al-Bader et al. 2020; Belizario et al. 2021; Liu et al. 2021;

Yousefzadeh et al. 2022; Alghamdi et al. 2023 and Nascimento et al. 2023). Fungi can produce toxic materials called mycotoxins, nonvolatile secondary metabolites that certain fungi can create. Humans can be exposed to mycotoxins through ingesting, inhalation, or skin contact. Several types of mycotoxins, such as aflatoxins, ochratoxins, and trichothecenes were studied in indoor air. (Zain 2011 and Zhang et al. 2016). Under specific conditions, fungi from the genera *Aspergillus* and *Fusarium* produce ochratoxin A (OTA), a poisonous secondary metabolite. This dangerous toxin causes kidney damage in both acute and chronic illnesses. It is also carcinogenic, genotoxic, and immunosuppressive (Khan and Karuppayil 2012). These toxins cause mucous membrane irritation syndrome, and its symptoms include sore throats, nasal congestion, rhinorrhea (running nose), and irritation of the eyes and nose (Lanier et al. 2010). Environmental monitoring in clinical settings should be regarded as an essential approach to discovering the variables contributing to developing healthcare-associated infections, particularly those produced by fungi. Thus taking into account how air contributes to the spread of fungal infections. The presence of toxinogenic fungi in ACS dust in clinics may cause adverse effects on indoor air and lead to health hazards to patients, so regular monitoring of IAQ must be carried out. In addition, there are not enough studies available worldwide that assess indoor air quality, so this study aims to isolate and identify the accumulated fungi in the dust of the ACS and fungi in the indoor air of clinics before and after the ACS is turned on. In addition, it examines the potential of these fungal species for producing some toxins such as AFs and OTA.

MATERIALS AND METHODS

Study Area

Damietta Governorate is situated in the northern part of the Delta, close to the coast at the mouth of the Nile River and is located between latitudes 31.26 N and longitudes 31.48 E. Situated 15 km from the Nile estuary; it is divided into two sections by the "Damietta sector" of the River Nile. The delta plains and fields are to the south and west, Al-Manzala Lake is to the east, and the Mediterranean Sea is to the north. Dakahleya Governorate surrounds it except in the north, which borders the Mediterranean Sea (Hasballah and El-Henawy 2019). Fourteen clinic sites (M1 to M14) were selected during this study in the Damietta Governorate (Figure 1 and Table 1).

Sampling strategy and collection of samples

Eighty-four samples were collected from filter dust, wing dust, indoor air without air conditioning, and indoor air with air conditioning (14 for each) for four months from May to August 2021 from 14 clinics. Dust from AC segments, filter screen, and wing were sampled manually to determine the total dust accumulation fungi number (DAF) and dust volume. The area of each segment was 100 cm^2; after collecting the dust, its weight was recorded. Next, the dust weight is divided by the area of the sampling location (100 cm^2) to get the dust volume (unit: g/m^2). Then, the dust samples were transferred directly in a sterile polythene bag to the lab and stored in the refrigerator at 4oC until plating out on appropriate media. According to Liu et al. (2021), the dilution plate technique isolated fungal taxa associated with AC dust. Three different media were used: Czapek's yeast extract agar, prepared by mixing 1 g K_2HPO_4, 10 ml Czapek concentrate, 5 g yeast extract, 30 g sucrose and 20 g agar in 1 L distilled water. Czapek concentrate was prepared by mixing 30 g $NaNO_3$, 5 g KCl, 5 g $MgSO_4.7H_2O$, 0.1g $FeSO_4.7H_2O$, 0.1 g $ZnSO_4.7H_2O$ and 50 mg $CuSO_4.5H_2O$ in 1 L distilled water), potato dextrose agar (200 g potato extract, 20 g glucose and 20 g agar in 1 L distilled water) (El-Fallal et al. 2025). Dichloran-Glycerol (DG18) agar media is prepared by mixing 5 g Casein Enzymatic Digest, 10 g D-Glucose, 1 g monopotassium Phosphate, 0.5 g Magnesium Sulfate, 0.002 g Dichloran, and 15 g Agar (Janet et al. 2003) amended with Chloraphenicol (50 mg/L) and Rose Bengal (1/15000) to inhibit bacterial growth (Smith and Dawson 1944).

At the peak occupancy hours, duplicate air samples were collected from each site. Samples were collected in the middle of each room, away from windows and doorways. The fungi can be collected by gravitational and filtration methods (Ruzer and Harley 2012). In the filtration method, the air samples were collected using personal samplers for 2 hours using a vacuum pump coupled with a holder with a flow rate of 1.5 l/min. Airborne particles were collected on Whatman 47 mm membrane filters with 2µm pores size (Harrison and Perry 1986). Filter papers were weighed and mixed individually with sterilized distilled water to a final volume of 10 ml. One ml of the diluted sample had been added to sterilized media and incubated for 3-5 days at 28-30 ºC. Subculturing was carried out repeatedly until pure fungi were obtained and were identified according to

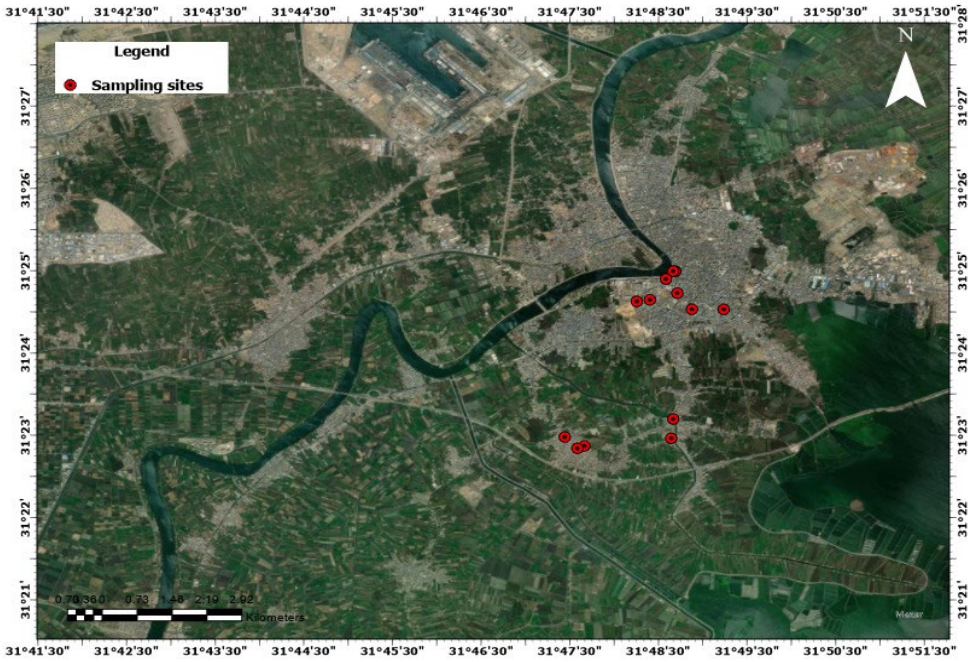

Figure 1. Location map of the selected clinics in Damietta Governorate, Egypt.

Table 1. Description of sample sites.

Site	Description	Coordination points of sampling sites	Site	Description	Coordination points of sampling sites
1M	Medical lab, 2nd floor, on a main road, AC in the lab room.	31.41041 N 31.80426 E	8M	Medical lab, 1st floor, on a side road, AC in reception.	31.38659 N 31.81109 E
2M	Radiology center,1st floor, on a main road, AC in doctor's room.	31.41209 N 31.81186 E	9M	Medical lab, 2nd floor, on a main road, AC in reception.	31.38269 N 31.81072 E
3M	Medical lab, 1st floor, on a side road, AC in reception.	31.4088 N 31.8206 E	10M	Dental clinic, 1st floor, on a side road, AC in doctor's room.	31.38114 N 31.7944 E
4M	Heart clinic, 2nd floor, on a main road, AC in doctor's room.	31.4088 N 31.81462 E	11M	Internal medicine clinic, 2nd floor, on a side road, AC in reception.	31.38064 N 31.79308 E
5M	Internal medicine clinic, 1st floor, on a main road, AC in doctor's room.	31.41072 N 31.80672 E	12M	Obstetrics & Gynecology clinic, 1st floor, on a side road, AC in doctor's room.	31.38294 N 31.79069 E
6M	Children's Clinic, 2nd floor, on a side road, AC in doctor's room.	31.41495 N 31.80974 E	13M	Dental clinic, 2nd floor, on a side road, AC in doctor's room.	31.41654 N 31.81109 E
7M	Medical lab, 3 rd floor, on a side road, AC in reception.	31.41651 N 31.81152 E	14M	Medical lab, 2nd floor, on a side road, AC in doctor's room.	31.37532 N 31.79104 E

their cultural morphology and spores description (Domsch et al. 1980 a, b). The most common technique of collecting airborne particles is gravitational settling, which involves putting up horizontal or vertical plates to study indoor surface contamination. In a gravitational method, air volume cannot be calculated (Ruzer and Harley 2012). The media used for fungi incubation was a Czapek's agar or potato dextrose agar media. Petri plates were 90 mm in diameter, and exposure time was 2 hours. At the end of sampling, the plates were transported to the Microbiology Laboratory of the Environmental Sciences and Botany Departments, Damietta University. Fungi were incubated at 28ºC for 3-5 days. Fungi were recognized microscopically to the genus level.

Calculation method

CFU/ m^3 for the gravitational method was determined using the equation provided by Omeliansky as the following: $N = 5a \times 10^4 (bt)^{-1}$

where N= microbial CFU/m^3 of indoor air; a= number of colonies per Petri dish. b= dish surface (cm^2); t= exposure time (min.) (Hayleeyesus and Manaye

2014). The number of CFU per cubic meter for the filtration process was determined as follows: number of colonies ×1000/sampling duration × airflow velocity (Fanga et al. 2005 and Osman et al. 2017).

Phenotypic identification of recovered taxa

Isolated species were morphologically recognized up to the species level. According to the phenotypic characters and relevant identification keys, Abdel-Azeem et al. (2020) for *Aspergillus* spp.; Samson (1981) for *Penicillium* spp.; Booth (1971) for *Fusarium* spp.; Woudenber (2013) for *Alternaria alternata*; Domsch et al. (2007) for *Mucur* sp. and *Cladosporium* sp. Kirk and Ansell (1992), The orderly setup adheres to the most recent classification scheme found in Ainsworth & Bisby's Dictionary of the Fungi, 10th edition (Kirk et al. 2008). All reported taxa name revisions, authorities, and taxonomic assignments were verified against the database on the www.indexfungorum.org website of the Index Fungorum. All fungi were identified in the Botany and Microbiology Department microbiology laboratory, Faculty of Science, Damietta University.

Preparation of fungal spore suspension

The purified fungal isolates were cultured for ten days, at 28 ºC on potato dextrose agar flasks. the spores were harvested using 20 mL of sterile saline solution containing 0.1% tween 80 after the incubation period, followed by 5ml maximum recovery diluent. The spores were gently scrapped off with a sterile inoculating loop and transferred into a sterile glass McCartney bottle; the suspended spores for each isolate were collected and adjusted to 10^5 CFU/mL by sterile saline solution.

Extraction of AFs and OTA from Yeast extracts sucrose (YES) medium

This experiment utilized YES medium (2% yeast extract and 15% sucrose/liter distilled water). After filling a 250 ml Erlenmeyer flask with the YES culture medium, it was autoclaved for 15 minutes at 120 ºC. It was then allowed to cool to room temperature and inoculated with roughly (10^5) spore suspension for strains of *Aspergillus* spp. Flasks were then incubated for 14 days at 28 °C (Davis et al. 1966). After incubation, 10 ml of culture medium was filtered before 20 ml of chloroform was used to extract the AFB_1 and OTA to separate AFs and OTA with HPLC. The chloroform phase was filtered through Whatman No. 3 filter paper, dried under nitrogen, and sodium sulfate anhydrous were added.

Determination of AFs by HPLC

Derivatization: 100 µl of trifluoroacetic acid (TFA) was added to the samples and standards, mixed thoroughly for 30 seconds, and left for 15 minutes. Thirty seconds were spent vortexing 900 µl of water acetonitrile (9:1 v/v) until thoroughly mixed. The HPLC analysis was performed using the produced combination. Aphenomenex C18 (250 x 4.6 mmi.d.), 5 µm from Waters Corporation (USA), a model Waters 1500 Rheodyne manual injector, a Waters 2475 Multi-Wavelength Fluorescence Detector, and a data workstation with software Breeze 2 comprise the HPLC system. Water, methanol, and acetonitrile (6:3:1 v/v/v) were utilized in an isocratic system. The separation was carried out at a 1.0 ml/min flow rate at room temperature. For both the sample extracts and standard solutions, the injection volume was 20 µl. The excitation and emission wavelengths for the fluorescence detector were 365 nm and 450 nm, respectively. By utilizing peak area for quantification, the standard curve was used to determine the concentrations of AFs in the samples (AOAC 2007).

Determination of OTA by HPLC

One milliliter of water, acetonitrile (3:1 v/v), was utilized, then vortexing for 30 seconds to dissolve the dry film of the sample. This mixture was subjected to an isocratic system containing acetonitrile, water, and acetic acid (55:43:2) for HPLC analysis. The separation was carried out at a flow rate of 1.0 milliliters per minute at room temperature. The injection volume for extracts and standard solutions was 20 µl. The excitation and emission wavelengths for the fluorescence detector were 335 nm and 465 nm, respectively. The peak area for quantification was utilized to get the OTA concentrations from the standard curve for each sample. The system used was a High-Performance Liquid Chromatography (HPLC) system (Waters) with the model 600 delivery system (Waters) (El-Desouky and Ammar 2016).

Determination of temperature (°C) and relative humidity (%)

The temperature (T) and relative humidity (RH) of clinic air before and after ACS operating were measured by the digital LCD thermometer and hygrometer, 2724445305121, China.

Statistical Analysis

The frequency ratio (%) for fungal isolates was calculated as: number of isolated species / total number of isolates×100 (Al-Bader et al. 2020). The

Shannon diversity index (H) was determined according to the following equation: - ∑ (Pi ln [Pi]); where Pi = ni/N, ni = number of individuals of the species i, and N = total number of individuals of all species, this index considers the relative abundance of taxa (Chauhan et al. 2019). One-way ANOVA with the Tuckey-B test was performed to find the significant differences between the collection methods. The correlations between fungi collected from the dust and the air samples collected by filtration and open plate methods, in addition to temperature and relative humidity, were investigated. IBM SPSS Statistics 25.0 program and Excel 2013 were utilized for all statistical analyses.

RESULTS

Fungal Species in air conditioning system dust

Through all dust samples of air-conditioning systems collected from clinics, five fungal genera with different numbers of species were recorded: *Aspergillus, Mucor, Fusarium, Penicillium*, and *Alternaria.* The most prevalent genus is *Aspergillus* and *Fusarium*. The most common species are *A. flavus* and *Fusarium equiseti,* with a percentage of 22% for each. While *A. niger*, *Mucor* sp., *A. fumigatus*, *Penicillium* sp., *A. flavipes*, and *Alternaria alternata* with percent of 17%, 14%, 12%, 7%, 5% and, 2%, respectively, (Figures 2, 5). The dust accumulated fungi number (DAF) for dust samples collected from AC's wings for sites (M1 to M14) was 500±0.06, 240±0.58, 220±1.0, 160±1.0, 280±1.0, 1240±1.0, 360±1.0, 120±0.58, 160±0.6, 500±1.0, 400±1.15, 1000±2.0, 200±1.0 and 140±1.53 CFU/cm^2, respectively. While for dust samples collected from AC's filters was 400±0.06, 60±0.58 and 80±1.15 CFU/cm^2 for the sites from (M1 to M3), respectively; 380±0.58, 480±1.0 and 360±1.0 CFU/cm^2 for sites from (M7 to M9), respectively; 400±2.52 and 200±1.5 CFU/cm^2 for sites (M11 and M12), respectively, and 220±2.52 CFU/cm^2 for site M14 (Table 2).

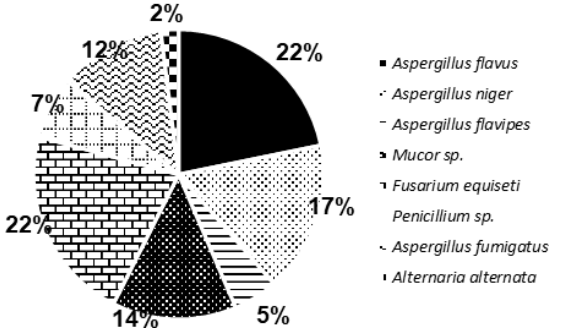

Figure 2. The frequency ratio (%) of fungal species in the air conditioning system dust through selected clinics.

Fungal species in indoor air of clinics before and after turning on AC (Open Plate Technique)

Results in Figures 3, 5, and Table 3 indicated that the air of the clinics before and after turning on ACS is contaminated with twelve species of fungi belonging to six genera: *Aspergillus, Mucor, Fusarium, Penicillium, Cladosporium*, and *Alternaria*. The most common species in indoor air before turning on AC were in the order of: *A. fumigatus* (17%)> *A.niger* & *Cladosporium sp.* (15%) for each> *Mucor* sp. (11%)> *A. flavus* & *Fusarium oxysporum* (9%) for each> *A. ochraceus* & *Alternaria alternata* (6%) for each> *A. nidulans* & *Fusarium equiseti* (4%) for each> *Penicillium* sp. (1%). The mean concentrations of the total fungal count of indoor air before turning on AC ranged between 46±1.0 and 256±1.0 CFU/m^3. On the other hand, the most abundant species in indoor air after turning on AC were in the order of: *A. niger* (16%)> *A. fumigatus* (14%)> *Mucor sp*. (12%)> *A. flavus* & *Cladosporium* (10%) for each > *Fusarium oxysporum* & *A. ochraceus* & *Alternaria alternata* (8%) for each> *Fusarium equiseti* & *A. nidulans* (6%) for each> *Penicillium* sp. (2%). The mean concentrations of total fungal count of indoor air after turning on ACS ranged between 52±0.0 and 347±2.0 CFU/m3. The highest concentration was recorded at site 1 representing a medical lab**.**

Fungal species in the indoor air of clinics before and after turning on AC (Filtration Technique)

Indoor air of the clinics before and after turning on ACS is contaminated with nine species of fungi belonging to six genera: *Aspergillus, Mucor, Fusarium, Penicillium, Alternaria,* and *Cladosporium.* The most common species in indoor air before turning on ACS were in the order of *Mucor sp*. (15%)> *A.niger* & *Fusarium equiseti* & *Cladosporium* sp. (13%) >*A. fumigatus* (12%) >*A. flavus* & *Alternaria alternata (*9%) > *A ochraceus* (8%)> *A. terreus* (5%) >*Penicillium* sp. (3%) (Figures 4 and 5) .The mean concentrations of the total fungal count in indoor air before turning on ACS ranged between 80.00±0.1 and 32.67×10^4 ±0.1 CFU/m^3. On the other hand, the most abundant species in indoor air after turning on ACS ordered as *A. fumigatus* & *Fusarium equiseti* (15%)> *A.niger* (14%) > *Cladosporium* sp. (13%)> *Mucor sp*. (11%)> *A. flavus* (10%)> *A. ochraceus* & *Alternaria alternata (*8%) > *A. terreus* & *Penicillium* sp. (3%) (Figure 4). The mean concentrations of the total fungal count of indoor air after turning on ACS ranged between 8.67×10^4±0.00 and 34.0×10^4 ±0.10 CFU/m^3 (Table 4).

Table 2. Isolated fungal species and the mean of total fungal count in air conditioning dust.

samples	AC segments	*A. fumigatus*	*A. niger*	*A. flavipes*	*A. flavus*	*Fusarium equiseti*	*Mucor* sp.	*Alternaria alternata*	*Penicillium* sp.	Mean of Total fungal count	Total DAF number CFU/gm	Total DAF number CFU/cm^2
M1	Wing			x	x					25	50×10^3±0.06	500
	Filter				x					20	40×10^3±0.06	400
M2	Wing				x	x	x		x	12	24×10^3±0.58	240
	Filter			x						13	6×10^3±0.58	60
M3	Wing				x		x		x	11	22×10^3±1.0	220
	Filter						x		x	4	8×10^3±1.15	80
M4	Wing				x		x			8	16×10^3±1.0	160
M5	Wing	X				x	x			14	28×10^3±1.0	280
M6	Wing	X				x				62	124.8×10^3±1.0	1240
M7	Wing		X		x					18	36×10^3±1.0	360
	Filter	X	X		x					19	38×10^3±0.58	380
M8	Wing		X		x	x				6	12×10^3±0.58	120
	Filter		X		x					12	4.8×10^4±1.0	480
M9	Wing				x	x	x		x	8	16×10^3±0.6	160
	Filter					x				18	36×10^3±1.0	360
M10	Wing		X							25	50×10^3±1.0	500
M11	Wing	X	X		x	x	x			20	40×10^3±1.15	400
	Filter		X							20	40×10^3±2.52	400
M12	Wing	X			x	x				50	100×10^3±2.0	1000
	Filter					x				10	20×10^3±1.5	200
M13	Wing		X							10	20×10^3±1.0	200
M14	Wing		X			x				7	14×10^3±1.53	140
	Filter		X					x		11	22×10^3±2.52	220
X= identified					Thresholds of the total number of fungi in dust CFU/cm^2 (MOH, 2012)							100

Table 3. Isolated fungal species and the mean of total fungal count in air before and after turning on ACS (Open plate technique).

Samples	AC work	*A. fumigatus*	*A. niger*	*A. ochraceus*	*A. flavus*	*Fusarium equiseti*	*Mucor* sp.	*Alternaria alternata*	*Penicillium* sp.	*Fusarium oxysporum*	*Cladosporium* sp.	*A. nidulans*	Mean of Total fungal count (CFU/m^3)
M1	Before	X							x				66±1.0
	After	X	x			x					x		347±2.0
M2	Before	X	x	x				x		x		x	98±1.0
	After	X			x					x			98±1.0
M3	Before	X			x								85±0.0
	After	X	x	x	x		X					x	164±1.0
M4	Before	X					X				x		79±1.0
	After						X	x				x	118±1.0
M5	Before						X			x	x		256±1.0
	After						X			x	x		262±1.0
M6	Before		x							x	x		98±1.0
	After	X	x							x			79±1.0
M7	Before	X	x		x						x		124±1.0
	After	X	x		x						x		118±1.0
M8	Before		x		x	x						x	72±2.0
	After		x		x	x							59±0.0
M9	Before	X			x		X						46±1.0
	After	X			x		X						79±1.0
M10	Before										x		131±1.0
	After			x				x					138±1.0
M11	Before	X		x			X		x				72±1.0
	After	X		x			X		x			x	105±3.0
M12	Before	X	x	x						x		x	98±2.0
	After		x	x			X	x		x			111±3.0
M13	Before		x			x					x		72±0.0
	After		x								x		66±1.0
M14	Before		x			x	X	x			x		85±1.0
	After		x			x		x			x		52±0.0

Table 5 shows the average calculated values of Shannon's diversity index (*H*) for the isolated fungal species in the samples collected. The highest values were observed in indoor air samples collected by open plate and filtration techniques before turning on AC (*H* = 2.26 and 2.24, respectively) followed by AC dust samples (*H* = 2.19).

Statistical analysis

Figure 6 illustrates the air temperature range without AC through selected clinics, which ranged from 24 to 34 ºC with a mean of 24 ºC. On the other hand, the air temperature with AC was nearly constant in all sites, with an average of 20 ºC. The relative humidity of air without AC ranged from 39 to 78 %, with a mean of

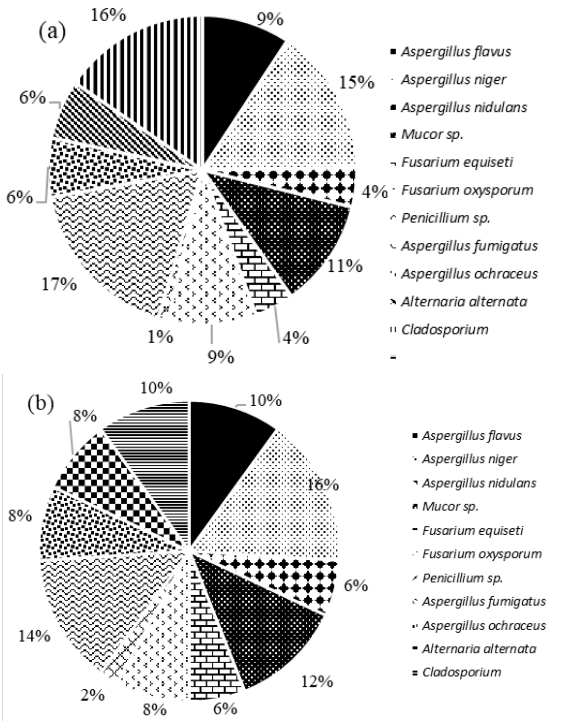

Figure 3. The frequency ratio (%) of fungal species in the air before (a) and after (b) turning on the air conditioning system (Open Plate Technique) through selected clinics.

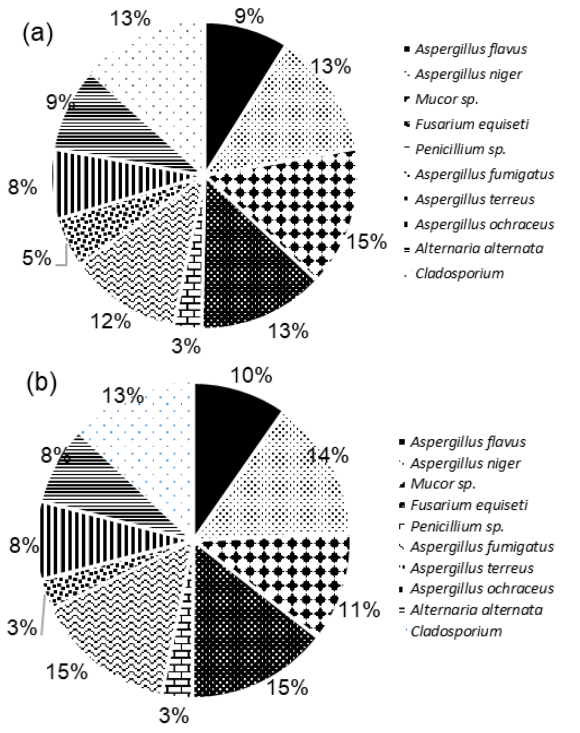

Figure 4. The frequency ratio (%) of fungal species in the air before (a) and after (b) turning on the air conditioning system (Filtration Technique) through selected clinics.

60.64%, while the relative humidity of air without AC ranged from 22 to 66 %, with a mean of 47.35 % by all studies clinics. The strong positive correlation between the relative humidity of air with and without AC (Table 6) indicates that air humidity is not affected by the air conditioner's turning; therefore, humidity change may not affect the growth of fungi in the air.

The correlations between fungi collected from the dust and the air samples collected by of filtration and open plate methods, in addition to temperature and relative humidity, were investigated (Table 6). The absence of significant relationships between fungal densities isolated from different locations may indicate that fungal growth in those places is not affected by each other. Although the absence of significant relationships between temperature, humidity, and fungal densities suggests that they are not affected by such variables, the positive linearity of fungal density with temperature and the negative linearity with humidity (the r values in Table 6) indicates a considerable effect of rising temperature and decreasing humidity on fungal growth. One Way Anova showed highly significant differences in fungal density between different isolation and collection methods, both with ($F=6.5$, $p<0.01$) and without ($F=67.8$, $p<0.001$) AC. The fungal density collected from filters was significantly higher than that collected from dust and open plates (Figure 7).

Detection of AFs and OCT A through AC dust and indoor air of clinics

The results in (Table 7) indicated the ability of some isolated fungi to produce AFs (AFB_1, AFB_2, AFG_1, and AFG_2). The isolate of *A. flavus* isolated from AC dust samples can produce AFB_1 with a value of 3.73 ng/ml. On the other hand, *A. flavus* isolated from indoor air samples collected by filtration technique before turning on AC produced AFB_1 and AFB_2 with values of 14.28 and 0.587 ng/ml, respectively. While *A. flavus* isolated from indoor air samples collected by filtration technique after turning on AC produced AFB1 and AFB_2 with values of 3.03 and 0.24 ng/ml, respectively. Whereas *A. flavus* isolated from indoor air samples collected by open plate technique before turning on AC produced AFG_1, AFB_1, AFG_2 ,and AFB_2 with values of 16.59, 1032.30, 95.29 ,and 13.23 ng/ml, respectively, while the same species isolated from indoor air samples collected by open plate technique after turning on AC produced only AFB_1 with the reduced value of 3.16 ng/ml. Other strains of *A. fumigatus* isolated from indoor air samples collected

Table 4. Isolated fungal species and the mean of total fungal count in air before and after turning on air conditioning system (Filtration Technique)

Samples	AC work	*A. fumigatus*	*A. niger*	*A. terreus*	*A. ochraceus*	*A. flavus*	*Fusarium equiseti*	*Mucor* sp.	*Alternaria alternata*	*Penicillium* sp.	*Cladosporium* sp.	Mean of Total fungal count (CFU/m^3)
M1	Before	x	x				x			x	x	$13.33\times10^4\pm0.0$
	After	x	x							x	x	$14.67\times10^4\pm0.30$
M2	Before		x	x	x		x	X	x			80.00±0.10
	After	x	x			x	x		x			$8.667\times10^4\pm0.10$
M3	Before	x	x	x		x		X			x	180.00±0.70
	After	x	x	x	x	x		X				$15.33\times10^4\pm0.10$
M4	Before	x		x				X	x		x	$11.33\times10^4\pm0.10$
	After			x				X	x		x	$12.67\times10^4\pm0.20$
M5	Before					X	x	X			x	$16.67\times10^4\pm0.20$
	After		x				x	X			x	$12.67\times10^4\pm0.20$
M6	Before		x				x	X			x	$9.33\backslash\times10^4\pm0.00$
	After	x	x				x				x	$12.67\times10^4\pm0.20$
M7	Before	x	x	x		X	x				x	$32.67\times10^4\pm0.10$
	After	x	x			X	x				x	$34\times10^4\pm0.10$
M8	Before		x	x	x	X	x					$21.33\times10^4\pm0.00$
	After		x	x	x	X	x					$26\times10^4\pm0.10$
M9	Before	x				X	x	X	x			$10.67\times10^4\pm0.20$
	After	x				X	x	X				$10.67\times10^4\pm0.40$
M10	Before				x			X	x		x	$13.33\times10^4\pm1.40$
	After				x				x		x	$18\times10^4\pm0.10$
M11	Before	x		x	x			X		x		$9.33\times10^4\pm0.00$
	After	x		x	x	X		X		x		$10\times10^4\pm0.10$
M12	Before	x	x	x	x		x	X	x			$16\times10^4\pm0.10$
	After	x	x	x	x			X	x			$8.67\times10^4\pm0.00$
M13	Before	x	x				x				x	$20\times10^4\pm0.80$
	After		x				x				x	$17.33\times10^4\pm0.30$
M14	Before		x				x	X	x		x	$22.667\times10^4\pm0.60$
	After		x				x	X	x		x	$23.33\times10^4\pm0.10$

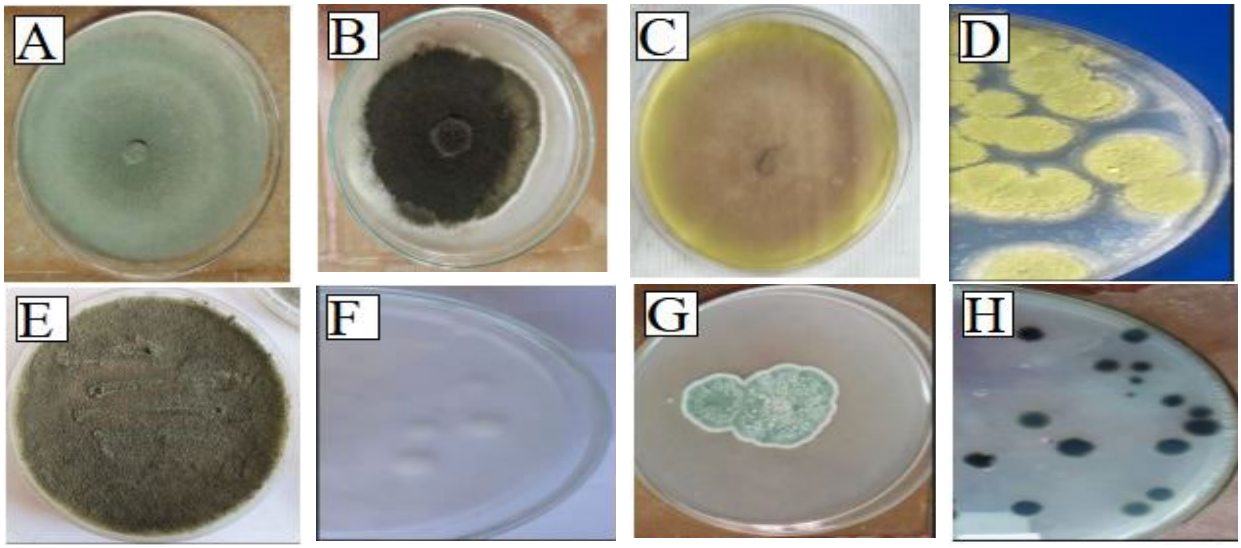

Figure 5. Cultures of some isolated fungal species growing at 25°C on Czapek's Yeast Extract Agar, Potato Dextrose Agar, and Dichloran-Glycerol-18 Agar media for 5 days: A) *Aspergillus fumigatus* B) *A. niger*, C), *A. terreus* D) *A. ochraceus*, E) *A. flavus,* F) *Fusarium equiseti* , G) *Penicillium* sp. H) *Cladosporium* sp.

Table 5. Shannon's diversity index of isolated fungal species from selected clinics.

Diversity Index	Dust of AC	Indoor air collected by (Open plate technique)		Indoor air collected by (Filtration technique)	
		Without AC	With AC	Without AC	With AC
Shannon (*H*)	2.19	2.26	2.07	2.24	2.17

Table 6. Sperman correlation between the fungal density, temperature (Temp) and relative humidity (RH).

		Dust	Open Plate	Filtration	Temp-B.	RH-B.	RH-A.
Dust	r	1	0.42	-0.43	0.16	0.00	-0.11
	p-value		0.14	0.12	0.59	0.99	0.72
Open Plate	r	0.42	1	0.37	0.37	-0.37	-0.43
	p-value	0.14		0.19	0.20	0.20	0.12
Filtration	r	-0.43	0.37	1	0.26	-0.19	-0.11
	p-value	0.12	0.19		0.38	0.52	0.70
Temp-B.	r	0.16	0.37	0.26	1	-0.783**	-0.817**
	p-value	0.59	0.20	0.38		<0.001	<0.001
RH-B.	r	0.00	-0.37	-0.19	-0.783**	1	0.925**
	p-value	0.99	0.20	0.52	0.00		<0.001
RH-A.	r	-0.11	-0.43	-0.11	-0.817**	0.925**	1
	p-value	0.72	0.12	0.70	<0.001	<0.001	

** Correlation is significant at 0.01 level. B=before AC, A= after AC

Table 7. Concentrations of aflatoxins (AFs) through ACS dust and indoor air of selected clinics.

Fungal Species	*Source*	Concentrations of AFs (ng/ml)				
		AFG_1	AFB_1	AFG_2	AFB_2	Total AFs
A. flavus	Dust of AC	ND	3.73	ND	ND	3.73
A. flavus	Indoor air after working of AC (Filtration technique)	ND	3.03	ND	0.243	3.273
A. flavus	Indoor air before working of AC (Filtration technique)	ND	14.28	ND	0.587	14.867
A. flavus	Indoor air before working of AC (Open plate technique)	16.59	1032.30	95.29	13.23	1140.82
A. fumigatus	Indoor air before working of AC (Open plate technique)	1390.76	13171.96	684.11	465.49	14321.56
A. flavus	Indoor air after working on AC (Open plate technique)	ND	3.16	ND	ND	3.16

ACS: Air Conditioning System; ND: Not Detected

Table 8. Concentrations of Ochratoxin A (OTA) through ACS dust and indoor air of selected clinics.

Fungal Species	Source	OTA (ng/ml)
A. niger	Dust	0.067
A. ochraceus	Indoor air after working of ACS (Filtration technique)	13.70
A. niger	Indoor air after working of ACS (Open plate technique)	6.55
A. niger	Indoor air before working of ACS (Open plate technique)	8.49
A. niger	Indoor air before working of ACS (Filtration technique)	12.37
A. ochraceus	Indoor air before working of ACS (Filtration technique)	17.97

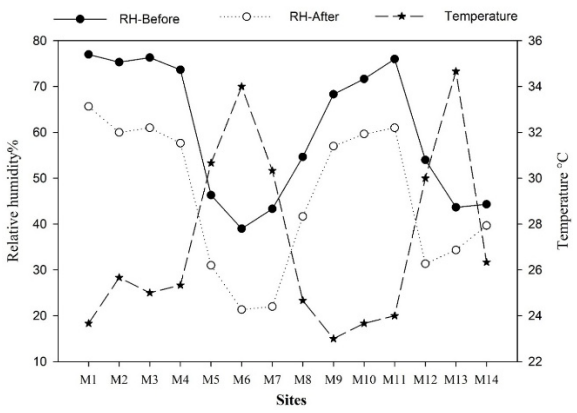

Figure 6. The average values of Temperature and relative humidity (RH) of air before and after turning on the air conditioning. Note: After turning on the air conditioning, the temperature curve has been removed because its values were nearly similar between sites, around 20ºC±1.

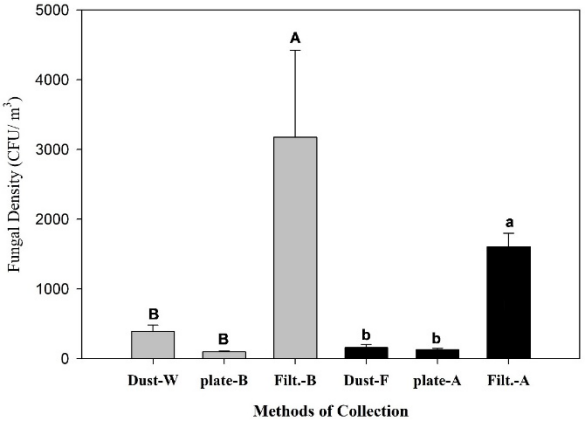

Figure 7. The fungal density between the different collection methods. W=wing, F= filter, B= before, and A= after. The values of filters (Filt.) are multiplied by 100 for the scale adjustment. The letters indicated significant differences based on the One Way Anova analysis with the Tuckey-b test, where a>b.

by open plate technique before turning on AC produced AFG_1, AFB_1, AFG_2 ,and AFB_2 with values of 1390.76, 13171.96, 684.11 ,and 465.49 ng/ ml, respectively. The results in (Table 8) indicated that *A. niger* isolated from dust samples was found to produce OTA with a concentration of 0.067 ng/ml. Also, *A. ochraceus* isolated from indoor air samples collected by filtration technique before and after turning on AC produced OTA with values of 17.97 and 13.70 ng/ml, respectively. Other strains of *A. niger* isolated from indoor air samples collected by open plate technique before and after turning on AC produced OTA with values of 8.49 and 6.55 ng/ml, respectively, while the same species isolated from indoor air samples collected by filtration technique before turning on AC produced OTA with value of 12.37 ng/ml.

DISCUSSION

The highest DAF number was observed in the following sites to M6>M12>M10>M1 as these sites represent the children's clinic on the 2nd floor, the obstetrics and gynecology clinic on 1st floor, the Dental clinic on 1st floor, and the Medical lab on 2nd floor, respectively. DAF number of all dust samples is higher than the allowable levels according to MOH (2012) except for dust samples collected from the AC's filter from Site M2 and Site M3. Since air conditioners maintain a comfortable temperature and humidity, they also contribute to the growth of indoor airborne fungi, which thrive best in environments with sufficient nutrients and little sunlight, especially in building materials (Khan and Karuppayil 2012 and Al-Bader et al. 2020). Outdoor air pollution affected indoor air quality (Hachimi et al. 2020). Moreover, fungi can grow on inorganic objects regularly colonized because they collect dust and provide suitable growth substrates for some *Aspergillus* species (Samet and Spengler, 2003). Higher relative humidity and temperature of study medical sites were conducive to fungal growth meaning the temperature of study sites ranged from 24±0.6 to 35±0.6 °C and humidity ranged between 39±0.7 and 78±0.7% through all medical sites. AC segments, such as filters are sources of bacteria and fungi (Liu et al. 2021). The results agree with Ljaljevic et al. (2008) who found that *Aspergillus* with five species was the most frequent, and its spores contaminate AC filters. Also (Liu et al. 2021) found that *Alternaria, Cladosporium*, and *Aspergillus* were the three most prevalent fungus species in AC dust. Additionally agree with Kim and Kim (2007) discovered *that Aspergillus*, *Penicillium* ,and *Cladosporium* were the most prevalent fungus species. Qudiesat et al. (2009) also discovered *Alternaria, Penicillium*, and *Aspergillus* fungal growth. In addition, Pimenta et al. (2022) revealed that *Aspergillus* sp., *Cladosporium* sp. ,and *Penicillium* sp. were the most abundant taxa discovered indoors (24 out of 56 healthcare facilities Coughing and sneezing are two ways people in medical places may increase the dispersion of fungi (Viegas et al. 2020b). They also contribute to the spread of numerous infectious diseases (Viegas et al. 2020a). Study results agree with Sham *et al.* (2021) who reported that *Aspergillus, Cladosporium,* and *Penicillium* were the most frequently found fungal taxa in hospital facilities with a percentage of 18.3% for each. Likewise, Yousefzadeh et al. (2022) found that the most frequently isolated fungi from hospital air were *Penicillium* (24.7%), *Cladosporium* (23.4%), *A. niger* (13.3%), and *A. flavus* (11.4%). *Cladosporium* sp. is the most dominant fungus in both indoor and outdoor environments (Aruj and Cabral, 2010 The highest total fungal count of indoor air before turning on ACS was recorded at site 5, an internal medicine clinic on the 1st floor of a main road. According to Marchand et al. (2016), several possible causes include medical operations, patient coughing and sneezing, improper clinic cleanliness, improper methods, and specific types of ventilation systems. In addition to the effect of outdoor sources such as dust and building structures (Taushiba et al. 2023). The air of site 5 with (T = 30±0.6 °C and RH = 47±0.7% provides good conditions for fungal growth. The study results of the most abundant species in indoor air after turning on AC were in agreement with (Mobin and Salmito 2006), who demonstrated that the most common fungi found in air conditioning filters in critical care units in Teresina, Piaul, were *A. niger* and *A. fumigatus.* The highest concentration was recorded at site 1, a medical lab visited by many patients daily and located on the 2nd floor of a main road. The AC in this site was presented in the lab room with its air with T of 20±0.6 °C and RH of 66±0.0%, which provide good conditions for fungal growth. Notably, the total fungal count of indoor air after turning on ACS was more than before turning on AC. That is in agreement with (Hamada and Fujita 2002) who noted that airborne fungal contamination in rooms with air conditioning was roughly two times higher than in rooms without air conditioning, peaked when the air conditioner was operating, and gradually decreased over time.

They also mentioned that the environmental factors in the rooms with air conditioning controlled the amount of airborne fungal contamination. That could

be because the air current passes through the air conditioner filter, which is contaminated with fungi. The fungi spread fungal spores that penetrated the room's atmosphere. When the air conditioner is turned on, it may appear as a cough or unpleasant odor (Hamada and Fujita 2002). According to Almoffarreh et al. (2016), the existence of these fungi is also brought on by water condensation and rises in temperature and relative humidity in the air conditioning system. According to Hsu et al. (2011), turning off air conditioners during off-peak hours to save power may result in water condensation increasing temperature and relative humidity and promoting the growth of fungi. The results agree with those of (Al-Abdalall et al. 2019), who reported that *Alternaria, A. flavus, A. fumigatus, A. niger, A. ochraceus,* and *Penicillium* sp. were all found to be sources of airborne contamination in air conditioning systems; of these, *A. fumigatus,* which has been isolated from air conditioning filters, is the cause of numerous serious illnesses (Al-Abdalall et al. 2019).

Table 4 showed that the highest mean levels of total fungal count in the indoor air before turning on the ACS were recorded at site 7, which represented a medical lab on the third floor on a side road and the AC in the reception room with different numbers and types of patients. The conditions of this site could be favorable for the growth of fungi with high exposure to outdoor contamination (Tang et al. 2015 and Najjar 2024). The lowest concentration was recorded at site 2, which represented a radiology center based on the 1st floor and the AC in the doctor's room; this may be attributed to the low density of patients and the daily cleaning of the room. Also, most air conditioners reduce the relative humidity as they reduce the temperature and, consequently, the evaporation rate that reduces fungal growth (Sekartaji et al. 2023). After turning on the AC, site 7 had the highest mean concentration of the total fungal count in indoor air. The total fungal count of indoor air after turning on ACS was higher than before turning on ACS in the in the open plate technique. Al-Bader et al. (2020) noted that spore growth and dryness during the AC shutdown time increase in fungal growth.

The results of this study agree with that of Arauj and Cabral (2010), who showed that fungi are widely distributed in all atmospheres and that species of *Cladosporium, Penicillium, Aspergillus*, and *Alternaria* predominate in both indoor and outdoor environments. Shannon's diversity index (*H*) indicates that the higher in the index, the more diverse in the species. The higher biodiversity of isolated fungal species in indoor air samples is collected by by open plate and filtration techniques before turning on ACS and the AC dust. The study results indicate that the indoor air and dust samples might have high species diversity.

Fungi in a structure do not necessarily indicate the presence of mycotoxins or their concentrations. Mycotoxins can be inhaled by airborne particulates inhalation, such as dust and fungal components (Halios and Helmis 2010). The production and decrease in AFs concentrations following AC turn-on may be caused by temperature effects, where the average temperature and relative humidity throughout all selected clinics were 27.4 ºC, 60.6%, and 20.0 ºC, 47.35%, respectively. That is in agreement with (Martí 2006), who indicated that moisture, pH, and temperature are the primary variables influencing the growth and synthesis of *Aspergillus* toxins. Strong hepatotoxic and hepatocarcinogenic effects are present in AFB_1. AFB_1 is categorized as a Group 1 carcinogen by the International Agency for Research on Cancer (Liu 2021). The results are consistent with those of Pardo et al. (2004, 2005a, 2005b), who reported that *A. ochraceus* might produce OTA better at temperatures between 20 and 30 ºC. According to Martí (2006), its concentration may have decreased because controlling moisture is the most effective and cost-effective way to regulate the environment and stop mold growth and mycotoxin formation. Temperature affects the variety of mycotoxins that the same species produces as well. The physicochemical conditions of the organism's environment for growth and its genotype influence the generation of mycotoxins. Any specific mycotoxin's ability to be produced depends on the strain rather than the species. Nonetheless, environmental factors affect the OTA biosynthesis of ochratoxigenic Aspergillus species more than the organism's innate capacity to synthesize OTA (Muhlencoert et al. 2004). Different quantities of sucrose (0 to 4%) and yeast extract (0 to 4%) in lab medium may cause variations in the amount of OTA produced by *A. ochraceus* strains (Atalla and El-Din 1993). Additionally, because this material will only hold particles at least three microns in size, AC filters do not eliminate microbiological contamination from the air. As a result, dust particles smaller than three microns will flow through without being blocked. Thus, if the filters get too wet, they may create an ideal habitat for the growth of fungus formaing mycotoxins (Al-abdalall et al. 2019).

The weak correlation between fungal concentrations

collected from dust and those obtained from air samples—using filtration and open plate methods—suggests that the different collection techniques variably influenced fungal levels. Furthermore, the findings indicated a weak correlation between each collecting method and temperature and relative humidity (RH), which suggests that temperature and RH had minimal influence on the fungal concentrations collected through these procedures. The significant value of the fungal concentration obtained using the various procedures was ($P<0.05$) indicating a significant difference in the fungal concentrations between the approaches.

CONCLUSIONS

Monitoring toxinogenic-producing fungi in ACS dust and indoor air environments controls IAQ and prevents fungal growth, especially in Damietta Governorate clinical settings. Shannon's diversity index investigates higher biodiversity of fungal species in ACS dust and indoor air. The most prevalent fungal genera found in ACS dust and indoor air at selected clinics were *Aspergillus, Mucor, Fusarium, Penicillium, Cladosporium* ,and *Alternaria*. The most common species were *A. flavus, A. niger, A. parasiticus, A. fumigatus, A.ochraceus, A. nidulans* ,and *A. terreus,* in addition to *Fusarium equiseti*, *Fusarium oxysporum* ,and *Alternaria alternata.* Results revealed the ability of these fungal species to produce mycotoxins such as *A. flavus* in dust (AFB_1) with a value of 3.73 ng/ml. *A. parasiticus* can produce (AFG_1, AFB_1, AFG_2 ,and AFB_2) with values of 1390.76, 13171.96, 684.11 ,and 465.49 ng/ ml, respectively. *A. ochraceus* can produce (OTA) with a value of 17.97 ng/ ml. It is important to mention that the total fungal count of indoor air after turning on ACS was more than before turning on ACS. The authors recommend the need to intensify such studies of the indoor air environment and ACS, and to establish restrictions related to environmental safety inside clinical settings. Since air filters are among the most often used air filtration techniques, it is advised that they must be checked frequently or changed at the start of each season.

LIST OF ABBREVIATIONS

AC**:** air condition
AFs: aflatoxins
OTA: ochratoxin A
IAQ: indoor air quality
DAF: dust accumulated fungi
CFU: colony formed unit
YES: yeast extracts sucrose
HPLC: high performance liquid chromatograph

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors. Omnya A. El-Batrawy suggested the point of research besides revising and editing the search. Amany F. Hasballah collected samples, carried out all the lab work regarding analysis and wrote the draft of the manuscript. All authors read and approved the manuscript.

CONSENT TO PUBLISH

All authors revised the manuscript and approved the publication in this respect journal.

AUTHORS CONTRIBUTIONS

Omnya A. El-Batrawy detects the point of research besides revising and editing the search. Amany F. Hasballah collected samples, carried out all the lab work regarding analysis and wrote the draft of the manuscript. All authors read and approved the manuscript.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that they have no conflict of interest.

DATA AVAILABILITY

The original data and materials will be ready on request.

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