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Airborne Fungi in Buildings of Nakhon Ratchasima College

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Abstract

The indoor building environments have become a rising concern about indoor air quality (IAQ) impacting people health. This study examined viable airborne fungi from 13 sampling sites in various buildings of Nakhon Ratchasima College, Thailand, between July and August 2020. In order to investigate fungal diversity and amount of fungi contaminated in the indoor air and determine the correlation of the findings with environmental factors such as room temperature and relative humidity as well as the number of attendants. An open plate technique was used as a sampling method. Totally 160 samples of potato dextrose agar (PDA) plates were carried for 1 hour and twice a day. Standard plate count was used to quantify colony counts and fungal concentrations. Results showed the highest fungal concentration was found in the anatomy laboratory. The lowest fungal concentration was found in the office (eighth floor). Both sampling sites were classified by index of microbial air contamination (IMA) level as very good and good respectively. Eight fungal genera were identified. The dominant genera were *Cladosporium* and *Aspergillus*, followed by *Penicillium, Fusarium, Alternaria*, *Rhizopus*, *Curvularia* and *Mucor*. Statistical analysis based on Pearson's correlation revealed the number of attendances had low negative correlation (r = -0.374) with the fungal concentrations (r = -0.374). Whereas relative humidity and room temperature had no correlation (r = -0.002 and -0.025 respectively). This study provides a profile of airborne fungi which benefit on indoor air quality assessments as fungal background data.

Keywords: Indoor air quality (IAQ), Airborne fungi, Open plate technique, potato dextrose agar (PDA), Standard plate count, Index of microbial air contamination (IMA)

1. Introduction

Fungal diseases kill over 1.5 million and impact more than a billion people worldwide. Thus, they are still important problems in global public health (1). For instance, inhalation of fungal spores is shown to contribute in symptoms of allergic rhinitis (2). In addition, serious fungal infections occur as a consequence of other underlying diseases including asthma, AIDS, cancer, and organ transplants. The Leading International Fungal Education (LIFE) estimates over 10 million cases of fungal asthma annually, over 3 million cases of chronic pulmonary aspergillosis, 1 million cases of fungal keratitis, 700 thousand cases of invasive candidiasis, 500 thousand cases of *Pneumocystis* pneumonia (PCP), 25 thousand cases of invasive aspergillosis, 200 thousand cases of cryptococcal meningitis and 100 thousand cases of disseminated histoplasmosis (1).

Nowadays, indoor building environments are fundamental factors impacting onto living of the people. Indoor air quality of indoor environments is one of the main factors affecting health of people spending 80% - 90% of their time in indoors environments by breathing on average 14 m^3 of air per day. These make people highly exposed to indoor air environments. One problem of indoor air quality is contamination of fungi. The most common fungi frequently found in building were *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp. (3). Among the fungal species, *Alternaria* spp. is the major allergen which have significant role in the induction of allergic

rhinitis (4). Additionally, *Aspergillus* spp. is the main cause of fungal respiratory tract infections (5).

In general fungi in buildings require physical factors to contribute for their growth and distribution. For example, the size of indoor fungal populations is dependent upon the relative humidity. Most species of fungi cannot grow unless the relative humidity exceeds 60% (6). In addition, air temperature of 20 - 30 °C and good ventilation is appropriate for the fungal growth and distribution as well (7).

For airborne fungi surveys, few methods are selected for isolation of atmospheric fungi. A study of Basilico et al. (8), samples were taken with a standard air sampler operating on air force impact onto an agar media. This method not only investigated fungal species but also revealed airborne fungal concentrations in houses of Santa Fe city, Argentina. The results showed the prevalence of *Cladosporium*, *Alternaria*, *Epicoccum*, *Fusarium*, *Curvularia*, *Acremonium*, *Drechslera*, *Penicillium*, *Aspergillus*, *Mucor*, *Ulocladium*, *Nigrospora*, *Chrysosporium* and yeasts for 58.9, 8.68, 5.74, 5.37, 3.50, 1.27, 1.26, 1.25, 1.14, 0.61, 0.57, 0.48, 0.42 and 3.74% respectively. While in Thailand, a study of Bunnag et al. (9) used the culture plate method for the indoor and outdoor sampling of airborne fungi in houses. The results revealed mainly occurrence of *Aspergillus*, *Hermodendrum*, non-sporulated white fungi and yeasts. This study indicated that season did not affect occurrence of *Aspergillus*, *Fusarium* and *Curvularia.*

The aims of this study were: 1) to identify the genera of fungi and their concentrations in the indoor air of buildings in Nakhon Ratchasima College, Thailand, 2) to evaluate three physical factors: room temperature and relative humidity as well as the number of attendants influencing over fungal concentrations.

2. Materials and Experiment

2.1 Study design

A cross-sectional descriptive study, sample collections were divided into four weeks in the rainy seasonal period in July and August (2020).

2.2 Sampling conditions

Potato dextrose agar (PDA) plates were used as open plate technique: opened and located at 40 sites in 13 rooms of various buildings of Nakhon Ratchasima College for totally 160 samples. The PDA plates put on tables at the height of 75 centimeter from the ground. Each sample was taken 1 hour twice a day in the morning (9:00 a.m.) and in the afternoon (2:00 p.m.).

2.3 Isolation and identification

After the sampling, the agar plates were incubated at 25 °C for 48 hours. Cultures were observed colony characteristics and microscopic morphology. Fungal identification was performed using A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain (10). Standard plate count was used to quantify the amount of fungi as colony count and calculated to be fungal concentrations as a unit of colony forming units per plate per hour (CFU/plate/h). In order to confirm fungal growth and identification, all cultures were re-incubated and daily observation was performed.

2.4 Physical parameters

Data collection of physical parameters on each time of sampling consisted measurement of air temperature using thermometer, relative humidity using hygrometer and the number of attendants by visual observation.

2.5 Statistical analysis

Descriptive statistics was used to show airborne fungal concentrations (CFU/plate/h) in each room as mean and standard deviation, and fungal count was presented with number of isolates and percentage of colony count. For comparative study, one way ANOVA for airborne fungal concentrations in each rooms (p=0.05) and Pearson's correlation coefficient analysis between airborne fungal concentrations and physical parameters was determined with significance at the 0.05 level 2-tailed. These statistical analyses were facilitated using IBM SPSS Statistics 23.0.

3. Results and Discussion

3.1 Airborne fungal concentrations

The results (in Table 1) showed anatomy laboratory room contained the highest average airborne fungal concentration was 19.70 ± 11.79 CFU/plate/h. The second, Lumtakong meeting hall, was 18.83 ± 2.96 CFU/plate/h. The lowest average airborne fungal concentration, office (8th floor), was 4.32 ± 1.41 CFU/plate/h. There were no significant differences between the fungal concentrations in each room (p=0.05).

Table 1 Airborne fungal concentration of each sampling sites in Nakhon Ratchasima College

Sampling sites	Airborne fungal concentrations (CFU/plate/h)
Anatomy laboratory	19.70 ± 11.79
Lumtakong meeting hall	18.83 ± 2.96
First aid room (underground)	15.73 ± 2.11
Classroom (1st floor)	12.04 ± 3.73
Basic science laboratory	9.83 ± 3.73
Office (1st floor)	9.53 ± 3.06
Computer laboratory	9.24 ± 3.70
Thai traditional medicine clinic	9.04 ± 3.17
Classroom (5th floor)	8.65 ± 2.29
Library	8.35 ± 1.02
Microbiology laboratory	6.88 ± 3.35
Trirattana meeting room	5.90 ± 1.41
Office (8th floor)	4.32 ± 1.41

Table 2 Fungal count classified by fungal genera in each sampling sites

Sampling sites	Colony count (number of isolates)								
	Fungal genera								Total
	Alternaria	*Aspergillus*	*Cladosporium*	*Curvularia*	*Fusarium*	*Mucor*	*Penicillium*	*Rhizopus*	
Anatomy laboratory	10	225	54	4	3	1	13	5	315
Lumtakong meeting hall	11	26	138	3	2	0	22	3	205
Classroom (1st floor)	8	31	77	4	2	1	9	6	68
Thai traditional medicine clinic	3	7	26	0	80	0	6	2	138
Basic science laboratory	10	18	29	6	8	0	18	11	100
Office (1st floor)	17	24	34	0	7	1	13	1	97
First aid room (underground)	14	12	29	0	6	1	4	2	36
Library	6	16	20	1	5	0	14	3	124
Classroom (5th floor)	3	10	10	9	3	1	6	2	44
Computer laboratory	3	23	6	0	1	0	2	1	65
Microbiology laboratory	6	10	4	1	0	0	10	4	35
Trirattana meeting room	4	2	2	0	0	1	7	14	30
Office (8th floor)	4	0	3	1	1	0	4	0	13
Total of colony count	99	404	432	29	118	6	128	54	1270
Percentage of colony count	7.80	31.81	34.02	2.28	9.29	0.47	10.08	4.25	100.00

3.2 Fungal identification

1,270 isolated fungal colonies were identified and revealed 8 genera of *Cladosporium, Aspergillus, Penicillium, Fusarium, Alternaria, Rhizopus, Curvularia* and *Mucor* that 432 (34.02%), 404 (31.81%), 128 (10.08%), 118 (9.29%), 99 (7.80%), 54 (4.25%), 29 (2.28%), and 6 (0.47%) colonies of each genus was found respectively as shown in Table 2.

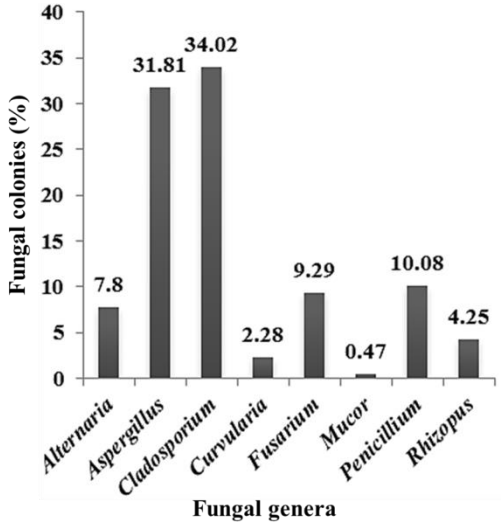

Figure 1 Percentage of fungal genera

The dominant fungi were *Cladosporium* and *Aspergillus* as shown in Figure 1. Microscopic examination revealed 2 species of *A. fumigatus* and *A. flavus* distinguished by spiny conidia in *A. flavus* same as appearance of uniseriate and biseriate phialides (11).

The *Cladosporium* spp, dematiaceous fungi, have a worldwide distribution (12). Most species are contaminants. However, some species are pathogenic and toxigenic to humans. These fungi are known to be the cause of cerebral and cutaneous phaehyphomycoses. In addition, they are strong aeroallergens and cause serious allergic diseases of the respiratory tract (12).

The *Aspergillus*, hyalohyphomycetes, is a ubiquitous organism. It can be found throughout soil, plants and dust. This fungus is able to produce airborne spores called conidia which people can inhale without getting illness. One of the most medically important species, *A. fumigatus* causes aspergillosis. Invasive pulmonary aspergillosis is a severe disease that can be mainly found in immunocompromised patients (5). Chronic necrotizing aspergillosis is locally invasive and is found in immunodeficiency patients or with a chronic lung disease. Aspergilloma and allergic bronchopulmonary aspergillosis are noninvasive, aspergilloma is a fungus ball that develops in lung, while another is a hypersensitivity in the lungs of asthma or cystic fibrosis patients (5). Whereas *A. flavus* is the second leading cause of invasive aspergillosis and it is the most common cause of fungal sinusitis and cutaneous infections. Moreover, *A. flavus* can produces aflatoxins in natural products such as maize and peanuts, and potentially causes hepatocellular carcinoma (11).

3.3 Environmental factors

The physical parameters such as room temperature, relative humidity including the number of attendances were shown in Table 3. The range of room temperature was 25.7 - 27.3 °C and the range of relative humidity was 48.1 - 62.6%. Both parameters represent as normal air temperature and relative humidity of the rainy season in Thailand. However, some sample sites have no attendances because of control measures in a period of COVID-19 outbreak in Thailand.

Average airborne fungal concentrations (CFU/plate/h) of each sample sites were conducted to determined correlation with physical parameters analyzed by Pearson's correlation as shown in Table 3.

Table 3 Correlation between average airborne fungal concentrations of each sample sites and physical parameters including comparison with index of microbial air contamination (IMA)

Sampling sites	Airborne fungal concentrations (CFU/plate/h)	Room temperature (°C)	Relative humidity (%)	The number of attendances (person/day)	IMA performance
Anatomy laboratory	19.70 ± 11.79	26.1	51.07	0	Good
Lumtakong meeting hall	18.83 ± 2.96	26.6	61.30	0	Good
First aid room	15.73 ± 2.11	26.0	57.60	1	Good
Classroom (1st floor)	12.04 ± 3.73	26.3	55.50	0	Good
Basic science laboratory	9.83 ± 3.73	27.3	62.57	0	Very good
Office (1st floor)	9.53 ± 3.06	26.6	48.12	30	Very good
Computer laboratory	9.24 ± 3.70	27.3	50.10	0	Very good
Thai traditional medicine	9.04 ± 3.17	26.1	55.10	4	Very good
Classroom (5th floor)	8.65 ± 2.29	26.2	60.22	28	Very good
Library	8.35 ± 1.02	26.2	50.00	15	Very good
Microbiology laboratory	6.88 ± 3.35	26.1	51.26	10	Very good
Trirattana meeting room	5.90 ± 1.41	26.0	57.82	12	Very good
Office (8th floor)	4.32 ± 1.41	25.7	51.20	10	Very good
Pearson's correlation (r) (n=160)		-0.002	-0.025	-0.374	
Sig. (2-tailed) 0.05		0.995	0.934	0.208	

Basically, fungi prefer grow in warmth and high humidity (13). But in this study, there were no correlation between airborne fungal concentrations with room temperature (r = -0.002) and relative humidity (r = -0.025). Nevertheless, the number of attendances displayed low negative correlation (r = -0.374) with the airborne fungal concentrations. Previous studies have indicated that the number of attendances is a positive factor affecting the fungal airborne concentrations in buildings (14, 15). But our study was negative correlation because the rooms used by the people had a daily cleaning that related to the reduction of fungal aerosols, whereas the rooms not used by the people were without the cleaning due to suspension using the rooms.

From this study, the data of average airborne fungal concentrations of each sample sites were allowed to compare with classes of the index of microbial air contamination (IMA) which is internationally accepted standard criteria about indoor air quality (16). Each sample sites indicated performance as very good or good as shown in the Table 3. Nevertheless, all sample also showed appropriate air temperature (25.7 - 27.3 °C) and humidity (48.1 - 62.6%) that support growth of most fungi. This happening related to a study of Pasanen et al. (17) that suggests the optimum of air temperature and relative humidity for fungal growth is 4 - 30 °C and 11- 96% respectively.

The fungi found in this work also broadly match with international findings. The most frequent reported airborne fungi in buildings have been *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Alternaria* spp. (18, 19, 20, 21). However, this study performed data collection just only rainy season which a study of Taylor et al. (22) stated that different seasons influence on airborne fungal profiles.

About some data conflicts of sample sites, the anatomy laboratory room was under construction might be a key reason of the high fungal airborne concentration were why. This circumstance is explained in a previous study (23). Mechanical ventilation systems typically have ducts carrying air through the building, which also provide sites for dust, including spores to accumulate. Furthermore, the suspended rooms from the COVID-19 situation whether Lumtakong meeting hall, Classroom (1st floor), Basic science laboratory and Computer laboratory also presented quite high amount of fungi because of without room cleaning.

4. Conclusions

This study obtains a background of airborne fungal profiles including airborne fungal concentrations and fungal diversity in various rooms in buildings of Nakhon Ratchasima College, Thailand. Although our study results cannot show relation of room temperature and relative humidity,

however this study indicates under construction rooms and suspended rooms tend to contain fungal spore accumulation more than available rooms.

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Declaration of conflicting interests

The authors declared that they have no conflicts of interest in the research, authorship, and this article's publication.

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