Patterns and Distribution of Airborne Mold Spores in Lower Northern Thailand: A 10-Year Comparison of Two 12-Month Surveys

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Abstract

Background: Fungal sensitization is strongly associated with asthma severity and increased outdoor spore exposure correlates with higher asthma-related hospitalization. However, data on outdoor airborne fungal spores in lower northern Thailand are limited.

Objective: To assess changes in outdoor airborne mold spore patterns and counts over two distinct periods, ten years apart, in Phitsanulok, lower northern Thailand, to optimize allergic disease management.

Materials and Methods: Mold spores were collected from August 2013 to July 2014 and from August 2022 to July 2023, using a Burkard seven-day volumetric spore trap positioned 15 meters above ground. Exposed slides were stained for microscopic analysis, and meteorological data were recorded.

Results: Monthly outdoor airborne mold spore levels ranged from 336.5 to 939.6 spores/m^3 (2013-2014) and 257.7 to 821.8 spores/m^3 (2022-2023), with peaks in June (2013-2014) and May to June (2022-2023). *Cladosporium* spp. was the most abundant spore type in both periods. Rankings for the sixth to eleventh spore types were consistent, while the top five varied. *Cladosporium* spp. peaked in July 2014 and May to June 2023, approaching 500 spores/m^3, a threshold for allergic risk. Overall, total spore concentrations were highest in the early rainy season, as rainfall increased from late April through June. In both periods, many spore types exhibited lower concentrations during months with the lowest relative humidity (March to April) and during peak rainfall (September).

Conclusion: *Cladosporium* spp. accounted for the majority of outdoor mold spores. Many spore types increased at the onset of the rainy season and decreased during months with the lowest humidity.

Keywords: Mold spores; Airborne; Aeroallergen; Allergic diseases; Lower northern Thailand

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Outdoor aeroallergens, including airborne pollen and fungal spores, play an important role in triggering allergic conditions such as allergic rhinitis, atopic conjunctivitis, and asthma. Patterns of aeroallergen sensitization vary widely according to geographic location, climate, and genetic background. In warm and humid Asian and tropical regions, sensitization to house dust mites predominates, with very high prevalence

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What is already known about this topic?

The distribution of outdoor airborne fungal spores varies markedly across climatic and geographic regions. Surveys in the United States have identified *Cladosporium* spp., *Penicillium* spp., non-sporulating fungi, and *Aspergillus* spp. as the most common airborne fungi. Studies in Europe have reported predominance of ascospores and *Cladosporium* spp. in outdoor environments. In China, *Cladosporium* spp., *Nigrospora* spp., and *Alternaria* spp. have been identified as the most prevalent airborne fungal genera. In Thailand, sensitization to fungal allergens in the lower northern region ranges from 5.2% to 19.1% among the asthmatic population, indicating a substantial prevalence. However, most surveys of outdoor airborne fungal spores have been conducted in Bangkok, with only one study performed in the northern and southern regions. These surveys, spanning from 1972-2018, identified various fungal spore types; nevertheless, *Cladosporium* spp. has consistently been reported as the dominant outdoor airborne fungal spores.

What does this study add?

This investigation constitutes the first assessment of airborne fungal spores in Phitsanulok, located in lower northern Thailand, and the first study in Thailand to employ a Burkard seven-day recording volumetric spore trap. Across both study periods (2013-2014 and 2022-2023), airborne fungal spores were present throughout the year, with peak levels consistently occurring between May and July over the 10-year span. *Cladosporium* spp. emerged as the dominant airborne fungal spore in both intervals, comprising roughly half of the total mold spore load. Spore concentrations were highest during the early rainy season and decreased during periods of heaviest rainfall. In contrast, *Alternaria* spp. were found at substantially lower concentrations than *Cladosporium* spp. Overall, these findings demonstrate that total airborne fungal spore concentrations and the proportional distribution of mold spore types did not differ significantly between the two 12-month periods studied, despite the 10-year interval.

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rates reported in countries such as Thailand and Singapore[1-4]. In contrast, temperate regions in Europe and North America demonstrate more heterogeneous pollen sensitization patterns or prevalence rates comparable to those of house dust mites[5-7].

Although sensitization to fungal spores is generally less common than sensitization to house dust mites and pollens, reported prevalence rates worldwide range from 0.5% to 22.5%[1-8]. Importantly, fungal sensitization has been strongly associated with asthma severity, with studies indicating that 35% to 75% of individuals with severe asthma are sensitized to fungal allergens[8]. Sensitization to *Alternaria* spp. and related fungal species has been specifically implicated in cases of fatal asthma[8]. Several epidemiological studies have demonstrated significant associations between elevated outdoor fungal spore exposure and increased asthma-related hospital admissions, particularly among pediatric and adolescent populations[9].

The composition and distribution of outdoor airborne fungal spores vary substantially across climatic and geographic regions. Surveys conducted in the United States have identified *Cladosporium* spp., *Penicillium* spp., non-sporulating fungi, and *Aspergillus* spp. as the most commonly detected airborne fungi[10]. In Europe, studies have reported predominance of ascospores and *Cladosporium* spp. in outdoor environments[11]. In China, *Cladosporium* spp., *Nigrospora* spp., and *Alternaria* spp. have been identified as the most prevalent airborne fungal genera[12]. In Southeast Asia, including Singapore and Bangkok, Thailand, *Cladosporium* spp. has consistently been reported as the dominant outdoor airborne fungal spore[13,14].

Routine monitoring of outdoor aeroallergens is beneficial for clinicians and individuals with allergic diseases, as it supports effective prevention strategies and informed avoidance of environmental triggers. However, data on outdoor airborne fungal spores in lower northern Thailand remain limited. This gap is notable given that sensitization rates to fungal allergens in this region range from 6.2% to 19.1% among asthmatic children and from 5.2% to 19.0% among adults with asthma[4,15].

Therefore, the aim of this study was to investigate patterns and concentrations of outdoor airborne mold spores over two distinct 12-month periods separated by ten years in Phitsanulok, lower northern Thailand. During this interval, the region has undergone increasing industrialization and may have experienced the effects of climate change. Characterizing long-term changes in airborne mold spore exposure may assist clinicians in selecting relevant allergens for diagnostic testing and in developing targeted allergen-specific immunotherapy strategies.

Materials and Methods

Aeroallergen sampling

Outdoor aeroallergen sampling was conducted during two 12-month periods: August 2013 to July 2014 and August 2022 to July 2023, in Phitsanulok, Thailand (16°44’55.1” N; 100°11’20.5” E). During the first survey period, airborne spores were collected using a Burkard seven-day recording volumetric spore trap installed on the terrace outside the fourth floor of Naresuan University Hospital, Phitsanulok, Thailand, approximately 15 meters above ground level. The sampler was operated according to the British Aerobiology Federation guidelines[16].

Adhesive tape mounted on a rotating drum advanced at a constant rate of 2 mm per hour, completing one full rotation every seven days. Air was drawn through a 14×2 mm inlet at a constant flow rate of 10 L/minute, approximating the average human inspiratory airflow, allowing airborne particles with sufficient inertia to adhere to the greased tape. At the end of each seven-day sampling cycle, the tape was removed, cut into seven consecutive 48-mm segments representing 24-hour sampling periods, and mounted onto glass slides for microscopic analysis[16-18]. Sampling was performed continuously throughout the entire study period.

During the second survey period, outdoor aeroallergen collection was repeated using the same Burkard sampler installed at the identical location and height, with identical sampling procedures.

Identification and quantification of airborne fungal spores

Microscopic glass slides obtained from both survey periods were stained with Calberla’s solution[14,19] and examined using a compound light microscope at 400x magnification. Fungal spores were identified primarily to the genus level based on morphological characteristics, following established aerobiological criteria[14,19]. Slide readings were performed by trained personnel experienced in airborne pollen and fungal spore identification. Counting and identification procedures were standardized to ensure consistency across both study periods.

Spore concentrations were calculated according to established aerobiological protocols and expressed as the number of spores per cubic meter of air (spores/m^3), averaged over 24 hours[16]. Comparative

Table 1. Comparison of meteorological parameters, including average monthly temperature, min-max temperature, average monthly relative humidity, average total monthly rainfall, and wind speed, during the two study periods: August 2013 to July 2014 and August 2022 to July 2023

Year	Min-max temperature (°C)	Mean temperature (°C)	Relative humidity (%)	Total rainfall (mm)	Wind speed (km/hour)
August 2013 to July 2014	22.4-33.2	27.6	74.9	102.0	19.2
August 2022 to July 2023	22.7-34.0	28.1	74.3	109.4	22.8

analyses of mold spore counts and distributions were performed between the two 12-month survey periods.

Meteorological data

Meteorological data for Phitsanulok were obtained from the Thai Northern Meteorological Center and included daily measurements of ambient temperature, relative humidity, total rainfall, and wind speed for both study periods. Monthly mean values were calculated for temperature, relative humidity, and wind speed, while total monthly rainfall was expressed as cumulative precipitation (mm). Wind speed was reported in kilometers per hour. A comparison of meteorological parameters between the two study periods is presented in Table 1.

This study was reviewed and approved for research and publication by the Faculty of Medicine, Naresuan University, and the Naresuan University Institutional Review Board. The study was exempt from human research ethics review because it focused exclusively on airborne fungal spore allergens (ethics approval certification number: NU-IRB 0354). It did not involve human participants; therefore, informed consent was not required. As this was not a clinical trial, registration was also not required.

Statistical analysis

Statistical analyses were performed using Stata Statistical Software, version 17 (StataCorp LLC, College Station, TX, USA). Descriptive statistics were used to summarize data as means, frequencies, and percentages. Because airborne fungal spore data were not normally distributed, monthly variations in total airborne mold spores and individual spore types within each study period were assessed using the Kruskal-Wallis test. Month-by-month comparisons between the two study periods were also performed using the Kruskal-Wallis test. Associations between the fungal spore counts and meteorological variables were evaluated using Spearman's rank-order correlation coefficient[20]. Statistical significance was defined as a two-tailed p-value less than 0.05.

Results

Overall airborne fungal spore concentrations

During the first survey period (August 2013 to July 2014), average monthly total airborne mold spore concentrations were calculated from daily records collected using the Burkard volumetric sampler. Mold spores dominated outdoor aeroallergens, accounting for 88.7% of the total, while pollens made up 11.3%[19]. Monthly total mold concentrations ranged from 336.5 to 939.6 spores/m³, with an annual mean of 521.3 spores/m³. The highest monthly level occurred in June (939.6 spores/m³), whereas the lowest were recorded in April, March, and February (336.5, 346.9, and 349.9 spores/m³, respectively) (Figure 1).

During the second survey period (August 2022 to July 2023), mold spores again remained the predominant outdoor aeroallergen, accounting for 82.7% of the total, while pollens accounted for 17.3%[19]. Monthly concentrations ranged from 257.7 to 821.8 spores/m³, with an annual mean of 493.5 spores/m³. Two peaks occurred in May and June (821.8 and 793.8 spores/m³, respectively), while the lowest concentrations were recorded in April, September, and December (375.6, 259.6, and 257.7 spores/m³, respectively) (Figure 1).

Comparing the two periods, the average annual mold spore concentration in 2022-2023 was slightly lower than in 2013-2014 (493.5 vs. 521.3 spores/m³), though this difference was not statistically significant ($p=0.873$). Significant monthly variations in total mold spores were observed within both periods (2013-2014: $p<0.001$ and 2022-2023: $p=0.012$). Month-by-month comparisons showed significantly higher concentrations in August and December during 2013-2014, and significantly lower levels in March, compared with the corresponding months in 2022-2023 ($p=0.001$, 0.030, and 0.007, respectively).

Distribution of outdoor airborne fungal spore types and their changes over 10 years

Outdoor airborne fungal spores were classified into 11 morphologically distinct groups based on light microscopic examination at 400x magnification: *Cladosporium* spp., unclassified one-celled spores,

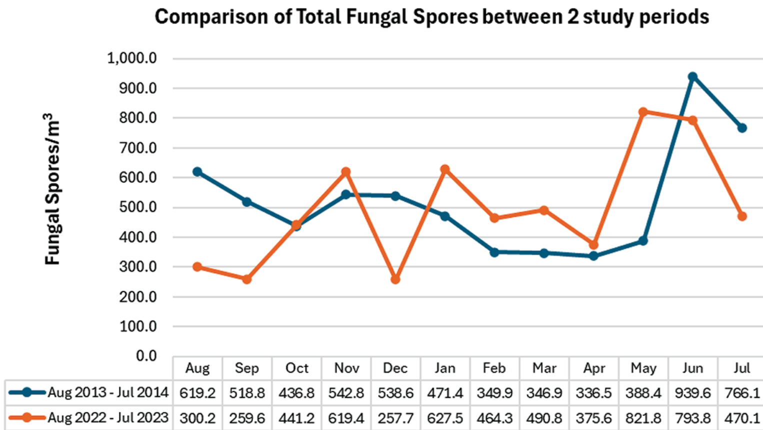

Figure 1. Comparison of total airborne fungal spore concentrations (spores/m^3) between two 12-month periods: August 2013 to July 2014 and August 2022 to July 2023. The graph presents the total fungal spore concentration for each month.

Table 2. Percentage distribution of outdoor fungal spore types per year during August 2013 to July 2014 and August 2022 to July 2023

	% of total mold spores per year		p-value*
	August 2013 to July 2014	August 2022 to July 2023	
Unclassified one-celled spores	40.60	13.78	<0.001
Cladosporium spp.	31.45	48.64	0.029
Yeast	11.02	4.28	<0.001
Rust	8.72	18.32	<0.001
Nigrospora spp.	4.25	7.34	<0.001
Fusarium spp.	1.54	3.06	<0.001
Alternaria spp.	1.35	2.77	<0.001
Helminthosporium spp.	0.71	0.76	0.491
Curvularia spp.	0.30	0.76	<0.001
Tetraploa spp.	0.06	0.17	<0.001
Penicillium spp.	0.01	0.11	0.080

* Comparisons of each fungal spore type between the two periods were analyzed using the Kruskal-Wallis test

yeast, rust, *Nigrospora* spp., *Fusarium* spp., *Alternaria* spp., *Helminthosporium* spp., *Curvularia* spp., *Tetraploa* spp., and *Penicillium* spp. Unclassified one-celled spores were defined as small, oval, single-celled spores that could not be reliably assigned to a genus. They measured 2-5 µm in diameter, similar to spores of *Cladosporium* spp. (3-7 µm), *Penicillium* spp. (2-3 µm), and *Aspergillus* spp. (3.5-5 µm)(21). This group likely consisted mainly of spores from these genera, which are known to release single-celled airborne spores(22,23).

During the first survey period (2013-2014), the most abundant spore types were unclassified one-celled spores (40.60%) and *Cladosporium* spp. (31.45%) (Table 2). Other commonly detected types included yeast (11.02%), rust (8.72%), *Nigrospora* spp. (4.25%), and *Fusarium* spp. (1.54%). Less frequent spores included *Alternaria* spp. (1.35%), *Helminthosporium* spp. (0.71%), *Curvularia* spp. (0.30%), *Tetraploa* spp. (0.06%), and *Penicillium* spp. (0.01%).

During the second survey period (2022-2023), the same major groups predominated, but their relative proportions changed markedly (Table 2). *Cladosporium* spp. became the dominant type (48.64%). Rust spores increased substantially and ranked second (18.32%). Unclassified one-celled spores declined to 13.78% and ranked third, followed by *Nigrospora* spp. (7.34%) and yeast (4.28%). Other spores showed similar ranking patterns to 2013-2014, including *Fusarium* spp. (3.06%), *Alternaria* spp. (2.77%), *Helminthosporium* spp. (0.76%), *Curvularia* spp. (0.76%), *Tetraploa* spp. (0.17%), and *Penicillium* spp. (0.11%).

Kruskal-Wallis analysis showed significant differences in concentrations of several spore types between the two periods. Unclassified one-celled spores and yeast were more abundant in 2013-2014 ($p<0.001$), whereas *Cladosporium* spp., rust, *Nigrospora* spp., *Fusarium* spp., *Alternaria* spp., *Curvularia* spp., and *Tetraploa* spp. were significantly more abundant in 2022-2023 ($p<0.05$). Significant monthly variation occurred for nearly all spore types within each period, except *Penicillium* spp. in 2013-2014 and *Alternaria* spp. and *Tetraploa* spp. in 2022-2023.

Seasonal variation and climatic influences on outdoor airborne mold spores

Seasonal variations in airborne fungal spores and meteorological parameters are illustrated in Figure 2-4. During 2013-2014, *Cladosporium* spp. concentrations

Figure 2. Seasonal variation of the six most prevalent outdoor airborne mold spore types during August 2013 to July 2014 and August 2022 to July 2023. Each graph presents the monthly concentrations (spores/m^3) of individual spore types, comparing the two 12-month periods using different colors.

peaked in July (236.9 spores/m³). Unclassified one-celled spores and yeast also reached high levels in June and July, with peak values of 544.0 and 335.5 spores/m³ for unclassified one-celled spores and 103.8 and 85.3 spores/m³ for yeast, respectively. Rust spores and *Nigrospora* spp. recorded their highest concentrations in August (150.0 spores/m³) and July (43.0 spores/m³), respectively.

During 2022-2023, *Cladosporium* spp. showed higher peaks than in the earlier period, with maxima in May and June (460.5 and 486.6 spores/m³). Rust spores also reached higher levels, peaking at 201.1 spores/m³ in October. In contrast, unclassified one-celled spores appeared at lower overall levels, with a maximum of 184.4 spores/m³ in May. Yeast spores were detected year-round but at much lower concentrations than in 2013-2014, peaking at 82.2 spores/m³ in February. Conversely, *Nigrospora* spp. and *Alternaria* spp. showed higher concentrations throughout the year compared with the earlier period, with peak values in November (61.3 spores/m³) and October (17.1 spores/m³).

Both *Fusarium* spp. and *Curvularia* spp. demonstrated similar seasonal patterns across periods. *Fusarium* spp. showed lower concentrations from January to May and higher levels from July to September. *Curvularia* spp. exhibited lower concentrations from December to April and higher levels from July to August.

Meteorological conditions were generally comparable between the two study periods, with no notable differences in monthly temperature or relative

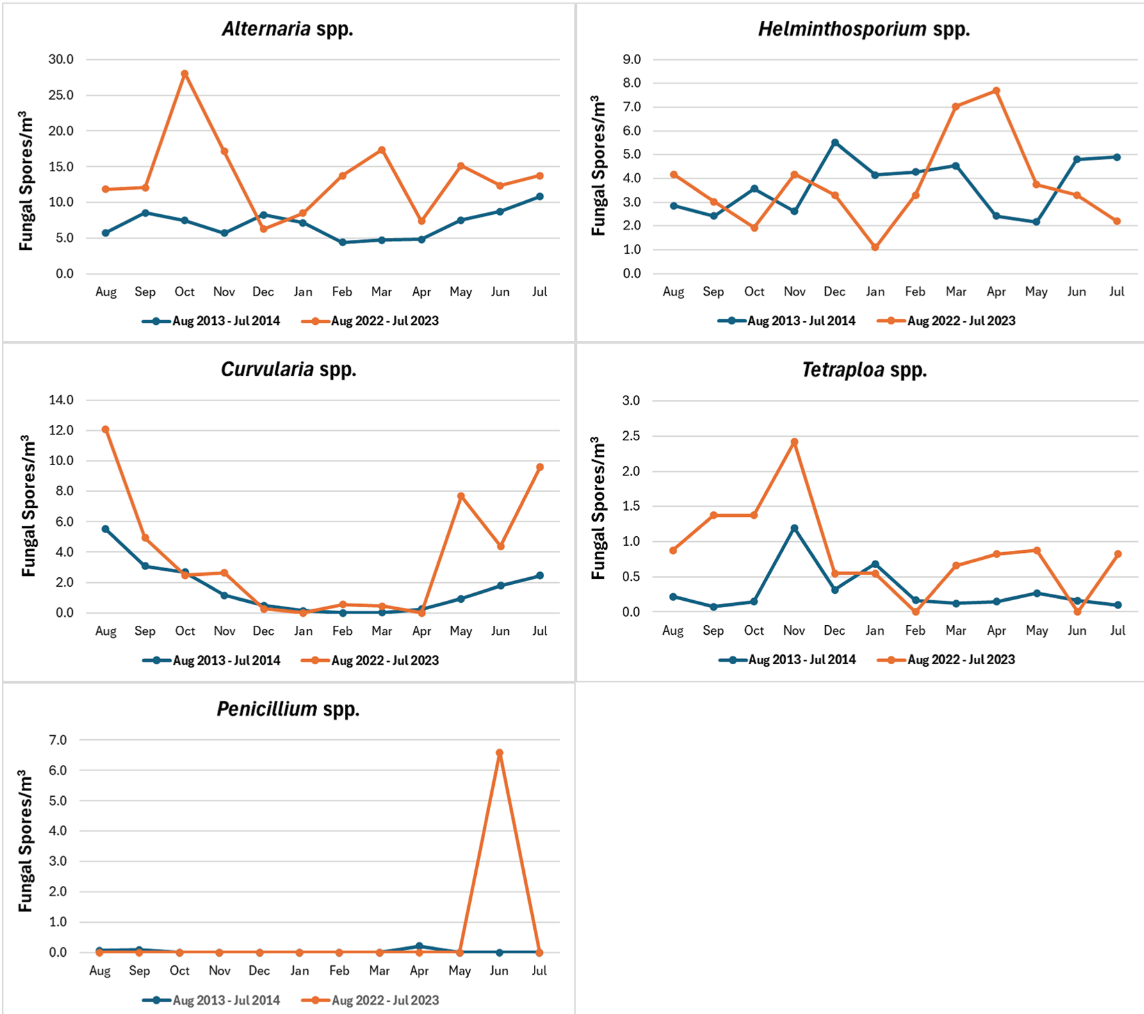

Figure 3. Seasonal variation of outdoor airborne mold spores ranked seventh to eleventh during August 2013 to July 2014 and August 2022 to July 2023. Each graph presents the monthly concentrations (spores/m^3) of individual spore types, comparing the two 12-month periods using different colors.

humidity. However, total annual rainfall and average wind speed were slightly higher during 2022-2023 (Table 1, Figure 4). In both periods, peak rainfall and relative humidity occurred in September, with additional high rainfall in August 2013, July 2014, and August 2022. Relative humidity remained elevated from July through November, while November to April showed minimal rainfall. April in both periods had the highest temperatures and lowest relative humidity.

This seasonal pattern corresponded to the lowest airborne mold spore levels in April 2014; however, a similar decrease was not noted in April 2023. High monthly rainfall did not demonstrate a direct temporal association with elevated mold spore concentrations in the same month. The highest total mold spore concentrations occurred in the early rainy season, from late April through June, as rainfall began to increase. This pattern remained consistent across both periods. Additionally, peak wind speeds aligned with the highest airborne fungal spore concentrations in June and July 2014 and in May and June 2023.

Correlation analyses between airborne fungal spores and meteorological variables are summarized in Table 3 and 4. During 2013-2014, total airborne mold spores showed a very weak negative correlation with temperature, whereas a weak positive correlation was documented during 2022-2023. *Nigrospora* spp. and *Helminthosporium* spp. demonstrated significant strong positive correlations with maximum temperature in 2022-2023.

Regarding relative humidity and rainfall, total airborne mold spore concentrations during 2013-2014

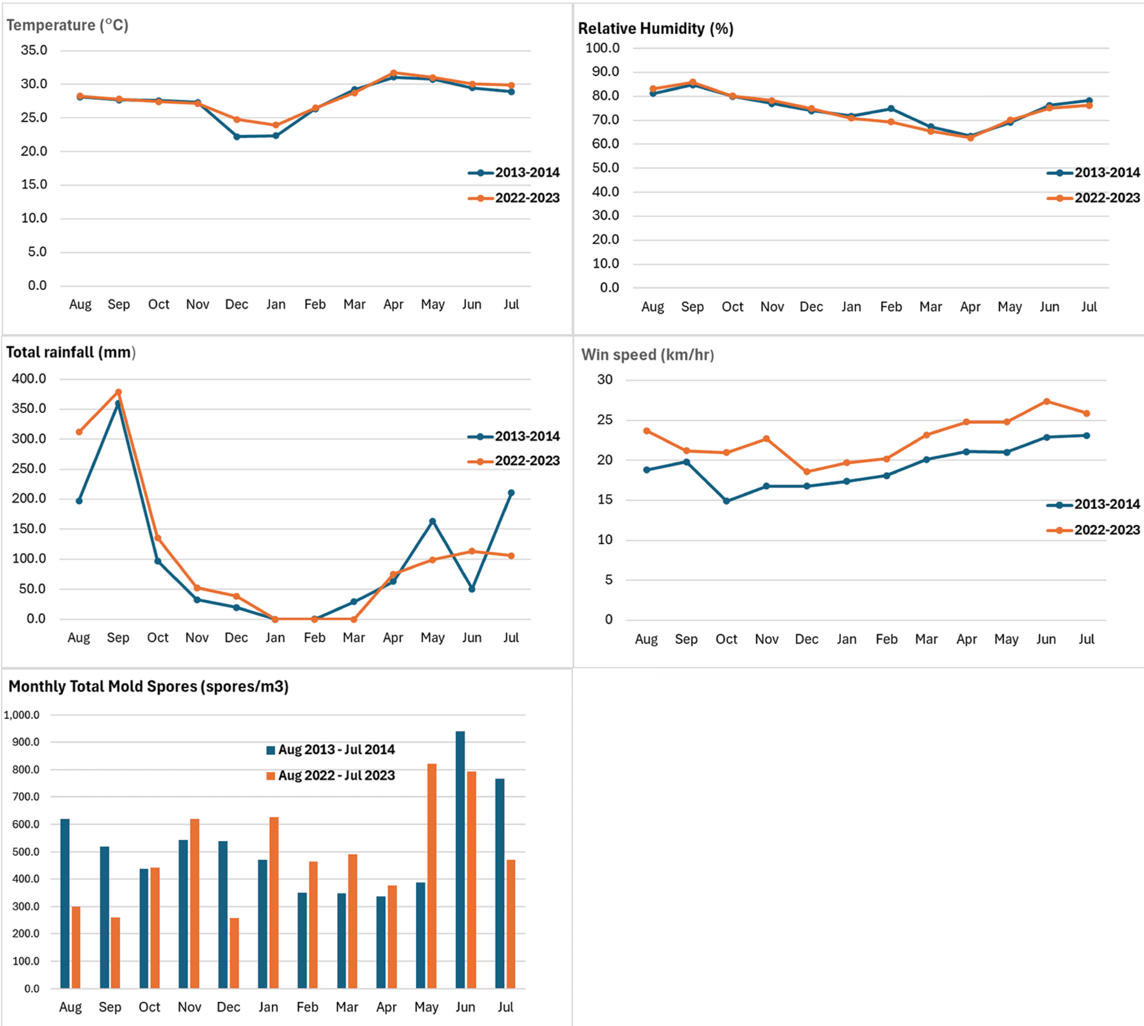

Figure 4. Comparison of meteorological parameters and total airborne fungal spore concentrations (spores/m^3) during August 2013 to July 2014 and August 2022 to July 2023, presented month by month to compare the two 12-month periods using different colors.

demonstrated a significant, strong positive correlation with relative humidity and a weak positive correlation with total rainfall. In contrast, weak negative correlations with both variables were observed in 2022-2023. For individual spore types, *Curvularia* spp. demonstrated very strong positive correlations with relative humidity and rainfall during 2013-2014 and strong positive correlations during 2022-2023 (Table 3, 4). Rust spores also exhibited significant moderate to very strong positive correlations with relative humidity and rainfall in both periods. *Fusarium* spp. showed significant moderate to strong positive correlations with relative humidity across both periods. Yeast and *Alternaria* spp. demonstrated moderate to strong correlations with relative humidity and rainfall during 2013-2014, but these associations were not detected during 2022-2023. Relative humidity and rainfall exhibited weak negative correlations with *Cladosporium* spp. in both periods. Wind speed demonstrated a very weak correlation with total airborne mold spores during 2013-2014 and a moderate positive correlation during 2022-2023.

Discussion

This study represents the first survey of outdoor airborne fungal spores conducted in Phitsanulok, a province in lower northern Thailand, and the first aeroallergen study in Thailand to utilize a Burkard seven-day recording volumetric spore trap, allowing simultaneous sampling of airborne fungal spores and pollens[19]. Previous Thai aeroallergen studies have primarily been conducted in Bangkok using Durham

Table 3. Spearman's rank correlation coefficients between meteorological variables and total airborne fungal spore concentrations and individual fungal spore types in August 2013 to July 2014

Mold spores	Tmin (°C)	Tmax (°C)	Mean temp (°C)	Relative humidity (%)	Total rainfall (mm)	Wind speed (km/hour)
Total mold spores	0.1399	−0.3257	−0.1608	0.6364*	0.2937	0.1016
Cladosporium spp.	0.0629	0.0666	−0.0629	−0.3566	−0.2308	0.1856
Rust	0.1469	−0.2977	−0.0070	0.8601*	0.5804*	−0.0385
Unclassified one-celled spores	0.1189	−0.3363	−0.1538	0.7552*	0.3566	0.1156
Nigrospora spp.	0.1509	−0.2214	−0.1088	0.5544	0.3404	0.0035
Yeast	0.4965	−0.0736	0.2587	0.5804*	0.6713*	0.4028
Fusarium spp.	0.2308	−0.2942	−0.0420	0.5804*	0.3566	−0.0525
Alternaria spp.	0.3222	−0.2351	0.0560	0.4518	0.5079	0.2842
Helminthosporium spp.	−0.1436	−0.2702	−0.3573	−0.0105	−0.3958	0.0175
Curvularia spp.	0.2697	−0.2386	0.1121	0.8476*	0.8126*	0.0246
Tetraploa spp.	−0.4136	−0.3190	−0.4612	−0.1537	−0.5124	−0.4088
Penicillium spp.	0.1611	0.1775	0.3545	0.1519	0.4880	0.2260

Tmin=minimum temperature; Tmax=maximum temperature; Mean temp=mean temperature
* Significant correlation, $p<0.05$

Table 4. Spearman's rank correlation coefficients between meteorological variables and total airborne fungal spore concentrations and individual fungal spore types in August 2022 to July 2023

Mold spores	Tmin (°C)	Tmax (°C)	Mean temp (°C)	Relative humidity (%)	Total rainfall (mm)	Wind speed (km/hour)
Total mold spores	0.1608	0.3538	0.2378	−0.3077	−0.2592	0.3993
Cladosporium spp.	0.0559	0.2102	0.1049	−0.2867	−0.3187	0.3082
Rust	0.3497	−0.0140	0.1958	0.6573*	0.8161*	0.3222
Unclassified one-celled spores	−0.0629	0.3082	0.1469	−0.8112*	−0.7250*	0.0841
Nigrospora spp.	0.4623	0.7140*	0.5744	−0.2417	−0.0561	0.6351*
Yeast	−0.0936	0.2500	0.1560	−0.8734*	−0.6640*	−0.0547
Fusarium spp.	−0.3776	−0.4203	−0.4825	0.6224*	0.2172	−0.2837
Alternaria spp.	0.0175	0.2667	0.1191	0.0876	−0.0228	0.1649
Helminthosporium spp.	0.2822	0.6679*	0.4903	−0.4902	−0.2014	0.3675
Curvularia spp.	0.5009	0.2246	0.3538	0.6305*	0.6807*	0.5281
Tetraploa spp.	0.2928	0.1731	0.1129	0.5750	0.5353	0.0671
Penicillium spp.	0.2184	0.2187	0.3057	0.0437	0.2187	0.4812

Tmin=minimum temperature; Tmax=maximum temperature; Mean temp=mean temperature
* Significant correlation, $p<0.05$

samplers[24,25] or Rotorod devices[14]. Across both survey periods, outdoor mold spore concentrations were approximately five to eight times higher than airborne pollen levels, consistent with prior findings from Thailand[24-26] and other regions worldwide[13,18,27].

In both study periods, total airborne mold spore concentrations increased and peaked between May and July, corresponding to the beginning of the rainy season in lower northern Thailand. As rainfall intensified and reached its maximum in September, total mold spore concentrations declined to their lowest levels in the same month. This inverse relationship between heavy rainfall and airborne spore concentrations has been documented previously in Chiang Mai and Songkhla, Thailand, and is likely attributable to heavy precipitation washing spores out of the atmosphere and depositing them on the ground[26].

Across the ten-year interval, *Cladosporium* spp. consistently represented the most abundant outdoor airborne mold spores, accounting for 31.45% of total mold spores in 2013-2014 and 48.64% in 2022-2023. However, during the first survey period, unclassified one-celled spores constituted a larger proportion of total spores (40.6%) than *Cladosporium* spp. *Cladosporium* spp. are microscopically characterized by conidiophores bearing long, fragile, branched conidial chains that are easily dispersed by wind turbulence[21,28]. Consequently, preserving intact conidial chains during microscopic slide preparation is challenging, suggesting that many of the unclassified one-celled spores identified in this survey were likely derived from fragmentation of *Cladosporium* spp. conidial chains into single conidia. Moreover, the decline in unclassified one-celled spores from 40.6% in 2013-2014 to 13.78% in 2022-2023 may partly reflect increased experience in slide preparation, reading, and interpretation by the same trained personnel. When unclassified one-celled spores and *Cladosporium* spp. are considered together, *Cladosporium* spp. may account for approximately 60% to 70% of total airborne mold spores, reinforcing its dominance in the atmospheric fungal profile of lower northern Thailand[14,24,25] and from other countries[9,12,18,29]. Although some unclassified one-celled spores may have originated from *Penicillium* spp. and *Aspergillus* spp., whose spore dimensions substantially overlap with those of unclassified one-celled spores[22,23].

The seasonal distribution of *Cladosporium* spp. demonstrated elevated concentrations during the early rainy season (May to July), followed by a decline in September when rainfall and relative humidity peaked, and a secondary increase during November through January as rainfall decreased. Statistical analyses supported these observations, revealing weak negative correlations between *Cladosporium* spp. concentrations and both total rainfall and relative humidity in both study periods. Similar seasonal trends have been reported in Croatia and other temperate and subtropical regions[18]. These findings likely reflect the release mechanism of *Cladosporium* spores, which are primarily dispersed by wind turbulence and tend to reach peak concentrations under relatively dry and windy conditions[21]. In the present study, however, wind velocity showed only weak correlations with *Cladosporium* spp. concentrations, suggesting that multiple interacting environmental factors influence spore dispersal. Global warming may contribute to higher concentrations of *Cladosporium* spp., as research in Poland showed significantly increased spore counts and markedly accelerated spore seasons with rising average air temperatures[30]. In contrast, our study did not reveal a correlation between temperature and *Cladosporium* spp. counts.

Rust spores ranked second in abundance during the 2022-2023 period, compared with fourth during 2013-2014. Rust fungi, primarily belonging to the genus *Puccinia* (division Basidiomycota), thrive under humid conditions and during periods of increased rainfall[21]. This observation was supported by moderate to very strong positive correlations between rust spore concentrations and both relative humidity and rainfall across both survey periods. As obligate plant pathogens, rust fungi may also benefit from increased host plant availability during wetter conditions, potentially contributing to their increased atmospheric presence.

Nigrospora spp. were detected at higher concentrations during the 2022-2023 period. These spores are primarily released through wind turbulence and typically reach peak concentrations under dry and windy conditions[21]. Consistent with this mechanism, higher wind speeds during 2022-2023, particularly in November, April, May, June, and July, corresponded with increased *Nigrospora* spp. concentrations, and a strong positive correlation with wind speed was observed during this period.

Although the relative ranking of mold spore types from sixth to eleventh remained unchanged across the two surveys, all six types exhibited higher counts during 2022-2023, potentially reflecting higher overall rainfall. Among these taxa, *Fusarium* spp. showed significant positive correlations with relative humidity, while *Curvularia* spp. demonstrated strong correlations with both humidity and rainfall. In contrast, *Alternaria* spp., *Helminthosporium* spp., *Penicillium* spp., and *Tetraploa* spp. did not exhibit consistent strong correlations with meteorological parameters.

Notably, *Penicillium* spp. and *Aspergillus* spp., clinically important fungal allergens were detected at very low levels in the outdoor atmosphere during both survey periods. This contrasts with results from Bangkok, where *Penicillium* spp. and *Aspergillus* spp. spores ranked among the most prevalent fungal taxa[14]. In that study, *Penicillium* spp. and *Aspergillus* spp. spores were grouped together because their morphology is indistinguishable under light microscopy[14]. In the present survey, a proportion of unclassified one-celled spores may have originated from *Penicillium* spp. or *Aspergillus* spp., potentially leading to an underestimation of these taxa and partly explaining this discrepancy.

Previous studies in Phitsanulok have demonstrated sensitization to fungal allergens among asthmatic children and adults, including *Alternaria tenuis*, *Cladosporium herbarum*, *Aspergillus* mix, and *Penicillium* mix[4,15]. Although house dust mites and other allergens predominate in Thailand[2-4,15,31], fungal allergens remain clinically relevant contributors to allergic disease and asthma severity[21,32-34]. *Alternaria* spp. has been identified as one of the most potent fungal allergens and is associated with asthma severity[35] and respiratory arrest events[33], while *Cladosporium* spp. sensitization has also been linked to severe or lifethreatening asthma[21]. While definitive atmospheric thresholds for symptom induction remain uncertain, concentrations exceeding 50 to 100 spores/m³ may provoke symptoms, with higher thresholds proposed for *Cladosporium* spp. (500-1,500 spores/m³)[36]. In this study, *Cladosporium* spp. concentrations approached 500 spores/m³ during several months, and when combined with unclassified one-celled spores, the effective atmospheric burden may have exceeded this level, underscoring potential clinical relevance during peak seasons. In contrast, *Alternaria* spp. concentrations remained below the suggested threshold of 50 spores/m³ during both surveys. Nevertheless, these levels should not be disregarded, as fungal fragments and submicron particles containing allergenic material may contribute substantially to total allergen exposure and occur in far greater numbers than intact spores, as demonstrated by respiratory deposition models[36]. Furthermore, airborne allergen concentrations do not always correlate with spore counts, as allergen release varies according to fungal developmental stage[36]. Year-round monitoring of airborne fungal spores in the area and public reporting of the findings would be highly beneficial for providing guidance and facilitating avoidance strategies for patients who are allergic to fungal allergens. Future studies should examine the relationship between spore counts and asthma-related emergency visits in this region.

This study did not assess correlations between airborne fungal spores and allergic disease outcomes, which represents an important limitation. Additional limitations include single-site sampling and reliance on light microscopy, which constrained differentiation among morphologically similar spores, particularly unclassified one-celled spores that were found in substantial concentrations during the first study period. Nevertheless, this study provides the first comparison of airborne mold spores in Phitsanulok, lower northern Thailand, across two 12-month periods separated by a 10-year interval, as well as the first application of a Burkard volumetric spore trap in Thailand, enabling reliable temporal comparisons and concurrent pollen monitoring[19].

Conclusion

During the 2013-2014 and 2022-2023 study periods, airborne fungal spores in Phitsanulok, lower northern Thailand, were recorded across all seasons, with maximum concentrations persistently observed between May and July across the 10-year interval. *Cladosporium* spp. was the predominant airborne fungal spore in both periods, accounting for approximately half of the total mold spore burden. Concentrations were highest during the early rainy season and declined during months of peak rainfall. *Alternaria* spp. concentrations were noticeably lower than those of *Cladosporium* spp., whereas *Aspergillus* spp. and *Penicillium* spp. were detected at very low levels. No significant differences were observed in overall total airborne fungal spore concentrations or in the percentage distribution of mold spore types between the two study periods. These findings highlight stable patterns in airborne fungal spore distribution in lower northern Thailand across two 12 month periods, 10 years apart, and support the value of continued aeroallergen monitoring to inform clinical management and seasonal risk awareness among individuals sensitized to mold allergens.

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Authors' contributions

Concept and design: SU and BD; Data collection: SU and SB; Analysis and interpretation: SU, BD, and SI; Writing the article: SU. All authors have read and approved the final manuscript.

Clinical trial registration

Not applicable. This study was not a clinical trial and was not registered as a clinical trial.

Conflicts of interest

The authors have no conflict of interest to disclose.

Data availability statement

The datasets used in this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was reviewed and approved for research by the Naresuan University Institutional Review Board. The study was exempt from human research ethics review, as it focused exclusively on airborne fungal spore allergens and did not involve human participants (ethics approval certification number: NU-IRB 0354); therefore, informed consent was not required.

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Use of artificial intelligence

Artificial intelligence was not used in the preparation of this manuscript.

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