

Assessment of Fungal Bioaerosols in Indoor and Outdoor Air Conditions of Child Development Centers of the Local Government in Northeast Thailand

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Received: 9 January 2026/ Revised: 15 April 2026/ Accepted: 19 April 2026

Abstract

This cross-sectional survey aimed to determine the concentration of fungal bioaerosols at the 6 child development centers in Loei province, Northeast Thailand. Eighty-two air samples were collected by using a BioStage. The Indoor and Outdoor (I/O) ratio was 1.81. Moreover, the fungi from the indoor air conditions were found belonging to 7 fungal groups. The predominant fungi were in the yeast group (9.81 ± 17.37 CFU/m³; 49.05%), followed by *Aspergillus* sp. (4.49 ± 4.71 CFU/m³; 22.45%). However, the fungi collected from outdoor air conditions belonged to 6 genera of filamentous fungi and 1 group of yeast. These fungi belonged predominantly to *Aspergillus* sp. (4.07 ± 4.31 CFU/m³; 38.00%), followed by the yeast group (2.23 ± 5.54 CFU/m³; 20.82%). This result could be useful in the monitoring or planning of environmental conditions in child development centers or public spaces to reduce the abundance of airborne microbes.

Keywords: Fungal bioaerosols, Indoor air, Outdoor air, Child development centers, Air quality

Introduction

The air quality in buildings and the environment has become a significant concern in public health monitoring, and more than 90% of the general public spends time in indoor areas. Microorganisms can be found in approximately 33% of indoor air in buildings, and pollution can be caused by one or more microorganisms, such as fungal bioaerosols (i.e., *Aspergillus* spp., *Alternaria* spp., *Penicillium* spp., *Cladosporium* spp., and yeast) [1-4]. Fungal bioaerosols are opportunistic pathogens that are present in the form of airborne fungal spores, which allows them to survive under environmental stress and spread through the air and in the environment [5, 6]. However, fungal bioaerosols might have serious effects associated with asthma, allergies, and acute toxicity in immunodeficient populations, such as infants and young children, because the majority of defects in the humoral immune response (HIR) system occur when



children are 1.5 to 2 years old, they are normally unable to produce antibodies against response antigens [7-11]. For all these reasons, young children enrolled at the child development centers of the local government and spending time there for more than 6 hours a day might be more susceptible to fungal bioaerosols than adults are. The child development centers of the local government should be monitoring locations because of the high population density of children per room, the lack of air conditioning maintenance and the lack of sufficient sanitation. Due to the importance of healthcare for young children, this study investigated the quantity of fungal bioaerosols in the child development centers of the local government in Loei province, northeast Thailand. These results could be used as information for evaluating the quality of children's physical growth.

Materials and Methods

Study Area

This study was performed in Loei province, northeast Thailand which has an approximate population of 0.64 million. The geographic coordinates of Loei province are 101°29'20.1" E, and the province is located in close proximity to the border with Laos [12]. The 6 child development centers of the local government were divided by terrain conditions into 3 groups. The first group included low plain areas, consisting of Na-O and BanTit-O child development centers; the second group included high mountain areas, consisting of Kaeng-Laen and Kok-Pho child development centers; and the last group included irrigation areas, consisting of Na-Sao and Ban-Mai child development centers. These centers were selected for air sample collection.

Ethical Approval

Ethical approval of research protocol was considered and certified as an exemption for research ethics based on The Declaration of Helsinki and the ICH Good Clinical Practice Guideline.

Sample Collection

In this study, eighty-two air samples were collected. For indoor air collection, sampling was conducted in duplicate according to room size as follows: in one room of Na-O child development center, samples were collected at seven points (14 samples), while in one room each of BanTit-O, Na-Sao and Ban-Mai child development centers, samples were collected at six points (36 samples). In one room of Kok-Pho and Kaeng-Laen child development centers, samples were collected at five points (20 samples). Additionally, the samples were collected at one point twice in outdoor areas per child development center (12 samples). Air samples were taken during the rainy season from June to August 2022.

Fungal bioaerosols were investigated using a BioStage (BioStage Impactor, Model 255-9611) (BBL Becton Dickinson, USA). The sampler was positioned 80 cm above the floor (respiratory height average children) [10, 13] the airflow rate was set at 28.3 L/min for 10 minutes, and the collector was sterilized with 70% ethyl alcohol before use. The collector allowed the air to pass over disposable Petri dishes (90 × 10 mm) containing Sabouraud dextrose agar (SDA) for fungal collection. The humidity and temperature were measured both indoors and outdoors via the same real-time sampling method (Hygrotherm Thermo/Hygrometer; Digicon TH-02), and the indoor and outdoor wind speeds were measured via a hot wire anemometer (Testo-425) [1].

Isolation and Identification of Fungi

The sample plates were stored in sterile plastic bags and transported to the laboratory within one hour. These plates were incubated at 35-37 °C for 48 hours as recommended by the American Society for Microbiology and Bailey & Scott's Diagnostic Microbiology. The number of fungal filamentous colonies was calculated and reported in colony-forming units per cubic meter of air (CFU/m³). In addition, identification of fungal genera was performed based on macroscopic and microscopic characteristics. The macroscopic characteristics were directly examined by eye observation on the upper and lower regions of the colonies. However, microscopic identification was performed by the slide culture method. After incubation, all of the fungal isolates were stained with lactophenol cotton blue to observe the spore arrangement and hyphal structure under a brightfield microscope [14]. The Identification of Pathogenic Fungi (2nd edition) was used as a standard key for identification [15].

Fungal DNA Extraction

Fungal genomic DNA was isolated by using a GF-1 fungal DNA extraction kit (Vivantis Technologies Sdn Bhd, Malaysia). A total of 30 mg of fungal sample was collected into a microcentrifuge tube, after which 280 µl of Buffer FuL was added to the microcentrifuge tube. The suspension was mixed well by vortexing for 30 seconds to make a homogenous solution. Then, 20 µl of Proteinase K was added, and the solution was mixed by inverting the tube several times, followed by incubation at 65 °C for 30 minutes (the sample was mixed every 5 minutes during incubation). The sample was then centrifuged at 14,000 rpm for 5 minutes. The supernatant was transferred to a clean microcentrifuge tube, and 2 volumes of Buffer FuB and mixed by inverting the tube several times. The mixture was incubated at 65 °C for 10 minutes, and 200 µl of absolute ethanol was added to the microcentrifuge tube. The sample was subsequently transferred to a column and centrifuged at 10,000 × g for 1 minute. Afterward, the column was washed with 650 µl of wash buffer and centrifuged at 10,000 × g for 1 minute. DNA was eluted by adding 50 µl of preheated Elution Buffer directly onto column membrane and allowing to stand for 2 minutes, followed by centrifugation at 10,000x g for 1 minute. The DNA was stored at 4 °C or -20 °C.

Polymerase Chain Reaction (PCR) for Fungi

To confirm that the major fungal groups found in this study were yeast and *Aspergillus* sp., the fungi were subjected to PCR-specific testing using the fungal universal primers ITS1/ITS4 (600 bp) [16] and the *Aspergillus* sp. specific primers Asp-ITS-F/Asp-ITS-R (170 bp) [16]. PCR was performed using the primer ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and the primer ITS4 (5'- TCCTCCGCTTATTGATATGC-3') for fungal confirmation [17]. The PCR protocol consisted of an initial denaturation at 95 °C for 4.5 minutes followed by 35 cycles of 95 °C for 30 seconds, 50 °C for 30 seconds, and 72 °C for 1 minute and a final extension at 72 °C for 3 minutes. The primers Asp-ITS forward (5'-ACCGAGTGTAGGGTTCCTAG-3') and Asp-ITS reverse (5'-GCGATACAATCAACTCAGAC-3') were used for *Aspergillus* sp. confirmation [16]. The PCR protocol consisted of an initial denaturation at 98 °C for 3 min followed by 28 cycles of 98 °C for 10 s, 55 °C for 10 s, and 72 °C for 30 s and a final extension at 72 °C for 1 min. The PCR products were evaluated via electrophoresis on 1.2% agarose gels in 1X TBE buffer and stained with SYBR Safe (Vivantis Technologies Sdn Bhd, Malaysia).



Statistical Analysis

The descriptive statistics (i.e., frequency, percentage, mean and standard deviation) are reported as the concentration of fungal bioaerosols, temperature, %RH, and wind speed. The dependent variable was the concentration of fungal bioaerosols. Moreover, the independent variables were the indoor temperature, %RH, wind speed, and the indoor/outdoor ratio (I/O ratio). The statistical analysis was conducted using the SPSS software program.

Results and Discussion

Concentrations of Fungal Bioaerosols in Different Sampling Area

The concentrations of fungal bioaerosols in the sampled indoor air of the child development centers are presented in Table 1. The mean concentration of fungal bioaerosols from the six child development centers was 20.83 ± 23.25 CFU/m³. Among these centers, the Ban-Mai child development centers had the highest concentration of fungal bioaerosols (37.02 ± 28.57 CFU/m³); however, this concentration did not exceed the standard quality requirements for cleanrooms set by the Department of Health Ministry of Public Health, Thailand (must be less than 50 CFU/m³). Moreover, the mean indoor temperature and indoor %RH were 27.90 ± 2.42 °C and $65.04 \pm 11.61\%$, respectively. These results showed that the temperature exceeded the recommended standard (24–26 °C), while the %RH was within the standard range (50–65% RH). For the fungal bioaerosols collected outdoors, the mean concentration of fungal bioaerosols was 11.45 ± 9.78 CFU/m³, and the Na-Sao child development centers had the highest concentration of fungal bioaerosols (22.28 ± 0.75 CFU/m³). However, the mean I/O ratio was 1.81 (Table 2), which indicates that fungal contaminants from the air outside enter the room, and the ratio exceeds the recommendations of the Bureau of Environmental Health (2015) (must be less than 1.0) [1].

Table 1 The mean concentrations of fungal bioaerosols and indoor environmental parameters.

The child development centers	Mean concentration of fungal bioaerosols (CFU/m ³) (S.D)	Temperature (°C) (S.D)	%RH (S.D)	Wind speed (m/s) (S.D)	Mean concentration of CO ₂ (ppm)
Na-O	$16.14 \pm 21.66^*$	25.48 ± 2.10	49.53 ± 3.03	0.17 ± 0.03	705.14 ± 13.84
BanTit-O	29.29 ± 26.83	25.90 ± 0.39	67.47 ± 2.20	0.08 ± 0.02	515.67 ± 14.06
Na-Sao	$9.83 \pm 23.37^*$	28.87 ± 0.36	77.97 ± 0.28	0.11 ± 0.04	440.67 ± 16.33
Ban-Mai	37.02 ± 28.57	31.97 ± 0.16	52.52 ± 0.44	0.10 ± 0.02	445.17 ± 10.22
Kok-Pho	10.29 ± 7.87	27.52 ± 0.08	76.58 ± 0.48	0.11 ± 0.09	489.20 ± 8.20
Kaeng-Laen	21.52 ± 9.69	28.04 ± 0.39	71.82 ± 0.17	0.20 ± 0.14	430.80 ± 2.44
Total mean	$20.83 \pm 23.25^*$	27.90 ± 2.42	65.04 ± 11.61	0.13 ± 0.08	512.71 ± 13.16

Remark: The mean concentration of fungal bioaerosols and indoor environments including temperature, %RH, wind speed, and CO₂ concentration were a summary of the concentration of fungal bioaerosols and indoor environments in each child development centers during June to August, 2022.

* The concentration of fungal bioaerosols could not be measured at every point, so the average concentration of fungal bioaerosols is not reported as the geometric mean. Thus, it is reported as the arithmetic mean.

**Table 2** The mean concentrations of fungal bioaerosols and outdoor environmental parameters.

The child development centers	Mean concentration of fungal bioaerosols (CFU/m ³) (S.D)	Temperature* (°C)	%RH*	Wind speed* (m/s)	Concentration of CO ₂ * (ppm)	I/O ratios
Na-O	1.52±0.72	28.70	72.40	0.16	395.00	10.61
BanTit-O	3.04±0.00	27.30	81.30	0.18	425.00	9.63
Na-Sao	22.28±0.75	28.90	78.60	0.12	421.00	0.44
Ban-Mai	13.93±11.06	32.20	53.30	0.19	399.00	2.66
Kok-Pho	8.68±2.19	29.50	77.10	0.43	386.00	1.19
Kaeng-Laen	19.22±14.18	29.00	74.70	0.08	386.00	1.12
Total mean	11.45±9.78	29.27±1.54	72.90±9.61	0.19±0.12	402.00±16.29	1.81

Remark: * The sampling was conducted in onetime.

Fungal Genera in Different Sampling Areas

The fungi were identified by using standard microbiological techniques, and the fungal type was investigated by checking with The Identification of Pathogenic Fungi (2nd edition). The results showed that all the samples collected from the 6 child development centers of the local government in Loei province were contaminated with different fungal genera. After identification, the fungi from indoor air condition were belonged to 7 fungal groups; 6 genera were filamentous fungi, and 1 yeast group was found. However, the yeast genera were not identified by this method (Table 3) (Figure 1). The yeast group was predominant among these fungi at 9.81±17.37 CFU/m³ (49.05%), followed by *Aspergillus* sp. 4.49±4.71 CFU/m³ (22.45%). The fungi collected from outdoor air conditions included six genera of filamentous fungi and one belonging to the yeast group. The predominant among these was *Aspergillus* sp. (4.07±4.31 CFU/m³; 38.00%), followed by the yeast group (2.23±5.54 CFU/m³; 20.82%) (Table 4).

Table 3 Characteristics of fungal isolates from the different child development centers of the local government in Loei province

Fungal Genera	Characteristics	
	Macroscopic	Microscopic
<i>Aspergillus</i> sp.	The colonies were initially white and then turning into dark green, rapidly and fast growth.	The conidia were radiated and contained in the vasicle; the spore walls were thick, hyaline, and smooth.
<i>Penicillium</i> sp.	The colonies were initially white and then turned into blue-green.	The conidiospores were branched and produced from the tips of phialides. Appear septate between conidiophores and branched.



Fungal Genera	Characteristics	
	Macroscopic	Microscopic
<i>Rhizopus</i> sp.	The colonies were initially cottony white and turn dark grayish-brown.	Sporangiophores, non-septate (coenocytic), stolon, and rhizoids.
<i>Phaeoacremonium</i> sp.	The colonies were suede-like, initially whitish-grey, and turn olivaceous-grey and slow-growing.	Phialides were tapered towards the tip with conidia.
<i>Neurospora</i> sp.	Orange colonies and fast-growing.	The conidia were orange-colored.
<i>Epidermophyton</i> sp.	Greenish-brown colonies, a velvety and folded center surface, and slow growing.	Appear septate between macroconidia, direct growth from the hyphae.
Yeast group	The colonies were white-creamy, round, and smooth.	Oval cell with budding.

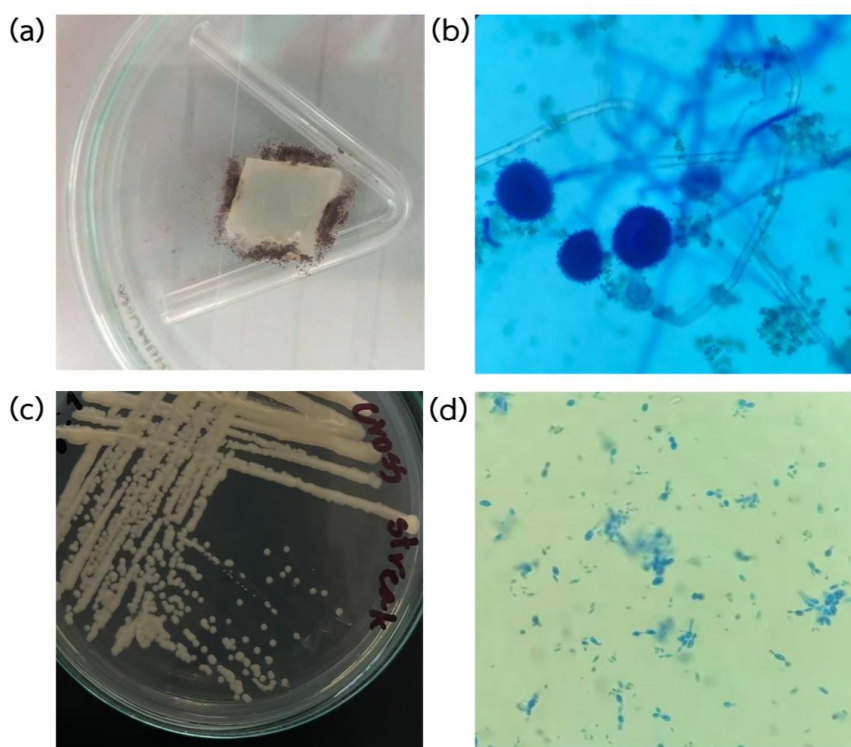


Figure 1 The Characteristics of *Aspergillus* sp. and yeast. (a) macroscopic morphology of *Aspergillus* sp.; (b) microscopic morphology of *Aspergillus* sp. under a microscope (400 x magnification); (c) macroscopic colony morphology of yeast; (d) microscopic morphology of yeast under a microscope (400 x magnification).

Table 4 Distribution and identification of airborne fungi (CFU/m³) in air samples from the different child development centers of the local government in Loei province

Genera	The mean concentration of isolated fungi				PCR primers	
	Indoor		Outdoor		(Percent positive)	
	CFU/m ³	%	CFU/m ³	%	ITS1/ITS4	Asp-ITS-F/Asp-ITS-R
<i>Aspergillus</i> sp.	4.49±4.71*	22.45	4.07±4.31*	38.00	100%	100%
<i>Penicillium</i> sp.	0.03±0.17*	0.15	1.18±4.09*	11.02	100%	-
<i>Rhizopus</i> sp.	1.02±2.66*	5.10	0.93±1.33*	8.68	100%	-
<i>Phaeoacremonium</i> sp.	4.19±7.04*	20.95	1.80±4.73*	16.81	100%	-
<i>Neurospora</i> sp.	0.39±0.50*	1.95	0.42±0.52*	3.92	100%	-
<i>Epidermophyton</i> sp.	0.07±0.40*	0.35	0.08±0.29*	0.75	100%	-
Yeast group	9.81±17.37*	49.05	2.23±5.54*	20.82	100%	-
Total	20.00±3.47	100.00	10.71±1.36	100	100%	-

Remark: *The concentration of fungal bioaerosols could not be measured at every point, so the average concentration of fungal bioaerosols is not reported as the geometric mean. Thus, it is reported as the arithmetic mean.

PCR method for confirmation of yeast and *Aspergillus* sp.

The majority of yeast group and *Aspergillus* sp. found in this study were confirmed. The *Aspergillus* sp. causes an aspergillosis [18], most infected people will develop a symptom related to the respiratory system. The fungi were thus subjected to PCR-specific testing using the fungal universal primers ITS1/ITS4 [16] and the *Aspergillus* sp. specific primers Asp-ITS-F/Asp-ITS-R [17]. All the yeast and *Aspergillus* sp. samples exhibited positive PCR results for the ITS1/ITS4 primers (600 bp) (data not shown); likewise, the *Aspergillus* sp. samples had positive PCR results for the Asp-ITS-F/Asp-ITS-R primers (170 bp) (Figure 2).

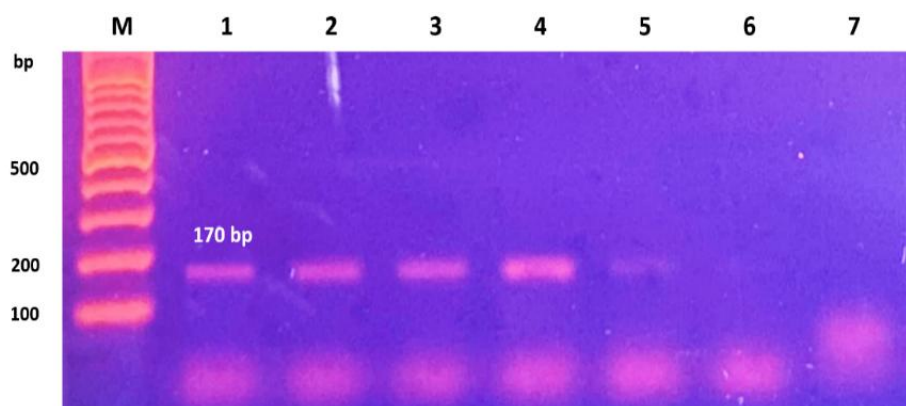


Figure 2 PCR confirmation of *Aspergillus* sp. by the *Aspergillus* sp.-specific primers Asp-ITS-F/Asp-ITS-R, yielding a 170 bp amplicon. Lane M, standard marker; lanes 1–6, *Aspergillus* sp. from child development centers 1–6; and lane 7, negative control.



The fungi from indoor air conditions were collected from 6 child development centers of the local government in Loei province, northeast Thailand and belonged to 7 fungal groups. The predominant fungi among these were the yeast group and *Aspergillus* sp. However, there are concerns regarding *Aspergillus* sp. as a cause of aspergillosis, and airborne *Aspergillus* sp. spores might cause serious problems associated with asthma or allergies in children or immunocompromised people. Therefore, *Aspergillus* sp. was confirmed by PCR with the fungal universal primers ITS1/ITS4 and the specific primers Asp-ITS-F/Asp-ITS-R. In the same way, although natural yeasts consist of beneficial types, they also include pathogenic types, such as *Candida* spp. and *Cryptococcus neoformans*. Therefore, all the yeast strains in this study were confirmed via PCR with the fungal universal primers ITS1/ITS4. The results from the present study were the same as those of Chotigawin R. who studied the microbial indoor air quality in early childhood education facilities in Chonburi, eastern Thailand. The results showed that the predominant fungus was *Aspergillus* sp. (50%) [19]. However, a previous study reported microbial counts and particulate matter levels in indoor air samples collected from a child home care center in Bangkok, Thailand, from 2006–2007. The total bacterial and fungal concentrations in the air were 527.8 ± 230.9 CFU/m³ and 514.6 ± 256.7 CFU/m³, respectively [20]. In 2018, Fang *et al.* studied assemblages of culturable airborne fungi in a typical urban, tourism-driven center of southeast China; the concentrations of fungi in the air in these areas ranged from < 12 CFU/m³ to 8,767 CFU/m³ (mean of 848 CFU/m³); and the predominant fungi were from the genera *Penicillium*, *Cladosporium*, *Alternaria*, *Aspergillus* and *Trichoderma* [21]. In 2019, Andualem *et al.* studied the indoor microbial load and found that it was correlated with physical indoor air quality parameters in public primary schools in Ethiopia; the predominant fungal pathogens were *Penicillium* sp., *Cladosporium* sp. and *Aspergillus* sp. at 40%, 19% and 16%, respectively [22]. In 2020, Szulc *et al.* assessed the microbiological indoor air quality of cattle breeding farms located in central Poland, and the samples were collected between February and April 2019. The results showed that the bacterial and fungal concentrations reduced between 7.14×10^3 and 3.88×10^4 CFU/m³ and between 5.53×10^1 and 1.30×10^4 CFU/m³, respectively, indicating that the predominant fungi were *Aspergillus* sp. However, the air circulation and the types of particles in the air may be important factors that help increase the amount of mold in the air [23]. In 2020, Ma studied blowing in the wind, bacteria and fungi are spreading from public restroom hand dryers. The results showed that 40 fungal species were found, and the predominant fungi genera were *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., and *Didymella* spp. [24]. In 2013, Augustyniuk-Kram and Dmowska studied the spectrum and concentration of culturable fungi in house dust from flats in Warsaw, Poland. The results showed that the highest fungal concentrations were observed in dust collected from shelves (median value 8.2×10^4 CFU/g of dust), and the lowest fungal concentrations were isolated from dust collected from beds (median value 8.0×10^3 CFU/g of dust) [25]. In 2020, Sepahvand *et al.* studied the quantity and quality of indoor and outdoor air fungus bioaerosols in Khorrambad day care child centers western of Iran, 2018. The results showed that a direct relationship was found between fungal and relative humidity (%RH) and temperature, based on water activity requirements [26]. The common fungi such as *Aspergillus* sp., *Rhizopus* sp. and *Mucor* sp. can divided the second colony at water activity about 0.8-0.9% [27]. In 2023, Jabeen *et al.* studied the bioaerosol assessment in indoor and outdoor environments: a case study from India, the most common

fungal species were *Cladosporium*, *Aspergillus niger*, *Penicillium*, *Rhizopus*, *Fusarium*, *Mucor*, and *Alternaria*, relative to weather conditions, such as temperature and relative humidity [28]. In the same way, the present study, the mean indoor relative humidity (%RH) in the child development centers was 65.04 ± 11.61 , while the average temperature was 27.90 ± 2.42 °C. These conditions are favorable for fungal growth. In particular, relative humidity exceeding 60% and temperatures ranging from 25–30 °C provide suitable environmental conditions for yeast survival [29]. Moreover, indoor air-conditioning systems with inadequate ventilation may act as reservoirs that facilitate yeast proliferation. In addition, *Aspergillus* spp., which are mesophilic fungi, exhibit optimal growth at temperatures ranging from 20 to 40 °C [30]. The predominant fungi are also yeast and *Aspergillus* sp. Although the source of airborne bacteria may be shedding of the skin and secretions from the respiratory tract transmitted via human breathing, airborne fungi may be widely distributed in soil, water, and decaying vegetation [31-35]. Moreover, the outdoor air quality is usually related to the environment directly. In the country, environmental laws are not strictly enforced, leading to air pollution [36]. For instance, the report of Pringsulaka et al. studied the assessment of indicator microorganisms and fungi: health risk in the Saen Saeb canal, Thailand. They found the amounts of the fungi and airborne bacteria were higher than usual and related to high levels of pathogenic microorganisms of water in the Saen Saeb canal [36].

Fungal bioaerosols are opportunistic pathogens that might have serious effects in immunodeficient populations, particularly young children who spend more than six hours per day in child development centers. The Department of Health Ministry of Public Health, Thailand, set the standard quality requirements for cleanrooms, the concentration of fungal bioaerosols must be less than 50 CFU/m³, due to concern for the health of young children, this study investigated the quantity of fungal bioaerosols in the child development centers of the local government in Loei province, northeast Thailand. The concentration of fungal bioaerosols from the six child development centers did not exceed the standard quality, the predominant fungi from the indoor air conditions were the yeast group, followed by *Aspergillus* sp., which can cause fungal diseases and allergies [37]. However, the predominant fungi collected from outdoor air conditions were *Aspergillus* sp. followed by the yeast groups, the results of the study were similar in all terrain conditions of the three groups. The results of this study can lead to the prevention and elimination of fungal bioaerosols, as well as being able to monitor and plan environmental operations in child development centers to further reduce microorganisms in the air.

The indoor environment of child development centers should be appropriately managed to maintain cleanliness and to prevent these facilities from becoming sources of fungal contamination. Particular emphasis should be placed on the management of indoor spaces that may serve as reservoirs for fungal growth. This includes the control of environmental parameters such as indoor temperature, relative humidity, and air velocity. In addition, the density of children relative to the available indoor space should be regulated. Ensure regular implementation of daily activities and systematic cleaning practices within child development centers. Furthermore, policy recommendations should be developed to promote the improvement of biosafety measures within child development centers. Such measures are important for



minimizing potential health risks to young children and to individuals who may be exposed to airborne fungal spores in these environments.

Conclusion

The present study aimed to determine the concentration of fungal bioaerosols in 6 child development centers of the local government in Loei province, Thailand. The mean concentrations of fungal bioaerosols indoor air condition (I) and outdoors air condition (O) were 20.83 ± 23.25 CFU/m³ and 11.45 ± 9.78 CFU/m³, respectively. The I/O ratio was 1.81. The predominant fungal bioaerosols were yeasts and *Aspergillus* sp. This result could be useful in surveillance and planning for proper environmental management at child development centers to reduce the abundances of airborne fungi.

Acknowledgements

This research was financially supported by Thailand Science Research and Innovation (2022) and Loei Rajabhat University. We also special thanks to Dr.Chantana Khensri and Dr.Pokkamol Laoraksawong, who helped in Polymerase chain reaction (PCR) process.

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