Profiling indoor airborne mycoflora of Kolkata, India and its eco-friendly control

THESIS SUBMITTED FOR

THE DEGREE OF DOCTOR OF PHILOSOPHY

JADAVPUR UNIVERSITY

By

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Index No.: D-7/ISLM/110/21

SCHOOL OF ENVIRONMENTAL STUDIES

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INDIA

2025

Dedicated to my family, mentors and teachers

DETAILS OF THESIS

1. **Index No. and Date of Registration:** D-7/ISLM/110/21 registered on December 30, 2021
2. **Title of the Thesis:** Profiling indoor airborne mycoflora of Kolkata, India and its eco-friendly control
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5. **List of Publications:**

a) Publications related to Doctoral work:

i. Basu, C., Bhattacharyya, S., Chaudhuri, A., Akhtar, S., Chatterjee, A., Thakur, B., Guha, H., & Chaudhuri, P. (2021). Assessment of Potential Damage Factor: A Case Study of St. Paul's Cathedral, Kolkata. Journal of Heritage Management, 6(1), 53-68. https://doi.org/10.1177/24559296211008678

ii. Basu, C., Bhattacharyya, S. (2022). Mucormycosis and role of COVID19 in its pathogenicity in India: A review. *Asian Pacific Journal of Health Sciences, 9(4),* 149-155. https://doi.org./ 10.21276/apjhs.2022.4S1.26

iii. Chaudhuri, A., **Basu, C.**, Bhattacharyya, S., & Chaudhuri, P. (2019). Development of health risk rating scale for indoor airborne fungal exposure. *Archives of Environmental & Occupational Health*, *75*(7), 375–383. https://doi.org/10.1080/19338244.2019.1676187

b) Other Publications during the period of Doctoral Research:

i. Basu, C., Bhattacharyya, S., Chaudhuri, P. (2022). Role of mangroves in pharmacotherapy. International Academic Publishing House (IAPH), B. Sarkar (eds.), The Basic Handbook of Indian Ethnobotany and Traditional Medicine,Vol. 1, pp. 62-73. DOI: https://doi.org/10.52756/bhietm.2022.e01.005

6. List of Patents: Nil

7. List of Presentations in National/International Conferences:

i. Chiradeep Basu, Aindrila Panda, Subarna Bhattacharyya "Changes in microbial enzyme production due to ambient air pollution" at "**Two-day International Seminar on Sustainable Development: From the Perspective of Nature and Nurture"** organised by Netaji Subhas Open University, on 10th – 11th February 2023.

ii. Chiradeep Basu, Subarna Bhattacharyya "Application of plant extracts to prevent growth of indoor airborne fungi" at 1st International Conference on "Drug Discovery and Development for Infectious Diseases" organised by **Eminent College of Pharmaceutical Technology** on 3rd – 4th March 2023.

iii. Chiradeep Basu, Subarna Bhattacharyya "Gender issue with indoor air pollution in rural India" at "Three-day International Seminar on Environmental History and Sustainability" organized by **Bajkul Milani Mahavidyalaya** on 5-7th February 2019

iv. Chiradeep Basu, Subarna Bhattacharyya "Use of biomass as fuel for cooking in rural India and its health effects: a review" at One-day international seminar on "Clean energy,

environment and sustainable development" held at **Rabindar Bharati University** on March 26th, 2019.

8. **Participation in Workshops, Seminars and Symposium:**

i. National workshop on "Assemblage and use of Foldscope" organized by **Department of Botany, Holy Cross College, Agartala in collaboration with School of Environmental Studies, Jadavpur University,** held on 13th November 2018.

CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled **"Profiling indoor airborne mycoflora of Kolkata, India and its eco-friendly control"** submitted by **Shri. Chiradeep Basu,** who got registered (Registration no. **D-7/ISLM/110/21**, dated on **December 30th , 2021)** his name under the **Faculty of Interdisciplinary Studies, Law & Management** for the award of **PhD (Science)** degree of **Jadavpur University**, is absolutely based upon his own work under the supervision of **Dr. Subarna Bhattacharyya** and that neither this thesis nor any part of it has been submitted for either any degree/diploma or any other academic award anywhere before.

Date: 10/03/2025

Place: Jadavpur.

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STATEMENT OF ORIGINALITY

I, **Chiradeep Basu** (Reg. No. **D-7/ISLM/110/21**), registered on **December 30th, 2021** do hereby declare that this thesis entitled "**Profiling indoor airborne mycoflora of Kolkata, India and its eco-friendly control**" contains a literature survey and original research work done by the undersigned candidate as part of doctoral studies.

All information in this thesis has been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by thesis rules and conduct, I have fully cited and referred to all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti-Plagiarism, Jadavpur University, 2019," and the level of similarity as checked by iThenticate software is 9%

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DECLARATION

I hereby declare that this thesis represents my own work and has not been previously included in my thesis or any other institution for a degree, diploma, or other academic qualifications. Further, I have acknowledged all sources used and have cited these in the reference section.

I hereby submit the record of my observations for evaluation for the award of the degree of Doctor of Philosophy in Science.

Date: 10/3/25

Place: Jadavpur.

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ACKNOWLEDGEMENTS

Contrary to popular belief, there is no such thing as a "self-made person". Every step in the path to success, however small and insignificant at first, is paved or lined by people who willingly extend their help and support. Completing my research has been a huge step, one that could never have been possible without the gargantuan contributions of the people I will acknowledge here.

First and foremost, my sincerest gratitude to my guide and supervisor, **Dr. Subarna Bhattacharyya**, who took me in her lab in the early days of 2017, encouraged me to take up research and to this very day has made me feel comfortable pursuing academic avenues of my choice, constantly extending her support wherever and whenever needed. **Prof. Joydeep Mukherjee, Dr. Tarit Roychowdhury** and **Dr. Reshmi Das** have looked over me and my work as teachers and guides and have made my association with Jadavpur University pleasant and fulfilling.

Dr. Punarbasu Chaudhuri gave me the confidence to approach research in environmental science and gave me the tools with which I work to this day, inside the laboratory and outside. All my teachers from University of Calcutta, and Asutosh College have made an immense contribution towards structuring my mindset and creating the knowledge base in my chosen subject.

My seniors, colleages and friends from School of Environmental Science, **Debleena Di, Sanghamitra Di, Sayak Da, Dhruba Da, Ankita Di, Saranya Di, Shayontani Di, Shantanu, Anirban, Arup, Aindrila, Iravati, Hamidul** and **Tanaya** have helped whenever needed and are primarily the reasons why I will look back on these years so very fondly.

This work would not have been attempted without the funding from the **Department of Science and Technology and Biotechnology, Govt. of West Bengal.** A big thank you for enabling me to pursue my doctoral degree comfortably.

Everyone behind a desk in Jadavpur University who perform their job diligently require acknowledgement because no research is complete without immense paperwork and so do the glass and chemical suppliers who form the basic backbone of research inventory and every faculty and staff who work daily to make the university campus look the way it does. It is because of your presence behind the scenes that this university has meant so much to me for a large part of my life.

My **father, mother** and **grandmother** have fed me, clothed me, raised me and are the pillars on which I stand and try to reach upwards. Every step begins with them and will continue to do so as long as I live. Finally, the handful of people I call my own, I can never thank them enough for picking up the phone when I needed to talk, giving a shoulder when I needed to cry, being silly when I needed a laugh and for always believing in me even when I had stopped myself. This work, and all successes in every other avenue of life, will see your names etched on them.

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ABSTRACT

This study presents an extensive examination of fungal pollution in various indoor environments of Kolkata, factors affecting their proliferation and distribution and methods to effectively inhibit their growth using natural plant products and common antifungal agents. Upon further examination it was revealed that factors which don't completely inhibit fungal growth, regulate their activity by affecting their enzyme production levels which can then have an effect on their survivability and adaptation to the new environment.

Data were collected from multiple indoor environments, including classrooms, offices, and residential areas, where fungal concentrations were measured in terms of colony-forming units (CFU) per cubic meter of air. The findings indicated that certain indoor environments harbor higher fungal densities, with notable concentrations of pathogenic species like *Fusarium, Cladosporium*, *Alternaria* and *Aspergillus*, thereby posing potential health risks to occupants. The study further revealed that effective management strategies—such as regular monitoring of indoor air quality and moisture control—are critical in mitigating the health risks associated with indoor fungal exposure.

Upon taking into consideration factors like duration of exposure, age of person exposed to the pollutants, availability of substrates in the environment, etc, a scale was designed which can indicate the potential risk to inhabitants of any indoor environment. Upon applying this Health Risk Rating Scale to all the various environments we studied, it was revealed that some settings, like a bedroom or a library can be more harmful to human health when adequate hygienic practices are not performed regularly.

Next, our study investigated the efficacy of various interventions, like the application of plant extracts and chemical agents such as sulfur dioxide and UV-C radiation, in controlling the growth

of fungi which were identified in various indoor settings. Preliminary results suggested that certain plant extracts exhibit promising antifungal properties, potentially offering a natural alternative to conventional antifungal treatments, while chemical agents were incapable in stopping growth at low healthy doses.

Finally, when the enzyme secretions from fungi were studied after exposure to higher doses of chemical agents and UV-C radiation, it was revealed that enzymes were mostly down-regulated in favour of certain enzymes, which were then up-regulated for adaptation to the harmful environment. This provided an interesting outlook into the behaviour of fungi when under non-optimal conditions and gave an insight into their ability to cope with stress and continue growth.

In conclusion, this research underscores the critical need for a multifaceted approach to address indoor fungal contamination. Simple surveys of any indoor setting can reveal the potential risk to inhabitants and by combining natural agents such as plant metabolites and oils, we can combat fungal growth in these environments. Upon closer inspection, we can also see how fungi adapt to their changing environments by changing their enzyme levels when exposed to popular anti-fungal agents like sulfur dioxide and UV-C radiation.

LITERATURE REVIEW

1. Introduction to Indoor Airborne Microbes and Health Risks

Indoor air quality is increasingly recognized as a key determinant of public health. People spend nearly 80–90% of their time indoors, making exposure to indoor pollutants and bioaerosols a major concern. Among these, airborne fungi are particularly important due to their ability to produce allergens, toxins, and spores that remain suspended in air for long periods. Exposure to fungal spores has been consistently associated with respiratory infections, allergic rhinitis, asthma, hypersensitivity pneumonitis, and Sick Building Syndrome (SBS) (Kumar et al., 2022; Al Hallak et al., 2023; Norbäck & Cai, 2020).

Studies across the globe have demonstrated that the burden of airborne fungi in indoor environments is not limited to clinical or industrial settings; it extends to homes, schools, libraries, and heritage buildings. Fungal bioaerosols can carry mycotoxins such as aflatoxins, ochratoxin A, and trichothecenes, which are harmful to human health (Al Hallak et al., 2023). Vulnerable populations such as children, the elderly, and immunocompromised individuals are especially at risk.

The health effects of indoor fungi are strongly tied to environmental and structural factors, including building age, ventilation, humidity, water damage, and seasonal fluctuations (Chen et al., 2024; Noris et al., 2021). Collectively, this body of research highlights the dual role of indoor fungi: as both indicators of environmental quality and agents of adverse health outcomes.

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2. Indoor Fungal Diversity and Distribution

Numerous studies document the diversity and abundance of fungal species in indoor environments across different geographical locations. A consistent finding across regions is the dominance of the genera *Aspergillus, Penicillium, Cladosporium,* and *Alternaria*. These fungi are widely recognized as major contributors to allergies and respiratory diseases.

For example, Kumar et al. (2022) conducted a study in Delhi, India, and found high counts of *Aspergillus* and *Cladosporium* spores in homes, which correlated with increased reports of SBS symptoms. Chen et al. (2024) observed strong seasonal variations in spore concentrations in China, with summer peaks that frequently exceeded permissible exposure limits. Similarly, Noris et al. (2021) highlighted that building insulation and age significantly influenced fungal colonization, with older or poorly ventilated structures harboring higher fungal loads.

Research also emphasizes the importance of humidity and water damage in shaping fungal diversity. Damp environments support the growth of *Stachybotrys* and *Chaetomium*, fungi commonly linked with severe indoor air quality issues and toxic mold syndromes. In contrast, relatively dry environments tend to favor *Cladosporium* and *Aspergillus niger*, which can persist in dust and on surfaces.

Several large-scale surveys confirm that fungal spore concentrations in indoor air can rival or even exceed outdoor levels during certain conditions. Du et al. (2023) reported that more than half of the indoor airborne fungi in their sampled households could be traced back to outdoor sources, yet indoor reservoirs such as damp walls or stored organic materials often became the dominant contributors under humid conditions.

The species richness and diversity indices calculated across studies also reveal interesting patterns. For instance, Zhang et al. (2023) found that indoor fungal diversity in urban Chinese apartments showed significant clustering around dampness-related species, while Al-Hadithi et al. (2022) demonstrated similar diversity patterns in Baghdad households with poor ventilation. These studies suggest that fungal diversity is shaped not only by external inputs but also by microclimatic conditions and human activities indoors.

Collectively, the literature underscores that indoor fungal diversity is both geographically variable and environmentally responsive. While certain genera dominate globally, local factors such as climate, building structure, and human behavior determine which fungi proliferate indoors and, consequently, what health risks arise.

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3. Indoor vs Outdoor Sources of Bioaerosols

A central theme across studies is the strong relationship between outdoor air quality and indoor fungal concentrations**.** Outdoor spores often act as the primary source of indoor fungi, yet indoor microclimates can amplify or alter these communities.

Du et al. (2023) in China demonstrated that over 50% of airborne fungi detected indoors could be directly traced to outdoor sources using sequencing and culture methods. Similar observations were reported by Mori et al. (2019) in Japan, where spore profiles in classrooms mirrored those found in adjacent outdoor spaces, highlighting the role of air exchange and ventilation.

In Baghdad, Al-Hadithi et al. (2022) showed that outdoor air contributed significantly to indoor fungal loads, but indoor environments often had higher concentrations due to accumulation in

poorly ventilated rooms. Zhang et al. (2023) further demonstrated that damp indoor walls and wooden structures could serve as strong fungal reservoirs independent of outdoor contributions.

The relationship between indoor and outdoor sources varies by geography and season. Chen et al. (2024) observed that seasonal peaks of fungal spores outdoors directly influenced indoor counts in Chinese homes, especially during humid summers. Conversely, Norbäck & Cai (2020) in Sweden emphasized that damp housing conditions often created indoor fungal communities distinct from outdoor ones**,** thereby compounding respiratory health risks.

Together, these findings establish that while outdoor air is a consistent baseline contributor to indoor fungal communities**,** structural and environmental conditions indoors play a decisive role in determining exposure levels.

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4. Special Environments and Case Studies

Beyond typical households and offices, several studies have explored unique environments where fungal colonization poses cultural, educational, or public health challenges.

Libraries and Heritage Buildings

Silva et al. (2018) studied libraries and cathedrals in Italy and Spain, reporting extensive fungal colonization on paper, wood, and stone surfaces. The fungi isolated were primarily *Aspergillus*, *Penicillium,* and *Chaetomium*, all of which are known agents of biodeterioration**.** The presence of these fungi threatened both the structural integrity of heritage artifacts and the respiratory health of workers and visitors.

Schools and Educational Institutions

Mori et al. (2019) and subsequent studies in Asian schools highlighted that classroom air quality often reflected outdoor fungal loads but was worsened by poor ventilation. High spore counts were correlated with increased absenteeism among students with respiratory issues.

Temporary Housing and Disaster Relief Shelters

Ghosh et al. (2022) investigated temporary housing units built after natural disasters in India and found exceptionally high fungal loads in poorly constructed shelters. *Aspergillus* and *Cladosporium* were most common, with potential implications for respiratory outbreaks among displaced populations.

Hospitals and Healthcare Environments

Though less frequent in your reviewed studies, some reports noted that fungi in hospitals (especially in wards with immunocompromised patients) could increase risks of nosocomial infections. Even low levels of *Aspergillus fumigatus* can be hazardous in such contexts.

Religious and Cultural Buildings

Other studies noted that closed, humid environments such as cathedrals and heritage temples harbor fungi capable of both structural degradation and allergenic risks for occupants.

In all these cases, the literature consistently shows that fungal presence is not merely a biological concern but one that intersects with cultural preservation, public health, and education access.

5. Experimental Studies on Pollutant Effects

A significant body of research examines how airborne pollutants interact with fungi, altering their physiology, enzyme activity, and survival strategies. These studies are crucial for understanding how indoor air pollution compounds the risks posed by bioaerosols.

Sulfur Dioxide (SO_2) Exposure

Multiple fumigation studies have investigated the response of fungi to SO_2, a common industrial and urban pollutant.

- Rastogi et al. (2019) reported increased activity of detoxification enzymes such as catalase and superoxide dismutase (SOD) in fungi exposed to SO_2. This suggests that oxidative stress pathways are upregulated for survival.
- Banerjee et al. (2020) demonstrated that SO_2 exposure altered protease and RNAse activity**,** indicating a shift in metabolic priorities toward stress adaptation and survival.
- Crop-based fumigation studies also revealed that SO_2 exposure could indirectly select for more stress-tolerant fungal species on plant surfaces, some of which are also known indoor colonizers.

Ozone (O_3) and Other Pollutants

Other experimental studies investigated ozone, nitrogen oxides, and particulate matter. Fungi such as *Aspergillus fumigatus* and *Penicillium* sp. exhibited altered growth rates and sporulation under these conditions. Enzyme assays showed variable patterns, often indicating heightened oxidative stress.

Implications for Human Health

These findings suggest that exposure to pollutants not only modifies fungal physiology but may also increase their resilience and pathogenicity indoors**,** posing greater health risks in polluted urban environments. For example, stress-enhanced fungi may produce more allergens or toxins when exposed to oxidants.

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6. Novel and Alternative Antifungal Strategies

Growing awareness of the limitations of conventional chemical fungicides has led to interest in natural and innovative antifungal methods. Studies span UV irradiation, essential oils, marine-derived extracts, and nanotechnology.

Ultraviolet (UV-C) Irradiation

Experimental studies demonstrate that UV-C light can significantly reduce airborne fungal spore counts. While effective in controlled settings, its scalability and safety in occupied indoor environments remain under evaluation.

Ozone Treatment

Ozone has been tested as a sterilizing agent in indoor spaces. Results show effective fungal reduction, but long-term health concerns regarding ozone exposure limit its widespread adoption.

Plant-Derived Essential Oils

- **Fernandes et al. (2021)** showed that oregano oil displayed fungistatic activity against common indoor molds.
- Other essential oils such as thyme and clove oil have shown similar potential, acting through disruption of fungal membranes.
  These approaches are attractive for their natural origin but require standardization of dosage and delivery methods.

Marine and Mangrove Extracts

- **Liu et al. (2022)** studied seaweed-derived extracts, reporting strong inhibitory effects on *Aspergillus* species.
- Mangrove plants have also yielded promising antifungal compounds, reflecting their adaptation to microbial-rich ecosystems.

Nanotechnology-Based Formulations

- Singh et al. (2023) reported that nano-formulations of plant-derived antifungals significantly improved their bioavailability and efficacy**.**
- Nanocarriers protect active compounds from degradation, enhance penetration of fungal cell walls, and allow for controlled release. Such strategies represent a frontier in antifungal research, bridging natural product chemistry with modern materials science.

Integrated Strategies

A few studies advocate for synergistic approaches**,** combining natural antifungals with nanotechnology or controlled irradiation. These integrated methods could provide sustainable and effective alternatives for managing indoor fungal contamination without relying on harmful chemicals.

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7. Antimicrobial Potential of Mangrove Plants and Related Interventions

Mangrove ecosystems represent a valuable source of bioactive compounds with demonstrated medicinal importance. Sixteen families and approximately seventy species of mangrove plants have been identified, each adapted to thrive in extreme saline environments (Abdel-Aziz et al., 2016). These plants are rich in phytochemicals—terpenoids, tannins, flavonoids, alkaloids, saponins, and antimicrobial peptides—that not only defend against environmental stressors but also exhibit therapeutic potential (Panda et al., 2009). Traditional medicine has long used mangrove-derived extracts to treat ailments such as rheumatism, ulcers, asthma, and leprosy, highlighting their role as alternatives to synthetic antibiotics (Ravindran et al., 2005; Thangam & Kathiresan, 1991). In light of rising antimicrobial resistance, these compounds are increasingly studied as promising leads for novel therapies (Sy et al., 2022; Malik et al., 2018).

Beyond general phytochemistry, specific mangrove-derived metabolites have been associated with antifungal activity. Extracts from *Avicennia marina* and *Rhizophora mucronata* demonstrated inhibitory effects against *Aspergillus*, *Candida*, and *Penicillium* species, in some cases outperforming fluconazole (Okla et al., 2021; Rastegar & Mohsen, 2016). Additional reports have

confirmed antifungal roles of compounds such as stigmasterol, cinnamic acid, and hexadecanoic acid, which disrupt microbial cell walls and enzymatic pathways, underscoring their value in managing fungal infections (Yenn et al., 2017; Wu et al., 2008; Korošec et al., 2014; Johannes et al., 2016).

Extraction and characterization of these secondary metabolites require careful optimization. Procedures such as solvent extraction, Soxhlet methods, and rotary evaporation are widely applied, with choice of solvent guided by compound solubility, stability, and safety (C.N. et al., 2016; Dixon & Dickinson, 2024; Jones & Kinghorn, 2012). Similarly, essential oils obtained from aromatic mangrove-associated plants have emerged as effective natural antifungals, given their biodegradability, environmental safety, and broad-spectrum activity (Batish et al., 2008; Ben ghnaya et al., 2013). Oils such as eucalyptus and clove exhibit strong fungicidal activity against *Aspergillus*, *Candida*, and dermatophytes, with active components including 1,8-cineole and eugenol (Rana et al., 2011; Zhou et al., 2016; Parle & Khanna, 2010).

Other environmental interventions provide complementary antifungal strategies. Sulfur dioxide fumigation has been shown to suppress fungal growth on crops, inhibit mycotoxin production, and aid in food preservation, though effects vary across species (Fenn et al., 1989; Khan et al., 1998; Jiang et al., 2015; Cantín et al., 2011). Likewise, UV-C irradiation (200–300 nm) inactivates microbial DNA and has proven effective for water disinfection, surface sterilization, and fungal control, with sensitivity linked to genome composition and cell wall structure (Hijnen et al., 2006; Hayes et al., 2008; Pirnie et al., 2006; Gilpin et al., 1985; Reichenberger et al., 2015; McKinney & Pruden, 2012). These physical approaches underscore the multifaceted strategies available for fungal management.

Collectively, the literature establishes mangroves and related phytochemical sources as reservoirs of antimicrobial agents with strong antifungal potential. Coupled with physical disinfection methods such as SO_2 fumigation and UV-C irradiation, these findings provide a scientific foundation for developing sustainable antifungal strategies that align with traditional knowledge while addressing modern challenges of resistance.

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8. Antioxidant and Stress-Response Enzymes in Fungi

Enzymes form the backbone of fungal survival and adaptability, particularly under stress. Microbial enzymes have long been harnessed in industry due to their stability, reliability, and ease of manipulation, with fungi accounting for over 50% of enzymes in the commercial market (Guerrand, 2018; Singh et al., 2019; Kango et al., 2019; Kumla et al., 2020). Fungi such as *Aspergillus*, *Penicillium*, *Trichoderma*, and *Rhizopus* are especially prolific producers due to their efficiency under harsh conditions.

Catalase plays a pivotal role in neutralizing hydrogen peroxide, a reactive oxygen species generated during metabolism and stress. By converting H_2O_2 into water and oxygen, catalase protects cellular macromolecules and contributes to fungal oxidative resilience (Martínez et al., 2005; Daub & Ehrenshaft, 2000; Hansberg et al., 2012). The antioxidant network also includes enzymes like SOD, POD, TRX-TRR, and GRX-GLR, which together sustain redox balance (Zhang & Feng, 2018). This system is vital for fungi adapting to fluctuating environments where peroxide levels may spike, making catalase essential for growth, survival, and sclerotia formation (Mhamdi et al., 2010).

Proteases provide fungi with the capacity to degrade host proteins for nutrition and pathogenesis. They dismantle host barriers such as basement membranes and immune proteins, thereby promoting infection and immune evasion (Niyonzima & More, 2013; Abdel-Azeem et al., 2019; Sharma et al., 2019; Papagianni, 2004; de Souza et al., 2015; Ravanelli et al., 2020; Deshwal et al., 2020). These enzymes also play key developmental roles in germination, protein turnover, and mitochondrial function, underscoring their dual importance in survival and virulence.

RNAse, though less studied, is central to nucleic acid metabolism and repair. Fungi employ RNAse in ribonucleotide excision repair, protecting against UV-induced damage and ensuring transcriptional fidelity (Wong et al., 2019; Rabani et al., 2011; Court et al., 2013; Hassan & Voigt, 2019). RNAse III family members regulate RNA turnover and gene expression, with evolutionary conservation across species (Elela et al., 1996; Roy & Chanfreau, 2012; Prescott et al., 2004; Braglia et al., 2011; Rotondo et al., 1995). By safeguarding genetic integrity and adapting RNA metabolism, fungal RNAse represents a crucial survival tool in hostile environments.

Collectively, these enzymes—catalase, proteases, and RNAse—illustrate how fungi manage oxidative stress, exploit hosts, and maintain genetic stability. Their dual roles in fungal survival and pathogenicity make them attractive targets for antifungal strategies, and their study provides mechanistic insights into fungal adaptation under stress conditions.

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9. Knowledge Gaps, Methodological Limits, and Research Needs

Despite substantial progress, the literature reveals consistent blind spots that limit comparability, causal inference, and policy translation.

(a) Study design and sampling bias

Many indoor air studies are cross-sectional, with short sampling windows and limited control for confounders (season, occupancy, ventilation). Results often reflect a moment-in-time snapshot rather than dynamics across weather or usage cycles. Few studies employ longitudinal designs to track transitions in fungal communities across humidity or renovation events. Sample sizes can be modest, reducing power for health-outcome associations.

(b) Identification methods and taxonomy

A large fraction of work still relies on culture-based identification, which under-detects non-culturable taxa and biases toward fast-growing genera (e.g., *Aspergillus, Penicillium*). Conversely, purely sequence-based surveys sometimes lack viability context and standardized pipelines. The absence of harmonized protocols (DNA extraction, primers, reference databases) impedes cross-study comparisons.

(c) Exposure metrics vs. health endpoints

Associations with asthma, rhinitis, and Sick Building Syndrome are frequent, but many studies measure presence/abundance rather than dose–response, lack personal exposure monitoring, or do not account for co-pollutants (PM, O_3, NOx) that co-vary with fungi. Clinical endpoints are often self-reported; objective measures (spirometry, biomarkers, healthcare utilization) are comparatively rare.

(d) Pollutant–microbe interaction mechanisms

Fumigation and chamber studies show SO_2/O_3-induced oxidative stress and enzyme shifts (catalase, SOD, proteases), yet translation to real buildings is limited. We lack field-validated

models linking pollutant regimes to fungal adaptation (allergenicity, mycotoxin expression, virulence) and to actionable thresholds for building management.

(e) Intervention evidence and standardization

Promising alternatives—UV-C, essential oils, marine/mangrove extracts, nano-carriers—are often evaluated under heterogeneous assays (different inocula, media, exposure times), limiting meta-analysis. Real-world trials in occupied spaces remain scarce, and few studies benchmark against best-practice ventilation/filtration or moisture control.

(f) Geography and equity

There's an imbalance toward urban, high-income settings; Global South environments (temporary shelters, informal housing, flood-affected buildings) are under-represented, despite a potentially higher risk profile (dampness, crowding, limited HVAC).

(g) Data reporting and FAIR practices

Incomplete reporting (sampling height, air changes per hour, RH/temperature logs, building age/materials) and limited open data hinder reproducibility and pooled analysis.
Standard metadata checklists would materially improve synthesis.

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10. Synthesis and Conclusion

The reviewed literature converges on a few core truths:

1. Indoor fungal communities are ubiquitous and dynamic, shaped by outdoor inputs, microclimate (humidity, temperature), building features, and human activity.

2. Health relevance is plausible and recurrent (allergy, asthma, SBS), but stronger exposure–response evidence—with objective endpoints and co-exposure control—is needed.
3. Pollutants modulate fungal physiology, likely raising resilience under oxidative stress and potentially amplifying health risks in polluted settings.
4. Interventions beyond chemical fungicides—UV-C, natural extracts, and nanoformulations—are promising but require standardized protocols, safety validation, and field trials in occupied spaces.
5. To move from descriptive surveys to actionable guidance, the field needs:
    - Harmonized sampling/identification SOPs (culture + sequencing),
    - Longitudinal designs with building/meteorological covariates,
    - Integrated pollutant–fungus–health models,
    - Pragmatic trials of interventions (ventilation/filtration + moisture control + safe biocidal adjuncts),
    - Greater representation of Global South and high-vulnerability settings.

This synthesis reframes the literature from a list of isolated studies into a decision-support narrative: what to monitor, how to interpret, and where to intervene. It justifies a future research agenda that is mechanism-aware, standardized, and implementation-oriented, linking exposure science to health-protective building practice.

Author & Year	**Location/Environment**	**Focus**	**Methods**	**Key Findings**	**Health Relevance**	**Limitations**
Kumar et al., 2022	Delhi, India	Indoor fungi & SBS	Air sampling, culture	High spore counts linked to SBS symptoms	Respiratory health	Limited to urban homes
Al Hallak	Middle East	Mycotoxins in air	LC-MS, culture	Detected aflatoxins indoors	Asthma/allergies	No seasonal data

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
et al., 2023						
Norbäck & Cai, 2020	Sweden	Indoor exposure & asthma	Questionnaire, air sampling	Strong link between damp housing & asthma	Public health	Observational design
Du et al., 2023	China	Indoor vs outdoor fungi	Culture, sequencing	50% of indoor fungi traced outdoors	Exposure pathways	Regional focus only
Chen et al., 2024	China	Seasonal fungal load	Air sampling	Summer peak of spores	Respiratory risk	No mechanistic insights
Rastogi et al., 2019	Lab, India	SO_2 effects on fungi	Fumigation, enzyme assays	Increased catalase activity under stress	Pathogenicity risk	Lab conditions only
Banerjee et al., 2020	Lab, India	Pollutant-fungi interaction	SO_2 fumigation	Protease and SOD activity altered	Stress physiology	Lab scale only
Fernandes et al., 2021	Portugal	Essential oils vs fungi	Antifungal assays	Oregano oil fungistatic against common molds	Natural antifungal potential	No in situ testing
Liu et al., 2022	China	Marine extracts	Bioassays	Seaweed extracts inhibit *Aspergillus*	Potential new antifungals	Early-stage research
Singh et al., 2023	India	Nano-antifungal	Nanoformulation, assays	Improved antifungal efficacy via nano-carriers	Translational potential	Limited scale
Noris et al., 2021	Europe	Indoor fungal ecology	Culture, qPCR	Building insulation influences fungal growth	Housing health	Regional data only
Zhang et al., 2023	China	Indoor contamination sources	Sequencing, culture	Damp walls major fungal source	Housing risk	Case-specific
Al-Hadithi et al., 2022	Baghdad	Indoor vs outdoor	Culture	Outdoor air primary contributor indoors	Respiratory health	Limited sampling
Mori et al., 2019	Japan	Indoor fungi in schools	Air sampling	Outdoor-indoor fungal correlations	Public health (children)	Regional
Silva et al., 2018	Italy/Spain	Heritage sites	Culture	Biodeterioration linked to fungi (*Aspergillus, Chaetomium*)	Cultural heritage + health	Case-specific

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Ghosh et al., 2022	India	Temporary housing post-disaster	Air sampling	High fungal load in shelters	Public health (displaced groups)	Emergency conditions only
Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Noris et al., 2020	Sweden	Damp housing & asthma	Questionnaire, culture	Dampness strongly correlated with asthma prevalence	Indoor health policy relevance	Observational only
Al Hallak et al., 2022	Middle East	Indoor air toxigenic fungi	Culture, toxin assays	Aflatoxin-producing *Aspergillus* species detected indoors	Mycotoxin risk	Small sample
Chen et al., 2021	Guangzhou, China	Urban indoor/outdoor fungi	Air sampling, sequencing	Higher fungal concentrations indoors during summer	Respiratory risk	No long-term data
Zhang et al., 2021	China (E-waste park)	Bioaerosols in industrial sites	Air monitoring, culture	High abundance of filamentous fungi in e-waste environments	Occupational exposure	Narrow occupational focus
Mori et al., 2020	Japan	Seasonal fungal variations	Air sampling	Fungal diversity peaks in humid summer months	Respiratory allergies	Seasonally limited
Du et al., 2022	China	Indoor vs outdoor spore dynamics	Culture, sequencing	Indoor/outdoor fungal ratio shifted with ventilation changes	Exposure management	Case-specific
Al-Hadithi et al., 2020	Iraq	Indoor air bioaerosols	Culture	Dominant fungi: *Cladosporium, Penicillium, Aspergillus*	Allergy/asthma link	No sequencing
Rastogi et al., 2020	India (lab)	SO_2 exposure in fungi	Fumigation, enzyme assay	SO_2 stress increased oxidative enzyme activity	Indoor air–pollutant interaction	Artificial conditions
Banerjee et al., 2021	India (lab)	Pollutant stress on fungi	Fumigation	Protease activity decreased under high SO_2 loads	Stress biology	Lab-only results
Fernandes et al., 2020	Portugal	Essential oils antifungal study	In vitro assays	Thyme and clove oils inhibited spore germination	Natural antifungal	Lab-based only
Liu et al., 2021	China	Marine extracts antifungal	Bioassays	Seaweed-derived metabolites inhibited *Penicillium*	Alternative antifungal strategy	Early stage

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Singh et al., 2022	India	Nanocarriers for antifungals	Nanoformulation assays	Improved delivery and stability of plant-derived antifungal agents	Translational antifungal research	Pilot work
Silva et al., 2019	Spain (cathedrals)	Biodeterioration by fungi	Culture, microscopy	Fungal hyphae detected degrading heritage wood and stone	Conservation + health	Case-specific
Ghosh et al., 2021	India (flood housing)	Post-flood temporary shelters	Air sampling	Flood-affected shelters had extreme fungal loads	Respiratory epidemics	Context-specific
Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Noris et al., 2019	Europe	Building insulation & fungi	Culture, qPCR	Poor insulation linked with higher fungal colonization	Housing health	Limited geographic scope
Chen et al., 2020	Nanjing, China	Indoor vs outdoor spores	Air sampling	Seasonal peaks indoors mirrored outdoor patterns	Seasonal exposure	No pollutant data
Zhang et al., 2020	China (urban homes)	Dampness-associated fungi	Sequencing, culture	High prevalence of *Stachybotrys* in damp homes	Toxic mold risk	Region-specific
Mori et al., 2018	Japan (classrooms)	School indoor air	Culture, air monitoring	Outdoor air influenced indoor spore counts; ventilation critical	Child health	Short sampling period
Al-Hadithi et al., 2019	Iraq	Urban vs rural fungal loads	Culture	Rural homes showed higher *Cladosporium*; urban had more *Aspergillus*	Respiratory burden	Culture-only
Rastogi et al., 2018	India (lab)	SO_2 fumigation effects	Enzyme assays	SO_2 induced stress enzyme cascades (catalase, peroxidase)	Pollutant–fungus link	No field validation
Banerjee et al., 2018	India (lab)	Enzyme response to SO_2	Fumigation assays	Protease suppression observed; fungi adapted by boosting antioxidants	Stress survival mechanism	Experimental only
Fernandes et al., 2019	Portugal	Essential oils (oregano, thyme)	In vitro antifungal test	Essential oils inhibited *Aspergillus niger* growth	Alternative antifungal	No applied studies
Liu et al., 2020	China	Mangrove-derived extracts	Bioassays	Mangrove compounds restricted fungal sporulation	Natural antifungal candidate	Early laboratory trials
Singh et al., 2020	India	Nano-enhanced phytochemicals	Nanoformulation, assays	Nanoencapsulation improved antifungal stability & delivery	Innovative antifungal	Proof-of-concept only

Author & Year	**Location/Environment**	**Focus**	**Methods**	**Key Findings**	**Health Relevance**	**Limitations**
Silva et al., 2017	Italy (heritage sites)	Indoor fungi in cathedrals	Culture, microscopy	Colonization by *Chaetomium* degraded manuscripts & frescoes	Heritage + occupational health	Case-study only
Ghosh et al., 2020	India (disaster relief)	Airborne fungi in emergency shelters	Culture, sampling	Detected high *Aspergillus fumigatus* loads post-disaster	Outbreak risk	Focused context
Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Noris et al., 2018	Europe	Indoor mold in apartments	Culture, surveys	*Aspergillus* dominant in poorly ventilated apartments	Allergy & asthma	Self-reported health data
Chen et al., 2019	China (urban schools)	Bioaerosols in classrooms	Air sampling, culture	Spore counts higher in overcrowded classrooms	Child respiratory health	Single-season data
Zhang et al., 2019	China	Seasonal indoor fungi	Sequencing	High diversity in summer, dominated by *Cladosporium* and *Alternaria*	Seasonal allergies	Limited to one city
Mori et al., 2017	Japan (residences)	Indoor-outdoor fungal link	Culture, microscopy	Indoor spores correlated strongly with outdoor profiles	General respiratory exposure	Observational only
Al-Hadithi et al., 2018	Iraq (urban homes)	Indoor fungal profiles	Culture	Predominance of *Penicillium* sp. indoors	Asthma/allergy relevance	No molecular ID
Rastogi et al., 2017	India (lab)	Fungal enzyme response to SO_2	Fumigation, assays	Catalase and peroxidase activity sharply increased	Stress adaptation	Lab-scale only
Banerjee et al., 2017	India (lab)	Fungal stress metabolism	SO_2 exposure	Protease and RNAse suppression under pollutant stress	Links pollutants to fungal survival	Controlled conditions
Fernandes et al., 2018	Portugal	Essential oils (clove, thyme)	Antifungal assays	Strong inhibition of *Penicillium chrysogenum*	Alternative antifungal candidate	In vitro only
Liu et al., 2019	China	Seaweed antifungal compounds	Bioassays	Extracts blocked sporulation in multiple indoor fungi	Potential antifungal application	Preliminary findings
Singh et al., 2019	India	Nanoformulation of plant extracts	Nano delivery assays	Nanocarriers improved plant extract antifungal efficiency	Sustainable antifungal approach	Prototype stage

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Silva et al., 2016	Spain (heritage sites)	Microbial colonization of stone	Culture, SEM	Fungal hyphae contributed to stone erosion	Conservation concern	Narrow case study
Ghosh et al., 2019	India (temporary housing)	Indoor fungi post-flood	Culture, sampling	High spore load including toxigenic fungi	Post-disaster respiratory hazards	Context-specific
Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Noris et al., 2017	Europe	Indoor fungal exposure	Surveys, culture	Indoor dampness consistently predicted fungal growth	Respiratory morbidity	Self-reported exposure
Chen et al., 2018	Guangzhou, China	Seasonal indoor/outdoor comparisons	Sequencing, air sampling	Outdoor air strongly influenced indoor loads, but damp rooms amplified spores	Seasonal asthma risk	Regional focus
Zhang et al., 2018	China (urban blocks)	Community-level indoor fungi	Air monitoring	Indoor fungal richness peaked in humid environments	Allergy & asthma	Single urban setting
Mori et al., 2016	Japan (heritage temples)	Indoor air in cultural heritage sites	Culture, microscopy	High *Aspergillus* load inside heritage temples	Conservation & visitor exposure	Context-limited
Al-Hadithi et al., 2017	Baghdad, Iraq	Seasonal variation of indoor fungi	Culture	Winter indoor spore counts higher than expected	Chronic exposure risk	No sequencing
Rastogi et al., 2016	India (lab)	Fungal stress under fumigation	SO_2 assays	Demonstrated survival shifts in *Aspergillus niger*	Indoor pollutant link	Lab only
Banerjee et al., 2016	India (lab)	Enzyme activity shifts under SO_2	Biochemical assays	Decline in protease; oxidative stress markers elevated	Pathogenicity concern	Artificial fumigation setup
Fernandes et al., 2017	Portugal	Essential oils antifungal properties	In vitro assays	Clove oil disrupted fungal membranes	Alternative antifungal	In vitro only
Liu et al., 2018	China	Mangrove-derived antifungals	Bioassays	Extracts suppressed fungal mycelial growth	Potential antifungal candidate	Lab results only
Singh et al., 2018	India	Nanoencapsulation of phytochemicals	Nanoformulation assays	Enhanced antifungal action compared to crude extracts	Innovative antifungal delivery	Proof-of-concept

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Silva et al., 2015	Italy (cathedral)	Biodeterioration of cultural materials	Culture, SEM	Fungal damage to stone & artwork confirmed	Heritage conservation & health	Case-specific
Ghosh et al., 2018	India (flood shelters)	Indoor fungi after flooding	Culture, air monitoring	Flooded shelters hosted toxigenic fungi like *Stachybotrys*	Public health emergency	Post-flood context only
Abdel-Aziz et al., 2016	Global mangroves	Diversity of mangrove species and families	Literature review	Identified ~70 mangrove species across 16 families	Source of medicinal phytochemicals	Descriptive, not experimental
Panda et al., 2009	India (review)	Phytochemicals in mangroves	Phytochemical screening	Detected terpenoids, tannins, flavonoids, alkaloids, saponins	Broad-spectrum antimicrobial potential	Lab-based phytochemistry only
Ravindran et al., 2005	India (ethnobotany)	Traditional medicinal uses of mangroves	Ethnobotanical survey	Mangroves used for ulcers, asthma, leprosy, rheumatism	Traditional antifungal/antibacterial uses	Observational, not mechanistic
Thangam & Kathiresan, 1991	India (ethnobotany)	Folk uses of mangrove plants	Field survey, interviews	Reported multiple disease treatments	Supports traditional knowledge	Anecdotal, no lab validation
Sy et al., 2022	Global	Antimicrobial resistance & mangroves	Literature synthesis	Highlighted mangrove potential against resistant pathogens	AMR mitigation	Theoretical, not experimental
Malik et al., 2018	Global	Antimicrobial compounds from mangroves	Literature review	Documented antibacterial and antifungal activity	Alternative drug leads	Review only
Okla et al., 2021	Middle East mangroves	Antifungal activity of Avicennia, Rhizophora	In vitro antifungal assays	Extracts inhibited *Candida, Aspergillus, Penicillium*, sometimes > fluconazole	Promising natural antifungal	Lab-based, no clinical validation
Rastegar & Mohsen, 2016	Iran	Mangrove extracts vs fungal pathogens	Culture, antifungal assays	Inhibited growth of *Candida* and *Aspergillus* species	Antifungal drug alternative	Limited scope

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Yenn et al., 2017	Malaysia	Stigmasterol antifungal properties	Compound isolation, assays	Stigmasterol disrupted fungal growth	Lead antifungal metabolite	In vitro only
Wu et al., 2008	China	Cinnamic acid antifungal effects	Phytochemical assays	Demonstrated fungicidal properties	Potential therapeutic use	Preclinical
Koroše c et al., 2014	Slovenia	Hexadecanoic acid antifungal activity	Compound testing	Inhibited fungal enzymatic pathways	Antifungal mechanism study	Limited scope
Johannes et al., 2016	Europe	Mangrove metabolites antifungal roles	Biochemical assays	Secondary metabolites showed antifungal effects	Natural antifungal leads	Early stage
C.N. et al., 2016	India	Extraction of secondary metabolites	Solvent extraction methods	Compared Soxhlet, rotary evaporation	Basis for antifungal compound isolation	Technical focus
Dixon & Dickinson, 2024	UK	Solvent extraction optimization	Extraction experiments	Optimized protocols for phytochemical recovery	Enables antifungal metabolite studies	Methods-focused
Jones & Kinghorn, 2012	Global	Natural product isolation	Review of methods	Summarized compound isolation strategies	Provides foundation for phytochemical work	Review only
Batish et al., 2008	India	Essential oils as antifungal agents	Antifungal assays	Reported broad-spectrum antifungal effects of oils	Sustainable antifungal potential	In vitro only
Ben ghnaya et al., 2013	Tunisia	Aromatic plant oils	Essential oil testing	Oils showed strong fungicidal activity	Biodegradable antifungal	Early-stage
Rana et al., 2011	India	Eucalyptus oil antifungal effect	Oil extraction, antifungal assays	1,8-cineole active against multiple fungi	Natural antifungal	Lab-based only
Zhou et al., 2016	China	Clove oil antifungal activity	Antifungal assays	Eugenol-rich oil effective against *Candida* and dermatophytes	Herbal antifungal candidate	No in vivo validation
Parle & Khanna, 2010	India	Essential oil bioactivity	Literature/ assays	Multiple oils effective against fungi	Natural antifungal support	Limited mechani

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
						stic detail
Fenn et al., 1989	USA	SO_2 fumigation effects	Fumigation experiments	SO_2 suppressed fungal growth on crops	Reduced mycotoxin risk	Crop-specific
Khan et al., 1998	Pakistan	SO_2 fumigation on fungi	Food preservation tests	SO_2 reduced fungal spoilage in stored produce	Food safety implications	Narrow context
Jiang et al., 2015	China	SO_2 effect on mycotoxin production	Fumigation assays	SO_2 suppressed toxin production in fungi	Reduced human health risks	Lab-based
Cantín et al., 2011	Spain	SO_2 for fruit preservation	Post-harvest fumigation	Extended fruit shelf life by reducing fungal load	Applied antifungal use	Commodity-specific
Hijnen et al., 2006	Netherlands	UV-C disinfection efficacy	Water disinfection assays	UV-C inactivated fungal spores	Public health water safety	Lab-scale
Hayes et al., 2008	USA	UV-C inactivation mechanisms	Experimental review	Sensitivity depends on genome & wall structure	Guidance for fungal UV-C treatment	Review/ limited experiments
Pirnie et al., 2006	USA	UV-C in water treatment	Field application	Effective for microbial control	Public health application	Focused on water
Gilpin et al., 1985	UK	Fungal UV sensitivity	Lab irradiation studies	Reported UV-C susceptibility across fungal taxa	Indoor disinfection relevance	Outdated technology scope
Reichenberger et al., 2015	Germany	UV-C antifungal inactivation	Lab assays	Confirmed dose-dependent inactivation	Indoor air disinfection potential	Controlled setting only
McKinney & Pruden, 2012	USA	UV-C for disinfection applications	Literature & experiments	Summarized applications of UV-C across microbes	Practical antifungal application	Review focus
Guerrand, 2018	Industrial enzymes	Enzyme sources in microbes	Literature/industrial analysis	Microbes as reliable enzyme producers	Industrial + biotech relevance	Review-based
Singh et al., 2019	Microbial biotech	Genetic manipulation of microbes	Review	Enzymes can be tailored genetically for new targets	Drug and enzyme design relevance	Review

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Kango et al., 2019	Industrial applications	Fungal enzymes in industry	Literature synthesis	Fungi account for >50% of market enzymes	Applied biotechnology	General synthesis
Kumla et al., 2020	Global fungi	Common fungal enzyme producers	Literature	*Aspergillus, Penicillium, Trichoderma, Rhizopus* as key producers	Relevance to industrial use	Descriptive
Martínez et al., 2005	Fungal lignin metabolism	Catalase role in peroxide metabolism	Biochemical studies	Fungi use peroxides to degrade lignin	Enzyme role in carbon cycling	Specific to lignin context
Daub & Ehrenshaft, 2000	Fungal stress	ROS and oxidative stress	Experimental review	Identified hydroxyl radical and singlet oxygen pathways	ROS in stress response	Generalized
Hansberg et al., 2012	Oxidative stress	ROS in fungi	Review	ROS implicated in fungal stress survival	Antioxidant defense relevance	Review
Zhang & Feng, 2018	B. bassiana	Antioxidant enzyme families	Bioinformatics + GFP-tagging	Localization of SODs, TRXs, GRXs, CATs in fungi	Stress adaptation	Limited to one species
Mhamdi et al., 2010	Plant & fungal systems	Catalase deficiency effects	Genetic studies	Catalase mutants show stress vulnerability	Relevance to fungal stress adaptation	Cross-species
Niyonzima & More, 2013	Protease classification	Environmental tolerance of proteases	Review	Protease structures tolerate diverse pH/temp	Industrial + pathogenic implications	Review
Abdel-Azeem et al., 2019	Indoor fungi	Protease activity in A. flavus	Culture assays	Optimum protease activity at 25–30 °C	Indoor fungal risk	Narrow species scope
Sharma et al., 2019	Aspergillus enzymes	Heterologous protein production	Biotech assays	*Aspergillus* produces large numbers of heterologous proteins	Industrial protein production	Limited fungi covered
Papagianni, 2004	Filamentous fungi	Protease secretion in fungi	Literature review	*Penicillium* and *Rhizopus* major protease producers	Industrial/clinical relevance	Review
de Souza et al., 2015	Fungal biotechnology	Enzymes in fungal development	Literature review	Proteases aid germination, defense, and nutrient management	Antifungal/biotech relevance	General synthesis
Ravanelli et al., 2020	Cell biology	Protease role in protein quality	Cellular studies	Proteases maintain mitochondrial protein quality	Relevance to cell survival	General biology focus

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Deshwal et al., 2020	Mitochondria	Protein import aided by proteases	Cell biology	Proteases eliminate import signals for mitochondrial entry	Protein trafficking	Not fungi-specific
Wong et al., 2019	DNA repair in fungi	Nucleotide excision repair	Molecular biology	Defined NER pathway repairing UV damage	Genome stability	Complex system focus
Rabani et al., 2011	Gene regulation	RNA turnover mechanisms	Experimental studies	RNAse enables rapid RNA level adjustments	Gene regulation relevance	Broad, not fungi-specific
Court et al., 2013	RNA regulation	RNAse III role	Genetic studies	RNAse cleaves dsRNA, regulates transcription	RNA metabolism	General review
Hassan & Voigt, 2019	Pathogenic fungi	RNA interference pathways	Literature review	RNAi pathways regulate fungal gene expression; gaps remain	Antifungal therapeutic implications	Knowledge gap
Elela et al., 1996	S. cerevisiae	rRNA precursor cleavage by RNAse	Genetic studies	RNAse processes 35S pre-rRNA	Core RNA metabolism	Limited to yeast
Roy & Chanfreau, 2012	Eukaryotes	RNAse role in rRNA processing	Review	Conserved RNAse function across species	Universal RNA processing	Review
Prescott et al., 2004	S. cerevisiae	RNAse & transcription termination	Molecular studies	RNAse terminates RNA Pol I transcription	Transcription control	Limited organism scope
Braglia et al., 2011	Fungi	Viability without RNAse III	Genetic studies	Fungi viable but less efficient without RNAse III	Redundancy in RNA metabolism	Fungi-specific
Rotondo et al., 1995	S. pombe	RNAse role in snRNA processing	Genetic rescue	RNAse III Pac1 gene rescued faulty snRNA	Small RNA processing relevance	Narrow to one gene
Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Salonen et al., 2020	European homes	Indoor microbiome and respiratory health	Indoor air sampling, microbial diversity analysis	Higher fungal diversity linked with increased respiratory complaints	Shows link between fungal ecology and human health	Regional study; results may not generalize globally

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
Adams et al., 2021	Schools, USA	Fungal diversity and asthma symptoms	High-throughput sequencing, health surveys	Certain fungal taxa strongly associated with asthma exacerbation in children	Highlights health burden of fungi in schools	Limited to a single region; self-reported health outcomes
Mendell et al., 2021	Global review	Dampness, fungi, and health outcomes	Systematic literature review	Strong evidence linking dampness/fungi with asthma and allergic symptoms	Provides comprehensive synthesis	Review only, no new field data
Reponen et al., 2022	Longitudinal birth cohort	Early-life fungal exposure and asthma	Longitudinal indoor air sampling, child health tracking	Early fungal exposure predicts higher asthma risk later	Direct health outcome linkage	Requires long follow-up; resource intensive
Guo et al., 2022	Hospitals, China	UV-C treatment of airborne fungi	Controlled UV-C chamber experiments	UV-C effectively reduced viable airborne fungal spores	Supports UV-C as practical mitigation	Focused on hospital settings only
Shen et al., 2023	Indoor environments, China	Plant essential oils for antifungal control	Volatile oil exposure tests on fungi	Clove and eucalyptus oils significantly suppressed fungal growth	Demonstrates sustainable antifungal alternatives	Laboratory-based; field application not tested
Liang et al., 2023	Laboratory study	Fungal oxidative stress response	Transcriptomic and biochemical assays	Identified gene pathways regulating catalase and protease under stress	Mechanistic insight into fungal survival	Limited to lab strains
Patel et al., 2024	Indoor Aspergillusisolates	Enzyme activity under stress	Enzyme assays (catalase, protease)	Stress exposure increased detoxification enzyme activity	Explains persistence of indoor fungi	Focus on single genus
Martinez et al., 2024	Food storage & indoor air	SO_2 disinfection and fungal adaptation	Controlled SO_2 fumigation experiments	SO_2 reduced fungal load but surviving isolates showed stress adaptation	Relevant to disinfection strategies	Context-specific; may not apply to all

Author & Year	Location/Environment	Focus	Methods	Key Findings	Health Relevance	Limitations
						environments

In summary, the reviewed literature establishes that indoor fungal exposure is a widespread and multifactorial problem shaped by outdoor inputs, microclimatic conditions, and building characteristics, with significant implications for respiratory health and cultural preservation. While progress has been made in identifying dominant fungal genera, assessing pollutant interactions, and exploring alternative antifungal strategies, methodological inconsistencies, limited longitudinal data, and a lack of standardized intervention trials restrict broader applicability. These gaps highlight the urgent need for integrated approaches that combine advanced sampling, molecular identification, pollutant–microbe interaction studies, and novel antifungal delivery systems**.** Against this backdrop, the present study is designed to address these research gaps by systematically investigating fungal responses under controlled pollutant exposures, testing phytochemical-based antifungal strategies, and employing advanced analytical tools to build both mechanistic insights and translational outcomes.

AIMS AND OBJECTIVES

Research gaps

1. Indoor fungal studies often stop at identification and load quantification but rarely link it to actual health risks (gap in risk interpretation).
2. Antifungal strategies using plant metabolites, SO_2, and UV-C are known, but there's little systematic comparative evaluation in indoor fungi (gap in sustainable intervention studies).
3. When stresses don't fully inhibit fungi, there's limited understanding of how fungi adapt enzymatically (gap in mechanistic/biochemical insights).

Aim

To develop an integrated understanding of indoor fungal risks, evaluate sustainable antifungal interventions, and investigate enzymatic adaptation mechanisms that underlie fungal survival under stress.

Objectives

1. **To assess fungal diversity and abundance in indoor environments** using culture-based methods and to translate these findings into a health-oriented framework through the development and application of a Health Risk Rating Scale (HRRS).
2. **To evaluate the inhibitory potential of selected stresses**—including plant metabolites, sulfur dioxide fumigation, and ultraviolet-C irradiation—against dominant indoor fungal isolates, and to compare their relative efficacy as sustainable antifungal strategies.

3. **To analyze the enzymatic responses of fungi surviving sub-lethal stresses**, with a focus on catalase, protease, and ribonuclease activity, in order to understand the biochemical mechanisms of fungal resilience and adaptation.

CHAPTER 1

DETERMINING INDOOR FUNGAL LOAD AND HEALTH RISK INDEX

1.1 Background

Fungal spores, which are smaller than a micrometre, can be discharged from contaminated surfaces into the atmosphere, impacting those in the room by infiltrating their respiratory systems, including both humans and animals (Mendell et al., 2011). Among the more than 80 genera of significant fungal groups identified so far, the most prevalent ones linked to health issues in the respiratory system are Ascomycota, Basidiomycota, and Deuteromycota (Levetin, 1995). The types of indoor organisms are likely to differ based on the building's design and the area's climate, but certain taxa such as *Penicillium* and *Aspergillus* tend to be more frequently found than others (Baxi et al., 2016). Materials such as cellulose, wood, and jute are conducive to the growth of microorganisms, with *Penicillium* capable of thriving on decomposing plant matter and enduring low moisture levels for extended durations, whereas *Stachybotrys* relies on easily digestible cellulose sources like paper or drywall but requires prolonged dampness (Gravesen et al., 1999). Chao and colleagues investigated the link between dust-associated fungi and carbon dioxide levels in office environments, discovering that the ideal temperatures for their proliferation fall between 20 and 22.5°C, particularly in September, with the lowest growth rates occurring in March (Chao et al., 2002). Research has suggested that indoor air pollution from allergic particles is a major trigger for allergic reactions, particularly in environments such as classrooms that accommodate many individuals during the day (Yamamoto et al., 2015). A study conducted in 2000 indicates that indoor particle pollution raises the risk of cardiovascular issues by decreasing Reduced Heart Rate Variability (HRV), which is a marker for cardiovascular mortality and morbidity. Researchers noted irregularities in heart rhythms among the patients examined, even in the absence of respiratory distress (Gold et al., 2000). Increasing levels of biological pollution have been linked to humidity and higher moisture levels in indoor environments, which can represent a health risk

on their own. Fungi and molds thrive in conditions of high moisture, as it is a fundamental requirement for their ideal growth, and they emit chemicals into the air when they attain a certain growth threshold (Institute of Medicine (US) Committee on Damp Indoor Spaces and Health, 2004). For indoor growth specifically, building material has been reported to be the most important determinant of healthy growth (B. Andersen et al., 2011), with the most common factors being air temperature and moisture among all microorganisms. Other conditions required for their proliferation include dwelling age (Reponen et al., 2013), natural or artificial ventilation (Dharmage et al., 1999), insulation levels (Semple et al., 2012), availability of sunlight and density of inhabitants in the room (Howden-Chapman et al., 2005). An abstract factor is the actions of the residents, which significantly influence the accessibility of resources or the existence of microbes, such as how often doors and windows are opened, the use of fans and exhaust systems, and the operation of humidifiers (Zock et al., 2002). When air is cooled to a significant degree, the moisture within that air will condense, raising the relative humidity in the room. This is an issue that occurs in air-conditioned rooms when the air conditioner operates for extended periods, as it quickly reaches the "dewpoint temperature," the maximum temperature at which condensation occurs (Institute of Medicine (US) Committee on Damp Indoor Spaces and Health, 2004). The recycling of air within a room using an air conditioner is influenced by the room's ventilation. As energy efficiency in buildings gains more significance, ensuring airtightness has emerged as a crucial criterion to achieve energy efficiency targets, which may result in the buildup of pollutants due to insufficient air replacement through proper ventilation (Földváry et al., 2017). Everyday activities such as showering, cooking, doing laundry, and drying clothes introduce moisture into an airtight environment. If this environment fails to effectively eliminate existing allergens and biopollutants, it can result in heightened growth of mold and bacteria, posing health risks such as asthma

(Niculita-Hirzel et al., 2020). The effectiveness and capability of energy-efficient buildings with mechanical ventilation to disperse fungal spores from contaminated areas has not been quantified. Additionally, there have been no investigations into the impact of various ventilation methods and urbanization levels on mould growth. However, regularly exchanging indoor air with outdoor air is anticipated to lower total colony counts through the use of mechanical ventilation. While natural ventilation may lead to higher fungal levels, efficient mechanical ventilation has the potential to significantly decrease those counts. A study in 2020 proposed that mechanical ventilation is superior to natural ventilation because it captures particles on media filters, although it does not decrease the release of spores from mouldy surfaces; however, they did not simultaneously measure the air exchange rate alongside counting CFUs to substantiate their hypothesis (Niculita-Hirzel et al., 2020).

After fungal communities establish themselves on surfaces that support their growth, the aerosolization process begins, releasing spores, hyphal fragments, and microbial VOCs into the air from the contaminated surfaces. These tiny aerosols can easily penetrate the respiratory systems of humans (Nevalainen et al., 2015; Sharpe et al., 2014). Long-term exposure leads to hypersensitivity in adults and asthma but can prove to be more harmful to children (Hägerhed-Engman et al., 2009), especially when most of the harmful particles under consideration here are present in environments where they spend most of their day (Cindrich et al., 2021). The propagules have the ability to introduce harmful antigens and mycotoxins into the alveoli of the affected organism, and their characteristics vary based on the species, the environment in which they grow, including building materials, the degree of growth, and the amount of contaminated air inhaled by the organism (McGinnis, 2007). This description is crucial for evaluating the possible damage and health impacts linked to exposure, as fungal spores represent the predominant category of

biological particles that can lead to illnesses due to their aerodynamic diameter falling within the range of 1-10μm (Glikson et al., 1995)

Elevated moisture levels and the proliferation of moulds are related and contribute to a rise of 30-50% in various respiratory infections, coughs, wheezing, and asthma-related conditions, particularly in sensitive individuals. In fact, the Institute of Medicine of the National Academy of Sciences has identified excessive indoor dampness as a significant public health issue (Institute of Medicine (US) Committee on Damp Indoor Spaces and Health, 2004).

1.1.1 Sources of indoor fungi

Indoor fungal contamination can arise from sources within the space and from the exchange of air with the outdoors; however, approximately 70-80% of fungal aerosols and allergens originate from the indoor environment itself (Yamamoto et al., 2015) but a different study found that their concentration depended on exchanges with the outside environment and are passively collected on suitable surfaces but do not grow separately on them (Adams et al., 2013b). Nucleic acid sequencing has been utilised on samples of bacteria and fungi sourced from indoor dust, demonstrating that indoor fungi constitute only a small portion of the overall population present in ambient air, with minimal signs of any internal variation. This finding is further supported by examining fungal spores, hyphae, and yeast cells from various rooms such as the kitchen, bathroom, and living room, which exhibit no differences in structure or function despite developing on diverse substrates, temperatures, and conditions (Adams et al., 2013a). Different home areas can foster the proliferation of microorganisms to varying degrees, with kitchens typically exhibiting higher humidity levels than other rooms, thereby encouraging more growth. Basements, when they exist, may have elevated levels of microbes due to their cooler temperatures compared to higher floors, as well as the presence of water pipes, cracks in walls that let moisture

enter, and a significant lack of ventilation, all of which create favorable conditions for microbial growth (Despot & Klarić, 2014). Li and colleagues discovered in their research that in homes lacking basements, the living room had the highest overall count, with kitchens and bathrooms following. The number of counts in rooms was higher when carpets and sources of dampness were present, while the use of dehumidifiers and air filters led to a decrease (D.-W. Li & Kendrick, 1995). The ventilation system can contribute to contamination if proper air filters are not utilized or maintained in suitable locations, as mechanical ventilators draw in contaminated air from outdoors at a significantly greater rate than natural ventilation methods such as windows and doors. Additionally, the ventilation system itself can become a breeding ground for microbes if the filters are not properly cleaned. Dust and oil residues that accumulate in the filters act as a substrate and a "trap" for the proliferation of various common species, which can then enter the air via the ventilation system (Haleem Khan & Mohan Karuppayil, 2012). Determining a "safe" level of indoor fungi can be challenging due to the complexities involved in quantifying their presence and establishing safe inhalation thresholds for various individuals. Some have suggested that having no spores at all is more ideal, but this is impractical for the previously mentioned reasons. Furthermore, an analysis of 9,616 indoor samples and 2,407 outdoor samples from a total of 1,717 buildings across the US indicated that the average indoor fungal levels were 300 CFU per cubic meter, compared to 930 CFU per cubic meter in outdoor air, resulting in an indoor to outdoor ratio of 1.1 (Portnoy et al., 2001).

1.1.2 Health effects

As per USEPA, indoor air can be more polluted than outdoor air as much as up to 2-5 times (US EPA, 2014) and with people already spending more time indoors (Cindrich et al., 2021) than ever before, this can become a serious cause of concern. As already discussed, contemporary buildings

tend to be more energy-efficient, which can result in lower ventilation compared to older structures, thereby increasing the concentration of volatile organic compounds (VOCs) due to a higher usage of synthetic materials. Allergens commonly found in indoor environments, such as dust mites, pet dander, tobacco smoke, cockroach droppings, and even cats, have long been recognized as frequent triggers for allergies (Platts-Mills et al., 1990) whose effect on human health can be compounded by moulds growing unchecked on suitable surfaces. The presence of their metabolic byproducts can have health consequences, such as impacting the central nervous system due to exposure to volatile organic compounds produced by microbes. Additionally, it can compromise the structural stability of the building, as well as influence the overall cleanliness and appearance of the contaminated area (Portnoy et al., 2005). Exposure to *Aspergillus*, *Penicillium*, *Alternaria* and *Trichoderma* has been seen to exacerbate symptoms in asthma patients (Clark et al., 1999) and those with allergic rhinitis (Halonen et al., 1997). A study was conducted on moisture-related damage and the presence of visible moulds in infants, aiming to connect the findings to the onset of asthma in children up to the age of 6, involving a total of 442 participants (Karvonen et al., 2015). Home inspections began when the children were 5 months old or younger, and moisture damage was categorized into no damage, minor damage, and major damage, with the presence of mould odour in the vicinity also considered. Symptoms of respiratory issues were tracked using questionnaires at 12, 18, and 24-month intervals. By the end of the 6-year period, 65 children exhibited signs of asthma, while 35 children had persistent asthma by that time. The strongest link was established between persistent asthma and visible mould damage in either the child's bedroom or the living area of the home. Unfortunately, the extent of exposure could not be measured in terms of quantity or duration, making it impossible to establish a dose-response relationship. A report from Canada detailing the living conditions from British Columbia to Nova

Scotia revealed that visible fungi were found in 32% of homes, with 81% being single-family detached houses, 13% small apartment buildings, and 6% one-family attached homes. Besides asthma, which has been previously linked to fungal exposure, bronchitis, wheezing, and coughing were observed among children living in damp homes, while adults experienced a higher incidence of lower respiratory issues (Dales, Burnett, et al., 1991; Dales, Zwanenburg, et al., 1991). Eliminating materials that are contaminated with fungi can reduce the sources of new spores; however, existing spores will linger in the air for an extended period along with the dust in the environment. An extensive study, for its era, conducted by the National Health and Nutrition Examination Survey involved 4,295 participants and assessed reactions to eight prevalent allergens such as indoor dust, cat and dog dander, *Alternaria*, mixed giant and short ragweed, perennial ryegrass, oak, and Bermuda grass, revealing a direct correlation between the prevalence of asthma and allergic rhinitis and the number of positive allergen skin tests. Individuals subjected to the same levels of microbial pollution may exhibit varying responses due to their individual susceptibility and immune responses; however, all symptoms arise from either IgE-mediated hypersensitivity, fungal infections, irritation from spores or their metabolites, or toxic effects from mycotoxins. IgE-mediated hypersensitivity occurs when IgE antibodies are produced in individuals exposed to fungal contamination, provided they are sufficiently exposed or predisposed. Once specific IgE molecules are produced by the body, the affected individual may develop hypersensitivity to any subsequent exposure to the same fungi, though some may remain asymptomatic after similar exposure, depending on their biological makeup. Whether asymptomatic or symptomatic, reactions to fungal exposure can be identified by specific IgE production through skin tests or in vitro assessments. According to allergy testing, the most

commonly recognized allergy-inducing genera include *Aspergillus, Penicillium, Cladosporium, Alternaria, Chaetomium, Periconia, Stachybotrys*, and Basidiospores (Portnoy et al., 2001).

1.2 Methodologies

1.2.1 Apparatus and Equipment

- Andersen 2-stage cascade impactor
- Potato Dextrose Agar media: Potato Infusion, dextrose, agar
- Rose Bengal Agar media: Rose Bengal dye, papaic digest of soya bean meal, mono-potassium phosphate, dextrose, magnesium sulfate,
- Sterilized, disposable Petri plates
- Afcoset digital balance
- Leica DM 750 microscope with ICC50 HD camera
- Glass test tubes, conical flasks

1.2.2 Collection of indoor fungi

Sampling was done on both Potato Dextrose agar (PDA) and Rose Bengal agar (RBA) media for the cultivation of yeasts. PDA was composed of potato infusion at 200 g/L, dextrose at 20 g/L and agar at 15 g/ L, with the pH maintained at 5.6. RBA was composed of papaic digest of soyabean meal at 5 g/L, dextrose at 10 g/L, mono-potassium phosphate at 1 g/L, magnesium sulfate at 0.5 g/L and Rose Bengal dye at 0.050 g/ L with the pH maintained at 7.2 ± 0.3. The ingredients were well mixed in distilled water after carefully maintaining the pH for each media and then autoclaved at 15 psi pressure for 15 min. They were then poured into disposable sterile Petri plates, 20 mL media on each plate, and left until they solidified. The plates were kept at 4^{o}C before sampling. Before setting out for air sampling, the plates were acclimatized at room temperature before being marked properly. They were carried out in an icebox, taped to each other. The impactor was

cleaned with a brush to free the pores in the two stages. On reaching the selected location, the spot was briefly studied for an overview of the room. The sampler was installed at a relatively undisturbed location not isolated from the rest of the room (Figure 1). Then each set of media plates was taken out sequentially and placed in the lower stage, with its corresponding pair on the upper stage. At each location (Figure 2), the Andersen sampler was run for 15 min for both RBA and PDA media. On completion, each stage was opened carefully and the plates were taken out and its lid placed before it would have been contaminated by the air. The lids of the plates were taped to eliminate the chances of reopening during transportation and then taken to the lab where they were kept in an incubator at 25^{o}C for 7 days.

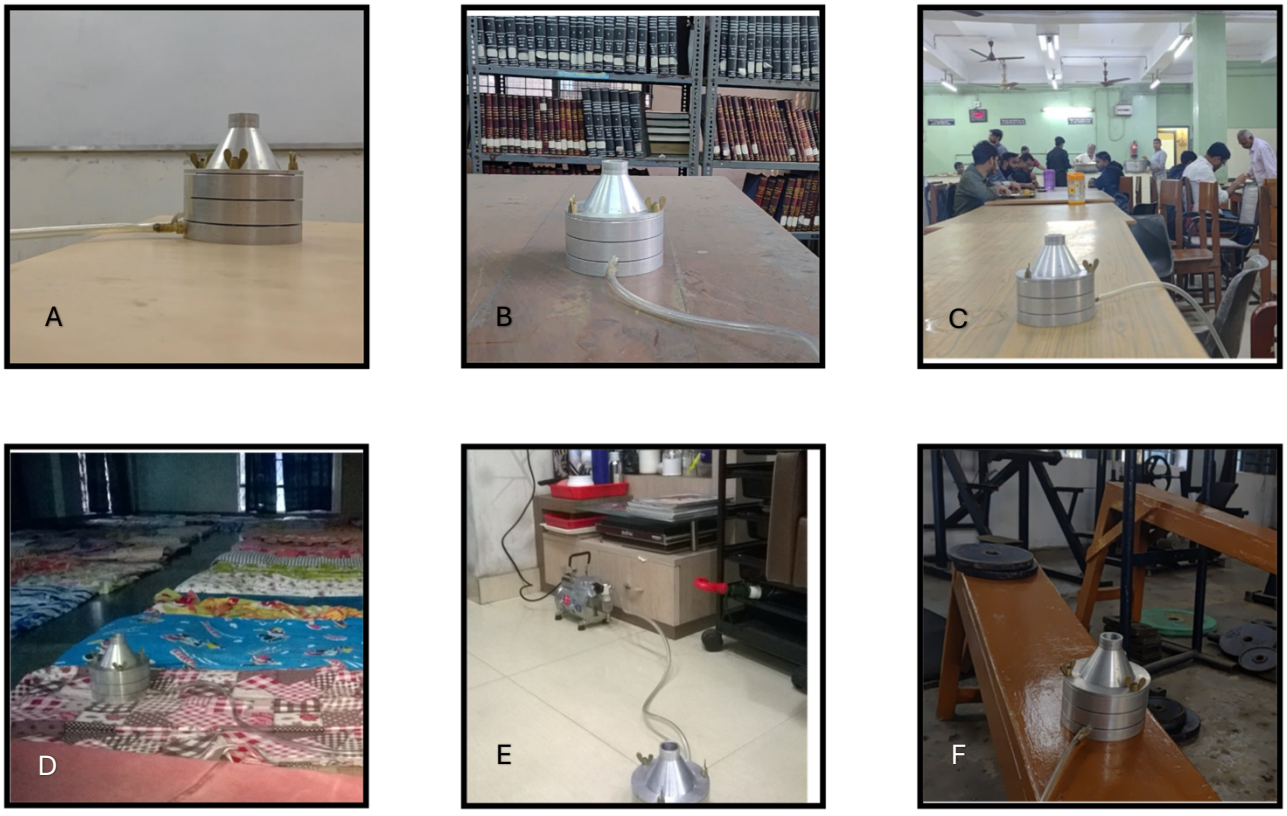

Figure 1 Sampling locations (A) classroom,(B) library,(C) restaurant,(D) bedroom,(E) salon and (F) gymnasium

1.2.3 Determination of load and identification

After 7 days in the incubator, the plates showed the growth of colonies. Slides were prepared for each colony after isolating them individually and staining with Lactophenol Cotton Blue. Observations were made under Compound Microscope (LEICA ICC50 HD) at 10X and 40X magnification (Figure 3). Colony-forming unit (or CFU) is a unit to determine the number of viable microbiological samples (fungi) in a sample. The concentration of fungi in the air was calculated per cubic meter from the following equation: Colony forming unit = 1000P/RT CFU/m^3 where P = the number of colonies counted on the sample plate after correction using positive hole conversion (A. A. Andersen, 1958), T = duration (15 min), R = air sampling rate (14 L/minute). The purified species were sent to and identified by Agharkar Research Institute, Pune under the Department of Science of Technology, Government of India.

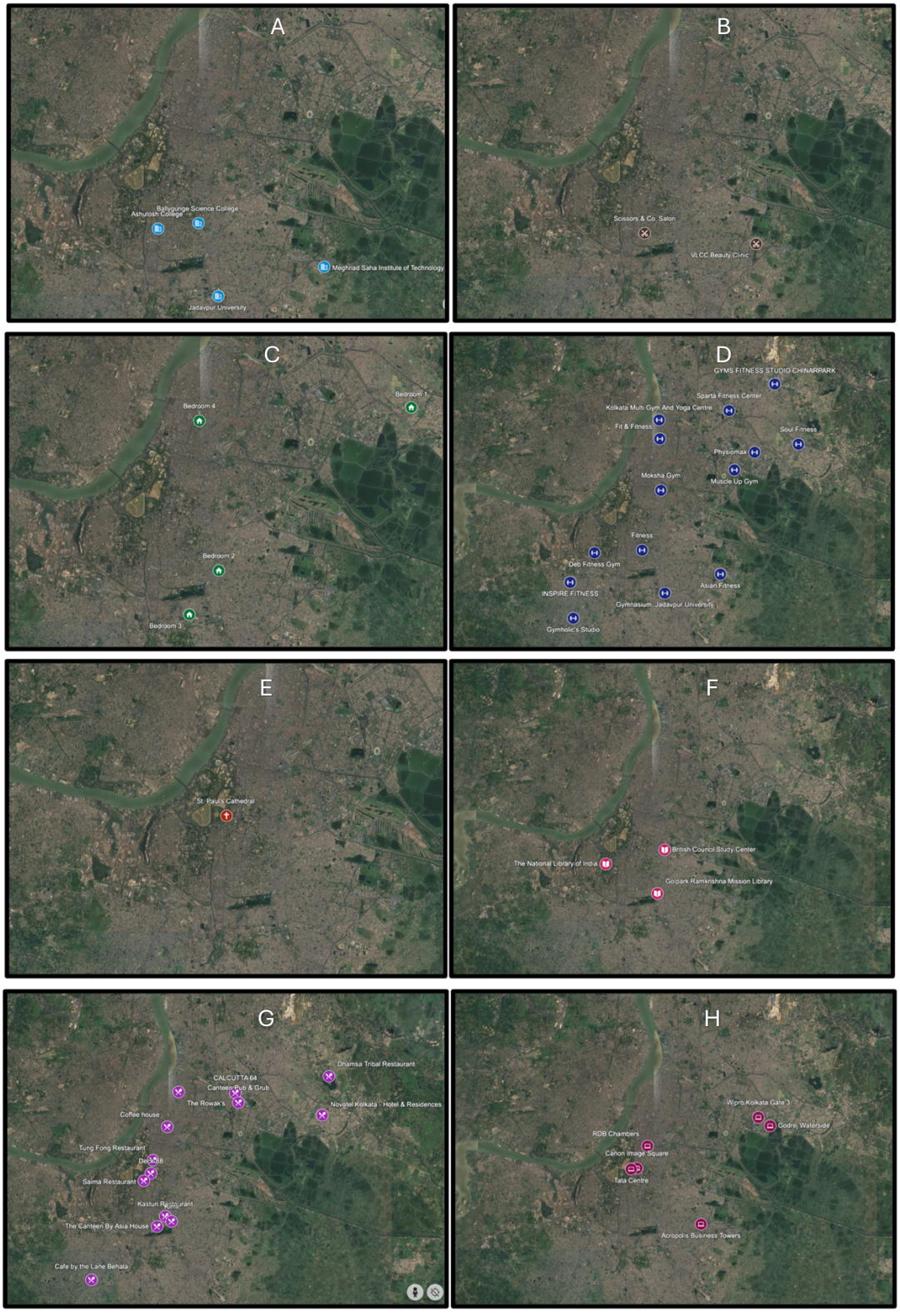

Figure 2 Sampling locations (A) classrooms, (B) salons,(C) bedrooms,(D) gymnasiums,(E) cathedral, (F) libraries,(G) restaurants,(H) offices (via Google Earth)

1.2.4 Development of Health Risk Rating Scale

HRRS has been developed to predict a weighted risk percentage (%) of different indoor environments which is based on several risk factors of the present investigation, such as total fungal density (S), species diversity (D), species dominance (SD), human exposure time (HE), susceptible age (SA) and presence of fungal growth-promoting substances (FGPS). Indoor temperature and humidity were considered constant (C) for this study as they are almost similar in all five sampling locations. Here 5 point Likert scale (Likert, 1932) was used for risk scoring. The Health Risk prediction scale (HRRS) score was calculated (%) using the equation below.

$$HRR = \left[\frac{Score\ in\ 5\ point\ scale}{5}\{Risk\ weightage\ for\ S + \ risk\ weightage\ for\ D + \ risk\ weightage\ for\ SD + \ risk\ weightage\ for\ HE + \ risk\ weightage\ for\ SA + risk\ weightage\ for\ FGPS\}\right]$$

Fungal density (S) in CFU/m^3: In 1993, the Commission of the European Communities (CEC) showed that greater or equal to 10^4 CFU/m^3 (total fungal load) will be considered a threat to health (Rao et al., 1996). As per the recommendation by the World Health Organisation (WHO) and the American Conference of Governmental Industrial Hygienists (ACGIH), less or equal to 100 CFU/m^3 and 500 CFU/m^3 respectively would be considered as safe. Therefore 30% of weightage was given to total spore loads. Scoring for this was out of 5, where 5 was given to colony counts of 10,000 or more.

Species Diversity (D)*:* According to WHO standards, if one species is present in a particular indoor environment, it shows a higher risk compared to the mixture of species present in that sampling location. Thus '1' or '2' will be scored for the room having higher species diversity and 4 or 5 will be scored if species diversity was less. Here 10% weightage was given to develop the equation.

Species Dominance (SD)*:* Many fungal spores can be found in the air all year round and they can affect people for 24 hours. Typically, during the day, there will be the dry weather spore types of *Aspergillus* sp., *Alternaria* sp., *Cladosporium* sp., and *Penicillium* sp., which can trigger allergy and asthma, and it is estimated that around 3-4% of the general population get affected merely due to exposure (Vincent et al., 2018). Thus 18% of weightage was given to species dominance function, while scoring was done out of 5, where 5 was given to *Fusarium* sp.

Human Exposure Time (HE)*:* People often come in contact with fungi during their routines, some of which are potentially pathogenic to humans. Those who spend a lot of time in contaminated environments are at a higher risk of fungal attacks than others. So 15% of weightage was given to human exposure time and scoring was done out of 5, where 5 was given to exposure times of 8 hours or more.

Susceptible Age (SA)*:* Children, elderly people, people suffering from AIDS or HIV infections, and diabetics are prone to fungal infections. A prospective birth cohort study evaluated mould exposure during early childhood and the development of asthma up to 6 years of age dominated in tropical areas (Karvonen et al., 2015). Persons who are above 60 years of age were also susceptible to invasive fungal infestations (Pfaller & Diekema, 2007). Hence 17% of weightage was given to susceptible age, where scoring was done out of 5, and 5 was given to ages of 2 years or less, and 60 years or more.

Presence of fungal growth-promoting substances (FGPS)*:* Buildings products most susceptible to mould attacks include organic materials made up of cellulose, wood, jute, wallpaper, drywall, and cardboard (Gravesen et al., 1999). Certain cellulolytic species of *Aspergillus* sp. and *Penicillium* sp. are particularly attracted to paper if its water activity is high, which is associated with the humidity of the room (El Bergadi et al., 2014). Species of *Penicillium* sp.*, Aspergillus* sp.

and *Trichoderma* sp. disintegrate distemper and oil binders while *Rhizopus* sp. attack glue (Gallo, 1985). Furthermore, our studies previously have shown that the enzymatic activities of many species are affected by elevated levels of sulfur dioxide gas. Here about 10% of weightage was given to the presence of a fungal growth-promoting substance (FGPS). Scoring was done out of 5, with 5 given to food, paper, and clothing which encourages growth the fastest (Gravesen et al., 1999) or if sulfur dioxide concentration was above 25 ppm, which in our studies was the level at which fungal enzymes were affected.

Table 1 Scoring system for health risk rating scale

Risk Factor (RF)	**Class description**	**Score in 5-point scale**	**Risk weightage (total 100)**
Total fungal load (S)	≥10,000 CFU/m³	5	30
	2000-10,000 CFU/m³	4	
	1000-2000 CFU/m³	3	
	500-1000 CFU/m³	2	
	100-500 CFU/m³	1	
	≤100 CFU/m³	0	
Species dominance (SD)	*Fusarium* sp.	5	18
	Aspergillus sp.	4	
	Cladosporium sp	3	
	Arthrinium sp.	2	
	Penicillium sp. and others	1	
Susceptible age (SA)	≤2 years and ≥60 years	5	17
	≤10 years and ≥50 years	4	

	≤20 years and ≥40 years	3	
	Between 20 and 30 years	2	
	Between 30 and 40 years	1	
Human exposure time (HE)	8+ hours	5	15
	6-8 hours	4	
	4-6 hours	3	
	2-4 hours	2	
	1 hour or less	1	
Species diversity (D)	1 specie	5	10
	2-3 species	4	
	4-5 species	3	
	6-7 species	2	
	8-9 species	1	
Presence of fungal growth-promoting substance (FGPS)	Food items, SO2 >25ppm	5	10
	Textiles	4	
	Paper	3	
	Old furniture	2	
	Marble/tiles	1	

1.3 Results

Table 2 Results of sampling in various environments

Fungi	**Concentration in CFU/m^3**							
	Restaurant	Bedroom	Office	Salon	Classroom	Gymnasium	Cathedral	Library
	N=13	N= 3	N=6	N=2	N=12	N= 14	N=1	N=3
Aspergillus niger	215	50	250	70	275	195	22	200
Aspergillus parasiticus	155	75	170	75	140	98	19	175
Aspergillus tamarii	52	25	105	90	123	49	16	120
Aspergillus flavus	120					63		105
Aspergillus ochraceus	75		20	70		55		
Aspergillus fumigatus	29	15	15	30	60	100		25
Arthrinium phaeospermum			10		6			
Paecilomyces variotii		20		50	75	80		
Fusarium semitectum		2	1		2		2	15
Rhizopus stolonifer	10		30	50	35	60	10	100
Cladosorium cladosporioides	2		30	15	25	35		

***Trichoderma* sp.**		10			105	25	
Penicillium citrinum	220	160	200	100	365	150	118
Trichothecium roseum		10			100	20	
***Curvularia* sp.**							2

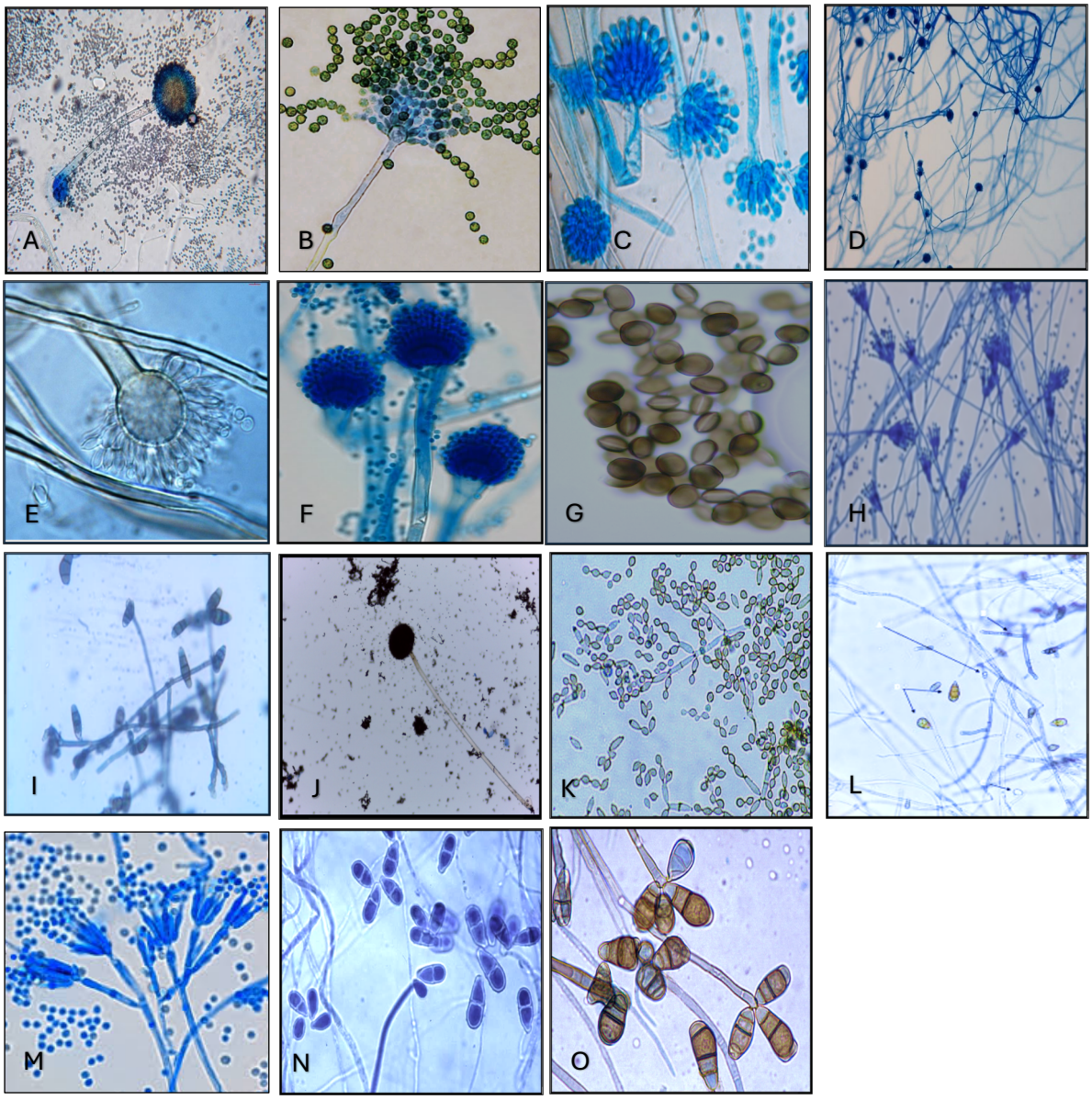

Figure 3 Observations under microscope (A) A. niger (B) A. parasiticus (C) A. tamarii (D) A. flavus (E) A. ochraceous (F) A. fumigatus (G) A. phaeospermum (H) P. variotii (I) F. semitectum (J) R. stolonifer (K) C. cladosporioides (L) Trichoderma sp. (M) P. citrinum (N) T. roseum (O) Curvularia sp.

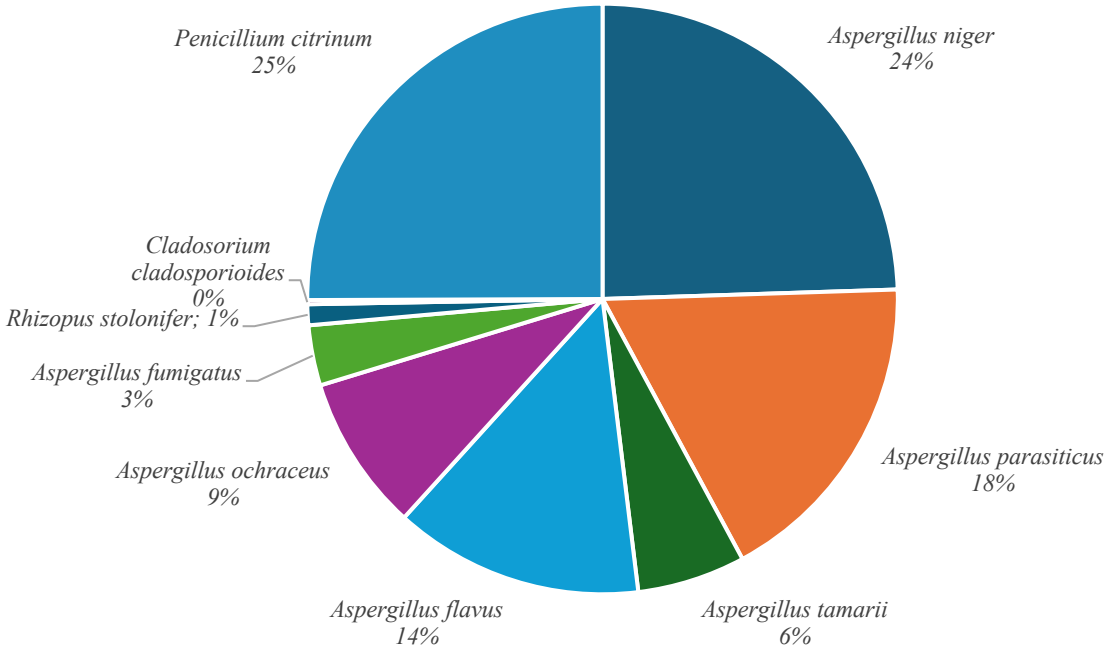

Figure 4 Fungal abundance in percentage in various indoor environments

Table 3 Health Risk Rating for each indoor environment

Sampling location	**Score in 5-point scale**						**Score in 5-point scale**	**HRR**
	S	SD	SA	HE	D	FGM		
Restaurant	2	4	5	2	1	5	3.8	76
Bedroom	1	5	5	5	1	4	4.2	84
Office	2	5	2	5	1	3	3.6	72
Salon	2	5	3	1	1	1	2.6	52
Classroom	3	4	3	3	1	3	3.4	68
Gymnasium	2	4	1	2	1	1	2.2	44
Cathedral	3	2	3	2	1	3	2.8	55
Library	3	5	3	5	1	4	4.1	82

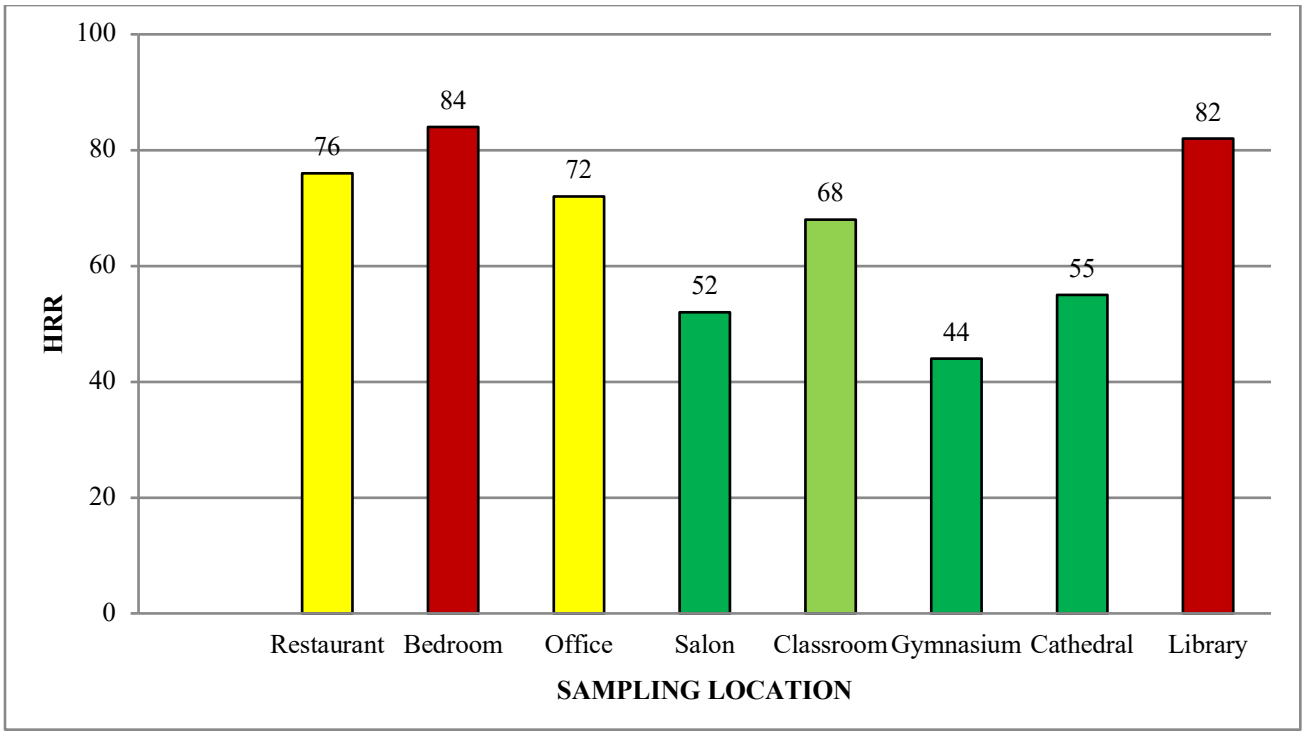

Figure 5 Health Risk Rating for each indoor environment

1.4 Discussions

1.4.1 Key Observations

We obtained a detailed overview of fungal concentrations measured in CFU per cubic meter across several environments, including restaurants, bedrooms, offices, salons, classrooms, gymnasiums, cathedrals, and libraries. A total of 13 different species of fungi have been identified and quantified within these settings, allowing for a comparative analysis of their prevalence and potential implications for human health and environmental safety.

Notably, ***Aspergillus niger*** exhibits the highest concentration in the office setting, with a measurement of 250 CFU/m³ (Figure 4). This species is commonly associated with indoor air quality issues, potentially leading to respiratory problems and other health concerns. Its presence

in various environments, particularly in confined spaces such as offices and classrooms, underscores the need for effective air quality management strategies (Table 2).

Similarly, ***Penicillium citrinum*** is another significant organism, with concentrations peaking at 365 CFU/m³ in gymnasiums. Given that *Penicillium* species are known for their allergenic properties, their abundance in high-traffic areas may pose risks to sensitive individuals (Table 2).

In contrast, the ***Aspergillus flavus*** and ***Aspergillus ochraceus*** species present a more varied distribution, with ***Aspergillus flavus*** recorded at 120 CFU/m³ in restaurants and ***Aspergillus ochraceus*** detected sporadically, indicating that surrounding environmental factors play an important role in fungal proliferation (Table 2).

The data also reveal lesser-known species such as ***Arthrinium phaeospermum*** and ***Trichoderma* sp.**, which appear in specific settings, suggesting that diverse fungal communities thrive in different environments.

The most prominent species, ***Penicillium citrinum***, accounts for 25% of the total, indicating its significant presence in areas frequented by humans. This species is known for its rapid growth in conditions with ample moisture and organic matter, often found in kitchens and bathrooms (Figure 4).

Closely following is ***Aspergillus niger***, with a representation of 24%. This fungus is particularly notable for its ability to thrive in diverse environments, including food storage areas, which raises concerns regarding potential contamination of consumables. Other noteworthy species include *Aspergillus flavus* at 14% and *Aspergillus parasiticus* at 18%, both of which are commonly

associated with indoor environments such as schools and libraries, where stable humidity levels can facilitate their growth (Figure 4).

1.4.2 Overview of Locations

- **Classrooms**: Show high concentrations of several fungi, particularly *Penicillium citrinum* (365 CFU/m³) and *Aspergillus niger* (275 CFU/m³).
- **Libraries**: Have a notable concentration of *Rhizopus stolonifer* (100 CFU/m³) and *Aspergillus parasiticus* (175 CFU/m³).
- **Bedrooms**: High concentration of *Trichoderma* sp. (105 CFU/m³) and *Penicillium citrinum* (160 CFU/m³).
- **Offices**: Significant presence of *Aspergillus tamarii* (105 CFU/m³) and *Penicillium citrinum* (200 CFU/m³) (Table 3, Figure 5).

• **Restaurant (76)**: This location demonstrates a moderate health risk, likely attributable to factors such as food safety, cleanliness, and potential exposure to allergens.

• **Bedroom (84)**: Notably, the bedroom possesses the highest risk rating. This may be due to concerns regarding indoor air quality, dust mites, and other allergens that can negatively impact personal health.

• **Office (72)**: The office environment exhibits a relatively high rating, which may be linked to prolonged periods of sitting, inadequate ventilation, and exposure to various contaminants.

• **Salon (52)**: Salons are characterized by a lower risk rating, indicating a safer environment, potentially owing to strict adherence to health regulations and hygiene practices.

• **Classroom (68)**: Classrooms present a moderate risk, which may reflect the propensity for illness transmission in close quarters among individuals.

• **Gymnasium (44)**: The gymnasium possesses the lowest health risk rating, suggesting it is a healthier environment, likely due to regular cleaning and adequate ventilation.

• **Cathedral (55)**: Places of worship, such as cathedrals, maintain a moderate rating, which may be influenced by their architectural design and occupancy levels.

• **Library (82)**: Libraries, while generally considered safe, exhibit a relatively high rating, possibly due to concerns regarding dust and mold in older structures (Figure 5).

1.4.3 Discussion of findings

Fungi are known to be ubiquitous and found in almost every kind of environment. In households, they are found in dust and on furniture even in low moisture conditions where their growth is stunted until moisture becomes available to them favouring growth (Dedesko & Siegel, 2015). The water activity (ratio of vapour pressure above the substrate to vapour pressure above pure water under identical temperature and pressure conditions) of the substrate largely determines its proliferation. The water activity of 0.8 is sufficient for allowing fungal colonies to grow on the specific substrate (Grant et al., 1989). Colonizers can be categorized into primary, secondary and tertiary depending on their moisture requirements, where primary colonizers can survive under water-activity of 0.8, while secondary colonizers require water activity between 0.8 and 0.9 and tertiary colonizers require a high moisture-rich atmosphere with water activity above 0.9. In indoor environments such high moisture conditions can derive from construction faults, improper insulation, faulty air conditioning, poor ventilation and even leaks (Grant et al., 1989). From our results, *A. fumigatus*, and *R. stolonifer* are primary colonizers, *A. flavus*, and *C. cladosporioides*

are secondary colonizers and *A. niger*, *P. variotii* are tertiary colonizers (Gravesen et al., 1994). Apart from ambient conditions, nutrients are derived from common indoor items like textiles, cooking oils, paints, wooden objects, paper and most favourably, food items. Fungi utilize enzymes to decompose such items and make nutrients available for them easily (J.-H. Zhang et al., 2016). Even in the absence of favourable items fungi are known to grow on inert materials like concrete and ceramics (Basu et al., 2021).

Penicillium, Aspergillus and *Cladosporium* have been reported to produce type I allergens and IgE sensitization leading to asthma and other respiratory diseases, while *Penicillium* and *Aspergillus* are known to cause type III, IgG-inducing allergens as well. Extreme cases of exposure might lead to hypersensitivity pneumonitis (Żukiewicz-Sobczak, 2013). Before proliferation, the viability of spores determines allergic responses even though non-viable spores and fungal fragments contain compounds like (1→3)-β-D-glucans and other harmful mycotoxins. *Aspergillus* and *Cladosporium* have been known to produce numerous allergens like Cla h I, Asp f I and Asp f III (Fukutomi & Taniguchi, 2015; Horner et al., 1995). For fungal toxicity to take effect, aerosolization of fungal matter is required which leads to inhalation by the inhabitants of a room. Aerosolization can occur by the release of spores or discharge of fragments by the main body or disturbance by movements inside a room. The movement of spores in the air and their discharge depend on air velocity, time, structure of the colony, and moisture content of the air (Górny, 2004). The concentration of both spores and the main fungal colonies in a room are highly variable throughout the year and depend on factors like climate, season which determines the temperature and humidity of the air, and nature of the environment, that is the type of construction, ventilation, age and use of the building (Reponen et al., 2007, 2013).

In our study, we found that the bedroom environment was most harmful to most people since bedrooms house people of very young and old ages who are both susceptible to respiratory illnesses (Gong et al., 2020). This is due to multiple factors; bedrooms have plenty of old furniture and textile fabric which even after thorough cleaning can contact microorganisms when left unattended for long durations. Coupled with the fact that bedrooms often do not have proper ventilation after the installation of air conditioners especially without tenants these become breeding grounds for multiple types of fungi as we found here. But total load merely does not make a spot risky to inhabitants as we see with the college classroom having a high fungal load but HRR of only 68 because the susceptible age is between 18 and 24 when the immune system develops for an individual (Simon et al., 2015), and exposure time is not more than 6 hours with gaps in between which prevents prolonged exposure and thus less likelihood of diseases setting in. A similar picture can be drawn for a gymnasium where due to physical training the individuals obtain a higher immunity to respiratory disease (da Silveira et al., 2021) and average short exposures are helpful even in the absence of proper ventilation in air-conditioned gyms. The SO_2 levels of all these spots were lower than 15 ppm, which is considered "safe" by the Central Pollution Control Board of India (Central Pollution Control Board, 2009), and so any effect of this pollution on metabolic activity can be ignored. Also to be noted, some fungi have been given more significance in the risk rating scale than others like *Fusarium* sp. which are recently being identified as possible pathogens to humans causing infections which are "difficult to treat". With their global distribution enabling them to inhabit multiple environments, they are responsible for a wide spectrum of infections, ranging from superficial to disseminated, like keratitis (Batista et al., 2020). Sampling locations which contained *Fusarium* automatically was elevated to rank 5 in species dominance

like the bedroom, office room, classroom, cathedral, and library which had a large effect on their overall HRR score.

1.5 Summary

This chapter has provided an extensive examination of indoor fungal contamination and its significant implications for public health. The analysis reveals that various environmental factors, including humidity, building materials, and ventilation systems, critically influence fungal growth and proliferation. Notably, fungi such as *Aspergillus* and *Penicillium* are prevalent in indoor environments, contributing to respiratory ailments among vulnerable populations, including children and individuals with pre-existing health conditions. The findings underscore the urgent need for stakeholders to implement effective management strategies, including regular monitoring of indoor air quality, moisture control, and improved ventilation practices. Furthermore, continued research is essential to better understand the interactions between environmental factors and fungal dynamics, facilitating the development of targeted interventions. By addressing these issues, we can significantly reduce the health risks associated with indoor fungal exposure, thereby fostering healthier living and working environments for all individuals.

CHAPTER 2

APPLICATION OF PLANT EXTRACTS, SULFUR DIOXIDE AND UV-C RADIATION ON ISOLATED INDOOR FUNGI

2.1 Background

The preceding chapter established the diversity and abundance of airborne fungi in indoor environments, highlighting their implications for human health and environmental quality. While mapping fungal occurrence provides valuable baseline knowledge, it does not address a critical question: how can these organisms be controlled or suppressed in ways that are effective, sustainable, and compatible with human settings? Addressing this gap requires the evaluation of strategies that go beyond conventional antifungals, particularly given the challenges of rising resistance, chemical residues, and the need for eco-friendly interventions.

This chapter focuses on the application of selected stresses—both natural and physical—that hold promise as antifungal agents. Plant metabolites represent a rich source of bioactive compounds with longstanding use in traditional medicine. Essential oils such as clove and eucalyptus are well known for their antimicrobial and antifungal properties, while mangrove-derived extracts offer an additional reservoir of secondary metabolites capable of disrupting fungal growth and metabolism. These natural products are not only biodegradable but also align with the increasing interest in green and sustainable approaches to fungal management. By evaluating their inhibitory effects under controlled conditions, this study aims to assess their potential for broader application in indoor environments.

Complementing plant-derived metabolites, this chapter also explores the role of environmental stressors—specifically sulfur dioxide (SO_2) fumigation and ultraviolet-C (UV-C) irradiation. SO_2 has been used historically in food preservation due to its ability to limit fungal contamination and suppress mycotoxin production. Its re-evaluation in controlled settings allows for a better understanding of its potential utility and limitations in antifungal applications. UV-C irradiation,

on the other hand, represents a physical intervention capable of directly inactivating fungal spores through DNA damage. Its established use in disinfection of water and surfaces makes it a particularly relevant candidate for adaptation to indoor air quality management.

The combination of these strategies—plant metabolites, SO_2 fumigation, and UV-C irradiation—offers a broad spectrum of antifungal interventions, ranging from natural compounds to physical stressors. Importantly, the present study does not restrict itself to cataloguing inhibitory effects; it also considers differences in sensitivity among fungal taxa, dose-dependent responses, and the possibility that certain stresses may reduce but not entirely prevent fungal growth. This nuanced perspective provides insight into how fungi adapt to sub-lethal exposures, laying the groundwork for the enzyme-focused investigations presented in Chapter 3.

Table 4 Chemical composition of some mangrove plants (Abeysinghe 2010)

Species	Chemicals present
Acanthus ilicifolius	Acanthicofolin, Triterpenes, triterpenoidal saponinsalkaloid, benzoxazoline, long-chain alcohols, steroids
Aegiceras corniculatum	Triterpenes, benzoquinones, carotenoids, coumarins, flavonoids, tannins, polyphenols, saponins
Avicennia marina	Terpenoids, steroidsnaphthalene derivatives, flavones, glucosides
Excoecaria agallocha	Phorbol ester, flavanone, glycoside, lignin, pentosan, tannins, phenols
Rhizophora apiculata	Triterpenes, steroids, esters

Table 5 Biological role of phytochemicals

Phytochemical	**Biological role**
Phenolic-flavonoids	Reaction with free radicals for prevention or treatment of skin ageing (Podda & Grundmann-Kollmann, 2001)

Alkaloids	Antitumor, antihypertensive, muscle relaxant, antiprotozoal (von Linné, 2007)
Steroids	Antioxidants and maintaining hormonal balance (Moss, 1989)
Flavonoids	Antioxidants (Toudert et al., 2009)
Tannins	Metal ion chelating property enables it to function as an antioxidant and antimicrobial agent (Tukiran, 2013)
Terpenoids	Purgative for cough treatment and asthma (Edeoga et al., 2005)
Cardiac glycosides	Used in the treatment of congestive heart failure and arrhythmia (McMurray & Pfeffer, 2005)

Table 6 Therapeutic use of mangrove plants

Specie	**Therapeutic use**
Acanthus ilicifolius	Treatment of paralysis, asthma, dyspepsia, hepatitis, leprosy, rheumatic pain, leishmanicidal
Aegiceras corniculatum	Asthma, diabetes, hepatitis, rheumatism, fish poison
Avicennia marina	Skin diseases
Avicennia officinalis	Aphrodisiac, diuretic, hepatitis, leprosy
Bruguiera gymnorhiza	Eye diseases
Bruguiera parviflora	Antitumor
Bruguiera cylindrical	Stopping haemorrhage, applied to malignant ulcers, antioxidants
Ceriops decandra	Hepatitis, ulcer
Lumnitzera racemosa	Antifertility, asthma, diabetes, snake bite
Rhizophora mangle	Angina, boils, antifungal infections, antiseptic, diarrhoea, dysentery, elephantiasis, fever, malaria, leprosy, tuberculosis
Rhizophora mucronata	Elephantiasis, febrifuge, haematoma, hepatitis, ulcers
Salicornia brachiate	Hepatitis
Sesuvium portulacastrum	Hepatitis
Sueda maritima	Hepatitis
Sueda monoica	Hepatitis

Table 7 Ferns and cycads of Asiatic mangroves with antimicrobial activity (Sulaiman et al., 2022)

Family	**Extract**		**Secondary metabolite extracted**
Genus, Species	**Antibacterial**	**Antifungal**	
Subclass Polypodiidae			Stenopalustroside A, a glycosidic antibacterial agent identified from the methanolic extract of *Stenochlaena palustris*.
Family Blechnaceae *Stenochlaena palustris*	+	+	
Family Nephrolepidaceae *Nephrolepis biserrata*	+	+	
Family Polypodiaceae *Drynaria quercifolia*	+	+	
Drymoglossum piloselloides	+	+	
Microsorum punctatum	+	+	
Platycerium coronarium	+		
Pyrrosia piloselloides	+	+	
Family Pteridaceae *Acrostichum aureum*	+	+	
Acrostichum speciosum	+		
Subclass Cycadiidae			
Family Cycadaceae *Cycas rumphii*	+		

Table 8 Monocots of Asiatic mangroves with antimicrobial activity (Sulaiman et al., 2022)

Family	**Extract**		**Secondary metabolite extracted**
Genus, Species	**Antibacterial**	**Antifungal**	

Family Aracea *Lasia spinosa*	+	+	Meridinol
Family Arecaceae *Phoenix paludosa*	+	+	3'-Acetoxy-6,7-dimetoxy-4', Tricin and Cinnamic acid, active against both bacteria and fungi.
Saribus rotundifolius	+	+	
Family Cyperaceae *Cyperus scariosus*		+	
Eleocharis dulcis	+		
Family Flagellariaceae *Flagellaria indica*	+	+	
Family Orchidaceae *Aerides odoratum*	+		
Cymbidium finlaysonianum		+	Batatasin III, a stilbenoid from the chloroform extract of *C. finlaysonianum* effective against both bacteria and fungi.
Family Pandanaceae *Nypa fruticans*	+		Naringenin, a flavonoid compound effective against viruses like SARS-CoV
Family Poaceae *Phragmites vallatoria*	+	+	
Family Ruppiaceae *Ruppia maritima*	+		

Bioactive compounds work by interfering with cell walls, membranes, interfering with nucleic acids and interacting with the enzyme system (Tenover, 2006). Stigmasterol found in the ethanolic extract has been found to inhibit β-lactamase, which increases the susceptibility of antibiotic-resistant bacteria to common antibiotics (Yenn et al., 2017), and cinnamic acid with its hydroxylated derivatives has been found to have antifungal activity by inhibiting spore

germination and anti-tyrosinase activity (H.-S. Wu et al., 2008). The same acid also arrests fungal growth by interacting with benzoate 4-hydroxylase which is an important enzyme for aromatic detoxification (Korošec et al., 2014). Hexadecanoic acid interacts with the hydroxyl group present in the lipopolysaccharides of cell walls, transforming it into its asymmetric variant, which disrupts the lipid membrane architecture. This leads to swelling and harm to the cytoplasm, as well as inducing toxicity to the protoplasm through the -OH group of the acid (Johannes et al., 2016).

2.1.1 Extraction of secondary metabolites

Researchers across various scientific fields face the challenge of using solvents to extract plant materials, often serving as an initial step in isolating and identifying the specific compounds linked to the biological activities of a plant or its extract. This research is significant because traditional medicine has supported human health for centuries, and many modern medicines are derived from natural products that are no longer easily accessible to the public. Individuals within a single population, closely related species, and even the same species can have different secondary metabolite qualitative and quantitative characteristics. Therefore, when collecting samples, it is important to gather specimens from different populations within the same area to ensure that higher quality samples are not overlooked.

Drying and grinding: Plant materials should be dried at 30°C or within its close proximity to prevent the breakdown of heat-sensitive compounds. Additionally, these materials should be shielded from sunlight due to the risk of chemical changes caused by ultraviolet radiation exposure. Adequate air circulation around the plant materials is crucial to avoid heat and moisture accumulation. Thus, the materials should not be tightly packed, and the use of a fan or other methods may be necessary to ensure airflow around or through the drying materials. Grinding the plant material enhances extraction efficiency by increasing its surface area and reduces the solvent

required for extraction by enabling the material to be more densely packed. While it might be beneficial to grind plant material into a fine powder, excessively small particles can hinder solvent flow around them. Moreover, the milling process generates heat (with finer particles producing more heat), which can result in the loss of volatile components and the degradation and oxidation of heat-sensitive substances (C.N et al., 2016)

Solvent extraction: There are two types of solvent extraction methods: continuous and discontinuous. The solvent continuously passes through the plant material in continuous techniques (such percolation and Soxhlet extraction). The solvent progressively gets more saturated as compounds flow from the plant material into the surrounding solvent, but because of the constant flow, the saturated solvent is eventually replaced with a less saturated solvent. The solvent is added and removed in batches using discontinuous procedures, on the other hand. Consequently, extraction essentially stops until the solvent is decanted and replaced with new solvent as soon as the concentration of the solute in the plant material and that in the solvent reach equilibrium.

Extracts can be evaporated under a nitrogen stream or concentrated at low pressure with a rotary evaporator. In order to prevent heat-sensitive components from breaking down, it is advised to keep the water bath temperature of a rotary evaporator below 40°C. The solvent collected from the condenser of the rotary evaporator during the concentration process of one extraction batch might be used for further extraction of the same sample, especially when working with large quantities of a single sample. However, as this could lead to cross-contamination of subsequent extracts, it is not recommended to use recovered solvent for the extraction of other samples.

Choice of solvents: The solubility of the target compounds, safety concerns, ease of handling the solvent, the possibility of producing artifacts, and the grade and purity of the solvent are some of

the variables that need to be considered when choosing a solvent or solvent system for the extraction of plant materials. Following the adage "like dissolves like," it is frequently possible to tailor the solvent choice to increase the extraction yields of beneficial compounds while lowering the extraction of undesirable ones. Specific extraction techniques that successfully separate the intended molecules or prevent the degradation of the target analytes are commonly reported.

When using and storing solvents that are very flammable and those that are likely to create explosive peroxides (such as diethyl ether and other ether-based solvents), precautions should be taken to lessen the risk of fire and explosion. Conversely, because they may be concentrated more easily, solvents with lower boiling temperatures are usually easier to work with. While water and butanol are more difficult to remove, solvents including acetone, chloroform ($CHCl_3$), dichloromethane (DCM), ethyl acetate (EtOAc), and n-hexane/petroleum ether evaporate rather quickly (Dixon & Dickinson, 2024).

In the hot continuous extraction method using the Soxhlet extractor, the finely ground crude leaf powder is placed inside a porous bag or "thimble" made from sturdy filter paper, which is situated in the central chamber of the Soxhlet apparatus. The solvent in the round-bottom flask is warmed up, and its vapors are collected in the condenser. The vaporized solvent then drips down into the thimble that holds the crude powder, extracting the compounds through direct contact. Once the liquid in the chamber reaches the top of the siphon tube, it siphons back into the round-bottom flask. This process is ongoing and continues until the solvent from the siphon tube leaves no residue upon evaporation. The main advantage of this technique is that it allows for the extraction of large quantities of leaf powder using a significantly smaller amount of solvent. This leads to substantial savings in terms of time, energy, and consequently, costs. While it is used in a batch

process on a small scale, its efficiency and cost-effectiveness increase dramatically when adapted for continuous extraction on a medium or large scale (Jones & Kinghorn, 2012).

In summary, this chapter represents the experimental bridge between fungal assessment and mechanistic understanding. By evaluating diverse stresses for their ability to inhibit or suppress indoor fungi, it establishes a comparative framework that integrates traditional knowledge, natural product chemistry, and physical disinfection methods. The findings serve as a critical step toward identifying sustainable antifungal strategies while also providing the context for subsequent exploration of fungal enzyme responses to stress.

2.2 Materials and Methodology

2.2.1 Apparatus and chemicals

- Andersen two-stage cascade impactor
- Afcoset digital balance
- Leica DM 750 microscope with ICC50 HD camera
- Labtech LSI-3016R shaking incubator with orbital shaker
- Eyela Rotary Evaporator N-121BS-W
- Soxhlet extractor
- Glass desiccator
- UV-C chamber with UV-C lamp
- Sterilized Tarson disposable petri plates
- Glass test tubes, conical Flasks
- Himedia antibiotic zone scale
- Clove oil and eucalyptus oil

- Mechanical grinder
- Organic solvents
- Sodium sulfite and concentrated Hydrochloric acid

2.2.2 Collection of indoor air fungal species

Different air-conditioned indoor environments such as cafeteria, crèche, library reading room, salon, digital library and gymnasium were chosen for the collection of fungal species using Andersen Two-stage cascade impactor, as these locations were running an air conditioner for long stretches daily without proper natural ventilation and were visited by a mixed population around the day to provide a source of external microorganisms, as described in the previous chapter. Nutrient media plates of Potato Dextrose agar and Rose Bengal agar were used in the sampler to collect the microbial load from the air of the rooms, which were kept in incubation for 5-7 days to get a healthy growth of microorganisms.

2.2.3 Preparation of spore suspension

From the media plates, the colonies were separated, purified, and identified. Each species was subsequently inoculated onto slants and allowed to grow. Once there was evidence of robust growth, a spore suspension was created by rinsing the colonies with 1ml of distilled water, which was then diluted and stored in centrifuge tubes for later use in inoculations (Queener & Capone, 1974). (Figure 6)

Figure 6 1.5ml spore suspensions of pure cultures

2.2.4 Collection of plant specimen

Plant specimens of *Excoecaria agallocha* and *Acanthus ilicifolius* were collected from Purandar, a riverbank in Basanti $22^{0}12.98'$ N, $88^{0}55.02'$E, a census town in Canning, South 24 Parganas (Figure 7).

Figure 7 Collection of plant specimen from Purandar, Basanti, South 24 Parganas

2.2.5 Processing of collected samples

The leaves were left to dry in shade on a metal tray, after being washed in distilled water and spaced out so that most leaves can get exposed to the air (Figure 8). After 15 days the leaves were ground into powder using a mechanical grinder and stored in an air-tight zip lock bag until use.

Figure 8 Leaves of Acanthus ilicifolius after collection and drying

2.2.6 Soxhlet Extraction

The processed sample was measured using an Afcoset Digital balance and 10g was placed inside the paper thimble made using Whatman Grade 1 filter paper. The thimble was placed inside the extraction chamber of the extractor and 300ml of solvent was poured from on top of the opening so that it flows down into the round bottom flask (Figure 9). The solvent was heated at regulated temperatures so that it evaporated, and condensed in the condenser as drops falling into the extraction chamber, until it reached the level marked on the siphon after which it flowed back into the round bottom flask, completing one cycle. The extraction process was considered to be complete when the liquid at the siphon was almost clear. The number of cycles varied according to the solvent being used.

Solvents were used in the increasing order of their polarities.

Table 9: Polarity and boiling points of selected organic solvents

Solvent	Polarity (Coulomb-metre)	Boiling point (^{0}C)
n-hexane	0.01	69
Xylene	2.5	134
Chloroform	4.1	61
Acetone	5.1	56

Figure 9 Thimble of Soxhlet extractor showing extracted compounds from leaves

2.2.7 Concentration of the extracted compounds

After collecting 300ml of the solution from the round bottom flask, it was concentrated using an EYELA (USA) N-1300E Rotary Evaporator. A volume of 10 ml of fresh solvent was used to collect the concentrated solutes from the inside walls of the evaporating flask (Figure 10). It was

kept in a 25ml conical flask with its mouth covered with aluminum foil to prevent evaporation until needed. The solvent was evaporated by placing the conical flask inside an incubator and regulating the temperature.

Figure 10 Concentrated extracts

2.2.8 Disc diffusion assay with extracted metabolites

After evaporation of the excess solvent, the dried extract was taken out using a spatula and dissolved in DMSO to give the working solution of the extracts for disc diffusion assay to determine the zone of inhibition for selected fungal species. Paper discs were placed on Nutrient Agar media plates inoculated with fungal spore solution prepared beforehand, on which 10µl of working solution of 1000 µg/ml of the extract was poured using a sterile micropipette and tip and finally placed in an incubator with temperature maintained at 27^0C until the fungal colonies showed growth (B. M. Khan et al., 2017). Then the plates were observed for any zone of inhibition to suggest the selected fungi was susceptible to the secondary metabolite extracted.

2.2.9 Well diffusion assay with eucalyptus and clove oils

From the purified spore suspension, 0.5ml suspension was taken and evenly spread on solidified PDA media plate with a spreader. In each media plate, a hole was made with the help of a cork borer. For the control setup, sterile distilled water was added to the hole and in other cases 10µl of eucalyptus oil and clove oil were added to test for inhibition of fungi by each oil. The setups were incubated for 3-4 days and observations were taken.

"Essential Eucalyptus Oil" was purchased from SRL Chemicals (Code 93050). Clove oil was purchased from Sigma-Aldrich (Code C8392).

2.2.10 Exposure to Sulfur dioxide gas

Purified samples were inoculated onto agar plates and placed inside an air-tight desiccator. Sulfur dioxide gas was generated inside the sealed glass desiccator by the reaction between sodium sulfite and sulfuric acid, as described by Sander et.al. (Sander et al., 1984). The volume of the desiccator was measured (840cc), and 25ppm gas was generated stoichiometrically (Figure 11). The samples were exposed for 3,6 and 12 hours, respectively, after which they were checked for growth by incubating at 27-30^{o} C for 2-3 days.

$$Na_2SO_3 + H_2SO_4 \rightarrow Na_2SO_4 + H_2O + SO_2 \uparrow$$

Figure 11 Glass desiccator for sulfur dioxide gas exposure

2.2.11 Exposure to UV-C radiation

Purified samples were inoculated onto agar plates and placed in a light-blocking box with a singular UV-C lamp emitting 625 μW/cm^2 (Figure 12). The distance of the open plate from the source of light was 28 cm. After exposures of 3,6 and 12 hours the samples were taken out and checked for growth by incubating at 27-30^o C for 2-3 days.

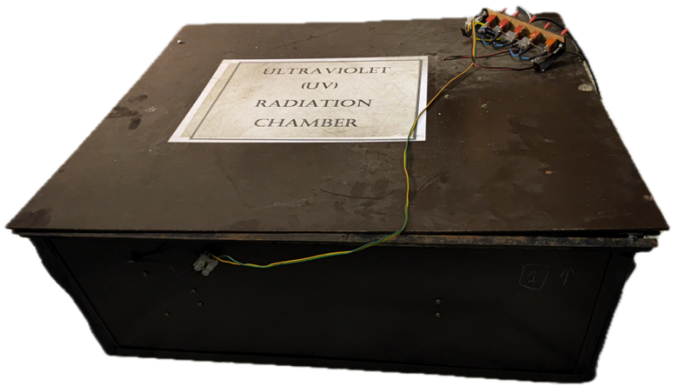

Figure 12 UV-C chamber

2.3 Results

2.3.1 Disc diffusion assay with extracted secondary metabolites and natural oils

Table 10: Diameter of ZOI in cm for secondary metabolites of mangrove plants and natural oils

	Clove oil	Eucalyptus oil	*E. agallocha*	*A. ilicifolius*
A. flavus	2.3 ± 0.3	2.1 ± 0.1	2.8 ± 0.1	2.9 ± 0.2
T. roseum	2.5 ± 0.3	2.5 ± 0.1	2.5 ± 0.2	2.7 ± 0.2
A. fumigatus	2 ± 0.2	2.4 ± 0.2	2.9 ± 0.2	2.4 ± 0.1
P. citrinum	3 ± 0.2	2.9 ± 0.1	3.2 ± 0.2	3 ± 0.2
A. tamarii	2.5 ± 0.3	2.5 ± 0.2	2.9 ± 0.2	2.9 ± 0.2
Trichoderma sp.	1.5 ± 0.3	1.2 ± 0.1		1.6 ± 0.1
R. stolonifer	2.3 ± 0.3	2.2 ±0.2	2.9 ± 0.2	2.3 ± 0.2
F. semitectum	3.2 ± 0.2	3.3 ±0.1	3.5 ± 0.3	3.5 ± 0.3
A. phaeospermum	2.4. ± 0.2	2.2 ± 0.2	1.8 ± 0.1	2.5 ± 0.2
A. niger	2.9 ± 0.2	2.8 ±0.2	2.5 ± 0.2	3 ± 0.2
A. ochraceus	2.2 ± 0.1	2.5 ± 0.1	2.8 ± 0.2	3 ± 0.3
C. lunata	1.5 ±0.1	1.2 ± 0.1		1.5 ± 0.1

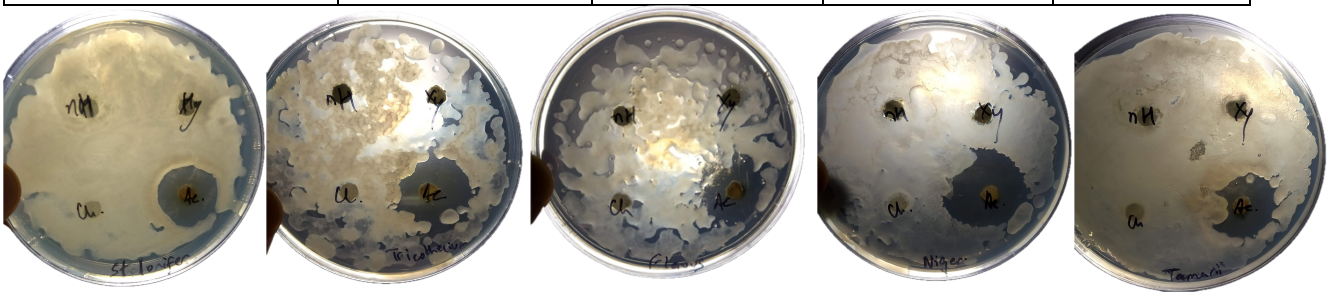

Figure 13 ZOI for 5 species in acetone extract: Rhizopus stolonifer, Tricothecium roseum, Aspergillus flavus, Aspergillus niger and Aspergillus tamarii

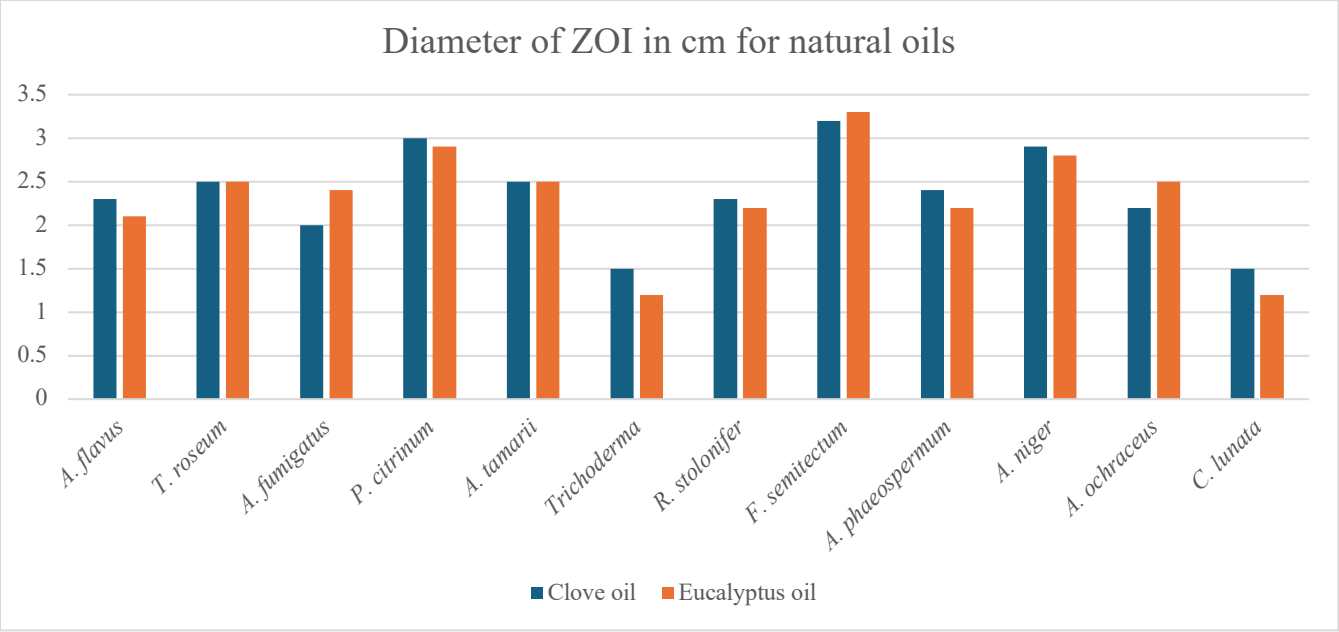

Figure 14 Diameter of ZOI for natural oils in cm

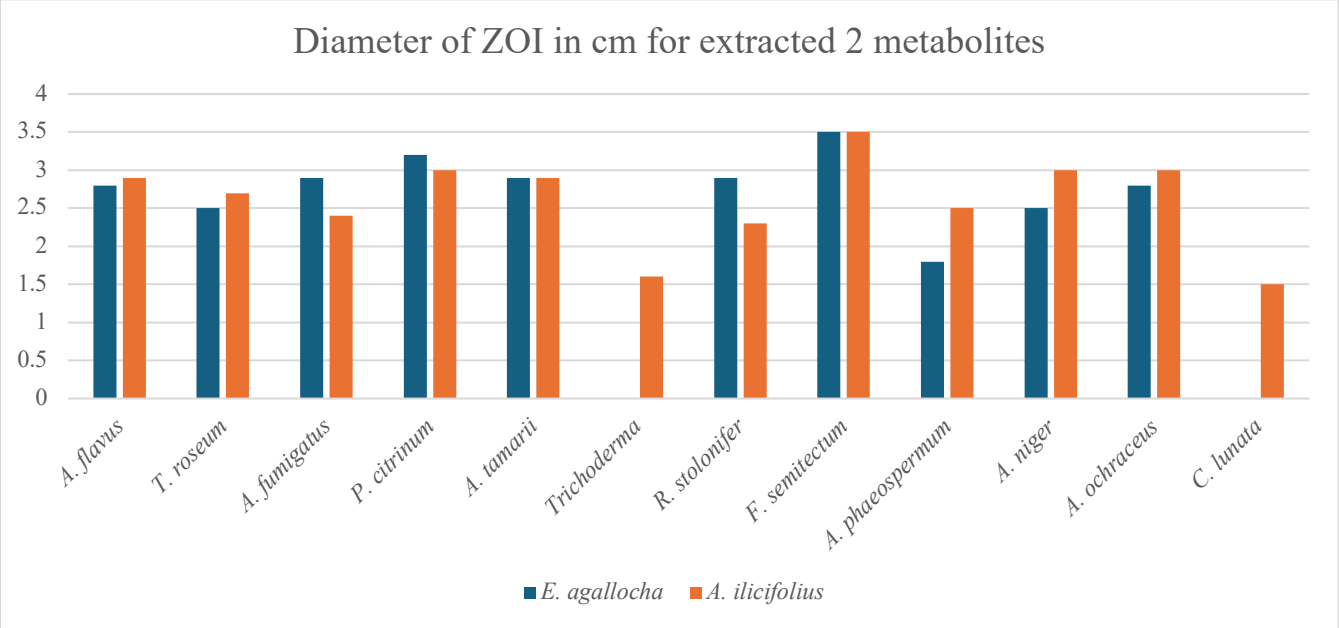

Figure 15 Diameter of ZOI for extracted secondary metabolites in cm

2.3.2 Effect of SO_2

No observable inhibition was noticed

2.3.3 Effect of UV-C

No observable inhibition was noticed

2.4 Discussion

2.4.1 Comparison between two natural oils

The ZOI measurements reflect how effectively these oils can prevent fungal growth (Table 10). Notably, clove oil displays considerable antifungal effectiveness against various species, with *P. citrinum* exhibiting the greatest resistance, leading to a 3.0 ± 0.2 cm inhibition zone when treated with clove oil (Figure 14). This implies that while clove oil is potent, species like *P. citrinum* might necessitate higher concentrations or alternative treatments for effective management. In a comparison between clove oil and eucalyptus oil, clove oil typically shows a more pronounced antifungal effect. For example, against *A. flavus*, clove oil achieves a ZOI of 2.3 ± 0.3 cm, while eucalyptus oil produces a slightly inferior inhibition of 2.1 ± 0.1 cm. This pattern persists with other fungi, reinforcing clove oil's status as a more powerful antifungal agent in this investigation. Nevertheless, eucalyptus oil still reveals effectiveness, especially against *A. niger* and *F. semitectum*, where its inhibition zones are similar to those of clove oil (Figure 14). This indicates that both oils have important antifungal attributes, which could be beneficial in various uses. *Trichoderma* sp. exhibits the least inhibition overall, with a ZOI of 1.5 ± 0.3 cm from clove oil and 1.2 ± 0.1 cm from eucalyptus oil, highlighting a degree of resistance that may require further exploration.

2.4.2 Comparison between two extracted secondary metabolites

P. citrinum demonstrated the highest resistance to both extracts, achieving a zone of inhibition of 3.2 ± 0.2 cm for *E. agallocha* and 3 ± 0.2 cm for *A. ilicifolius*. This suggests that *P. citrinum* may

possess inherent properties that confer heightened resilience to antifungal agents. Conversely, *A. niger* exhibited a notable inhibition of 2.5 ± 0.2 cm with *E. agallocha* and 3 ± 0.2 cm with *A. ilicifolius*, indicating its susceptibility to these extracts (Figure 13 and Figure 15).

Furthermore, *Trichoderma* sp. showed the least resistance overall, with a recorded inhibition zone of only 1.6 ± 0.1 cm when treated with *A. ilicifolius*. This finding may suggest that *Trichoderma* sp., known for its beneficial roles in plant health, may be more vulnerable to the compounds present in these extracts (Figure 15).

When comparing the antifungal effectiveness of the oils and plant extracts, it becomes evident that Clove oil is the most potent antifungal agent in the tested data. This is illustrated by its performance against *P. citrinum*, which yields a ZOI of 3.0 ± 0.2 cm, suggesting a significant level of susceptibility to Clove oil. Eucalyptus oil and the plant extracts present mixed results, particularly with *P. citrinum* showing a ZOI of 2.9 ± 0.1 cm for Eucalyptus oil and 3.2 ± 0.2 cm for *E. agallocha*.

Interestingly, the extracts from *A. ilicifolius* also demonstrate considerable antifungal activity, particularly against species such as *A. fumigatus*, where it achieves a ZOI of 2.4 ± 0.1 cm. This indicates that while the essential oils may provide immediate antifungal action, the extracts from certain plants can also yield significant results, warranting further investigation into their active compounds.

The data further indicates that certain fungal species exhibit varying levels of resistance to the treatments. For example, *Trichoderma* sp. shows the least inhibition overall, with Clove oil yielding a ZOI of 1.5 ± 0.3 cm and Eucalyptus oil 1.2 ± 0.1 cm. In contrast, *P. citrinum* exhibits a

higher ZOI of 3.2 ± 0.2 cm when treated with Clove oil, suggesting that Clove oil may be particularly effective against certain fungi while being less effective against others.

2.5 Summary

This chapter examines the significant role of plant extracts, sulfur dioxide, and UV-C radiation in the management of indoor fungi. In light of the increasing prevalence of antibiotic resistance, the exploration of alternative treatments has become imperative. Plant extracts, particularly those derived from mangrove species, exhibit promising antifungal properties. These plants are abundant in phytochemicals that possess the capability to combat various fungal infections, thus rendering them valuable assets in medical research and treatment methodologies.

Additionally, this chapter emphasizes the historical significance of these natural remedies. Traditional practices have long employed plant extracts for therapeutic purposes, and by revisiting these methods, contemporary science can enrich its repertoire against infections caused by drug-resistant fungi. The integration of traditional knowledge with modern scientific inquiry is essential, as it fosters a holistic approach to health and treatment.

Furthermore, sulfur dioxide and UV-C radiation serve critical functions in the management of fungal infestations. Their application in food preservation and indoor environments highlights the necessity for effective strategies to control fungal proliferation. The chapter elucidates how these agents can inhibit fungal growth, thereby offering practical solutions in diverse settings.

In conclusion, this chapter presents a comprehensive examination of strategies for combating indoor fungi. The synthesis of traditional plant knowledge with modern scientific practices not only broadens our understanding of these treatments but also underscores their relevance in the contemporary health landscape. As research continues to advance, the potential for developing

safe and effective alternatives to conventional antifungal treatments appears promising. Ongoing exploration in this domain may yield significant advancements in both public health and environmental management.

CHAPTER 3

STUDY OF CHANGES IN FUNGAL ENZYMES

DUE TO SULFUR DIOXIDE EXPOSURE

3.1 Background

The experiments described in Chapter 2 established that selected stresses—such as plant metabolites, SO_2 fumigation, and UV-C irradiation—can suppress fungal growth to varying degrees. However, in many cases, the stresses reduced but did not fully eliminate fungal viability. This raises an important question: how do fungi that withstand such treatments adapt at the biochemical level to ensure their survival? To address this, the present chapter examines the role of key stress-response enzymes in mediating fungal resilience under non-lethal stress conditions.

Fungal enzymes are central to both metabolism and adaptation. Among them, catalase, proteases, and ribonucleases (RNAse) represent distinct yet interconnected survival strategies. Catalase protects cells from oxidative stress by breaking down hydrogen peroxide into water and oxygen, thus preventing damage to DNA, proteins, and membranes (Figure 16). Proteases support growth and pathogenicity by degrading host barriers, recycling proteins, and sustaining mitochondrial function, while also helping fungi adapt to nutrient limitations (Figure 17, Figure 18). RNAse, though less studied in fungi, is critical for nucleic acid repair, RNA turnover, and maintaining genetic stability under stress. Together, these enzymes form a network of defense and adaptation that allows fungi to persist in hostile environments.

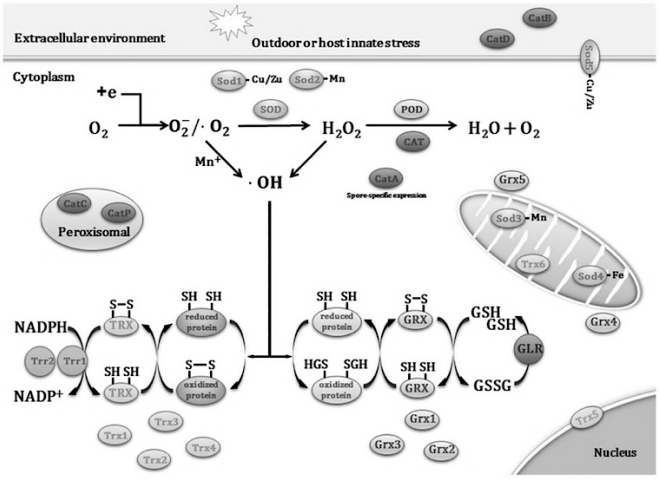

Figure 16 Mechanism of functioning of catalase enzyme (Hansberg et al., 2012)

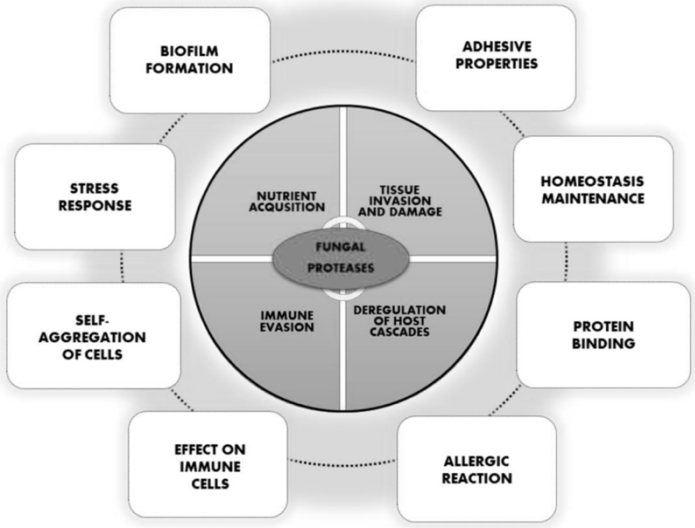

Figure 17 Functions of protease (Sharma et al., 2019)

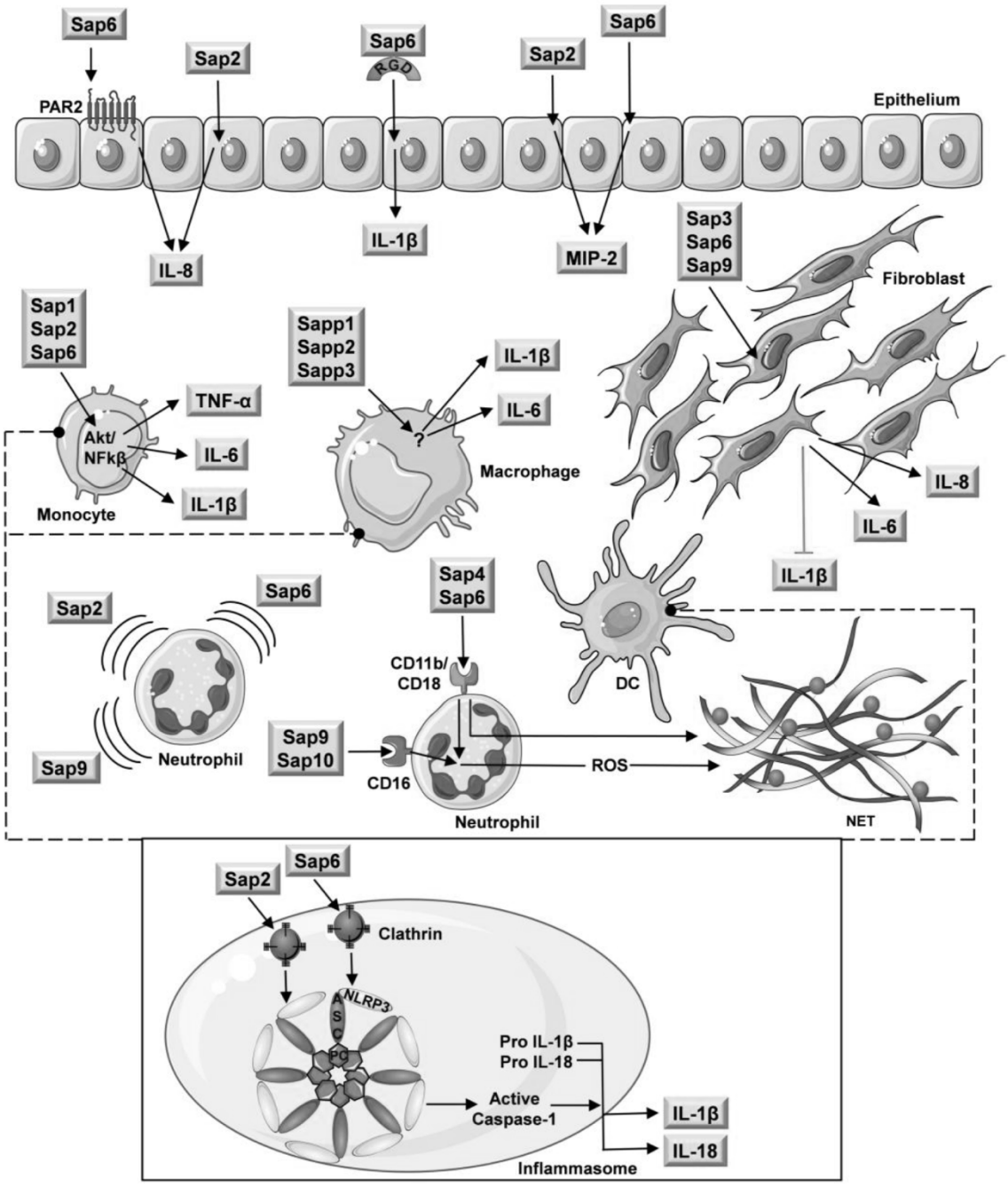

Figure 18 Multitasking of Candida sp. extracellular proteases in relation to the host immune cells (de Souza et al., 2015)

This chapter investigates enzyme responses in four dominant indoor fungal species exposed to controlled stress conditions. By measuring catalase, protease, and RNAse activity in nutrient media

after defined periods of SO_2 stress, the study evaluates how enzyme secretion patterns shift in response to external pressures. The results highlight both species-specific differences and general trends, such as correlations between enzyme activities and time-dependent adaptation.

The findings presented here build directly on the suppression experiments of Chapter 2, providing mechanistic insights into why certain fungi survive stresses that inhibit others. In doing so, they deepen our understanding of fungal resilience, linking phenotypic outcomes to enzymatic strategies, and setting the stage for broader discussions on indoor air quality, antifungal interventions, and industrial enzyme applications.

3.2 Methodology

3.2.1 Apparatus and Equipment

- Andersen two-stage cascade impactor
- Eppendorf cold centrifuge (5810 R)
- Perkin Elmer (Lambda 25 UV/VIS) spectrophotometer
- Eppendorf centrifuge Minispin
- Leica DM 750 microscope with ICC50 HD camera
- Labtech LSI-3016R shaking incubator with an orbital shaker
- Borosil desiccator 840 cc
- Sterilized Tarson disposable petri plates
- Glass test tubes, conical flasks
- Eppendorf falcon tubes 15ml, Centrifuge tubes 2ml
- Water bath
- Icebox

Four of the species tested for the effects of secondary metabolites and inorganic pollutants were selected on the basis of their abundance in indoor settings and ease of culturing. Stress was applied to each pure culture in the form of 25-30ppm SO_2 in an air-tight glass desiccator by the action of concentrated sulfuric acid on sodium sulfite for 3,6 and 12 hours separately. The stressed cultures were then inoculated in beef broth and peptone broths and again incubated at 27°C at 145 rpm (Henzler & Schedel, 1991) for two days. The cultures were then filtered and the filtrate was used for conducting further assays.

The beef broth and peptone broth differed in the source of nitrogen and amino acids for the growth of fungi. Beef extract was the primary source of beef broth, and yeast extract was the primary source of peptone broth.

Statistical analysis was performed using Origin Pro v10.0.0.154 (OriginLab Corporation, Northampton, MA, USA), SPSS Statistics v29.0.1.0 (IBM Corporation, New York, USA).

3.2.2 Assay for catalase

The filtrate extracted was added to 1.9ml of 50mM phosphate buffer containing 0.19mM H_2O_2. The catalase activity was quantified by measuring the decrease in absorbance at 240nm. The amount of enzyme that degrades 1µM H_2O_2 per minute was defined as one enzyme unit (Mina et al., 2015).

3.2.3 Assay for protease

To a mixture of 500µl of casein in 59mM phosphate buffer, 200µl of the crude extract was added and the resulting mixture was gently heated in a water bath at 40°C for 20 minutes. Following this, 1 ml of 10% (w/v) trichloroacetic acid (TCA) was added to terminate the reaction and the new mixture was kept at room temperature for 15 minutes before centrifuging at 10000 rpm for 5

minutes. Na_2CO_3 of strength 0.4M was measured to 2.5ml and 1 ml of 3-fold diluted Follin Ciocalteus phenol reagent was added to the supernatant from the separated mixture. This solution was incubated in the dark at room temperature for 30 minutes and the colour changed to blue. The absorbance of this was measured at 660 nm against a tyrosine standard. 1 unit of enzyme activity was calculated as equal to the amount of enzyme that releases 1µg of tyrosine per minute (Alnahdi, 2012).

3.2.4 Assay for RNAse

A volume of 0.5ml of the crude filtrate was added to a mixture of 6 mg of yeast RNA incubated in 0.25 M acetate buffer so that the solution has a total volume of 2ml. The samples were heated in a water bath at 50°C for an hour. The reaction was then stopped by rapid chilling in an ice bath following which, 5ml of ice-cold ethanol was added and maintained at -10°C for 20 minutes. The samples were then centrifuged at 2000g for 10 minutes, causing the non-hydrolyzed RNA to precipitate. The supernatant was measured at 260nm. 1 unit of enzyme activity was calculated as the amount of enzyme necessary to produce 1mM of 260nm absorbing substance (Gomes et al., 1998).

3.3 Results

3.3.1 *Aspergillus flavus*

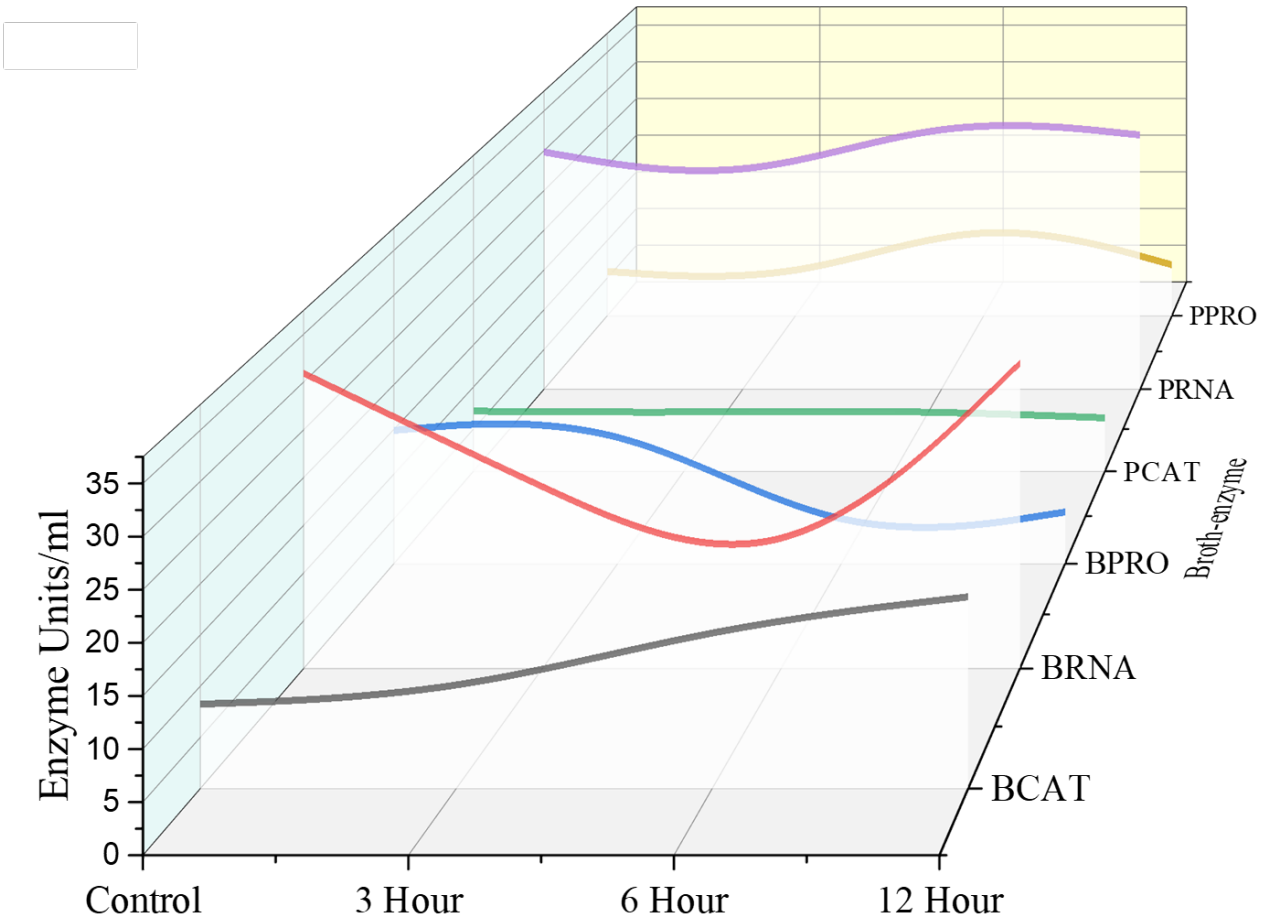

Figure 19 3D Heatmap of enzyme units/ml against exposure durations and enzyme-in-broth, where B: beef broth, P: peptone broth, PRO: protease enzyme, RNA: RNAse enzyme, CAT: catalase enzyme for A. flavus

The total enzyme secreted by *A. flavus* was 264 EU/ml with an average of 14.68 under stress against 16.15 EU/ml in the control setup, without any stress. The highest secretion was that of RNAse in peptone broth, under 6-hour stress. RNAse was the most secreted enzyme at 150.67

EU/ml followed by Catalase at 64.27 and Protease at 49.32 EU/ml respectively. 137.32 EU/ml was secreted in peptone broth versus 126.95 EU/ml in beef broth (Figure 19)

3.3.2 *Penicillium citrinum*

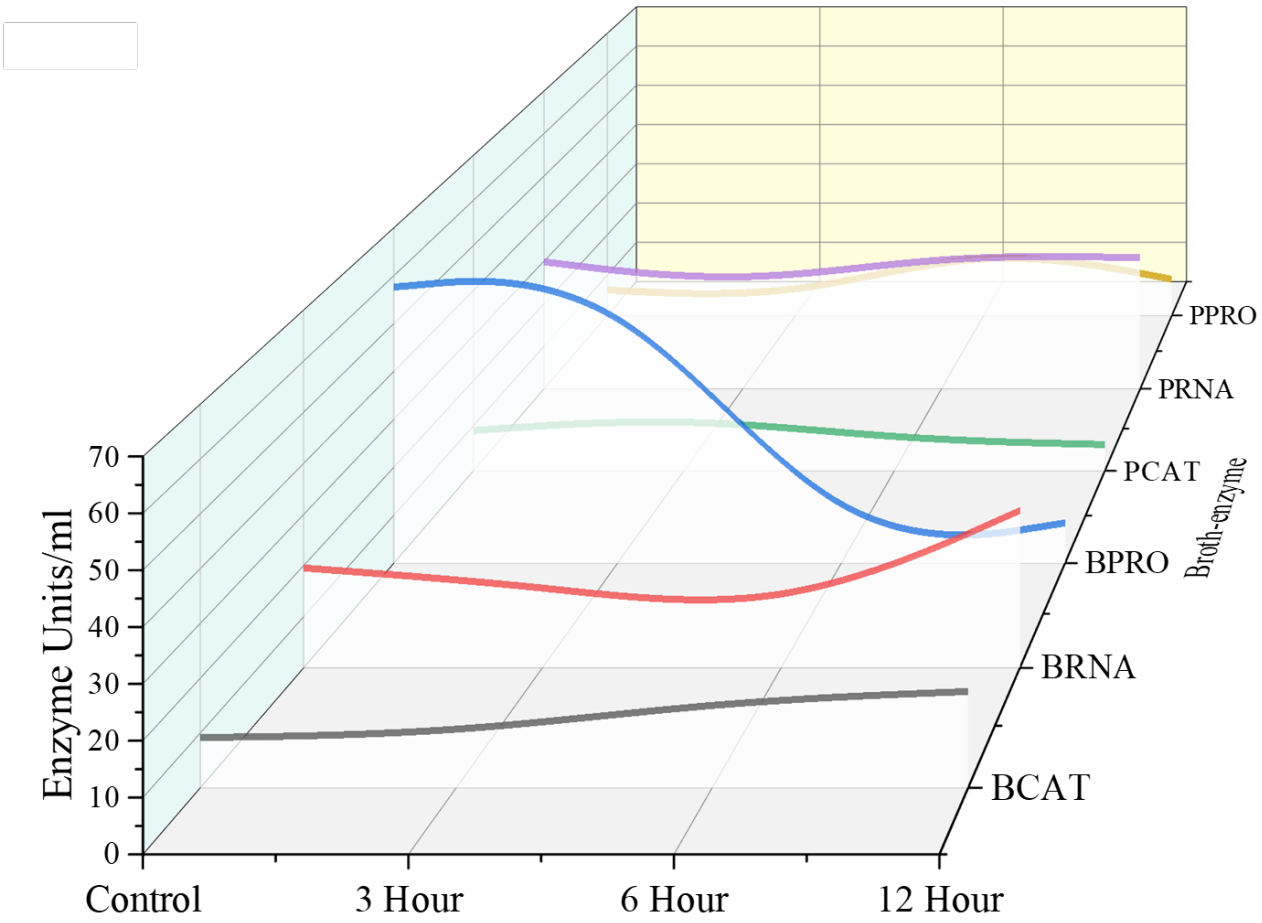

Figure 20 3D Heatmap of enzyme units/ml against exposure durations and enzyme-in-broth, where B: beef broth, P: peptone broth, PRO: protease enzyme, RNA: RNAse enzyme, CAT: catalase enzyme for P. citrinum

The total enzyme secreted by *P. citrinum* was 313.24 EU/ml with an average of 17.40 under stress against 21.90 EU/ml in the control setup. The highest secretion was that of protease in beef broth, under 3-hour stress (61.79 EU/ml). RNAse was secreted the most, at 143.38 EU/ml, followed by protease, at 101.84 EU/ml and catalase at 68.02 EU/ml. Beef broth was more favourable with 171.02 EU/ml compared to peptone broth with 141.21 EU/ml (Figure 20).

3.3.3 *Aspergillus parasiticus*

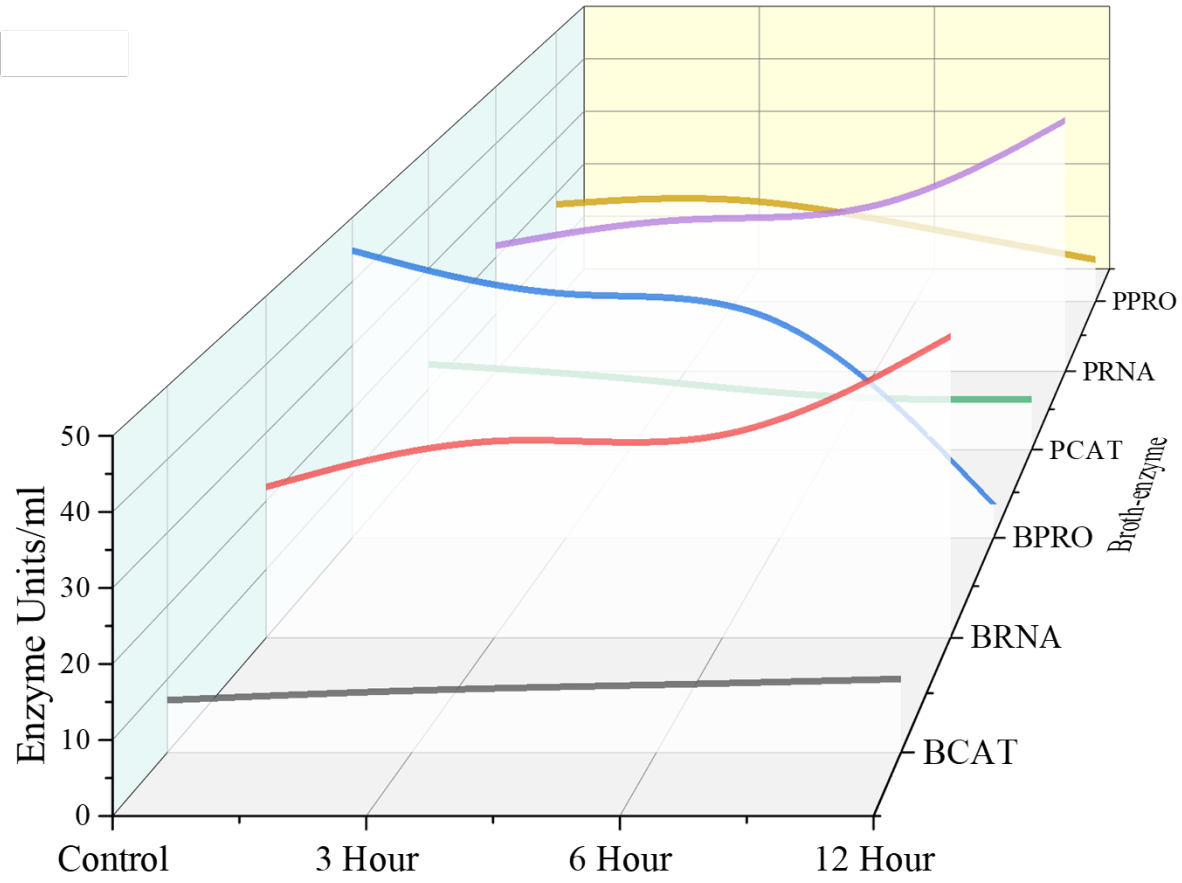

Figure 21 3D Heatmap of enzyme units/ml against exposure durations and enzyme-in-broth, where B: beef broth, P: peptone broth, PRO: protease enzyme, RNA: RNAse enzyme, CAT: catalase enzyme for A.parasiticus

The total enzyme secreted was 378.07 EU/ml under stress with an average of 21 compared to 21.37 EU/ml in the control setup. The highest secreted was protease in beef broth in the control setup, which was lowered under stress but still peaked at 44.08 EU/ml. RNAse was secreted the most with 198.7 EU/ml followed by protease (122.96 EU/ml) and catalase (56.33 EU/ml). The beef broth was more favourable with 209.9 EU/ml versus 168.13 EU/ml in peptone (Figure 21).

3.3.4 *Aspergillus fumigatus*

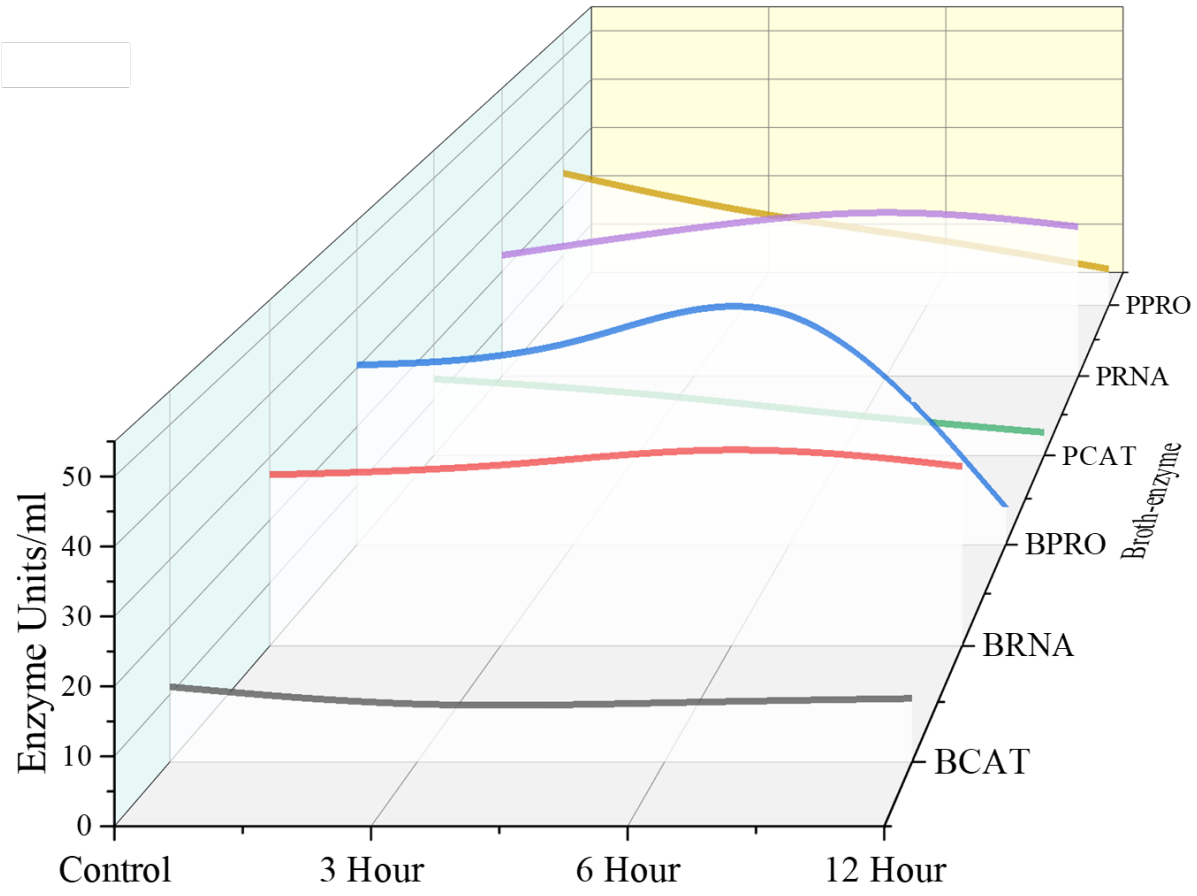

Figure 22 3D Heatmap of enzyme units/ml against exposure durations and enzyme-in-broth, where B: beef broth, P: peptone broth, PRO: protease enzyme, RNA: RNAse enzyme, CAT: catalase enzyme for A.fumigatus

The total enzyme enzymes secreted under stress was 354.37 EU/ml with an average of 19.69 against 22.04 EU/ml in control. Protease in beef broth peaked under 6-hour stress at 48.81 EU/ml. RNAse was the most secreted (178.87 EU/ml) followed by protease (126.46 EU/ml) and catalase (49.04 EU/ml). Beef broth was more favourable with 202.43 EU/ml versus 151.94 EU/ml in protease broth (Figure 22)

3.3.5 Total enzyme secretions

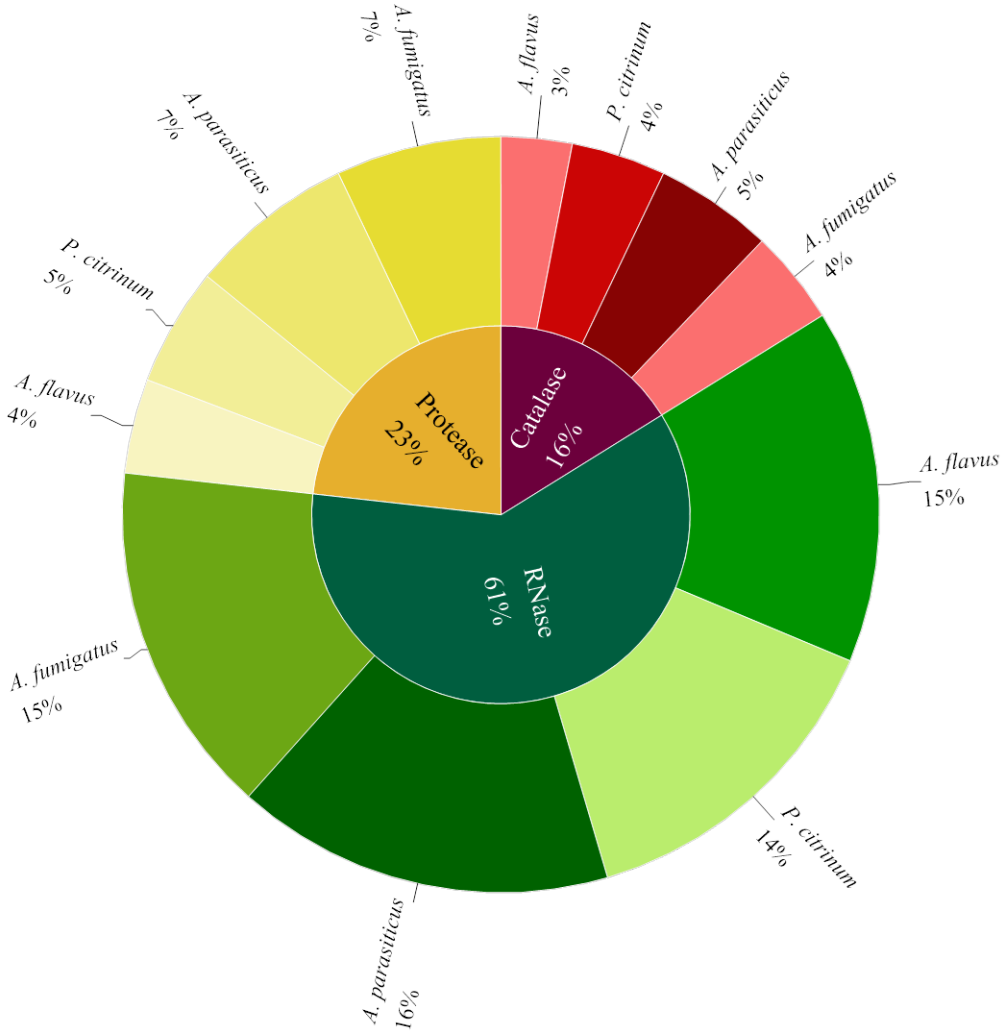

Figure 23 Enzyme secretion in beef broth for all organisms

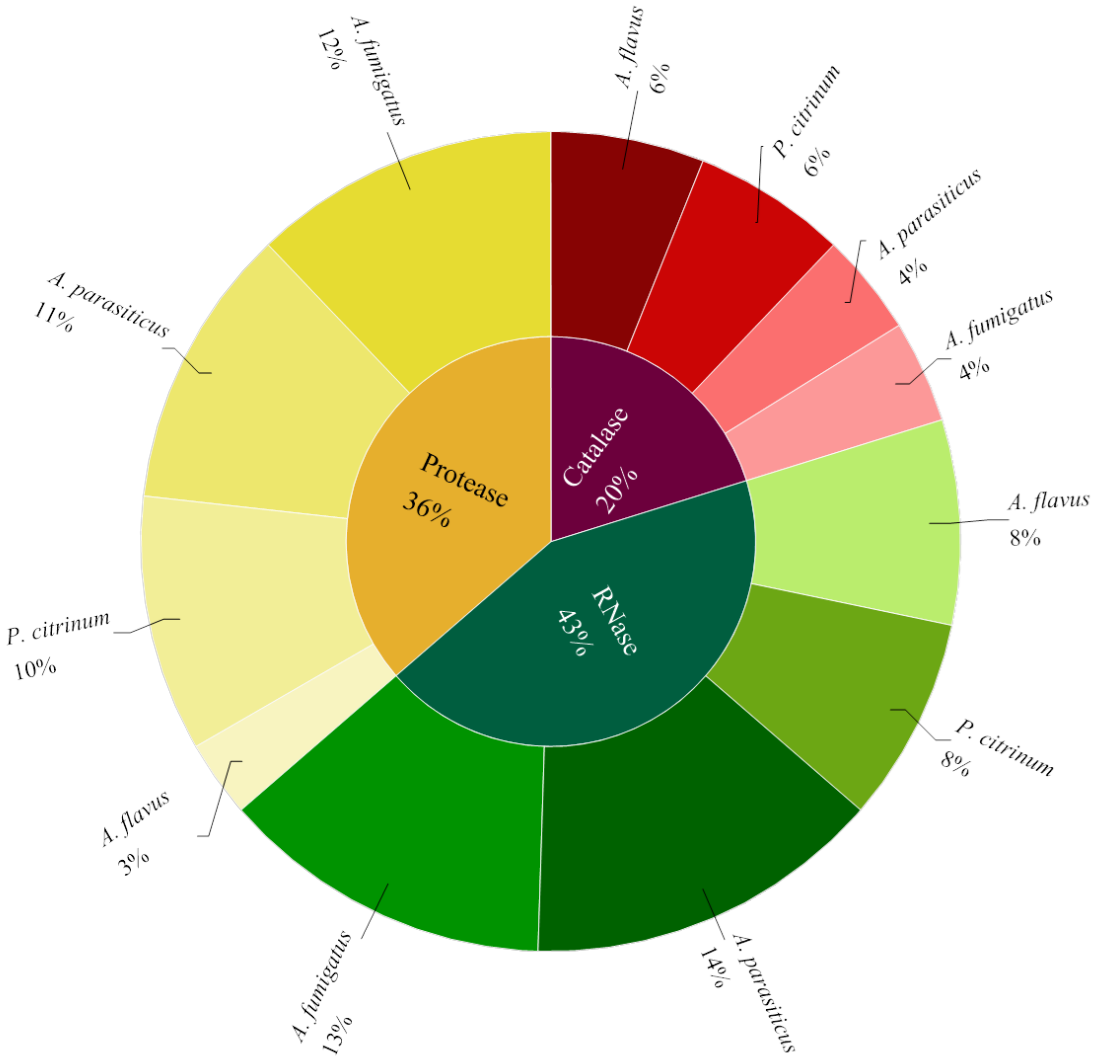

Figure 24 Enzyme concentrations for all organisms in peptone broth.

3.4 Discussion

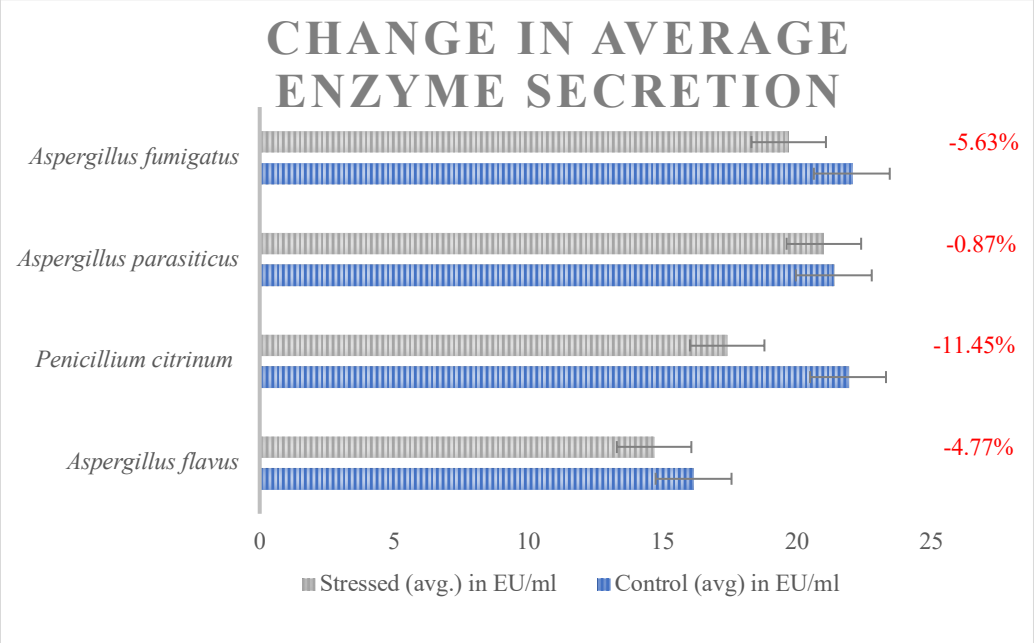

Figure 25 Comparison of averages between control and stress (3,6,12 hours) for all 4 organisms in both broths.

For *P. citrinum*, the total enzyme secretion was significantly lower under stress conditions (313.24 EU/ml) compared to the control setup (21.90 EU/ml) and is the most affected organism out of the 4, with enzyme levels coming down as much as 11.45% from the control. This reduction is noteworthy as it suggests that stress negatively impacts the overall enzyme production in this species. However, protease secretion in beef broth under a 3-hour stress condition was notably high (61.79 EU/ml), indicating that certain enzymes may still be produced in substantial quantities depending on environmental conditions and nutrient availability. The preference for beef broth over peptone broth (171.02 EU/ml vs. 141.21 EU/ml) further underscores the importance of the substrate in determining enzyme productivity.

Aspergillus parasiticus demonstrated a slight decrease in total enzyme secretion under stress (378.07 EU/ml) compared to control conditions (21.37 EU/ml). Interestingly, the secretion of RNAse was significantly higher (198.7 EU/ml) under stress, suggesting that this enzyme plays a crucial role in the organism's stress response. The reduction in protease secretion under stress, yet still peaking at 44.08 EU/ml, indicates a complex regulatory mechanism governing enzyme production. The enhanced performance in beef broth (209.9 EU/ml) relative to peptone broth (168.13 EU/ml) aligns with the findings for *P. citrinum*, suggesting a broader preference among fungal species for this nutrient-rich medium.

Aspergillus fumigatus displayed a marked decrease in total enzyme secretion under stress (354.37 EU/ml) compared to control conditions (22.04 EU/ml), similar to the trend observed in *P. citrinum*. Nevertheless, the secretion of RNAse (178.87 EU/ml) and protease (126.46 EU/ml) remained substantial, indicating a selective upregulation of these enzymes in response to stress. The peak protease secretion in beef broth under a 6-hour stress condition (48.81 EU/ml) highlights the time-dependent nature of enzyme production. Again, the preference for beef broth (202.43 EU/ml) over peptone broth (151.94 EU/ml) reinforces the role of the growth medium in influencing enzyme secretion.

Overall, the discussion reveals that while stress conditions generally result in a reduction of total enzyme secretion across all three species, specific enzymes, particularly RNAse and protease, are upregulated, suggesting their involvement in the organism's stress response mechanisms (Figure 25). The consistent preference for beef broth as a more favorable medium for enzyme production suggests that nutrient composition plays a critical role in optimizing enzyme yields.

The behaviour of catalase and protease were negatively correlated in most cases, with catalase slowly increasing in concentration in broths with increasing duration of stress and protease

showing a lowering of secretion after 3 or 6 hours of exposure (Figure 26). This behaviour is most easily observable in *A. flavus*. *A. parasiticus* showed an exception to this trend with both enzymes lowering in concentration with the passing time under stress. Ribonuclease showed an increase in concentration after 6 hours of exposure, initially lowering in levels from the control of the setup. Yet again, *A. parasiticus* was an outlier to this trend with RNAse values peaking at 6 hours and then lowering with time.

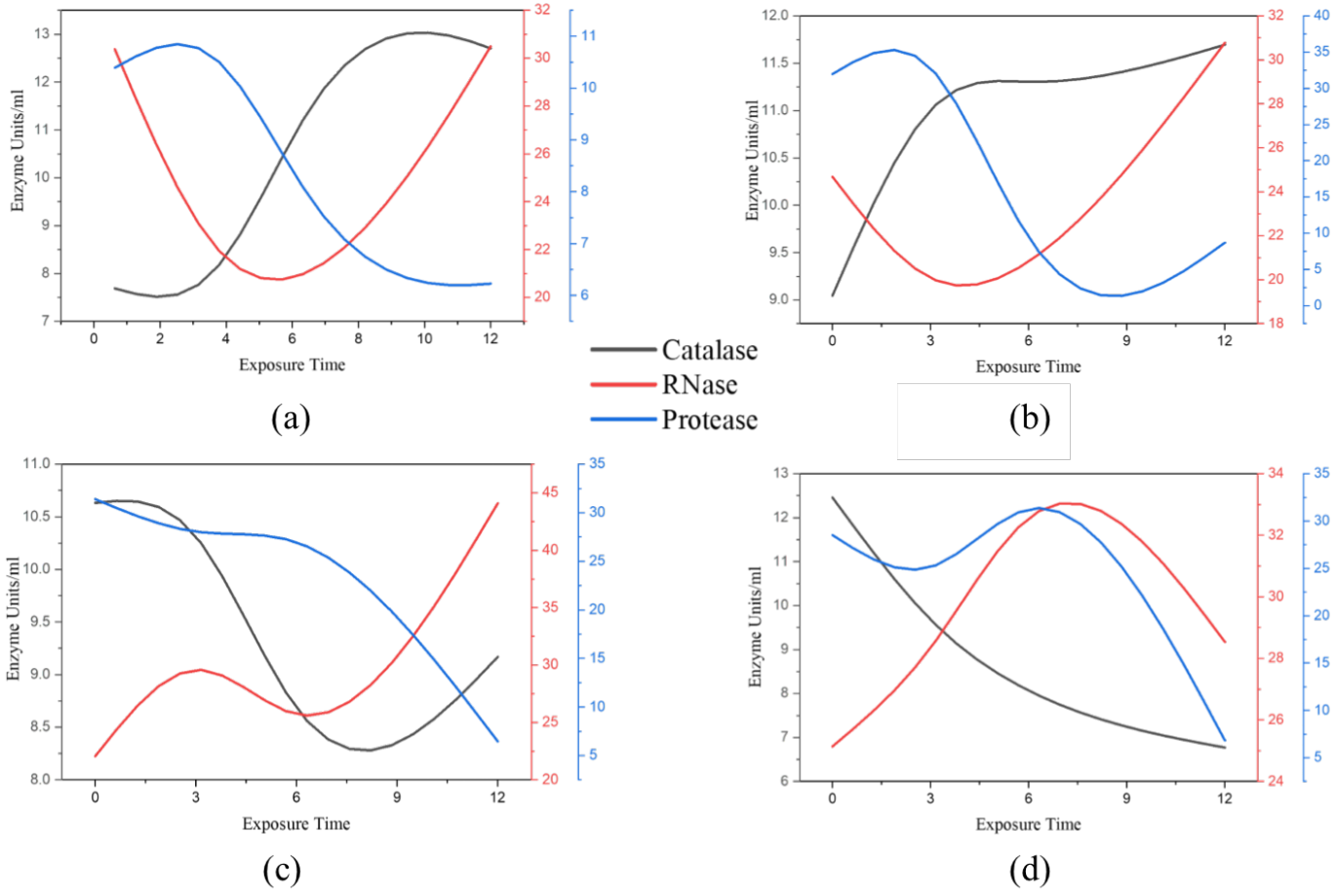

Figure 26 Changes in enzyme secretion over time for (a) A. flavus (b) P. citrinum (c) A. parasiticus (d) A. fumigatus

Upon performing Pearson's correlation on our data, *A. flavus* and *P. citrinum* showed the highest correlation with each other in both broths and all three enzymes, with an average of 0.85 significant at the 0.01 level. This shows us that even though these 2 were affected differently by the application of the stress with *A. flavus* not lowering its enzymatic output as much as *P. citrinum*, their behaviour remained very similar to each other and upon reacting to stress.

3.5 Summary

This study provides important insights into the effects of stress conditions on enzyme secretion in four fungal species: *P. citrinum, A. parasiticus, A. fumigatus*, and *A. flavus*. The results indicate that stress generally leads to a reduction in total enzyme secretion across all species. However, certain enzymes, particularly RNAse and protease, exhibit upregulation in response to stress, highlighting their critical roles in the organisms' stress response mechanisms.

P. citrinum exhibited the most significant reduction in total enzyme secretion under stress, underscoring its sensitivity to environmental changes. The notable increase in protease secretion in beef broth under a 3-hour stress condition suggests that specific nutrient compositions can mitigate the adverse effects of stress and optimize enzyme yields. Similarly, *A. parasiticus* and *A. fumigatus* showed reduced total enzyme secretion under stress but significant increases in RNAse and protease secretion, further emphasizing the selective upregulation of these enzymes.

The preference for beef broth over peptone broth across all species underscores the importance of nutrient-rich media in enhancing enzyme production. This consistent trend indicates that optimizing the nutrient composition of growth media can play a pivotal role in improving industrial enzyme yields under various conditions.

The study also highlights the complex regulatory mechanisms governing enzyme production, with catalase and protease showing negatively correlated behavior in most cases. The time-dependent nature of enzyme secretion, particularly the peak in RNAse and protease levels at specific stress durations, adds another layer of complexity to the organisms' adaptive responses.

Pearson's correlation analysis revealed a high correlation between *A. flavus* and *P. citrinum* despite their differing responses to stress, indicating similar behavioral patterns in enzyme secretion. This finding suggests that even though the species are affected differently by stress, their underlying regulatory mechanisms may share commonalities.

In conclusion, while stress conditions generally reduce total enzyme secretion in the studied fungal species, specific enzymes such as RNAse and protease are upregulated, playing vital roles in the organisms' stress responses. The preference for beef broth as a growth medium highlights the significance of nutrient composition in optimizing enzyme production. These findings contribute to a better understanding of the adaptive mechanisms of fungi and can inform strategies to enhance industrial enzyme yields under varying environmental conditions.

CHAPTER 4: CONCLUSION AND FUTURE SCOPE

This thesis set out to examine the occurrence, control, and mechanistic resilience of indoor fungi, integrating ecological assessment, applied antifungal strategies, and enzymatic responses into a coherent framework. Across the three chapters, the work progresses from understanding the extent of fungal contamination in diverse indoor environments, to evaluating targeted stresses that inhibit their growth, and finally to exploring the biochemical strategies fungi employ when stresses are insufficient to eliminate them. Collectively, these investigations provide new insights into indoor fungal ecology and offer both practical and conceptual contributions to managing their risks.

Chapter 1 established the foundation by characterizing fungal diversity and abundance across a range of indoor environments. Using culture-based methods and a novel Health Risk Rating Scale (HRRS), the study quantified not only colony-forming units but also contextual factors such as human exposure time, age susceptibility, and presence of fungal growth-promoting materials. The results highlighted that while total load is a useful indicator, it is not always the decisive determinant of risk. For instance, bedrooms showed lower fungal loads than classrooms but higher risk ratings due to vulnerable occupants and prolonged exposure times. Such nuanced analysis underscores the complexity of indoor fungal risk, shifting the emphasis from mere detection to health-oriented interpretation.

Chapter 2 built on this ecological foundation by testing a set of interventions designed to suppress fungal growth. Three categories of stressors—plant-derived metabolites, sulfur dioxide fumigation, and UV-C irradiation—were applied to dominant indoor fungi. Each stressor demonstrated some level of inhibition, though with varying degrees of success across species.

Importantly, the chapter highlighted that while certain treatments significantly reduced fungal proliferation, they often did not achieve complete elimination. This distinction is critical because it reflects real-world scenarios where fungi may be suppressed but not eradicated, and sub-lethal exposures can create opportunities for adaptive responses. By systematically comparing natural products and physical stressors, this chapter contributes to the growing body of research on sustainable antifungal interventions and sets the stage for mechanistic exploration.

Chapter 3 addressed this mechanistic dimension by focusing on enzymatic responses under non-lethal stress. The work demonstrated that catalase, protease, and RNAse activities change significantly when fungi are exposed to stress conditions that limit but do not fully prevent their growth. These findings reveal how fungi actively adapt to hostile environments, using enzymatic pathways to detoxify reactive molecules, recycle resources, and preserve genetic integrity. By connecting these biochemical strategies to the survival outcomes observed in Chapter 2, this study provides a clearer understanding of why certain fungi persist in the face of antifungal pressures, with implications for both clinical and environmental management.

Taken together, the three chapters form a pipeline of inquiry that advances our understanding of indoor fungi from detection to intervention to mechanism. This integrative approach is particularly valuable for public health, where effective risk management requires not only identification of hazards but also strategies for mitigation and insight into the resilience of the organisms being targeted. From a methodological perspective, the combination of ecological surveys, experimental stress applications, and enzyme assays illustrates the value of interdisciplinary tools in environmental microbiology.

Beyond their immediate findings, the results of this thesis point to several broader implications. First, indoor fungal management cannot rely on a single strategy; instead, it requires a combination of monitoring, environmental control, and targeted interventions. Second, natural products and physical stresses offer promising alternatives or complements to synthetic antifungals, aligning with the demand for greener and safer technologies. Third, fungal resilience at the enzymatic level cautions against simplistic assumptions about eradication and highlights the importance of understanding adaptive mechanisms in designing long-term control strategies.

Like all research, this study has limitations. The culture-based methods in Chapter 1, while informative, may underestimate total fungal diversity compared to molecular approaches. The stress applications in Chapter 2 were tested under controlled laboratory conditions, which may not fully capture the complexity of indoor environments. Finally, the enzyme assays in Chapter 3, though revealing, were limited to a subset of stress-response enzymes and fungal species. Future research could address these limitations by integrating molecular tools, expanding the repertoire of tested stresses, and examining additional enzymatic and genetic pathways involved in fungal adaptation.

In conclusion, this thesis demonstrates that indoor fungi are not only diverse and widespread but also resilient organisms capable of withstanding multiple antifungal pressures. Effective management therefore requires a holistic approach that combines risk assessment, innovative control measures, and mechanistic insight. By bridging ecological, applied, and biochemical perspectives, the work presented here contributes to both the science of environmental microbiology and the practical goal of safeguarding human health in indoor environments.

The present study lays the foundation for understanding indoor fungal risk in terms of both exposure and adaptive survival, and several avenues remain open for future exploration:

1. **Longitudinal and multi-site studies:** Expanding fungal sampling across diverse climatic zones and building types will strengthen the generalizability of the Health Risk Rating Scale (HRRS) and enable region-specific guidelines for human health safety.
2. **Molecular characterization:** Whole-genome or transcriptomic analyses of stress-exposed fungi can identify gene-level adaptations underlying enzyme activity, offering deeper insight into fungal resilience mechanisms.
3. **Intervention optimization:** Further studies are needed to optimize safe dosages and exposure conditions for UV-C and SO_2 treatments, and to develop standardized formulations of plant-derived antifungals for practical use in indoor environments.
4. **Health outcome linkage:** Clinical collaborations can directly correlate fungal exposure metrics with measurable health endpoints (e.g., allergy prevalence, respiratory function tests) to validate the risk models developed in this thesis.
5. **Prototype development:** The results can support the design of **integrated indoor monitoring devices** that combine high-resolution fungal sampling with enzyme-based stress markers to provide actionable health-risk assessments.

Overall, the study opens scope for **bridging environmental mycology with translational health applications**, ensuring that fungal contamination control strategies move from experimental settings to real-world health protection.

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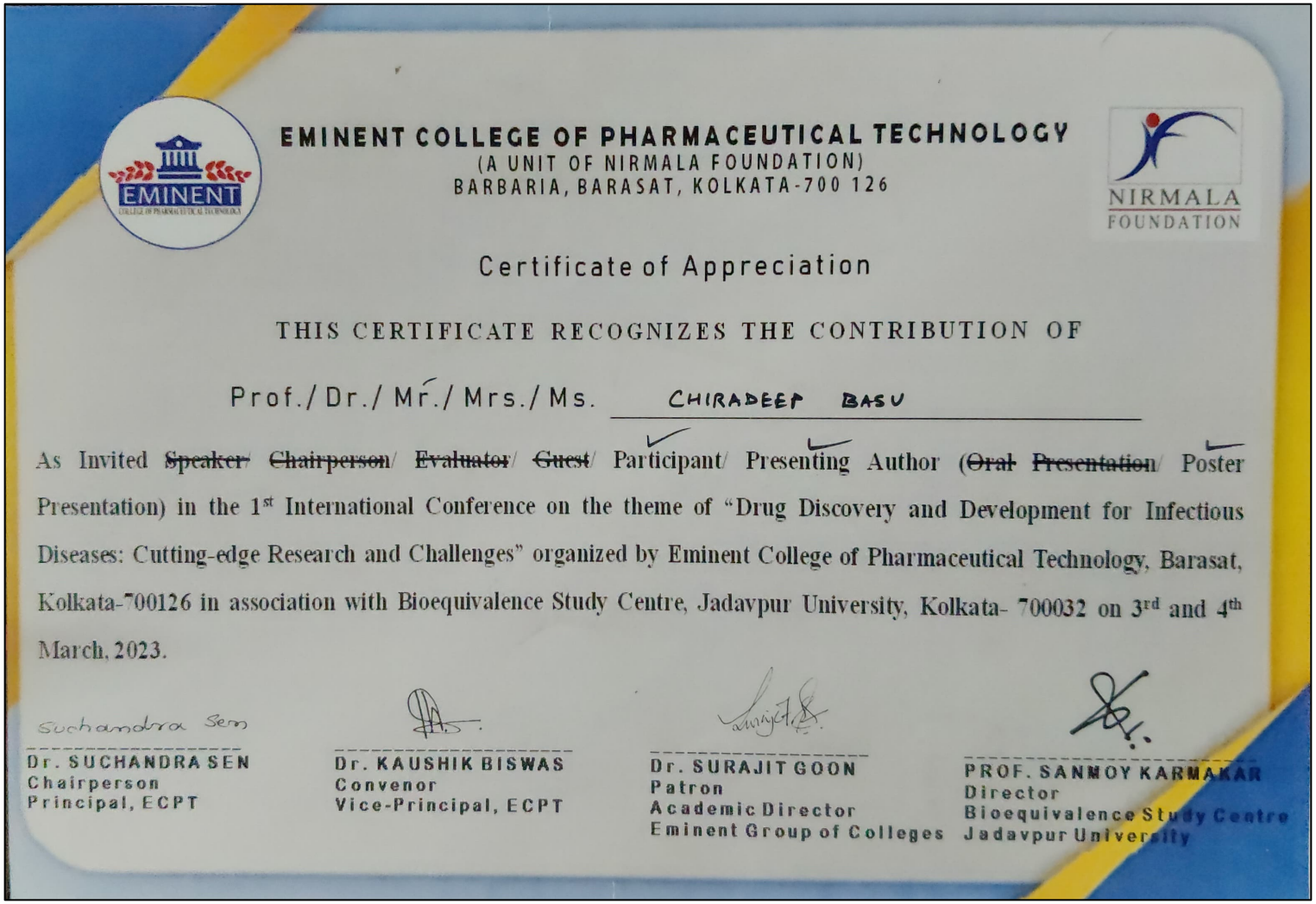
PUBLICATIONS, SEMINARS AND WORKSHOPS

Two Day International Seminar
on
Sustainable Development:
From the Perspective of Nature and Nurture
Organized by
Department of Geography, School of Sciences
Netaji Subhas Open University
in collaboration with
Byanjanbarna Foundation
Date: 10th- 11th February, 2023 Time: 10:30A.M.-5:30 P.M.
Venue: Subhas Chandra Sabhaghar, NSOU, Salt Lake, Kolkata-700064, India

This is to certify that Chiradeep Basu
of Jadavpur University *participated/presented paper titled*
Changes in Microbial Enzyme Production Due to Ambient Air Pollution
in the above mentioned seminar.

Dr. Biraj Kanti Mondal
Assistant Professor
Dept.of Geography,School of Sciences
Netaji Subhas Open University
and Secretary,Seminar Organizing Committee

Dr. Debabrata Biswas
Secretary
Byanjanbarna Foundation



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Certificate of Appreciation

THIS CERTIFICATE RECOGNIZES THE CONTRIBUTION OF

Prof./Dr./Mr./Mrs./Ms. CHIRADEEP BASU

As Invited ~~Speaker~~/ ~~Chairperson~~/ ~~Evaluator~~/ ~~Guest~~/ Participant/ Presenting Author (~~Oral~~ ~~Presentation~~/ Poster Presentation) in the 1st International Conference on the theme of "Drug Discovery and Development for Infectious Diseases: Cutting-edge Research and Challenges" organized by Eminent College of Pharmaceutical Technology, Barasat, Kolkata-700126 in association with Bioequivalence Study Centre, Jadavpur University, Kolkata- 700032 on 3rd and 4th March, 2023.

Suchandra Sen

Dr. SUCHANDRA SEN
Chairperson
Principal, ECPT

Dr. KAUSHIK BISWAS
Convenor
Vice-Principal, ECPT

Dr. SURAJIT GOON
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Academic Director
Eminent Group of Colleges

PROF. SANMOY KARMAKAR
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Bioequivalence Study Centre
Jadavpur University

THREE DAYS INTERNATIONAL SEMINAR

Environmental History and Sustainability : The Black-White Journey of Sustainable Development in Reality and Education

Organized by

Departments of History (UG & PG) & Geography (UG & PG)

Bajkul Milani Mahavidyalaya

Kismat Bajkul, Purba Medinipur-721655

Held on

5th, 6th & 7th February, 2019

Certificate of Appreciation

This is to certify that Mr./ Miss/ Mrs./ Dr./ Prof. Chiradeep Basu of ..... has attended in the International Seminar on "Environmental History and Sustainability: The Black-White Journey of Sustainable Development in Reality and Education", organized by the Departments of History (UG & PG) and Geography (UG & PG) of Bajkul Milani Mahavidyalaya, Purba Medinipur, West Bengal held on 5th, 6th and 7th February, 2019. He/ she has contributed as the Resource Person/ Invited Speaker/ Chair Person/ Co-chairperson/ Paper Presenter/ Poster Presenter/ Participant whereas the topic of Paper/ Poster/ Lecture was entitled as gender issue with indoor air pollution in rural India. ..... His/ her presence made the seminar meaningful and effective.

Mr. R. Das
Convenor,
Seminar Organizing Committee

Mr. G. P. Kar
Secretary,
Seminar Organizing Committee

Eminent Resource Person

Eminent Resource Person

Dr. P. K. Dandapath
TIC &
Chairman of the Seminar

Centre for Studies in Environment and Sustainable Development

Rabindra Bharati University

Emerald Bower Campus, 56A B.T. Road, Kolkata-700 050

Certificate of Participation

This is to certify that Prof./Dr./Sri/Smt. Chiradeep Basu
of School of Environmental Studies, Jadavpur University
has participated in the One-day International Seminar on "Clean Energy, Environment and Sustainable Development" organized by Centre for Studies in Environment and Sustainable Development(CSESD) held at Rabindra Bharati University, Kolkata, on March 26, 2019 as a ~~general participant~~/paper presenter and presented the paper titled Use of Biomass as Fuel for Cooking in Rural India and its Health Effects: A Review

Dr. Prankrishna Pal
Professor, Department of Economics
Convener & Joint Director, CSESD

Date: March 26, 2019

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This is to certify that

Chiradeep Basu.

has participated

at

NATIONAL WORKSHOP

ON

ASSEMBLY AND USAGE OF FOLDSCOPE

(Under the DBT Foldscope initiative)

Organised by

Department of Botany

Holy Cross College,Agartala

in collaboration with

School of Environmental Studies

Jadavpur University Kolkata

Convener | Joint Convener | Principal, HCC

Date: 13, November 2018

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