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Research Article

Indoor Airborne Fungal Community in an Experimental Animal Hall and their Risks and Control Using Essential Oil Vapors

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ABSTRACT

Airborne fungi are a major concern in indoor environments, particularly in experimental animal housing facilities, where they pose significant health risks to both animals and humans. This study investigates the diversity, concentration, and health implications of airborne fungal species present in an experimental animal house at Cihan University-Erbil. Air samples were collected from both indoor and outdoor environments to assess fungal contamination levels. The results revealed that indoor air had a significantly higher fungal concentration (401 colony-forming unit [CFU]/m^3) compared to outdoor air (202 CFU/m^3), with dominant species including *Aspergillus fumigatus*, *Alternaria* sp., and white yeast. The study also evaluated the antifungal efficacy of Lemongrass (*Cymbopogon citratus*) oil vapor, which demonstrated significant inhibitory effects on *Aspergillus terreus*, *Alternaraia* sp., and *Rhodotorula mucilaginosa*, suggesting its potential as a natural antifungal agent. The findings highlight the importance of proper ventilation and fungal control measures in animal housing environments to mitigate health risks. Future research should explore the long-term effectiveness of natural antifungal agents and optimize air quality management strategies.

Keywords: Airborne fungi, animal house, oil vapor, health impact, *Aspergillus*

INTRODUCTION

The past 20 years have seen a rise in interest in indoor airborne fungi as people have become more conscious of their possible health effects and the amount of time they spend indoors. Research connecting fungi to a number of health conditions, such as allergies, asthma, and other respiratory disorders, as well as the realization that people spend a large amount of their lives indoors, have led to this increased attention.[1] Indoor air quality issues are recognized as a globally significant risk concern for human health. People spend a significant portion of their time indoors, which makes indoor air quality crucial. Population groups that are more susceptible because of their age or health status are impacted by indoor air pollution in homes, daycare facilities, retirement homes, and other unique settings.[2] Many types of fungi can flourish and cause microbial contamination in the moist indoor environment that supports their development. Clinically, respiratory issues, allergies, asthma, and immunological responses are linked to exposure to bioaerosol pollution.[3] Due to a number of contributing factors, animal housing environments, including those for both large and small animals, are acknowledged as important sources of indoor airborne fungal contamination. It has been demonstrated that a range of fungal aerosols are present in chicken breeding facilities, in particular. *Trichosporon* sp., *Candida* sp., *Aspergillus* sp., *Cladosporium* sp., and *Alternaria* sp., are the most common fungal species found in chicken homes.[4] In rabbit breeding environments, *Aspergillus* sp., *Alternaria* sp., and *Fusarium* sp., were the predominant in air samples.[5] From duck breeding house *Aspergillus* sp., *Acrophialophora* sp., *Byssochlamy* sp., *Fusarium* sp., *Lichtheimia* sp., *Paecilomyces* sp., *Penicillium* sp., *Polycephalomyces* sp., *Rhizomucor* sp., *Scopulariopsis* sp.

Talaromyces, and *Thermoascus* sp. were recorded from air samples.[5] The degree of mycological air contamination and the taxonomic diversity of airborne fungi were investigated in the air of 20 different animal facilities within a zoological garden. A total of 10 fungal genera were isolated. *Penicillium* sp. was the dominant genus, accounting for 58.9% of the total fungal strains, followed by *Aspergillus* sp., *Cladosporium* sp., *Talaromyces* sp., *Mucor* sp., *Schizophyllum* sp., *Syncephalastrum*

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sp., *Alternaria* sp., *Absidia* sp., and *Cunninghamella* sp.[6] In contrast to domestic animals, the airborne fungi in the houses of experimental small animals did not receive enough attention. The current study aims to estimate the fungal bioaerosols in the experimental animal house of Cihan University-Erbil and evaluate *in vitro* activity of a friendly environment vapor on *Aspergillus terreus*, *Alternaria* sp., and *Rhodotorula mucilaginosa*.

MATERIALS AND METHODS

Study Location and Sampling

This study was conducted to evaluate airborne fungal diversity and density across two distinct environments: an indoor controlled animal housing facility and a natural outdoor field. On March 18, 2025, 20 air samples were collected from each site. The indoor air sampling was carried out in the experimental animal house at Cihan University-Erbil in a room housing albino mice and rats maintained under standard laboratory conditions. Environmental parameters included a temperature range of 22 ± 2°C, relative humidity of about 60%, and controlled ventilation with moderate airflow.

Airborne fungal spores were sampled using the settle plate method (passive exposure). In this method, sterile Petri dishes containing 15 mL of Sabouraud Dextrose Agar (SDA) supplement with 50 mg/L[7] were exposed to ambient air at a height of approximately 1 m above ground level for fungal spores were sampled using the settle plate method (passive exposure). In this method, sterile Petri dishes containing 15 mL of SDA were exposed to ambient air at a height of approximately 1 m above ground level for a duration of minutes to allow gravitational settling of fungal spores onto the agar surface.

To minimize contamination bias, samples were collected from the central area of the room, away from potential fungal sources such as bedding, feed, and waste disposal areas, and 1 m above the ground surface. The housing conditions adhered to established guidelines for the care and use of laboratory rodents, ensuring animal welfare and consistency in environmental exposure. Outdoor air samples were collected concurrently at a location carefully selected to minimize environmental disturbances, situated away from buildings, trees, vehicular traffic, and other potential sources of interference.

Fungal Community Analysis

The absolute number colony-forming units (CFUs) for each plate was modified to CFU/m^3 by the equation followed by[5] ($N = 5a \times 10^4 (b\ t)^{-1}$).

N = CFU/m^3 of air, a = number of colonies/plates, b = area of dish surface (cm^2), t: exposure time (minutes).

The percentage of occurrence and the frequency of occurrence% were calculated for each genus by the following equations, followed by:[8]

Occurrence% (O%) = (no. of samples in which the genus occurred)/(no. of total samples) × 100.

Frequency% (F%) = (no. of genus colonies/no. of total genera colonies × 100.

Importance value index = (O+T)/2.

Antifungal Effect of Lemongrass *Cymbopogon citratus* Oil Vapor

A disk volatilization method[9] was used to estimate the antifungal activity of lemongrass (*C. citratus*) oil vapor on the selected isolates. The experiment was applied to three isolates. *A. terreus*, *Alternaria* sp. and *R. mucilaginosa.* Petri dishes containing 15 mL of SDA supplemented by the antibiotic were prepared. Plates were inoculated from 7-day-old fungal cultures. A sterile needle was used to transfer the molds, and a sterile loop was used for yeast. A filter paper disk (2 cm in diameter) was saturated with 150 µL oil and was placed on the inner surface of the Petri dish lid. Each plate was sealed with parafilm tape and incubated at 25 ± 2°C. The antifungal activity is determined by measuring the growth diameter after 4 days. Triplicate assays were carried out for each essential oil and the control to validate the reliability of the experimental outcome.

Fungal Identification

The isolated fungi were identified based on the morphology feature of the colonies, followed by the microscopic examination for the microscopic characteristics such as the asexual spores forming structures, shape of conidiophores, and yeasts properties as fully described by Domsch *et al.*,[10] De Hoog and Guarro.[11]

RESULTS AND DISCUSSION

Fungal colonies became visibly observed on the Petri dishes, enabling reliable quantification during the incubation period. The distinct colonies aid in the primary identification and preparation of pure cultures [Figure 1].

Analysis of Fungal Communities

A 138 CFU related to eight isolates for the indoor air samples were recovered from indoor air samples, representing eight distinct fungal taxa [Table 1]. White yeasts were the most abundant, with 58 CFU, corresponding to a concentration of 136 CFU/m^3. The second most prevalent group was the *Aspergillus* species

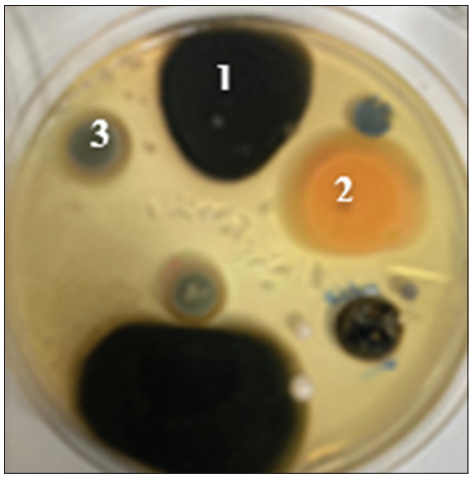

Figure 1: Representative plate of the culture plates after air exposure and incubation for 7 days. *Aspergillus niger* (1), *Rhodotorula muceligenosa* (2), *Alternaria alternata* (3)

Table 1: Fungal isolates of indoor air samples: (CFU)=colony forming units, (O%)=occurrence%, (F%)=frequency%, (IVI)=Importance Value Index

No.	Fungi	CFU	(O%)	(F%)	IVI	CFU/m³
1	*Aspergillus fumigatus*	34	66	24.28	90.28	89
2	*Alternaria* sp.	18	50	12.85	62.85	47
3	*Rhodotorula mucilaginosa*	8	33	5.71	38.71	21
4	*Aspergillus niger*	8	15	4.28	19.28	21
5	*Aspergillus ochraceus*	6	20	2.85	22.85	16
6	*Aspergillus terreus*	3	10	1.42	11.42	8
7	White Yeast	58	50	41.42	91.24	136
8	White mycelium	10	33	7.14	40.14	26
Total		145		100		362

Table 2: Fungal isolates of outdoor air samples: (CFU)=Colony-forming units, (O%)=Occurrence%, (F%)=Frequency%, (IVI)=Importance value index

No.	Fungi	CFU	(O%)	(F%)	IVI	CFU/m³
1	*Alternaria* sp.	16	80	23.5	51.75	41
2	*Aspergillus niger*	10	60	14.7	37.35	26
3	*Aspergillus fumigatus*	10	60	14.7	37.35	26
4	*Aspergillus terreus*	5	40	7.3	23.65	13
5	*Cladosporium* sp.	3	20	4.0	12.0	8
6	*Penicillium* sp.	4	20	5.8	12.9	11
7	*Rhodotorula mucilaginosa*	2	20	2.9	11.45	5
8	*Rhizopus* sp.	2	20	2.9	11.45	5
9	White mycelium	8	60	11	35.5	21
10	Whie Yeast	8	60	11.7	35.85	21
Totally		68		100		178

complex, comprising *Aspergillus niger*, *A. fumigatus*, *A. terreus*, and *Aspergillus ochraceus*, which collectively accounted for 51 CFU, equivalent to 134 CFU/m³ [Table 1].

In contrast, a total of 68 CFU were recovered from outdoor air samples, demonstrating ten distinct fungal isolates (6 genera besides whit mycelium and white yeast) [Table 2]. *Aspergillus* spp. (*A. niger*, *A. fumigatus*, *A. terreus*) were the most abundant 25 CFU, conforming to a concentration of 65 CFU/m³. The second most prevalent was *Alternaria* sp. 16 CFU and 51 CFU/m³ [Table 2].

The comparison between the total CFU of common isolates – occurred in both sites – showed a high difference after Chi-square analysis [Table 3].

The overall Chi-square test clarifies a highly significant difference in fungal composition between indoor and outdoor air ($\chi^2 = 26.61$, $P < 0.001$). *Post hoc* analysis shows that white yeast, *Alternaria* sp., and *A. niger* differed significantly between environments [Table 4].

Remarkably, white yeast counts were much higher indoors, likely reflecting favorable microclimatic conditions in the animal house. *Aspergillus fumigatus*, despite a higher indoor count, did not reach statistical significance due to variation relative to total colony counts.

Table 3: Comparison of total mean CFU counts between outdoor and indoor air of the animal house (common species)

Environment	Total mean CFU (common species)
Outdoor	68
Indoor	145
Chi-square (χ^2)	26.61
P-value	0.000068
Significance	Highly significant

CFU: Colony-forming units

The predominance of *Aspergillus* belongs to biological and environmental factors. The genus grew in a variety of settings, including soil, air, and decomposing organic materials. Their broad existence and survival are attributed to a number of traits. It produces a large number of tiny conidia that are easily airborne and dominate,[12] in addition, *A. fumigatus* is a thermotolerant fungus that thrives and multiplies in temperatures ranging from 0°C to 45°C. From another perspective, the fungus exhibits high enzymatic activity that facilitates its growth on various types of substrates.[13] The persistence of conidia in indoor and outdoor air, especially in humid or dusty environments, increases exposure risk.[12]

Health Impact of Predominant Isolates

The total CFUs in both locations are less than the risk level; they represent low level (178 CFU/m^3) and (362 CFU/m^3) in the outdoor and indoor air, respectively [Tables 1 and 2]. *Aspergillus* and *Alternaria* are among the most common fungal allergens. Recent studies indicate that occupational exposure in environments such as animal houses and farms is associated with an increased prevalence of respiratory symptoms, primarily due to the inhalation of *Aspergillus* aerosols.[14] Regarding *Aspergillus*, the genus was represented by 25 CFUs in outdoor air samples and 51 CFUs in indoor samples. Airborne *Aspergillus* species, particularly *A. fumigatus* pose significant health risks to humans. These filamentous fungi release conidia that are easily aerosolized due to their small size (~2–3 μm), allowing deep penetration into the respiratory tract upon inhalation.[15] In indoor settings, such as animal houses lodging mice and rats, *Aspergillus* thrives in organic bedding, feed, and poorly ventilated spaces. The presence of Asp f- 1, a major allergen released during spore germination, has been linked to inflammatory responses and airway remodeling.[16] The concentration of the outdoor total airborne fungal colony count and its composition are affected by environmental factors. *Aspergillus* spores, one of the predominant isolates here, are influenced by temperature, humidity, and vegetation, and several previous studies have shown that outdoor air contains significant levels of *Aspergillus* spore types, which may exacerbate respiratory symptoms during seasonal and daily peaks.[17,18]

From the other side of the view in the two sample locations, *Alternaria* sp. is listed as a common isolate; it has several properties that lead to its use as a significant indicator in aeromycological monitoring and public health risk assessments. *Alternaria* sp. is frequently isolated from indoor and outdoor environments. It produces large quantities of dry, lightweight spores that are easily dispersed by wind, making it a dominant component of bioaerosols.[19]

Alternaria sp. concentration was used to point out the total fungal air pollution,[20] and in this study, *Alternaria* was detected at concentrations of 41 CFU/m^3 in outdoor air and 47 CFU/m^3 in indoor samples from the animal house, which are less than the risk level (100 spores/m^3). Its presence at these levels suggests moderate fungal air contamination.[21] Due to the size of the spores and their constituents, *Alternaria* air spores are more harmful to human health at lower

Table 4: Comparison of mean CFU counts for common airborne fungal species between outdoor and indoor air of the animal house, with Chi-square statistical analysis

Species	Outdoor (Mean CFU)	Indoor (Mean CFU)	χ^2 (species-specific)	*P*-value	Significance
Alternaria sp.	16	18	6.15	0.013	Significant
Aspergillus niger	10	8	5.89	0.015	Significant
Aspergillus fumigatus	10	34	0.57	0.449	NS
Aspergillus terreus	5	3	3.21	0.073	NS
White yeast	8	58	12.26	0.00046	Significant
Rhodotorula mucilaginosa	2	8	0.06	0.810	NS
Overall χ^2	26.61			0.000068	Highly significant

CFU: Colony-forming units

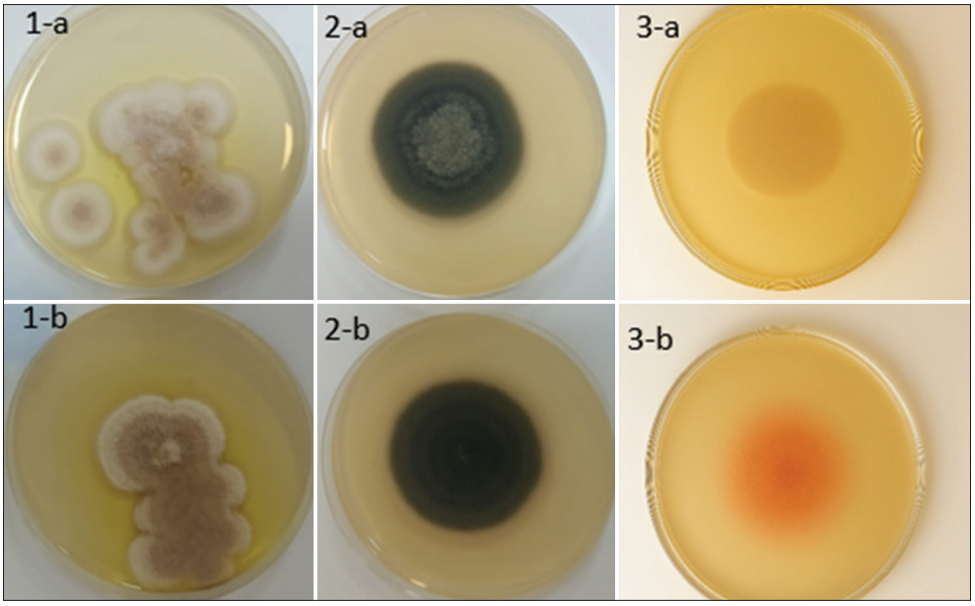

Figure 2: 1 = *Aspergillus terreus*. 2 = *Alternaria* sp. 3 = *Rhodotorula mucilaginosa* (a=treated; b=untreated)

concentrations than *Aspergillus, Penicillium*, and *Cladosporium*, the primary causes of fungal respiratory illnesses.[22]

The concentration of *Alternaria* in outdoor samples is higher than in indoor samples, primarily due to its ecological role as a plant pathogen and phylloplane-associated genus.[23] Moreover, outdoor environmental factors – particularly sunlight and elevated temperatures – significantly influence the survival of fungal propagules. The multicellular, thick-walled, and pigmented spores of *Alternaria* exhibit greater resistance to desiccation and ultraviolet radiation compared to the smaller, unicellular spores of *Aspergillus* sp.

Antifungal Activity of Lemongrass (*C. citratus*) Oil Vapor

Lemongrass oil vapor was used based on initial screening tests reported by Al-Bader *et al*.,[24] Bakkali *et al*.[25] The vapor demonstrated a distinct inhibitory effect on both molds and yeasts. The relative inhibition RI = 43%, 71%, and 8% for *A. terreus*, *R. mucilaginosa*, and *Alternaria* sp., respectively [Figure 2].

The vapor markedly reduces mycelial growth and the abundance of conidia (lighter colony color) of *A. terreus* and *Alternaria sp*., and significantly inhibits the development and density of *R. mucilaginosa*. The high antifungal activity of lemongrass oil (on mold and yeast) is largely attributed to citral, the main biochemical constituent, which is easily absorbed through fungal cell membranes, causing a disruption of membrane structure and functions.[26]

CONCLUSION

This study revealed a diverse fungal community within the indoor air of an experimental animal hall with. *Aspergillus* spp. and *Alternaria* sp. as the dominant genera. Although total CFU counts were below critical health thresholds, the indoor fungal load exceeded outdoor levels, emphasizing the need for regular environmental monitoring. Vaporized lemongrass (*C. citratus*) essential oil demonstrated significant antifungal activity, effectively inhibiting growth and sporulation of filamentous fungi such as *A. niger*, *A. fumigatus*, and *Alternaria* spp., as well as yeasts such as *R. mucilaginosa*. These findings highlight lemongrass oil vapor as a promising, eco-friendly alternative to synthetic chemical agents for improving air quality in animal housing environments. Its natural origin, low toxicity, and broad-spectrum efficacy support its potential for sustainable fungal control. Future research should focus on optimizing application parameters, including concentration, exposure duration, and treatment frequency. In addition, investigating other essential oils, evaluating synergistic effects, and assessing long-term impacts on air quality and animal health will be crucial for advancing the practical use of plant-based volatiles in maintaining safe and hygienic indoor environments.

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