MINISTRY OF EDUCATION AND TRAINING MINISTRY OF HEALTH
NATIONAL INSTITUTE OF MALARIOLOGY, PARASITOLOGY AND ENTOMOLOGY

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SOME CHARACTERISTICS OF FUNGAL CONTAMINATION AND MYCOTOXINS IN TRADITIONAL MEDICINAL MATERIALS AND THE OUTCOMES OF AN INTERVENTION AT NGHE AN PROVINCIAL TRADITIONAL MEDICINE HOSPITAL (2023–2025)

Major : Epidemiology

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SUMMARY OF THE DOCTORAL THESIS IN MEDICINE

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The thesis will be defended before the Institute-level Thesis Examination Committee convened at the National Institute of Malariology, Parasitology and Entomology at *8:30 a.m. on July 29, 2026.*

The thesis is available at:
- National Library of Vietnam
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INTRODUCTION

Traditional medicinal herbs comprise Vietnamese medicinal herbs and Chinese medicinal herbs, the former originating in Vietnam and the latter originating from China. These materials are commonly processed by sun-drying, oven-drying, or treatment with sugar, honey, and other excipients. Under Vietnam's hot and humid tropical climate, together with inadequate storage, transportation, and distribution conditions resulting from limited preservation facilities and techniques, traditional medicinal materials are highly susceptible to mold growth caused by fungal species such as *Aspergillus flavus and Aspergillus niger*.

Parasitic fungi contaminating traditional medicinal materials produce aflatoxins, including aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). In 1988, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommended that aflatoxins should be strictly controlled in agricultural commodities and post-harvest products. Countries are encouraged to establish comprehensive regulatory frameworks for monitoring aflatoxin levels and to develop reliable techniques for the detection of fungal contamination and mycotoxins throughout the production, storage, and distribution chain in order to protect public health. In Vietnam, epidemiological studies on fungal contamination of traditional medicinal materials remain limited. In Nghe An Province, Dau Huy Hoan (2018) investigated the prevalence of fungal contamination and aflatoxin B1 (AFB1) in traditional medicinal materials. However, under the hot and humid climatic conditions and the inadequate storage facilities commonly encountered in Vietnam, several important questions remain unanswered: What is the prevalence of fungal contamination in traditional medicinal materials? What is the prevalence of aflatoxin contamination? In addition to aflatoxin B1, what are the contamination rates of aflatoxin B2, aflatoxin G1, and aflatoxin G2, given the persistent risk factors for fungal growth and mycotoxin production? To address these gaps and provide scientific evidence for the prevention and control of fungal and mycotoxin contamination in traditional medicinal materials, we conducted the study entitled *"Some Characteristics of Fungal Contamination and Mycotoxins in Traditional Medicinal Materials and the Outcomes of an Intervention at Nghe An Provincial Traditional Medicine Hospital (2023–2025)"* with the following objectives:

1. To determine the prevalence and species composition of fungal contamination in selected traditional medicinal materials at Nghe An Traditional Medicine Hospital during (2023–2025).

2. To analyze the prevalence, composition, and concentrations of aflatoxins, including aflatoxin B_1 (AFB1), aflatoxin B_2 (AFB2), aflatoxin G_1 (AFG1), and aflatoxin G_2 (AFG2), in traditional medicinal material samples collected at Nghe An Traditional Medicine Hospital during (2023–2025).

3. To evaluate the outcomes of an intervention through improving the microclimatic conditions in the traditional medicinal materials storage facility.

SCIENTIFIC MERIT, NOVELTY, AND RELIABILITY OF THE THESIS

The thesis employed contemporary scientific research methods that are widely accepted in Vietnam and internationally, including descriptive epidemiological research and laboratory-based experimental investigations. Fungal species were identified using both morphological and molecular techniques, while aflatoxins were quantified by high-performance liquid chromatography (HPLC). Data were analyzed using validated statistical software packages, including Stata, Epi Info, and SPSS, under the supervision of experienced researchers. The rigorous study design, standardized laboratory procedures, and appropriate statistical analyses ensured the validity and reliability of the study findings.

THESIS STRUCTURE

The thesis comprises 126 pages, including: Introduction (2 pages), Literature Review (32 pages), Research Methods (24 pages), Results (35 pages), Discussion (30 pages), Conclusions (2 pages), and Recommendations (1 page). The thesis contains 43 tables, including 38 tables presenting the study results, 18 figures, and 137 references, of which 37 were published within the last five years.

Chapter 1: LITERATURE REVIEW

1.1. Basic Concepts and Research History of Fungi

Concept of fungi: Fungi are eukaryotic organisms with true nuclei and well-defined cell walls. They are heterotrophic, reproduce by spores, and are widely distributed in nature. Most fungi are saprophytic and inhabit soil, whereas a relatively small proportion are parasitic and capable of causing diseases in plants and animals. Fungi constitute one of the five major kingdoms of life on Earth.

Research history of Aspergillus spp.: *Aspergillus* spp. was first recognized as a medically important fungus in 1729 by the Italian priest and biologist Pier Antonio Micheli. *Aspergillus* spp. comprises approximately 700 species, of which 19 have been reported to cause human disease. The most common pathogenic species include *Aspergillus* flavus, *Aspergillus* moninus, and others. *Aspergillus* spp., together with several other fungal species, plays an important role in the production of aflatoxins.

1.2. Epidemiological Characteristics of Fungal Contamination in Traditional Chinese Medicinal Materials

1.2.1. Fungal Agents

The most common fungal agents are *Aspergillus flavus*. In storage facilities, the predominant fungi are *Aspergillus* spp. and *Penicillium* spp., whereas *Fusarium* spp. and *Trichothecenes* spp. are commonly found in the field. *Aspergillus* spp. has the greatest capacity to produce toxins among medically important fungi, with aflatoxins accounting for up to 90% of the toxins produced by these fungi.

1.2.2. Routes of Fungal Transmission

In nature, fungal spores are dispersed through the air by wind. Under favorable conditions, such as suitable temperature, humidity, and nutrient availability, the spores develop into yeast cells or fungal hyphae.

1.2.3. Susceptible Population

Fungal spores are dispersed through the air by wind. When they come into

contact with cereals, food products, and post-harvest products under favorable conditions, they develop into fungal hyphae and colonies and subsequently produce aflatoxins that contaminate food products.

1.2.4. Factors Associated with Fungal Contamination

Several studies conducted in Vietnam and other countries have investigated factors associated with fungal contamination. These studies have concluded that the combination of three microclimatic factors, namely high temperature, high humidity, and low ventilation rate, provides favorable conditions for fungal growth. Anima J. Sirma et al. (2015) investigated aflatoxin contamination in cereals and the knowledge, attitudes, and practices regarding fungal contamination prevention among households in Kenya. A total of 408 food samples were analyzed using the ELISA technique, and household interviews were conducted. The results showed that 77.9% of maize samples and 92.9% of millet samples were contaminated with aflatoxins.

1.2.5. Fungal Contamination and Aflatoxin Contamination in Foods, Traditional Chinese Medicinal Materials, and Post-harvest Agricultural Products

According to the Food and Agriculture Organization (FAO), approximately 25% of cereals intended for human consumption are contaminated with mycotoxins, among which aflatoxin B1 is the most prevalent, accounting for approximately 75% of fungal toxins. The incidence of mycotoxin contamination has shown an increasing trend worldwide. In Europe and the United States, the annual economic losses attributable to mycotoxin contamination are estimated to reach approximately USD 100 million.

1.3.2. In Asia and Africa

In Africa and Asia, mycotoxin contamination poses serious public health and economic challenges. In 2022, Korean researchers identified the presence of aflatoxins, alternariol, alternariol monomethyl ether, ochratoxin A, fumonisins, and other mycotoxins in raw materials, intermediate products, and final products. A study conducted in five African countries across four ecological zones showed that 76% of maize samples, 64% of millet samples, and 60% of rice samples were positive for aflatoxin B1. Among these, aflatoxin B1 concentrations exceeded the permissible limits in 26% of maize samples, 10% of millet samples, and 11% of rice samples.

1.3.4. In Vietnam

In Vietnam, numerous studies have investigated mycotoxin contamination. Nguyen Thi Cam Vy (2010) reported that more than 40 samples of oilseeds and related products were contaminated with aflatoxins. In particular, the aflatoxin concentration in peanuts was 263 times higher than the national permissible limit, while peanut candy exceeded the national standard by 138-fold. Dau Huy Hoan (2018) analyzed 505 traditional medicinal material samples collected from hospitals in Nghe An Province and reported an overall fungal contamination rate of 51.8% based on culture on Sabouraud medium. The contamination rate was 45.53% in Chinese medicinal materials and 72.8% in Vietnamese medicinal materials. Filamentous fungi accounted for 92.0% of the isolates, whereas yeasts accounted for

8.0%. High-performance liquid chromatography showed an overall aflatoxin contamination rate of 4.75% (24/505). Among the 24 traditional Chinese medicinal material samples contaminated with aflatoxins, 66.66% (16/24) exceeded the maximum permissible limits specified in QCVN 8-1:2011/BYT.

1.4. Techniques for the Detection of Fungal Contamination and Aflatoxins

1.4.1. Techniques for the Detection of Fungal Contamination

Direct microscopic examination: Direct microscopic examination is performed using 20% KOH or normal saline. The specimen is examined under a ×40 objective lens to identify characteristic fungal hyphae. *Aspergillus* spp. is characterized by short, septate hyphae with dichotomous branching at an angle of approximately 45°, together with characteristic conidial heads.

Fungal culture on Sabouraud medium: Sabouraud medium is the most commonly used culture medium for fungal isolation. The medium has a pH of <5.5 and is commonly supplemented with antibiotics during culture. To date, fungal culture on Sabouraud medium remains the gold standard for the diagnosis of fungal contamination.

Polymerase chain reaction (PCR): Margit Hummel et al. (2004) used nested PCR to diagnose invasive A. *fumigatus* (ATCC 9197) infection in mice. Their study laid the foundation for the subsequent application of nested PCR for the detection of A. *fumigatus* DNA by other investigators, including L. Klingspor (2006), Loeffler, and White P.L. (2006).

1.4.3. Techniques for the Detection of Aflatoxins

The detection of fungal toxins in food indicates "*fungal contamination*". Previous studies have used high-performance liquid chromatography (HPLC) to determine aflatoxin contamination.

1.5. Prevention of Fungal and Aflatoxin Contamination in Traditional Chinese Medicinal Materials

1.5.1. Regulatory Measures

Strict monitoring of mycotoxin levels, particularly aflatoxins B1, B2, G1, and G2, in food products and surveillance of food storage environments are essential. In Vietnam, the National Technical Regulation on the Limits of Mycotoxin Contamination in Foods (QCVN 8-1:2011/BYT) has been promulgated. Many countries have also established their own national regulations to control mycotoxin contamination in food.

1.5.2. Technical Measures

Technical measures aim to create unfavorable conditions for fungal growth. Storage facilities should maintain appropriate microclimatic conditions, including a temperature below 25°C, relative humidity below 70%, and an air velocity of 0.5–1.0 m/s. Chemical preservatives may also be used. Nguyen Thi Hai (2017) investigated the reduction of aflatoxin contamination in food using sorbic acid combined with moist heat treatment of agricultural products. The results showed reductions of 90.8% for aflatoxin B1, 84.1% for aflatoxin B2, and 89.21% for aflatoxins G1 and G2.

1.5.3. Studies on Fungi and Their Harmful Effects on Traditional

Chinese Medicinal Materials at Nghe An Traditional Medicine Hospital

In Nghe An Province, studies on fungal contamination in food and traditional medicinal materials remain limited. In 2018, Dau Huy Hoan analyzed 505 traditional medicinal material samples collected from hospitals in Nghe An Province and reported an overall fungal contamination rate of 51.8% based on culture on Sabouraud medium. The contamination rate was 45.53% in Chinese medicinal materials and 72.8% in Vietnamese medicinal materials. Filamentous fungi accounted for 92.0% of the isolates, whereas yeasts accounted for 8.0%. The overall aflatoxin contamination rate was 4.75% (24/505). Among the 24 traditional medicinal material samples contaminated with aflatoxins, 66.66% (16/24) exceeded the maximum permissible limits specified in QCVN 8-1:2011/BYT.

Chapter 2: RESEARCH METHODS

2.1. Research Methods for Objective 1: *Determining the Prevalence and Species Composition of Fungal Contamination in Selected Traditional Medicinal Material Samples at Nghe An Traditional Medicine Hospital in 2023*

2.1.1. Research Subjects

- Vietnamese medicinal material samples and Chinese medicinal material samples.
- Fungal isolates cultured from traditional medicinal materials.

2.1.2. Research Sites

- Cross-sectional survey: Nghe An Traditional Medicine Hospital.

- Fungal species identification: High-Technology Laboratory, Department of Parasitology and Entomology, Vietnam Military Medical Academy.

2.1.3. Research Period

From January 2023 to December 2024.

2.1.4. Research Design

Cross-sectional descriptive study.

2.1.5. Sample Size and Sampling Method

- Sample size: The minimum sample size was calculated using the formula for estimating the prevalence of fungal contamination in traditional medicinal material samples.

$$n = Z^2_{1-\alpha/2} \frac{1-p}{p\varepsilon^2}$$

Where: p: Prevalence of fungal contamination in traditional medicinal material samples; p = 0.51, according to Dau Huy Hoan (2018). $Z_{1-\alpha/2}$: Standard normal deviate corresponding to a 95% confidence level ($Z_{1-\alpha/2}$ = 1.96). ε: Desired relative precision, set at 0.15. Based on these parameters, the calculated sample size was 165. In this study, a total of 170 traditional medicinal material samples were included.

- Sampling Method:

Based on the list of traditional medicinal materials available at Nghe An Traditional Medicine Hospital, there were 171 traditional medicinal material samples in the hospital storage facility at the time of the study, including 81

Vietnamese medicinal material samples and 90 Chinese medicinal material samples. With a calculated sample size of 170, and because one Vietnamese medicinal material sample was no longer available at the time of sampling, all remaining Vietnamese and Chinese medicinal material samples were selected, totaling 170 samples.

+ Sample selection for PCR amplification and gene sequencing

Based on fungal-positive samples cultured on Sabouraud medium, PCR and gene sequencing were performed using ITS1, ITS4, ITS5, and NL4 primers to determine the prevalence and species composition of fungi in traditional medicinal material samples.

2.1.6. Research Contents

Traditional medicinal material samples and accompanying information were collected. Fungi were isolated from traditional Chinese medicinal material samples. The prevalence of fungal contamination was determined. Fungal species composition in traditional medicinal material samples was identified using morphological and molecular biological methods. Factors associated with fungal contamination were analyzed and determined.

2.1.7. Research Variables

Prevalence of fungal contamination determined by culture on Sabouraud medium supplemented with antibiotics at pH <5.5. Prevalence and species composition of fungi identified by morphological methods. Prevalence and species composition of fungi identified by molecular biological methods.

2.1.8. Techniques Used in the Research

- ***Fungal culture on Sabouraud medium***: Fungal culture was performed on Sabouraud medium (pH <4.5) supplemented with antibiotics and manufactured by Merck (Germany). The culture procedure was as follows:

- ***Methylene blue staining***: Fungal hyphae and spores were stained with Methylene blue for morphological identification.

- PCR technique

PCR primers: The forward primer ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and the reverse primer NL4 (5′-GGT CCG TGT TTC AAG ACG G-3′) were selected for PCR amplification and gene sequencing according to Irobi J. et al. (1999).

- Gene sequencing and fungal species identification

PCR product purification for sequencing: The remaining PCR products were purified using the GeneJET PCR Purification Kit (Cat. #K0701, Thermo Scientific).

Gene sequencing: PCR products showing a single, distinct band of the expected size (1,000–1,200 bp) were sent to First BASE Laboratories Sdn. Bhd. (Kembangan 43300, Selangor, Malaysia) for direct sequencing using the ITS5 and NL4 primers.

- Fungal species identification based on gene sequence analysis

The sequences obtained from the two primers were assembled and edited. The final consensus sequence was compared with sequences in the GenBank

database for fungal species identification using the BLAST tool available at http://blast.ncbi.nlm.nih.gov.

2.1.9. Research Indicators

Culture results on Sabouraud medium. Results of PCR-based fungal species identification. Overall prevalence of fungal contamination, prevalence by fungal group, prevalence by fungal species, and prevalence of fungal contamination in traditional medicinal material samples.

2.2. Research Methods for Objective 2: *Analysis of the Prevalence, Composition, and Concentrations of Aflatoxins B_1, B_2, G_1, and G_2 in Traditional Medicinal Material Samples at Nghe An Traditional Medicine Hospital in 2023*

2.2.1. Research Subjects

Collected traditional medicinal material samples.

2.2.2. Research Sites

- Laboratory Department, Nghe An Traditional Medicine Hospital.
- National Institute of Drug Quality Control.

2.2.3. Research Period

From January 2023 to December 2024.

2.2.4. Research Design

Laboratory-based descriptive experimental study.

2.2.5. Sample Size

All traditional medicinal material samples collected under Objective 1.

2.2.6. Research Contents

The research determined the prevalence of aflatoxin contamination in traditional medicinal material samples using high-performance liquid chromatography, as well as the proportion of aflatoxin-positive samples exceeding the permissible limits specified in QCVN 8-1:2011/BYT.

2.2.7. Research Variables

The research variables for Objective 2 included: overall prevalence of aflatoxin contamination (%); prevalence of contamination with each type of aflatoxin (%); prevalence of contamination with one, two, or three types of aflatoxins; prevalence of aflatoxin contamination at concentrations ranging from 0.5 ppb to <20 ppb/g (%); and the proportion of samples in which aflatoxins B_1, B_2, G_1, and G_2 exceeded the permissible limits.

2.2.8. Techniques Used in the Research

- Procedure for Quantitative Determination of Aflatoxins (B_1, B_2, G_1, G_2)

In this study, high-performance liquid chromatography (HPLC) was used for the quantitative determination of aflatoxins according to AOAC Official Method 990.33.

Calculation of results: A calibration curve equation, $y = ax + b$, was established, with the concentration of aflatoxins $B_1/B_2/G_1/ G_2$ (ng/mL) plotted on the x-axis and the response signal (S) plotted on the y-axis. The concentrations of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2 (C) in the test solution were calculated using the following formula:

$$C\ (ng/ml) = \frac{\mathrm{S} - \mathrm{b}}{a}$$

The contents of aflatoxins B_1, B_2, G_1, and G_2 in the medicinal materials, expressed as ng/g, were calculated using the following formula:

$$X\ (ng/g) = \frac{\mathrm{V1}\ \times \mathrm{V2} \times \mathrm{C}}{m\ \times Vi}$$

Where: m = mass of the medicinal material sample used for analysis (g); V1 = volume of extraction solvent used (mL); Vi = volume of the extract purified using the immunoaffinity chromatography column (mL); V2 = final volume of the solution after elution from the immunoaffinity chromatography column and dilution (mL); C = aflatoxin concentration measured in the test solution (ng/mL).

- Interpretation of results: Aflatoxin B1 contamination was evaluated according to the National Technical Regulation QCVN 8-1:2011/BYT [102], as follows:

Table 2.3. Maximum Limits of Aflatoxin Contamination in Selected Food Products According to QCVN 8-1:2011/BYT [112]

Food Product	**Permissible Limit (mg/kg)**	
	aflatoxin B1	Total aflatoxins
Peanuts and oilseeds - Intended for further processing before consumption - Ready for direct consumption	8 2	15 4
Almonds, pistachios, etc. - Intended for further processing before consumption - Ready for direct consumption	12 8	15 10
Hazelnuts - Intended for further processing before consumption - Ready for direct consumption	12 8	15 10
Almonds - Intended for further processing before consumption - Ready for direct consumption	8 5	15 10
Maize and rice intended for further processing before consumption	5	10
Spices	5	10
Cereal-based foods	0.1	1

2.2.9. Research Indicators

Overall prevalence of aflatoxin contamination in traditional medicinal material samples (%); proportion of samples with aflatoxin concentrations >0.5 ppb/g (%); proportion of samples with aflatoxin concentrations ranging from 0.5 to <20 ppb/g (%); proportion of samples with aflatoxin concentrations >20 ppb/g (%); and the mean aflatoxin concentration in traditional medicinal material samples

contaminated with aflatoxins.

2.3. Research Methods for Objective 3: *Evaluation of the Effectiveness of the Intervention Through Improvement of the Microclimatic Conditions in the Traditional Medicinal Material Storage Facility*

2.3.1. Research Subjects

Equipment and instruments used for medicine storage in the two storage facilities; microclimatic factors such as temperature, humidity, and air velocity; and the prevalence of fungal contamination after the intervention in medicine samples included in the list of medicines covered by health insurance.

2.3.2. Research Sites

Nghe An Traditional Medicine Hospital.

2.3.3. Research Period

From January 2024 to October 2024.

2.3.4. Research Design

Descriptive study.

2.3.5. Sample Size and Sampling Method

- Sample size: Two medicine storage facilities at Nghe An Traditional Medicine Hospital; description of the prevalence of fungal contamination after the intervention to improve microclimatic conditions.

- Sampling method:

All two traditional medicinal material storage facilities were selected.

Selection of traditional medicinal material samples: Based on the list of traditional medicinal materials covered by health insurance at Nghe An Traditional Medicine Hospital. The calculated sample size was 170, and the ratio of the number of available medicinal material samples in the hospital to the calculated sample size was 1:1. Therefore, all 170 traditional medicinal material samples, including both traditional Vietnamese medicinal materials and traditional Chinese medicinal materials, were included in the study.

2.3.6. Research Content

Research on the prevalence of fungal contamination by fungal culture on Sabouraud medium supplemented with antibiotics at pH 4.5; overall prevalence of fungal contamination and prevalence of fungal contamination in each medicinal material. Evaluation of the effectiveness of the intervention in reducing the prevalence of fungal contamination in traditional medicinal materials after the intervention.

2.3.7. Research Variables

Fungal contamination and fungal species composition in traditional Chinese medicinal materials and traditional Vietnamese medicinal materials determined by morphological techniques; mean relative humidity in the medicine storage facility; mean storage temperature; and air velocity.

2.3.8. Research Techniques

Morphological diagnosis of fungal contamination: Fungal culture on Sabouraud medium as described under Objective 1.

2.3.9. Research Indicators

Overall prevalence of fungal contamination among the 170 traditional medicinal material samples before and after the intervention (%); prevalence of fungal contamination in traditional Chinese medicinal materials and traditional Vietnamese medicinal materials before and after the intervention.

2.4. Data Processing and Statistical Analysis

Data were entered and analyzed using Epi Info 6.04 and Stata 20.0 software.

2.5. Sources of Error and Error Control

Compliance with the study inclusion and sample selection criteria; data cleaning prior to analysis; and use of appropriate statistical software and analytical methods for each type of variable.

2.6. Research Ethics

The research samples were randomly collected in accordance with the study procedures. The research protocol was approved by the Ethics Committee of the National Institute of Malariology, Parasitology and Entomology.

Chapter 3: RESEARCH RESULTS

3.1. Prevalence and Species Composition of Fungal Contamination in Selected Traditional Medicinal Materials at Nghe An Traditional Medicine Hospital in 2023

3.1.1. Prevalence of Fungal Contamination in Selected Traditional Medicinal Materials

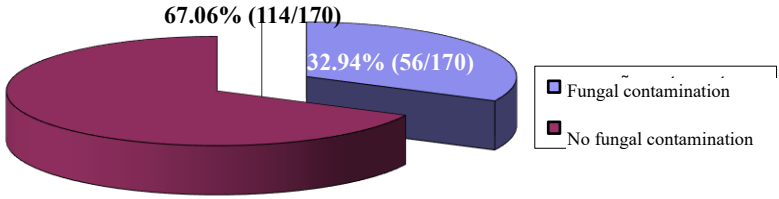

Figure 3.1. Prevalence of Fungal Contamination in Traditional Medicinal Material Samples (n = 170)

The prevalence of fungal contamination in traditional medicinal material samples at Nghe An Traditional Medicine Hospital was 32.94%.

Table 3.2. Prevalence of Fungal Contamination in Traditional Medicinal Material Samples (n = 170)

Origin of medicinal material samples	No. of Samples Tested	No. of positive samples	Prevalence (%)
Traditional Chinese medicinal materials (1)	89	33	37.10
Traditional Vietnamese medicinal materials (2)	81	23	28.40
Total	170	56	32.94

There was no statistically significant difference in the prevalence of fungal contamination between traditional Vietnamese medicinal materials and traditional Chinese medicinal materials ($p > 0.05$).

Table 3.3. Prevalence of Fungal Contamination in Traditional Medicinal Material Samples Derived from Roots, Stems, Leaves, Rhizomes, and Fruits (n = 170)

Origin of Medicinal Material Samples	No. of Samples Tested	No. of Fungal-Positive Samples	Prevalence (%)
Root (1)	67	27	40.30
Stem (2)	38	9	23.68
Branches, Leaves, Flowers, Fruits and Seeds (3)	53	17	32.07
Others (4)	12	3	25.00
Total	170	56	32.94

The highest prevalence of fungal contamination was observed in root-derived medicinal materials (40.30%), while the lowest prevalence was found in stem-derived medicinal materials (23.68%).

3.1.2. Identification of Fungal Species Composition

- Identification by Morphological Characteristics

Table 3.4. Number of Fungal Isolates with Distinct Morphological Characteristics Identified by Morphological Examination (n = 56)

No. of Fungal Species Detected	No. of Positive Samples	Prevalence (%)
1	50	89.29
2	5	8.93
3	1	1.79
Total	56	100.0

Among the 56 fungal-positive samples, 50 (89.29%) were contaminated with a single fungal species, 5 (8.93%) were contaminated with two fungal species, and 1 (1.79%) was contaminated with three fungal species.

Table 3.5. Identification of Fungal Genera Isolated from Traditional Medicinal Material Samples Based on Morphological Characteristics (n = 56)

Fungal Species	Single-Species Contamination	Two-Species Contamination	Three-Species Contamination
Aspergillus	18		
Rhizopus	16	2	1
Penicillium	9	0	0
Fusarium	4	0	0
Cladosporium	1	0	0
Phoma spp	1	0	0
Aspergillus+Penicillium	1	3	0
Total Number of Contaminated Samples	50	5	1

Among the 56 fungal-contaminated samples, 50 were contaminated with a

single fungal species, 5 with two fungal species, and 1 with three fungal species, yielding a total of 63 fungal isolates. Of these, Aspergillus accounted for 28 isolates (44.44%), Rhizopus for 16 isolates (25.40%), and Penicillium for 13 isolates (20.63%).

Identification of Fungal Species by Molecular Biological Techniques

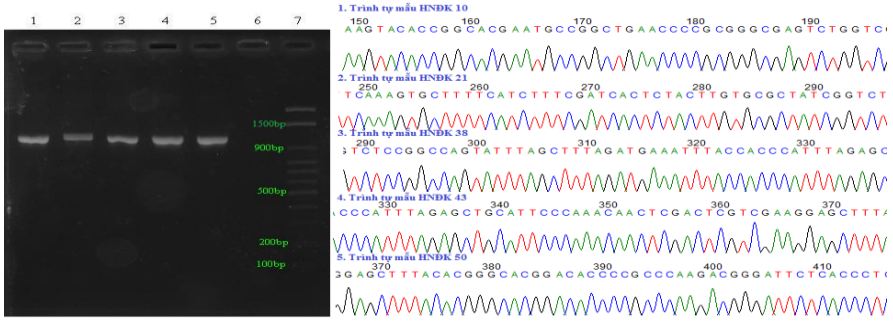

Figure 3.7. Agarose Gel Electrophoresis and DNA Sequencing Results

The obtained DNA sequences were subsequently submitted to the GenBank database and assigned accession numbers.

Table 3.7. Distribution of Aspergillus Species Identified Among Aspergillus Isolates (n = 28)

Fungal Species	No. of Isolates	Prevalence (%)	
		Among *Aspergillus* Isolates (n=28)	Among All Fungal Isolates (n=63)
Aspergillus niger	10	35.72	15.87
Aspergillus flavus	05	17.86	7.94
Aspergillus chevalieri	05	17.86	7.94
Aspergillus terreus	02	7.14	3.17
Aspergillus sydowii	02	7.14	3.17
Aspergillus fijiensis	01	3.57	1.59
Aspergillus restrictus	01	3.57	1.59
Aspergillus neotritici	01	3.57	1.59
Aspergillus spp.	01	3.57	1.59
Total	**28**	**100**	**44.44**

Aspergillus accounted for 44.44% of all fungal isolates. Eight Aspergillus species were identified, of which *Aspergillus niger* was the most prevalent, accounting for 35.72% (10/28) of the Aspergillus isolates.

Table 3.10. Prevalence of Aspergillus Contamination in Traditional Chinese and Traditional Vietnamese Medicinal Material Samples

Medicinal Material	No. of Samples Tested	No. of Aspergillus-Positive Samples	Prevalence (%)	p
Traditional Chinese medicinal materials	89	16	17.98	0.18

Traditional Vietnamese medicinal materials	81	8	9.88	5
Total	170	24	14.12	

The prevalence of *Aspergillus* contamination in traditional medicinal material samples was 14.12% (24/170).

3.2. Prevalence of Aflatoxin Contamination, Composition, and Concentrations of Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, and Aflatoxin G_2 in Traditional Medicinal Material Samples at Nghe An Traditional Medicine Hospital

3.2.1. Prevalence of Aflatoxin Contamination

Table 3.15. Distribution of Aflatoxin Contamination According to the Number of Aflatoxin Types Detected in Traditional Medicinal Material Samples (n = 16)

Number of Aflatoxin Types Detected	No. of Samples	Prevalence (%)
1	9	56.25
2	5	31.25
3	2	12.50
Total	16	100

Among the 16 aflatoxin-contaminated samples, 9 (56.25%) were contaminated with a single type of aflatoxin, 5 (31.25%) with two types, and 2 (12.50%) with three types. A total of 25 aflatoxin detections were recorded.

Table 3.16. Prevalence of Individual Aflatoxin Types (Aflatoxin G_1, G_2, B_1, and B_2) in Traditional Medicinal Material Samples (n = 170)

Type of Aflatoxin Detected	Aflatoxin Contamination Status		
	No.of Samples Tested	No.of Positive Samples	Percentage (%)
No. of Samples Positive for Aflatoxin G1 (1)	170	6	3.53
No. of Samples Positive for Aflatoxin G2 (2)	170	3	1.76
No. of Samples Positive for Aflatoxin B1 (3)	170	4	2.35
No. of Samples Positive for Aflatoxin B2 (4)	170	12	7.06
Total	170	25	14.71

The overall prevalence of aflatoxin detections was 14.71% (25/170). Among the four aflatoxin types, aflatoxin B2 was the most frequently detected, with a prevalence of 7.06% (12/170).

Table 3.17. Distribution of Individual Aflatoxin Types (Aflatoxin G1, G2, B1, and B2) Among Aflatoxin Detections (n = 25)

Type of Aflatoxin Detected	Aflatoxin Contamination Status		
	No. of Positive Detections	Percentage (%)	***p value***
No. of Samples Positive for	6	24.0	

Aflatoxin G1 (1)			0.047
No. of Samples Positive for Aflatoxin G2 (2)	3	12.0	
No. of Samples Positive for Aflatoxin B1 (3)	4	16.0	
No. of Samples Positive for Aflatoxin B2 (4)	12	48.0	
Total	25	100	

The prevalence of aflatoxin B2 contamination was significantly higher than that of aflatoxins G1, G2, and B1 ($p < 0.05$).

3.2.3. Association Between Fungal Contamination and Aflatoxin Contamination

Table 3.20. Association Between Fungal Contamination and Aflatoxin Contamination

Fungal Contamination	*Aflatoxin Contamination Status*			OR (CI 95%)	p Value
	Aflatoxin -Positive	Aflatoxin -Negative	Total		
Fungal contamination present	11	45	56	5.165 (1.69-15.70)	0.004
No fungal contamination	5	109	114		
Total	16	114	170		

There was a significant association between fungal contamination and aflatoxin contamination in traditional medicinal material samples (OR = 5.165, 95% CI: 1.69–15.70, $p < 0.01$).

Table 3.23. Association Between Fungal Contamination and Aflatoxin Contamination in Traditional Vietnamese and Traditional Chinese Medicinal Material Samples

Fungal Contamination	*Aflatoxin Contamination Status*			OR (CI 95%)	p - value
	Fungal contamination present	No fungal contamination	Total		
Traditional Chinese Medicinal Material	14	75	89	7.373 (1.621-33.542)	0.003
Traditional Vietnamese Medicinal Material	2	79	81		
Total	**16**	**154**	**170**		

There was a significant association between fungal contamination and aflatoxin contamination in traditional Chinese medicinal material samples (OR = 7.373, 95% CI: 1.621–33.542, $p < 0.01$).

3.2.5. Proportion of Traditional Medicinal Material Samples with Aflatoxin Levels Exceeding the Limits Specified in QCVN 8-1:2011/BYT

High-performance liquid chromatography analysis of 170 traditional medicinal material samples identified a total of 25 aflatoxin-positive detections.

Table 3.30. Proportion of Traditional Medicinal Material Samples with Aflatoxin Levels Exceeding the Limits Specified in QCVN 8-1:2011/BYT (n = 16)

Aflatoxin Contamination Status					
No. of Samples with Aflatoxin Levels < 0.5 ppb	Percentage (%)	No. of Samples with Aflatoxin Levels ≥ 0.5 ppb	Percentage (%)	No. of Samples with Aflatoxin Levels ≥ 30 ppb	Percentage (%)
11	44.0	14	56.0	0	0.0

Among the aflatoxin-contaminated traditional medicinal material samples, 56.0% had aflatoxin levels ≥ 0.5 ppb, while none had aflatoxin levels ≥ 30 ppb (0.0%).

3.3. Results of the Intervention Through Improvement of the Microclimatic Conditions in the Traditional Medicinal Material Storage Facility

3.3.1. Medicine Storage Equipment and Microclimatic Conditions Before and After the Intervention

Table 3.34. Changes in the Microclimatic Conditions of Storage Facility No. 1 After 12 Months

Time of Assessment	Storage Facility Characteristics and Medicine Storage Equipment	Mean Value	Recommended Standard	Compliance
Before the Intervention	Mean temperature at five locations (the four corners and the center of the storage facility) (°C)	27ºC±3.1ºC	< 25ºC	Not compliant
	Relative humidity (%)	75.91%±4.6 %	< 70%	Not compliant
	Mean air velocity at five locations (the four corners and the center of the storage facility) (m/s)	0.45 m/s ±0.05m/s	0.5 -1.0m/s	Not compliant
12 Months After the Intervention	Mean temperature at five locations (the four corners and the center of the storage facility) (°C)	23.5ºC±1.5º C	< 25ºC	Compliant
	Relative humidity (%)	65.91%±3.6 %	< 70%	Compliant
	Mean air velocity at five locations (the four corners and the center of the storage facility) (m/s)	0.75 m/s	0.5 -1.0 m/s	Compliant

Before the intervention, the mean temperature in Storage Facility No. 1 was

27.0 ± 3.1°C (>25°C), the mean relative humidity was 75.91 ± 4.6% (>70%), and the air velocity was 0.45 m/s (<0.5 m/s), indicating that the storage conditions did not meet the recommended requirements for medicinal material storage.

Twelve months after the intervention, the mean temperature measured at the four corners and the center of the storage facility was 23.5 ± 1.5°C (<25°C), the mean relative humidity was 65.91 ± 3.6% (<70%), and the air velocity was 0.75 m/s (within the recommended range of 0.5–1.0 m/s). These conditions met the recommended requirements for medicinal material storage.

Table 3.35. Changes in the Microclimatic Conditions of Storage Facility No. 2 After 12 Months

Time of Assessment	Storage Facility Characteristics and Medicine Storage Equipment	Mean Value	Recommended Standard	Compliance
Before the Intervention	Mean temperature at five locations (the four corners and the center of the storage facility) (°C)	26ºC±4.1ºC	<25ºC	Not compliant
	Relative humidity (%)	(77.15±3.06) %	< 70%	Not compliant
	Mean air velocity at five locations (the four corners and the center of the storage facility) (m/s)	(0.42 ± 0.03) m/s	0.5 -1.0m/s	Not compliant
12 Months After the Intervention	Mean temperature at five locations (the four corners and the center of the storage facility) (°C)	(23.8±1.5)ºC	< 25º	Compliant
	Relative humidity (%)	(66.01±3.7) %	< 70%	Compliant
	Mean air velocity at five locations (the four corners and the center of the storage facility) (m/s)	0.72 m/s	0.5 -1.0m/s	Compliant

Before the intervention, the mean temperature was 27.0 ± 3.1°C (>25°C), the mean relative humidity was 75.91 ± 4.6% (>70%), and the mean air velocity was 0.45 m/s (<0.5 m/s), indicating that the storage conditions did not meet the recommended requirements for medicinal material storage.

Twelve months after the intervention, Storage Facility No. 2 had a mean temperature of 23.5 ± 1.5°C (<25°C), a mean relative humidity of 65.91 ± 3.6%

(<70%), and a mean air velocity of 0.75 m/s. These conditions met the recommended requirements for medicinal material storage.

3.3.2. Prevalence of Fungal Contamination Before and After the Intervention

All 170 traditional medicinal material samples at Nghe An Traditional Medicine Hospital were examined for fungal contamination by culture on Sabouraud medium. The results are presented below.

Table 3.36. Prevalence of Fungal Contamination in Traditional Medicinal Material Samples Before and 12 Months After the Intervention in the Two Storage Facilities

Storage Facility	Before the Intervention			After the Intervention			p Value
	No. of Samples Tested	No. of Positive Samples	Prevalence (%)	No. of Samples Tested	No. of Positive Samples	Prevalence (%)	
Storage Facility No.1	84	27	32.14	84	18	21.43	< 0.05
Storage Facility No.2	86	29	33.72	86	20	23.26	< 0.05
Chung	170	56	32.94	170	38	22.35	< 0.05
Effectiveness (%)	32.15%						

The prevalence of fungal contamination decreased significantly after 12 months, from 32.94% to 22.35% ($p < 0.05$). The intervention effectiveness was 32.15%.

Chapter 4: DISCUSSION

4.1. Prevalence and Species Composition of Fungal Contamination in Traditional Medicinal Material Samples at Nghe An Traditional Medicine Hospital in 2023

4.1.1. Prevalence of Fungal Contamination in Traditional Medicinal Material Samples

The results presented in Figure 3.1 showed that the prevalence of fungal contamination in traditional medicinal material samples was 32.94% (56/170). This prevalence was lower than that reported by Dau Huy Hoan (2018), who investigated 505 traditional medicinal material samples collected from hospitals in Nghe An Province and found an overall fungal contamination rate of 51.8% based on culture on Sabouraud medium, including 45.53% among traditional Chinese medicinal materials and 72.8% among traditional Vietnamese medicinal materials.

According to the Food and Agriculture Organization (FAO), approximately 50.0% of agricultural products become contaminated with fungi after harvest. Microbial contamination of medicinal herbs is a serious problem not only in Asia but also in the Americas and many other parts of the world. In 2020, Carolina Miranda de Sousa Lima et al. investigated microbial contamination in medicinal herbs and reported that older adults were the most frequent users of herbal medicines and were also the population at greatest risk of diseases caused by microorganisms

contaminating these products. The study found that 35.6% of herbal samples were contaminated with fungi, 51.5% with bacteria, 31.8% exceeded the acceptable microbial limit (>10^5 CFU/g), and 31.5% were stored under inadequate conditions.

As is well known, fungi disseminate by producing airborne spores. When these spores settle on suitable substrates or new hosts under favorable environmental conditions, such as relative humidity >70%, temperature >25°C, air velocity <0.5 m/s or >1 m/s, and adequate nutrients, they rapidly germinate and develop into fungal hyphae and mycelia. The prevalence of fungal contamination observed in the present study was lower than that reported by Dau Huy Hoan in 2018, who found an overall contamination rate of 51.8% compared with 32.35% in the present study. Specifically, at Nghe An Traditional Medicine Hospital, Dau Huy Hoan reported a fungal contamination rate of 48.53%, which was also higher than that observed in the present study (32.35%).

Previous studies have demonstrated that each fungal species is capable of producing one or more mycotoxins. More than 18 species of Aspergillus have been identified as aflatoxin producers, with *Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus niger* being the most important species. The principal aflatoxins produced are aflatoxins B1, B2, G1, and G2. Species of *Fusarium* are known to produce fumonisins and other mycotoxins. In the present study, the highest prevalence of fungal contamination was observed in root-derived medicinal materials (40.30%), whereas stem-derived medicinal materials had the lowest prevalence (23.68%). These findings are consistent with the biological characteristics of medicinal plant materials. Root-derived medicinal materials are particularly susceptible to fungal contamination if not properly preserved after harvest because they remain in prolonged contact with soil, where fungal contamination may already occur. In addition, their relatively high moisture content and abundant nutrients provide favorable conditions for fungal growth.

4.1.2. Fungal Species Composition

In the present study, fungal culture on Sabouraud medium followed by morphological identification was used to characterize fungal species. As shown in Table 3.5, among the 56 fungal-contaminated traditional medicinal material samples, 50 were contaminated with a single fungal species, 2 were co-contaminated with two *Aspergillus* species, 3 were co-contaminated with *Aspergillus* and *Penicillium*, and 1 sample was contaminated with three different *Aspergillus* species. A total of 63 fungal isolates were identified, including *Aspergillus* (28 isolates, 44.44%), *Rhizopus* (16 isolates, 25.40%), *Penicillium* (13 isolates, 20.63%), *Fusarium* (4 isolates, 6.35%), and one isolate each of *Phoma* spp. and *Cladosporidium* (1.59% each). These findings demonstrate the considerable diversity of fungal species contaminating traditional medicinal materials.

PCR amplification and DNA sequencing further confirmed the fungal identification results. As presented in Tables 3.7, 3.8, and 3.9, six fungal genera were identified, namely *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp., *Phoma* sp., and *Cladosporium* sp. These findings demonstrate the diversity of fungal genera contaminating traditional medicinal materials; however, *Aspergillus*

was the predominant genus, accounting for 28 of the 63 fungal isolates (44.44%). The results of the present study are consistent with those reported by Le Quang Hanh Thu et al. (2021), Ta Thu Lan (2024), and Nguyen Thu Hoai et al. (2015).

4.1.2.1. Identification of Fungal Genera

Among the 56 traditional medicinal material samples contaminated with fungi, a total of 63 fungal isolates were recovered. Species-level identification was successfully achieved for 61 isolates, while two isolates could only be identified to the genus level: isolate VT98 as *Aspergillus* spp. and isolate VT162 as *Phoma* spp. (Table 3.6). Comparison of the obtained sequences with those in the GenBank database showed sequence similarities ranging from 99.9% to 100% for all 63 isolates. These findings demonstrate the diversity of fungal species contaminating traditional medicinal materials, with *Aspergillus* being the predominant genus. The results are consistent with those reported by Dau Huy Hoan (2018), who identified seven filamentous fungal species and four yeast species belonging to six genera, of which *Aspergillus* was the most prevalent (82.0%), followed by Rhizopus oryzae (6.0%).

4.1.2.2. Identification of Fungal Species

From the fungal isolates that had been identified to the genus level, gene sequencing was performed and the obtained sequences were compared with the reference ITS1 and ITS2 sequences available in international GenBank databases. The results, summarized in Table 3.9, revealed a total of 16 fungal species belonging to five genera. Specifically, the genus *Aspergillus* comprised nine species, *Rhizopus* two species, *Penicillium* three species, and *Fusarium* one species. In addition, one isolate belonged to the genus *Phoma* (Phoma spp.), but its species identity could not be determined.

The findings of this study demonstrate a high diversity in the species composition of fungi associated with traditional medicinal materials. However, the predominant genera were *Aspergillus*, followed by *Penicillium* and *Fusarium*, whereas *Rhizopus* and *Phoma* spp. accounted for only a very small proportion. The number of fungal species identified in our study was lower than that reported by the following authors:

Dau Huy Hoan (2018) investigated 505 traditional medicinal material samples collected from hospitals in Nghe An Province using fungal culture on Sabouraud medium. The study identified seven filamentous fungal species and four yeast species. Specifically, *A. niger* was the most prevalent species, accounting for 50/50 isolates (100.00%), followed by *A. tubingensis* (9/50, 18.00%), *A. flavus* (1/50, 2.00%), *A. oryzae* (1/50, 2.00%), *Rhizopus oryzae* (3/50, 6.00%), *Candida parapsilosis* (1/50, 2.00%), *Kodamaea ohmeri* (1/50, 2.00%), *Corynespora smithii* (1/50, 2.00%), and other fungal species (1/50, 2.00%).

Nguyen Thi Xuan Sam et al. (2022) used PCR to characterize fungal isolates and found that four of the 32 isolates tested positive for the *aflR*, *avfA*, *omtA*, and *ver-1* genes, which are involved in aflatoxin biosynthesis. In another study, Tran Trinh Cong et al. (2025) investigated 10 lotus seed samples collected in Hanoi and reported that *Aspergillus flavus* was the most frequently detected fungal species, being isolated from 3 of the 10 samples.

4.2. Prevalence of Aflatoxin Contamination, Composition, and Concentrations of Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, and Aflatoxin G_2 in Traditional Medicinal Material Samples at Nghe An Traditional Medicine Hospital

Among the 170 traditional medicinal material samples analyzed for aflatoxin by quantitative assay, aflatoxins were detected in 19 fungal-contaminated samples and in 6 samples without detectable fungal contamination.

Samrand Reshadi et al. (2024) determined the concentrations of aflatoxins B1, B2, G1, and G2 in cheese puffs made from corn flour in Iran using high-performance liquid chromatography (HPLC). Twenty-seven cheese puff samples were analyzed. The highest aflatoxin concentrations among brands A, B, and C were observed in samples A1 (1.35 ng/mL), B2 (0.95 ng/mL), and C2 (1.22 ng/mL), respectively. In contrast, the lowest aflatoxin concentrations were found in samples A3 (0.93 ng/mL), B3 (0.80 ng/mL), and C1 (0.75 ng/mL).

Studies on Aflatoxin Contamination Using High-Performance Liquid Chromatography

In Asia, medicinal plants and animal-derived medicinal materials are widely used in traditional medicine. During harvesting, processing, and storage, a considerable proportion of these materials become contaminated with fungi and their secondary metabolites, particularly aflatoxins. Therefore, establishing standardized procedures for the detection and quantification of aflatoxins is essential for ensuring the quality and safety of herbal medicinal products. In 2021, Chinese researchers reviewed domestic studies on fungal contamination and reported that *Aspergillus flavus* was the most prevalent aflatoxin-producing species. More than 50% of the food samples analyzed contained aflatoxin levels exceeding the permitted safety limits. In the present study, 48.0% of aflatoxin-contaminated samples had aflatoxin concentrations exceeding the permissible limit (>0.5 ppb) specified in QCVN 8-1:2011/BYT. This proportion was higher than that reported by Phan Van Manh et al. (2024), who found that 31.7% (34/80) of samples exceeded the permissible limit using the same high-performance liquid chromatography (HPLC) method.

Prevalence and Distribution of Aflatoxin Types

The results presented in Figures 3.12 and Tables 3.15 and 3.16 showed that the overall prevalence of aflatoxin contamination was 14.71%. Among the contaminated samples, aflatoxin B2 was detected most frequently, accounting for 7.06% (12/170), followed by aflatoxin G1 at 3.53% (6/170), while aflatoxin G2 was detected least frequently at 1.76% (3/170). Among the 16 aflatoxin-contaminated samples, individual samples contained one, two, or three types of aflatoxins, resulting in a total of 25 aflatoxin-positive detections. Of these, aflatoxin B2 accounted for 52.00% (13/25), aflatoxin B1 for 12.00% (3/25), aflatoxin G1 for 24.00% (6/25), and aflatoxin G2 for 12.00% (3/25). The prevalence of aflatoxin B2 contamination was significantly higher than that of aflatoxin B1, aflatoxin G1, and aflatoxin G2 (52.00% [13/25] vs. 12.00% [3/25], 24.00% [6/25], and 12.00% [3/25], respectively; $p < 0.05$). These findings indicate the important role of aflatoxin B2 in toxin contamination of traditional herbal medicines. As shown in Table 3.18, there was a statistically significant difference in the prevalence of aflatoxin contamination

between fungal-contaminated and non-contaminated traditional medicinal materials, with contamination rates of 19.64% (11/56) and 4.39% (5/114), respectively ($p < 0.05$). These findings further support the role of fungi in the production of aflatoxins.

To date, most studies have reported that aflatoxin B1 is the most prevalent aflatoxin, particularly in cereals such as maize, rice, peanuts, and sesame. Other aflatoxins, including aflatoxin B2, G1, and G2, are more commonly detected in post-harvest processed products, such as confectionery, fruit juices, dried foods, and traditional herbal medicines. In contrast, the present study found that aflatoxin B2 was the predominant aflatoxin, accounting for 52.00% of all aflatoxin-positive detections. This finding differs from those of several previous studies, which reported aflatoxin B1 as the most frequently detected aflatoxin. For example, Saba Shabeer et al. (2022) reported that *Aspergillus flavus* and *Aspergillus parasiticus* predominantly produced aflatoxin B1, followed by aflatoxin B2, aflatoxin G1, and aflatoxin G2. According to the Food and Agriculture Organization (FAO), approximately 25% of cereals consumed in African countries are contaminated with mycotoxins, among which aflatoxin B1 is the most common and hazardous, accounting for up to 75% of all fungal toxins. Foods contaminated with aflatoxin include maize, millet, and rice, with aflatoxin B1 detected in 76% of maize samples, 64% of millet samples, and 60% of rice samples. Furthermore, aflatoxin B1 concentrations exceeded the permissible limits in 26% of maize samples, 10% of millet samples, and 11% of rice samples.

4.2.3. Risk Factors Associated with Aflatoxin Contamination

The results presented in Tables 3.20, 3.21, and 3.23 showed a significant association between fungal contamination and overall aflatoxin contamination in traditional medicinal materials (OR = 5.165, 95% CI: 1.69–15.70, $p < 0.01$). Traditional medicinal materials contaminated with fungi had a 5.165-fold higher risk of aflatoxin contamination than those without fungal contamination. A significant association was also observed between fungal contamination and aflatoxin contamination in Chinese medicinal herbs (OR = 7.373, 95% CI: 1.621–33.542, $p < 0.01$). Fungal-contaminated Chinese medicinal herb samples had a 7.373-fold higher risk of aflatoxin contamination than non-contaminated samples. These findings are consistent with the well-documented occurrence of fungal contamination in post-harvest agricultural products and traditional medicinal materials and can be explained by the following scientific evidence:

Fungal contamination of medicinal materials sold in District 5, Ho Chi Minh City, has been attributed primarily to inadequate storage conditions. Similarly, Dau Huy Hoan (2018) reported that all traditional medicinal material storage facilities surveyed in Nghe An lacked air-conditioning systems, dehumidifiers, ventilation fans, and moisture-absorbing agents, all of which are essential for preventing fungal growth and mold contamination.

Hayrettin ÖZER et al. (2025) investigated the occurrence of aflatoxin B1 and total aflatoxins (B1, B2, G1, and G2) in pistachios and dried figs using high-performance liquid chromatography (HPLC). Aflatoxins were detected in 16 pistachio samples and

five dried fig samples. Among the pistachio samples, aflatoxins G1 and G2 were detected in three samples, aflatoxin B1 (AFB1) was detected in four samples at concentrations ranging from 0.21 to 404.62 ppb, and aflatoxin B2 (AFB2) was detected in three samples at concentrations ranging from 0.10 to 0.96 ppb. Total aflatoxin concentrations ranged from 5.04 to 405.58 ppb, with a mean concentration of 108.53 ppb. In the dried fig samples, AFB1, AFB2, AFG1, and AFG2 were also detected. AFG1 (0.03 ppb) and AFG2 (0.05–0.06 ppb) were detected in one sample, whereas AFB1 and AFB2 were detected in three samples. AFB1 concentrations ranged from 25.16 to 44.45 ppb, and AFB2 concentrations ranged from 0.01 to 6.07 ppb. The total aflatoxin concentration ranged from 0.56 to 51.15 ppb, with a mean concentration of 25.105 ppb.

4.3. Results of the Intervention Through Improvement of the Microclimatic Conditions in the Traditional Medicinal Material Storage Facility

Aflatoxin contamination in agricultural products has become a global public health concern. Numerous factors influence fungal growth and subsequent aflatoxin production, among which hot and humid climatic conditions and inadequate storage facilities and preservation techniques are the most important. In addition, post-harvest processing methods, transportation conditions, and traditional practices related to the use and storage of agricultural products also play significant roles in fungal contamination and aflatoxin production.

Fungal growth is favored under suitable environmental conditions, particularly when the relative humidity exceeds 70%, the temperature is above 25°C, the air velocity is below 0.5 m/s or above 1 m/s, and sufficient nutrients are available. Therefore, effective preservation of traditional medicinal materials requires maintaining appropriate microclimatic conditions, including a temperature below 25°C, relative humidity below 70%, and a clean, well-ventilated storage environment. Appropriate storage facilities should be equipped with dehumidifiers, ventilation fans, and air-conditioning systems. In addition, ultraviolet (UV) irradiation may be used to reduce airborne fungal spores and minimize fungal contamination.

The results presented in Table 3.32 showed that, before the intervention, neither of the two storage facilities met any of the required criteria for proper medicinal material storage. Following the intervention, which included the installation of equipment to improve the microclimatic conditions in both storage facilities and the implementation of 12 rounds of periodic inspections to strengthen compliance with standard operating procedures by warehouse staff, both facilities achieved Good Storage Practice (GSP) standards and met the required safety criteria (Table 3.33). The post-intervention evaluation of storage facility quality, together with the standardization of procedures for receiving, storing, and dispensing medicinal materials, is presented in Tables 3.36, 3.37, and 3.38. The overall fungal contamination rate decreased significantly after 12 months of intervention, from 32.94% to 22.35% ($p < 0.05$), corresponding to an intervention effectiveness of 32.15%. A statistically significant reduction in fungal contamination was observed in both storage facilities. In Storage Facility No. 1, the contamination rate decreased from 32.14% to 21.43% ($p < 0.05$), while in Storage Facility No. 2, it decreased from 33.72% to 23.26% ($p < 0.05$).

CONCLUSIONS

1. Fungal Species Composition in Traditional Medicinal Material Samples at Nghe An Traditional Medicine Hospital in 2023

A study of 170 traditional medicinal material samples collected at Nghe An Provincial Hospital of Traditional Medicine during 2023–2024 found an overall fungal contamination rate of 32.94%. The fungal contamination rates were 28.40% in Vietnamese medicinal herbs and 37.10% in Chinese medicinal herbs. Among medicinal materials classified by plant part, the highest fungal contamination rate was observed in root-derived materials (40.30%), followed by materials derived from stems (23.68%) and branches, leaves, flowers, and fruits (32.07%).

Among the 56 fungal-contaminated samples, 50 were contaminated with a single fungal species, five with two species, and one with three species, yielding a total of 63 fungal isolates belonging to 16 species. Species-level identification was successfully achieved for 61 isolates, whereas two isolates could not be identified to the species level. Of the 63 isolates, 28 (44.44%) belonged to the genus *Aspergillus*, comprising eight species. Among these, *A. niger* was the most frequently isolated species, accounting for 10/63 isolates (15.87%), followed by *A. flavus* and *A. chevalieri*, each accounting for 5/63 isolates (7.94%). The remaining 35 isolates (55.56%) belonged to five other genera, namely *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp., *Phoma* sp., and *Cladosporium*. *Rhizopus* spp. was the most prevalent, accounting for 16/63 isolates (25.39%). Three species of *Penicillium*, one species of *Fusarium*, and one species of *Cladosporium* were identified, whereas one *Phoma* sp. isolate could not be identified to the species level.

2. Prevalence of Aflatoxin Contamination, Composition, and Concentrations of Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2 in Traditional Medicinal Material Samples at Nghe An Traditional Medicine Hospital

The overall prevalence of aflatoxin contamination was 9.41% (16/170). Among the 16 aflatoxin-contaminated samples, nine (56.25%) were contaminated with a single aflatoxin, five (31.25%) with two aflatoxins, and two (12.50%) with three aflatoxins. In total, 25 aflatoxin-positive detections were identified, corresponding to 14.71% (25/170) of all samples. Among these, aflatoxin B2 was detected most frequently, accounting for 7.06% (12/170), followed by aflatoxin G1 at 3.53% (6/170), while aflatoxin G2 was detected least frequently at 1.76% (3/170). Of the 25 aflatoxin-positive detections, aflatoxin B2 accounted for the highest

proportion (48.0%, 12/25), followed by aflatoxin G1 (24.0%, 6/25), aflatoxin B1 (16.0%, 4/25), and aflatoxin G2 (12.0%, 3/25). The prevalence of aflatoxin contamination was 19.64% (11/56) among fungal-contaminated traditional medicinal material samples, compared with 4.39% (5/114) among samples without fungal contamination.

A significant association was observed between fungal contamination and aflatoxin contamination in traditional medicinal material samples (OR = 5.165, 95% CI: 1.69–15.70). A significant association was also found between contamination with *Aspergillus* spp. and aflatoxin contamination (OR = 4.533, 95% CI: 1.47–13.97), as well as between contamination with other fungal species and aflatoxin contamination (OR = 4.704, 95% CI: 1.623–13.636). In addition, fungal contamination was significantly associated with aflatoxin contamination in Chinese medicinal herb samples (OR = 7.373, 95% CI: 1.621–33.542).

The mean aflatoxin concentration was 1.89 ng/g. Aflatoxin B1 had the highest mean concentration (2.38 ng/g), followed by aflatoxin G1 (2.05 ng/g), whereas aflatoxins G2 and B2 were detected at lower concentrations. Overall, 62.5% (10/16) of the aflatoxin-contaminated traditional medicinal material samples exceeded the permissible limit of 0.5 ppb.

3. Results of the Intervention for the Prevention of Fungal Contamination in Traditional Medicinal Material Samples

Before the intervention, neither of the two storage facilities met the required conditions for medicinal material storage, failing to comply with the recommended standards for temperature, relative humidity, and air velocity. After 12 months of intervention, both storage facilities achieved appropriate microclimatic conditions, with a mean temperature of <25°C measured at the four corners and the center of each storage facility, relative humidity of <70%, and air velocity ranging from 0.5 to 1.0 m/s, thereby meeting the recommended storage requirements. The overall fungal contamination rate in the two storage facilities decreased significantly after the 12-month intervention, from 32.94% to 22.35% ($p < 0.05$), corresponding to an intervention effectiveness of 32.15%. The contamination rate of *Aspergillus* spp. also decreased markedly, from 16.47% (28/170) before the intervention to 6.47% (11/170) after 12 months, corresponding to an intervention effectiveness of 54.15%.

THESIS-RELATED PUBLICATIONS

1 **Ho Van Thang**, Nguyen Thi Kim Dinh, Nguyen Thi Nga, Duong Dinh Chinh and Cao Ba Loi (2025). Current Status of Fungal Contamination in Traditional Medicinal Materials at Nghe An Traditional Medicine Hospital. *Journal of Community Medicine,* No. 67(2), pp. 206–211.

2 **Ho Van Thang**, Nguyen Thi Kim Dinh, Nguyen Thi Nga, Duong Dinh Chinh and Cao Ba Loi (2025). Characteristics of Aflatoxin Contamination in Traditional Medicinal Materials at Nghe An Traditional Medicine Hospital in 2023. *Journal of Community Medicine,* No. 67(2), pp.128–132.

3 **Ho Van Thang**, Nguyen Thi Kim Dinh, Nguyen Thi Nga, Duong Dinh Chinh and Cao Ba Loi (2025). Some Factors Associated with Aflatoxin Contamination in Traditional Medicinal Materials at Nghe An Traditional Medicine Hospital in 2023. *Journal of Community Medicine,* No. 67(2), pp. 154–158.