ORIGINAL RESEARCH ARTICLE

Airborne Fungal Exposure and Associated Respiratory Symptoms among Mushroom Farm Workers in Rural Mountainous Communities of Northern Thailand: A Cross-Sectional Study

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DOI: 10.55489/njcm.170720266307

ABSTRACT

Background: Mushroom cultivation in humid, high-altitude rural areas of northern Thailand may expose workers to elevated levels of airborne fungi. However, quantitative evidence on fungal workplace environments and associated respiratory symptoms among mushroom workers remains limited.

Methods: A cross-sectional study not registered in a trial registry, was conducted among 350 mushroom workers from 35 rural mountain farms in northern Thailand. Workplace environmental conditions, including temperature, relative humidity, air velocity, carbon dioxide concentration, and airborne fungal counts, were measured using standardized instruments and a single-stage impactor. Worker characteristics and respiratory symptoms were collected using a structured questionnaire. Multiple logistic regression was performed to identify factors associated with upper and lower respiratory symptoms.

Results: Workers spent an average of 4.20±2.29 hours per day inside mushroom cultivation areas. Airborne fungal concentrations ranged from 515.67 to 5,708.00 CFU/m^3. Upper respiratory symptoms were reported in 33.1% (n=116/350) of workers, while lower respiratory symptoms were reported in 2.6% (n=9/350). Upper respiratory symptoms were significantly associated with age 31-60 years (aOR = 2.58; 95% CI: 1.08-6.17) and fungal concentrations of 2,001-4,000 CFU/m^3 (aOR = 15.03; 95% CI: 1.53-147.52). A history of respiratory disease was significantly associated with lower respiratory symptoms (aOR = 5.68; 95% CI: 1.10-31.95).

Conclusions: Airborne fungal exposure was significantly associated with respiratory symptoms, with workers exposed to fungal concentrations of 2,001-4,000 CFU/m^3 showing markedly increased risk at 15.03 times. Strengthening ventilation systems and respiratory protection practices may help reduce occupational respiratory risks.

Keywords: Airborne Fungi, Bioaerosols, Occupational, Respiratory Symptoms, Mushroom Workers, Thailand

ARTICLE INFO

Financial Support: None declared
Conflict of Interest: The authors have declared that no conflict of interest exists.
Received: 30-12-2025, **Accepted**: 08-05-2026, **Published**: 01-07-2026
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How to cite this article: Yenjai P, Chotigawin R, Buadaeng P, Polyong CP. Airborne Fungal Exposure and Associated Respiratory Symptoms among Mushroom Farm Workers in Rural Mountainous Communities of Northern Thailand: A Cross-Sectional Study. Natl J Community Med 2026;17(7):536-545. DOI: 10.55489/njcm.170720266307

www.njcmindia.com | pISSN: 0976-3325 | eISSN: 2229-6816 | Published by Medsci Publications

Introduction

Mushroom cultivation has expanded rapidly in Thailand due to its favorable tropical and humid climate, especially in high-altitude northern regions.[1] Mushrooms are widely consumed for their nutritional value and are an important source of income for rural households.[2] Despite its economic importance, mushroom farming in small-scale production systems often involves limited environmental control and occupational health protection. Workers typically operate in enclosed cultivation houses containing organic substrates that create humid micro-environments favorable for fungal growth and spore dispersal. Agricultural environments involving organic material handling are recognized as important sources of airborne bioaerosols, while elevated humidity and inadequate ventilation in enclosed indoor environments further promote fungal proliferation and airborne contamination.[3] Previous research has shown that exposure to airborne fungi can contribute to respiratory irritation, allergic reactions, or chronic respiratory symptoms, particularly among individuals who work in damp or poorly ventilated environments.[4-5]

In Thailand, studies on occupational exposure to bioaerosols have mainly focused on agricultural sectors such as rice farming, poultry operations, and composting facilities.[6] These investigations have documented elevated concentrations of fungal spores and associated respiratory risks, yet mushroom workers a group that closely interacts with organic substrates under high-humidity conditions remain understudied. The unique microenvironment of mushroom houses, characterized by constant moisture, decomposing organic matter, and limited ventilation, creates conditions conducive to microbial proliferation.[7,8] Such environments may expose workers to considerable fungal loads, with potential implications for both upper and lower respiratory health.[9]

Although several international studies have measured airborne fungi in agricultural workplaces, few have addressed the specific exposure patterns of mushroom workers, particularly in rural or mountainous locations where environmental monitoring and health services are limited.[5] Workers in these settings may be at heightened risk due to prolonged exposure periods, inadequate personal protective equipment, and pre-existing health vulnerabilities. Moreover, environmental parameters such as temperature, humidity, air velocity, and carbon dioxide concentration are known to influence fungal growth and dispersal,[10-11] yet these factors have not been systematically assessed in mushroom farms in Thailand.

There is a lack of baseline data regarding the fungal levels in mushroom cultivation environments in rural mountainous communities of northern Thailand. Additionally, limited information exists on how worker characteristics and environmental conditions collectively contribute to respiratory symptoms in this population. Understanding these relationships is essential for developing appropriate risk-reduction strategies and guiding preventive public health measures for informal agricultural workers.

Northern Thailand, particularly Chiang Rai Province, was selected as the study site due to its mountainous geography, high humidity, and widespread small-scale mushroom cultivation practices. These environmental conditions create favorable microclimates for fungal growth and bioaerosol accumulation, potentially increasing occupational exposure among workers. Furthermore, mushroom cultivation has been actively promoted in this region by governmental and private sectors, resulting in the establishment of more than 60 registered mushroom-related community enterprises in Chiang Rai Province. The province is recognized as one of the major mushroom-producing and exporting areas in northern Thailand,[12] highlighting its importance as a relevant public health setting for investigating airborne fungal exposure and respiratory health risks among mushroom workers.

Therefore, this study aimed to assess the fungal workplace environment and identify factors associated with upper and lower respiratory symptoms among mushroom workers in small-scale rural mountain farms in northern Thailand. Findings from this study are expected to provide essential evidence for improving occupational health practices, enhancing environmental control measures, and informing targeted screening and monitoring for high-risk worker groups.

Methodology

Sample and Sampling: The sample size was calculated using the single population proportion formula described by Cochran: $n = Z^2P(1-P)/d^2$, where n is the required sample size, Z is the standard normal deviate corresponding to a 95% confidence level (1.96), P is the estimated prevalence of respiratory symptoms (P = 0.633) based on a previous cross-sectional study among mushroom workers in Ireland conducted by Hayes and Rooney,[13] and d is the margin of error (0.05). Based on, the minimum required sample size was 356 participants. However, due to field feasibility constraints, a total of 350 participants were included in the study.

A multi-stage cluster sampling method was employed in this study. Chiang Rai Province was purposively selected as the study area due to its mountainous geography, humid climate, and widespread small-scale mushroom cultivation. From the total of 18 districts in the province, three districts (Phaya Mengrai, Phan, and Thoeng) were randomly selected using simple random sampling. Within the selected districts, mushroom farms were identified, and farms served as the primary sampling units (clusters). All eligible workers from participating farms were invited to participate in the study. On average, approximately 10 workers were available per farm, based on field enumeration conducted during farm visits prior to participant

recruitment. A total of 35 farms and 350 workers were included in the final sample.

Data collection involved environmental measurements of working conditions, assessment of airborne fungi, and structured interviews with workers. Inclusion criteria required participants to have at least one year of mushroom cultivation experience, the ability to communicate and understand Thai, and willingness to participate voluntarily. Exclusion criteria included workers who were absent on the scheduled data collection date.

Research Tools and Quality: The research instruments were categorized into four types: an airborne fungal test, workplace environment measurement tools, a carbon dioxide meter, and an interview questionnaire.

i) The airborne fungal test utilized a portable air pump (air suction rate: 28.3 liters/minute), an Andersen single-stage sampler, and potato dextrose agar (PDA) in a 9-cm Petri dish as the culture medium. Other materials included sterile cotton pads, 70% isopropanol alcohol, and ice packs for temperature maintenance during transport. Samples were incubated at 35-37°C for 48 hours. Fungal counts were measured in colony-forming units per cubic meter (CFU/m^3). Sample collection followed the NIOSH Method 0800 standard.

ii) For workplace environment measurements, a VelociCalc® Air Velocity Meter, Model 966, was used. This device measures air velocity (in meters per second), temperature (in degrees Celsius), and relative humidity (in percent). The instrument was calibrated by the supplier, with calibration certificate number P16440029.

iii) A Rotronic Carbon Dioxide Meter, Model CP11, was used to measure carbon dioxide levels in parts per million (ppm). The device was calibrated by the supplier, and its calibration certificate number was 71405146.

iv) The interview questionnaire was comprised of four sections: personal information (7 items, including age, smoking status, and past medical history), work experience (7 items, including job description, length of employment, and work frequency), personal protective equipment use (3 items, including the use, type, and frequency of respiratory personal protective equipment), and respiratory symptoms (23 items), which was adapted from American Thoracic Society's Division of Lung Disease Questionnaire (ATS-DLD).

The respiratory symptom section of the questionnaire was adapted from the ATS-DLD questionnaire, which remains widely used in respiratory epidemiological and occupational health studies. Recent occupational studies continue to apply standardized ATS-DLD based respiratory symptom questionnaires, supporting their continued relevance in contemporary research.[14] Therefore, additional reliability testing was not conducted, as the respiratory symptom items were derived from a previously validated standardized instrument.

The main outcome variable was respiratory symptoms were assessed using an adapted version of the ATS-DLD questionnaire. A total of 23 items were included and categorized into five domains: nasal symptoms (3 items), cough (6 items), phlegm (6 items), wheezing (4 items), and shortness of breath (4 items).

Upper respiratory symptoms included nasal symptoms, cough, and phlegm (15 items), while lower respiratory symptoms included wheezing and shortness of breath (8 items). Each symptom was recorded as a dichotomous response (yes/no). Participants were classified as having upper or lower respiratory symptoms if they reported at least one symptom within the respective category during the past 1 month. For data analysis, symptoms were dichotomized as either "yes" or "no," with a score of 1 for the presence (yes) of at least one symptom and 0 for no symptoms. The questionnaire content validity was assessed using the Index of Item-Objective Congruence (IOC) evaluated by three experts, in occupational health, safety, and occupational medicine. The IOC values ranged from 0.67 to 1.00, confirming its quality.

Data Collection: Fungal samples were collected using area sampling within the mushroom farms. Sampling points were chosen in areas with high worker activity and in farms with the most mushrooms ready for harvesting. The air suction pump was calibrated before and after each collection. All sampling equipment was cleaned with 70% alcohol. Petri dishes were placed on the Andersen single-stage sampler, which was set at a height of 1.5 meters from the ground, corresponding to the breathing zone. The pump was operated at an air suction rate of 28.3 liters per minute for 3 minutes. Samples were collected at one central point per farm, with three replicate samples taken at each point. After collection, the Petri dish lid was closed, labeled on the bottom with the sample number, and stored medium-side up to prevent condensation from dripping onto the medium. Samples were then placed in sterilized plastic bags, packed in a cooler with ice at 4°C, and transported to the laboratory. Fungal measurements for each farm were reported as the average of the total fungal counts.

Airborne fungal samples were collected using a single-stage Andersen impactor operated at a flow rate of 28.3 L/min for 3 minutes (≈84.9 L). Short-duration sampling protocols (2-5 minutes) are commonly used in bioaerosol monitoring to prevent overloading of agar plates and ensure accurate colony enumeration.[15] Unlike particulate dust sampling methods that require large air volumes (NIOSH Method 0800), bioaerosol sampling using impactors typically employs shorter sampling durations due to the risk of colony overgrowth and particle overload on agar media.

Workplace environmental data were collected, including temperature, relative humidity, air velocity, and carbon dioxide levels. One measurement was taken

per greenhouse area, at the same location as the fungal sample collection. The procedure involved setting up the instrument, holding it at a height of 1.5 meters, and extending the probe to the measurement point. After waiting for approximately one minute for stabilization, a reading was taken. This process was repeated three times per point, and the results were recorded on a data form.

For the questionnaire interviews, researchers first explained the study procedures and obtained informed consent from the mushroom workers. Interviews were conducted face-to-face at the farms after the workers had completed their tasks. Each interview, using the custom-developed questionnaire, lasted approximately 10-15 minutes. To verify the accuracy of the self-reported data, the research team observed participants' clothing and their use of respiratory protection equipment during the interviews.

All data collection procedures were conducted after obtaining ethical approval from the Faculty of Public Health, Burapha University (Human Research Ethics Clearance Certificate No. 001/2560). Written informed consent was obtained from all participants prior to data collection. All collected data were anonymised to ensure participant confidentiality. This cross-sectional study was not registered in a clinical trial or study registry. Data collection was conducted between 2017 and 2018.

Data Analysis: Data analysis began with the Kolmogorov-Smirnov test to determine the distribution of the data. For continuous variables with a normal distribution, descriptive statistics were reported as mean and standard deviation (SD). For non-normal distributions, the median, minimum, and maximum values were used. Discrete variables were analyzed using frequencies and percentages. Binary logistic regression was used to examine associations between independent variables and respiratory symptoms. Variables with $p < 0.20$ in bivariate analysis were considered for inclusion in the multivariable logistic regression model,[16] consistent with recommended statistical practices. No formal correction for multiple testing was applied because the analyses were conducted using multivariable logistic regression models to identify independent predictors rather than performing multiple independent hypothesis tests. A statistical significance level of $p < 0.05$ was used for all analyses.

Working hours per day contained missing values for 86 participants. In regression analyses, cases with missing working hours data were excluded only from analyses involving that variable, while other variables were analyzed using all available observations (n=350). Thus, the effective sample size varied depending on the availability of data for each variable.

Results

A total of 350 mushroom workers from 35 farms were assessed for eligibility following the multi-stage cluster sampling procedure described in the methods section. All workers met the predefined inclusion criteria and voluntarily agreed to participate in the study. Questionnaire interviews and environmental sampling were successfully completed for all participants. No missing questionnaire data were identified, as the questionnaires were interviewer-administered and reviewed for completeness at the time of data collection.

A flow diagram illustrating the selection of study areas, recruitment of mushroom farms, and inclusion of participants in the final analysis is presented in Figure 1.

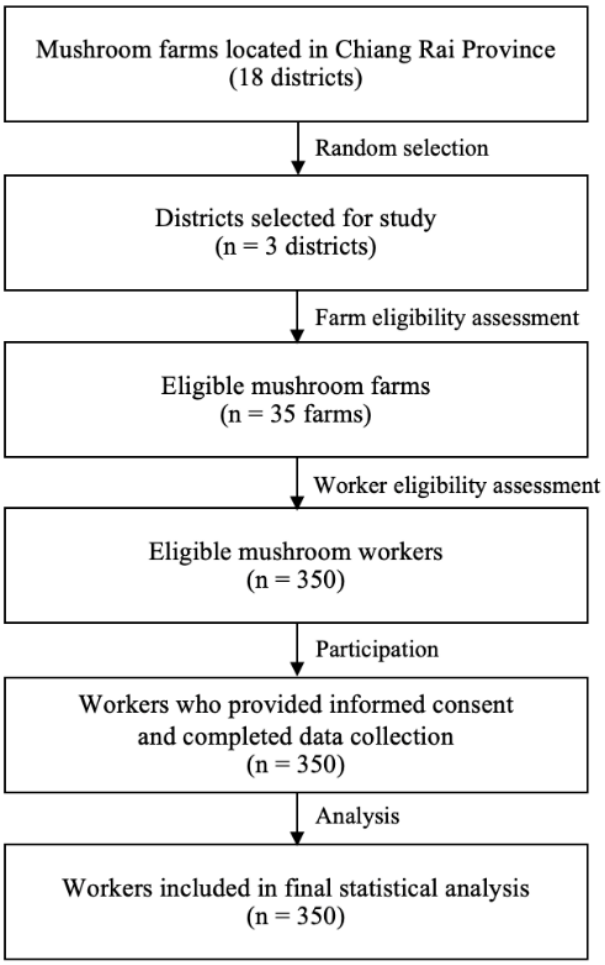

Figure 1: Flow diagram of study area selection, farm recruitment, and participant inclusion

Participants were predominantly female and middle-aged, with a relatively low prevalence of smoking and respiratory disease history. More than half reported using respiratory protective equipment; however, the use was largely limited to cloth masks and was not consistent. Most workers had less than 10 years of work experience and spent relatively short daily periods inside mushroom cultivation areas, although the majority worked throughout the week. Detailed characteristics are presented in Table 1.

Table 1: General information and work history of mushroom workers

Variables	Workers (%)
Gender (n=350)	
Male	131 (37.4)
Female	219 (62.6)
Age (n=350)	
≤ 30 years	41 (11.7)
31-60 years	264 (75.4)
> 60 years	45 (12.9)
Smoking history (n=350)	
No	298 (85.1)
Yes, current smoking	52 (14.9)
History of respiratory disease (n=350)	
No	325 (92.9)
Yes	25 (7.1)
Respiratory protective equipment used (n=350)	
No	163 (46.6)
Yes	187 (53.4)
Type of respiratory protective equipment (n=187)	
Cloth mask	124 (66.3)
Paper mask	53 (28.4)
Face mask hat	9 (4.8)
Particulate filter mask	1 (0.5)
Frequency of respiratory personal protective equipment used (n=187)	
Every time	35 (18.7)
Often	31 (16.6)
Sometimes	121 (64.7)
Longevity in the mushroom plant (year) (n=350)	
1-10	257 (73.4)
11-20	75 (21.4)
>20	18 (5.1)
Mean=9.51, SD=6.89, Min=1, Max=32	
Working hours per day in the mushroom plant (hours per day) (n=264)*	
1-4	196 (74.2)
5-8	59 (22.4)
>8	9 (3.4)
Mean=4.20, SD=2.29, Min=1, Max=12	
Working days per week in the mushroom plant (day per week) (n=350)	
1-2	3 (0.9)
3-4	12 (3.4)
5-7	335 (95.7)
Mean=6.76, SD=0.88, Min=1, Max=7	

Note: Values are presented as number (%). Mean (SD) and range are provided for continuous variables; *Working hour data were available for 264 workers; 86 workers (24.5%) were unable to specify daily working hours.

Table 2: **Workplace environment of mushroom farms**

Workplace environment of mushroom farms	Frequency (%)
Size of mushroom plant (m^3) (n=35)	
Very small (≤50 m^3)	1 (2.9)
Small (51-100 m^3)	7 (20)
Medium (101-150 m^3)	16 (45.7)
Large (>150 m^3)	11 (31.4)
Mean=138.74, SD=49.14, Min=42, Max=200	
Temperature in mushroom plant (°C) (n=35)	
≤35 °C	26 (74.3)
>35 °C	9 (25.7)
Mean=33.23, SD=4.13, Min=20.27, Max=41.0	
Humidity in mushroom plant (%) (n=35)	
≤70	32 (91.4)
>70	3 (8.6)
Mean=51.15, SD=12.46, Min=28.7, Max=81.1	
Concentration of Fungi (CFU/m^3) (n=350)	
<2,000	270 (77.1)
2,000-4,000	30 (8.6)
>4,000	50 (14.3)
Mean=1,237.39, SD=974.00, Min=515.67, Max=5,708.00	

Note: Measurements taken at breathing-zone height (1.5 m). Values shown as number (%) and mean (SD); range.

Most mushroom houses were of medium to large size, with indoor temperatures generally maintained at or below 35°C and humidity levels below 70% in the majority of farms. Airborne fungal concentrations were predominantly below 2,000 CFU/m^3, although a smaller proportion of farms showed moderate to high fungal levels. These findings indicate generally humid and enclosed working environments with variable fungal exposure levels across farms. Detailed environmental characteristics are presented in Table 2.

Overall, upper respiratory symptoms were reported in 33.1% of workers (116/350), while lower respiratory symptoms were reported in 2.6% of workers (9/350).

The analysis of upper respiratory symptoms revealed that workers aged 31-60 years had a significantly higher risk, with an adjusted odds ratio (aOR) of 2.58 (95% CI 1.08-6.17) compared to workers aged 30 or younger. Furthermore, an airborne fungal concentration of 2,001-4,000 CFU/m^3 was a significant factor, showing a considerably elevated risk with an aOR of 15.03 (95% CI 1.53-147.52) relative to concentrations of <2,000 CFU/m^3.

The prevalence of upper respiratory symptoms increased across fungal exposure categories, from 30.7% in workers exposed to <2,000 CFU/m^3 (83/270) to 56.7% in those exposed to 2,000-4,000 CFU/m^3 (17/30), while the prevalence in the >4,000 CFU/m^3 group was 32.0% (16/50). This pattern suggests a non-linear relationship between fungal exposure and upper respiratory symptoms.

Regarding lower respiratory symptoms, workers with a history of respiratory illness were at substantially higher risk, with an aOR of 5.68 (95% CI 1.10-31.95) compared to those without such a history (Table 3).

Discussion

This study provides empirical evidence of airborne fungal concentrations and related respiratory symptoms among mushroom workers in rural mountainous farms in northern Thailand. The findings demonstrate that workers are exposed to elevated fungal levels within cultivation houses and that both environmental and individual factors contribute to upper and lower respiratory symptoms. Older age, a history of respiratory disease, and exposure to moderately high fungal concentrations were key predictors of symptoms.

Table 3: Factors affecting respiratory symptoms (n=350)

Variables	Upper respiratory symptoms		OR (95% CI)		Lower respiratory symptoms		OR (95% CI)	
	Yes (n=116)	No (n=234)	Crude	Adjusted	Yes (n=9)	No (n=341)	Crude	Adjusted
Age (year)								
≤ 30	7(6.0)	34(14.5)	Ref.		0(0.0)	41(12.0)	Ref.	
31-60	95(81.9)	169(72.2)	2.73 (1.17-6.40)	2.58 (1.08-6.17)*	8(88.9)	256(75.1)	2.75 (0.16-48.56)	-
>60	14(12.1)	31(13.3)	2.19 (0.78-3.18)	1.94 (0.66-5.68)	1(11.1)	44(12.9)	0.72 (0.11-70.62)	-
Current smoker (Ref 'No')	23(19.8)	29(12.4)	1.74 (0.96-3.18)	1.47 (0.78-2.79)	2(22.2)	50(14.7)	1.66 (0.34-8.16)	-
Smoking duration (years)								
1-15	7(6.0)	9(3.9)	Ref.		0(0.0)	16(4.7)	Ref.	
16-30	13(11.2)	16(6.8)	1.04 (0.31-3.47)	-	2(22.2)	27(7.9)	3.00 (0.14-66.40)	-
>30	3(2.6)	4(1.7)	0.96 (0.16-5.57)	-	0(0.0)	7(2.1)	2.20 (0.04-121.80)	-
Number of cigarettes smoked per day								
1-10	18(15.5)	26(11.1)	Ref.		2(22.2)	42(12.3)	Ref.	
11-20	4(3.5)	2(0.9)	2.88 (0.50-16.88)	-	0(0.0)	6(1.8)	1.31 (0.06-30.41)	-
>20	1(0.9)	1(0.4)	1.44 (0.08-25.0)	-	0(0.0)	2(0.6)	3.40 (0.13-91.58)	-
History of respiratory disease (Ref 'No')	2(1.7)	1(0.4)	4.08 (0.37-45.20)	-	3(33.3)	22(6.5)	7.25 (1.70-30.98)	5.68 (1.10-31.95)*
Used respiratory PPE(Ref 'No')	60(51.7)	127(54.3)	0.90 (0.58-1.39)	-	5(55.6)	182(53.4)	1.09 (0.29-4.07)	-
Type of mask respiratory PPE								
Paper	18(15.1)	35(15.0)	Ref.	-	1(11.1)	52(15.2)	Ref.	-
Particulate filter	0(0.0)	1(0.4)	0.65 (0.02-18.00)	-	0(0.0)	1(0.3)	0.55 (0.01-26.30)	-
Cloth	39(33.6)	85(36.3)	0.89 (0.45-1.76)	-	4(44.4)	120(35.2)	1.73 (0.19-15.50)	-
Longevity in the mushroom plant (Year)								
1-10	78(67.2)	179(76.5)	Ref.		5(55.6)	252(73.9)	Ref.	
11-20	31(26.7)	44(18.8)	1.61 (0.92-2.85)	1.39 (0.79-2.45)	2(22.2)	73(21.4)	1.38 (0.26-7.20)	1.21 (0.21-6.93)
>20	7(6.0)	11(4.7)	1.46 (0.52-4.13)	1.31 (0.47-3.67)	2(22.2)	16(4.7)	6.33 (1.16-34.18)	4.52 (0.73-27.86)
Working hours per day in the mushroom plant (Hours)								
1-4	61(52.6)	135(57.7)	Ref.		3(33.3)	193(56.6)	Ref.	
5-8	19(16.4)	40(17.1)	1.05 (0.57-1.95)	1.01 (0.55-1.85)	2(22.2)	57(16.7)	2.25 (0.37-13.88)	1.92 (0.29-12.71)
>8	5(4.3)	4(1.7)	2.76 (0.72-10.66)	2.08 (0.51-8.43)	2(22.2)	7(2.1)	18.38 (2.65-127.57)	9.64 (0.39-239.22)
Size of plant (m3)								
<50	5(4.3)	5(2.1)	Ref.		0(0.0)	10(2.9)	Ref.	
50-100	36(31.0)	34(14.5)	1.05 (0.27-4.13)	1.11 (0.34-3.66)	2(22.2)	68(19.9)	1.06 (0.28-4.03)	-
100-150	41(35.3)	119 (50.9)	0.34 (0.09-1.28)	1.48 (0.73-3.02)	4(44.4)	156 (45.7)	0.34 (0.09-1.29)	-
>150	34(29.3)	76(32.5)	0.44 (0.12-1.72)	0.56 (0.14-2.19)	3(33.3)	107(31.4)	0.45 (0.12-1.72)	-
>35°C Temp in mushroom plant (Ref ≤35°C)	38(32.8)	52(22.2)	1.70 (1.00-2.90)	1.64 (0.96-2.81)	5(55.6)	85(24.9)	3.75	2.89 (0.70-11.86)
>70% Humidity in mushroom plant (Ref ≤70%)	7(6.0)	23(9.8)	0.58 (0.24-1.43)	-	0(0.0)	30(8.8)	0.54 (0.03-9.46)	-
No. of fungi in mushroom plant (CFU/m3)								
<2,000	83(71.6)	187(80.0)	Ref.		7(77.8)	263(77.1)	Ref.	
2,000-4,000	17(14.7)	13(5.6)	2.94 (1.33-6.57)	15.03 (1.53-147.52)*	1(11.1)	29(8.5)	1.29 (0.15-11.11)	-
>4,000	16(13.8)	34(14.5)	1.06 (0.53-2.10)	1.58 (0.71-3.52)	1(11.1)	49(14.4)	0.76 (0.09-6.59)	-

Note: Variables with p<0.20 in bivariate analysis were entered into the multivariate logistic regression model. Report crude OR with 95% CI for all variables, and adjusted OR with 95% CI only for variables retained in each final model; OR = Odds Ratio; Ref = Reference category. *Statistically significant at p < 0.05. Working hours variable contained missing data (n=86). Cases with missing working hours were excluded only from analyses involving this variable.

These results highlight potential occupational health risks in small-scale mushroom production, a sector that remains largely unregulated and understudied.

The airborne fungal concentrations observed in this study ranged from 515.67 to 5,708 CFU/m^3, which fall within ranges previously reported in agricultural and environmental settings. Airborne fungal concentrations in rural agricultural areas have been reported by Adhikari et al.[17] range from 72 to 1,796 CFU/m^3, while occupational and farming environments have shown levels ranging from 250 to 3,300 CFU/m^3, particularly in areas involving organic material handling.[18] Farming and mushroom cultivation environments are recognized as important sources of airborne fungal contamination due to the presence of decomposing organic substrates and contaminated materials.[19] The concentrations observed in mushroom houses in the present study may be explained by sustained moisture, limited ventilation, and the use of organic substrates that promote fungal growth. Environmental parameters such as temperature and humidity observed in this study further support this explanation, as increased humidity and moderate temperatures have been shown to enhance fungal survival and airborne dispersion.[20] Overall, these findings indicate that the fungal concentrations observed in this study are consistent with previously documented levels in environments characterized by high organic matter and humid conditions.

The association between moderate-to-high fungal concentrations (2,001-4,000 CFU/m^3) and upper respiratory symptoms aligns with prior studies linking fungal exposure to rhinitis, throat irritation, and allergic responses.[20,21] Previous research has demonstrated that exposure to airborne fungi and other bioaerosols is associated with irritation of the upper respiratory tract, allergic rhinitis, and respiratory complaints in occupational settings,[22] while broader epidemiological evidence indicates that fungal bioaerosol exposure can induce allergic and inflammatory responses affecting the nose and throat.[20] Although concentrations above 4,000 CFU/m^3 were also recorded, the stronger association at moderate levels may reflect differential exposure durations or variations in fungal species present, which were not assessed in this study. Previous research has indicated that cumulative exposure and fungal diversity can influence symptom patterns, even when total spore counts are comparable.[21] Future studies using species-level identification could help clarify these relationships. The prevalence of upper respiratory symptoms was highest in the 2,000-4,000 CFU/m^3 exposure group rather than in the >4,000 CFU/m^3 group, suggesting that the association may not follow a simple linear dose-response pattern. This finding should be interpreted cautiously, as subgroup sizes were relatively small and exposure was assessed using area-based measurements at the farm level. Possible explanations include exposure misclassification, heterogeneity in fungal species composition, differences in work practices or ventilation, and the healthy worker effect.

The absence of a statistically significant association between fungal concentrations >4,000 CFU/m^3 and upper respiratory symptoms, despite a significant association observed in the 2,001-4,000 CFU/m^3 range, represents a counterintuitive exposure-response pattern that warrants further consideration. Several alternative explanations may account for this finding. First, the healthy worker effect may have influenced the observed pattern, as workers experiencing severe respiratory symptoms may transfer to lower-exposure areas or leave employment, resulting in a relatively healthier subgroup remaining in higher-exposure settings and attenuating observable associations. Second, differences in PPE use patterns may have modified effective exposure levels, as workers in visibly contaminated environments are more likely to adopt respiratory protective measures, thereby reducing inhaled fungal exposure despite high environmental concentrations. Third, exposure misclassification may have occurred due to reliance on area-based fungal sampling at a single time point, which may not adequately capture temporal variability in fungal concentrations or individual-level exposure differences. Previous studies have highlighted that airborne bioaerosol exposure is highly variable over time and influenced by environmental conditions, work practices, and microbial composition, which may lead to misclassification of exposure levels in occupational studies.[4,23] Additionally, variations in fungal species composition and allergenic potential may influence respiratory outcomes independently of total colony counts, as different fungal taxa exhibit varying pathogenicity and immunogenicity.[4] Another possible explanation is limited statistical power in the highest exposure category, as relatively small subgroup sizes may reduce the ability to detect statistically significant associations. Future studies incorporating repeated measurements, personal sampling strategies, and fungal species identification are recommended to better characterize exposure-response relationships in mushroom cultivation environments.[4,22] Moreover, the relatively small number of workers in the highest exposure category (>4,000 CFU/m^3) may have limited statistical power to detect significant associations.

Older workers (31-60 years) had significantly higher odds of reporting upper respiratory symptoms, which corresponds with literature indicating age-related vulnerability due to immune changes, chronic inflammation, or cumulative occupational exposure.[24] Likewise, workers with a history of respiratory disease were significantly more likely to report lower respiratory symptoms, supporting findings from studies involving agricultural and industrial workers with pre-existing health conditions.[25] These observations emphasize the importance of medical surveillance and early screening for high-risk groups in informal work settings.

The average time spent inside mushroom houses (4.20 hours/day) suggests meaningful exposure duration, though shorter than in some agricultural

contexts. Longer exposure periods have been associated with higher risks of chronic respiratory irritation in previous studies.[5,26] While duration in this study did not independently predict symptoms, time indoors likely contributes cumulatively to exposure intensity, particularly in high-humidity environments where airborne fungal loads can fluctuate throughout the day.

The ventilation characteristics of the mushroom houses also merits consideration. Many structures relied on natural ventilation without mechanical airflow, a practice common across rural agricultural operations. Limited ventilation can trap airborne spores and carbon dioxide, creating a stagnant microenvironment that may exacerbate respiratory irritation.[26] Interventions such as improving air circulation, modifying structural openings, or reducing humidity levels may have measurable effects on reducing airborne fungal loads.

This study contributes important new insights into an occupation rarely examined in Thailand. Mushroom cultivation is largely informal and typically managed within households or small cooperatives, which often lack occupational health training or protective measures. Although PPE such as masks could minimize inhalation exposure, consistent use was not systematically assessed. Previous studies in similar agricultural contexts have shown low compliance with PPE in informal workforces due to discomfort, cost, limited accessibility, and lack of awareness.[27,28]

The study has several strengths. It is one of the first to quantify airborne fungi in mushroom houses in high-altitude rural communities, generating baseline exposure data for a previously undocumented population. The use of environmental measurements alongside worker-reported symptoms strengthens the assessment of exposure-response relationships. The large sample size covering 35 farms also enhances the representativeness of findings for similar rural settings.

Several limitations should be acknowledged. First, the cross-sectional design limits causal interpretation of the observed associations. Respiratory symptoms were self-reported using standardized instruments (ATS-DLD), which may be subject to recall bias. Environmental measurements were based on short-duration, point-in-time sampling and may not fully capture temporal or spatial variability in fungal concentrations within farms, potentially resulting in exposure misclassification. Furthermore, daily working hours could not be consistently reported by some participants due to variable work schedules, which may have introduced additional exposure misclassification. Future studies may benefit from collecting task-specific working durations (e.g., preparation, maintenance, and harvesting phases) to better reflect variability in work activities. In addition, fungal identification was limited to total colony counts without species-level characterization, restricting the ability to differentiate allergenic or pathogenic fungi.

The very low prevalence of lower respiratory symptoms (2.6%, n=9) represents another important limitation. The small number of outcome events may have reduced statistical power and contributed to the wide confidence interval observed in the multivariate analysis (aOR=5.68, 95% CI: 1.10-31.95), indicating uncertainty in the effect estimates.[29] These findings should therefore be interpreted with caution. Firth's penalized logistic regression was not applied; therefore, standard logistic regression may produce biased estimates due to the small number of lower respiratory symptom events.

Additional factors such as other environmental exposures (e.g., dust or ventilation patterns) and potential intra-farm similarity among workers may also have influenced the results but were not explicitly modeled. In addition, the clustered sampling design may have introduced intra-farm correlation among participants. However, design effect adjustment was not applied during sample size calculation or analysis, which may have influenced the precision of the estimates. Data collection was conducted between 2017 and 2018, and although delays in analysis occurred due to COVID-19-related disruptions, working conditions in mushroom cultivation environments have remained relatively stable, supporting the continued relevance of these findings.

Despite these limitations, the findings suggest potential occupational respiratory risks among mushroom workers and highlight the importance of preventive measures such as improved ventilation and consistent PPE use. Future research should employ longitudinal designs, incorporate spirometry assessment, and include fungal species identification to strengthen causal inference and exposure characterization.

Conclusion

These findings suggest a potential relationship between environmental fungal exposure and respiratory symptoms among mushroom workers. However, given the cross-sectional design and the limited number of lower respiratory symptom cases observed in this study, the results should be interpreted with caution. Further longitudinal studies with larger sample sizes are needed to confirm these findings and to better inform targeted health monitoring and preventive strategies. These findings should therefore be considered preliminary and interpreted within the context of the study limitations.

Acknowledgement: The authors would like to thank all mushroom workers who voluntarily participated in this study.

Individual Authors' Contributions: PY and **RC** were responsible for the conceptualization and study design. Data collection was conducted by **PY** and **PB**. Data analysis and interpretation were carried out by **PY**, **PB**, and **CPP**. **CPP** drafted the manuscript. All authors contributed to the critical revision of the manuscript and

provided final approval of the manuscript before submission.

Availability of Data: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Non-use of Generative AI Tools: This article was prepared without the use of generative AI tools for content creation, analysis, or data generation. All findings and interpretations are based solely on the authors' independent work and expertise.

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