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ISOLATION AND IDENTIFICATION OF AERIAL FUNGAL SPORES FOR BIOCHEMICAL STUDIES FROM THE UNIVERSITY OF SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract

This research work was conducted in March-April, 2022. This research aims to isolate aerial fungal spores in order to understand the presence of respiratory disorders caused by fungi in the future in certain areas. In this study, it was identified and isolated a total of 6 genera containing 7 species from 17 sampling sites. The medium used to capture fungal spores was potato dextrose agar. Fungal species were identified based on microscope characteristics. This study revealed that 7 species of fungi were identified and isolated from Swabi University, Khyber Pakhtunkhwa Pakistan. These fungi including Aspergillus flavus, Aspergillus niger, Cladosporium, Rhizopus oryzae, Alternaria alternata, Penicillium chrysogenum and Fusarium oxysporum. The maximum fungal colony was counted from the old canteen (Gate side) (25CFU) and the minimum was counted from the old door 2 (8CFU). Research also revealed that aerial sports were frequently practiced in various sampling areas of the University of Swabi. These spores can cause several serious fungal infections in students and faculty. Further research will be carried out to assess the status of fungal spores at Swabi University.

Keywords: Fungi, Fungal Isolation, Characterizations, Swabi.

Introduction

Airborne microfungi are one of the important indoor air bio contaminants. The main source of mold and indoor airborne fungi is usually the outdoor air (Liao *et al.,* 2004). Indoor mold growth releases infectious agents such as mold spores and mycotoxins into the indoor air of buildings. Airborne spores in the air form a significant part of the particulate matter in the atmosphere and are a long-standing cause of allergens and infectious diseases (Lang-Yona *et al.,* 2012). Many researcher examines about the potential risks to human health caused by toxins produced by the

metabolic activity of certain species, such as *Aspergillus* or *Fusarium*, and also about the relationship between these substances and air quality. Various research methods have been used in aeromycological studies; Sampling methods can be weight or volume (Kasprzyk, 2008). A significant portion of airborne spores come from agricultural and outdoor environments (Odebode, 2017). Fungal spores are a permanent part of the atmosphere. Over the last two decades, approximately 150 fungal allergens have been identified from approximately 80 fungal species. Among them, *Alternaria, Aspergillus, Cladosporium*, and *Penicillium* are responsible for IgE-mediated allergic reactions in atopic patients, and exposure can occur indoors, outdoors, or both (Bush & Prochnau, 2004). Microorganisms, especially airborne microbes, have tendency to pose health risks and cause problems *e.g*. pneumonia, asthma, rhinitis colds, hay fever and respiratory tract infections (Grinn-Gofron & Mika, 2008). Only airborne microorganisms can be infectious components or products of microorganisms can cause toxic and allergic reactions, such as mycotoxins and endotoxins (Fabian *et al.,* 2005). Indoor microbes are responsible to produce various pathogens like mycotoxins and other biologically active metabolites, such as carcinogenic mycotoxins, aflatoxin B1, austocystins, and sterigmatocystins (Fabian *et al.,* 2005).

Methods and Materials

Study Area: The district Swabi, Khyber, Pakhtunkhwa, Pakistan's is located between the Indus and Kabul Rivers (Anwar *et al.,* 2015). It is the fourth largest populated district. Swabi is located between 34.70 N and 72.280 E, with a total area of 1543 km^2. The district is primarily mountainous (78.0%), with the remaining portion (21.0%) being plain, dry ground. May and June show significant rises in temperature, with July, August, and September showing almost constant temperatures. June was the month with the highest recorded temperature of 41.5°C. January had the lowest temperature ever recorded, which was 2°C. The woody vegetation includes Quercus sp. *Vachellia nilotica, Pinus roxburgii*, *Tamarix aphylla* and *Senegalia modesta* Dominant shrubs include *Dodonaea viscose Justacia adhatoda*, and while *Saccharrum spontanum, etc.* are common.

Preparation of culture media and sterilization of Glassware's: Potato dextrose agar (PDA) was prepared by adding 20g of PDA to 250ml of distilled water in a flask, sterilized in an autoclave at 121°C for 15 minutes at 15-lb/inch pressure, then cooled in a laminar flow chamber. Ciprofloxacin antibiotic solution (20ml in 200ml distilled water) was added to the cooled PDA to prevent bacterial growth. The media was poured into Petri plates after shaking. All glassware, including flasks and Petri plates, was sterilized in an autoclave at 121°C and 15 $lb/inch^2$ pressure for 15 minutes.

Data collection: Samples were collected in the months of March and April (2022) from the various sites at Swab University using a gravity sedimentation method, which involved opening Petri dishes at 1.5 m intervals for 5 minutes and adding the samples to an antimicrobial solution in PDA medium to prevent bacterial growth. Samples were collected during sunny days between

11am and 4pm, focusing on the direction of wind movement. Sampling locations including pharmacy, English department, canteen, VC office, flag area, girls Hostels, both side of the road. The boys' hostel, rose garden, old canteen, ground and blocks A, B, C, D were selected. The research design aims to isolate the maximum number of fungal spores from the air at 17 selective locations of Swabi University (Figure 1).

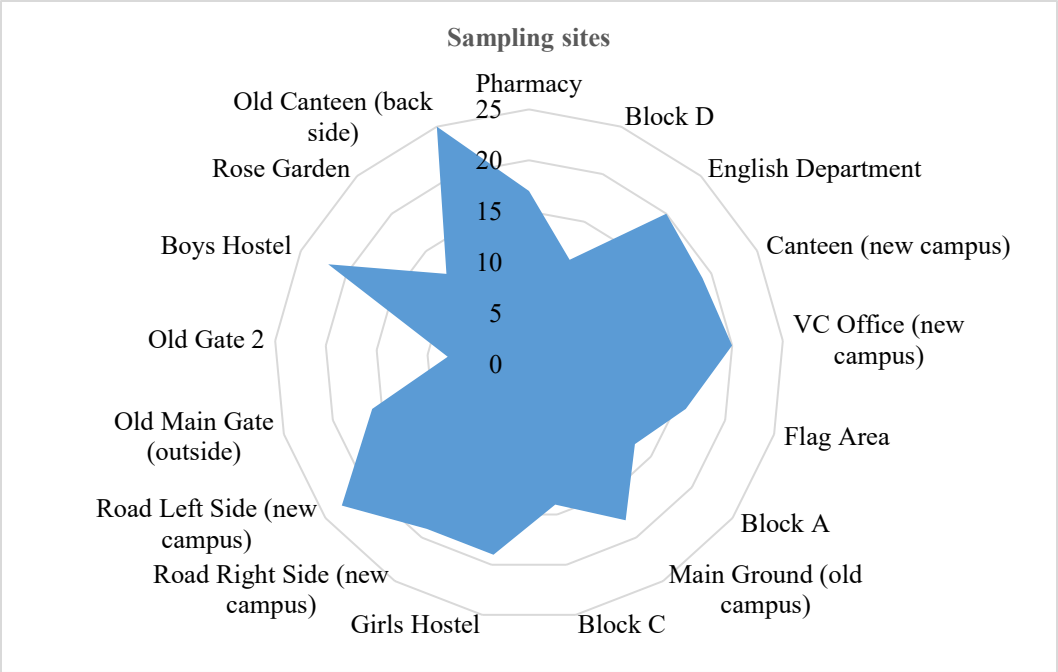

Figure 1. Represents the sampling sites of University of Swabi.

Air sampling: The spore trapping method involved exposing Petri plates containing potato dextrose agar for 5 minutes to collect spores through sedimentation under gravity. Subsequently, the plates were placed in an incubator at 27°C for 3-4 days for incubation.

Incubation period: Petri plates were sealed with tape and incubated at 27°C, approximately 3 days.

Calculation of CFU: After the incubation period, when fungal growth occurred on the plates, the colony-forming units (CFUs) were counted. Microscopic observation of the slides at 10x and 40x magnification showed fungal growth. The plates were divided into four equal parts using a marker, and the total number of colonies in each square was counted and calculated.

Identification of fungi: Slides were prepared by placing a drop of water followed by picking up a colony from the fungal plates using a wire loop, then adding lactophenol blue for visibility and

covering with a coverslip. The slides were examined under a microscope and fungi were identified based on morphology, color, hyphae, and spores.

Preparation of pure culture of fungi: Culture of pure Dextrose agar (PDA) was used to obtained individual fungal colonies in plates. These pure fungal strains were utilized for detailed taxonomic studies of isolated fungi up to the species level.

Extraction of fungal metabolite: Selected fungal species, including *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus oryzae*, and *Cladosporium* were chosen for the extraction of fungal metabolites. Potato dextrose broth was utilized in 500ml flasks, each containing 450ml of broth media, which were inoculated with 5 loops of pure culture from the selected fungal species. The flasks were sealed with cotton plugs and paraffin tape and then incubated in a shaker incubator set at 37°C with 180 RPM for 3 days. After reaching physical saturation of fungal metabolites and mycelial balls, the shaker was turned off, and the flasks were removed.

Filtration of fungal metabolites: Fungal metabolites and mycelium were separated using Whatman filter paper number 100 in a conical flask. The filtrate underwent further processing with ethyl acetate to eliminate impurities from the fungal mycelium through a separating funnel. The separating funnel yielded two phases: one clear and one foggy. The foggy material was then separated and stored in labeled glass vials according to the fungal species.

Results

In the present study, fungal species were collected, identified and isolated, a total of 7 fungal species from the study area district Swabi, University of Swabi. These fungal species belong to 6 genera including *Aspergillus flavus, Aspergillus niger, Cladosporium, Rhizopus oryzae, Alternaria alternate, Penicillium chrysogenum* and *Fusarium oxysporum.*

Figure 2 shows the colony-forming units (CFU) of various fungal genera isolated from air samples collected at different locations of the University of Swabi. Among the identified fungi, *Aspergillus* species exhibited the highest CFU count, followed by *Rhizopus*, *Alternaria*, *Penicillium*, *Cladosporium*, and *Fusarium*. The predominance of *Aspergillus* indicates its wide distribution and ability to disperse easily through air, suggesting it as a major component of the airborne mycoflora in the study area.

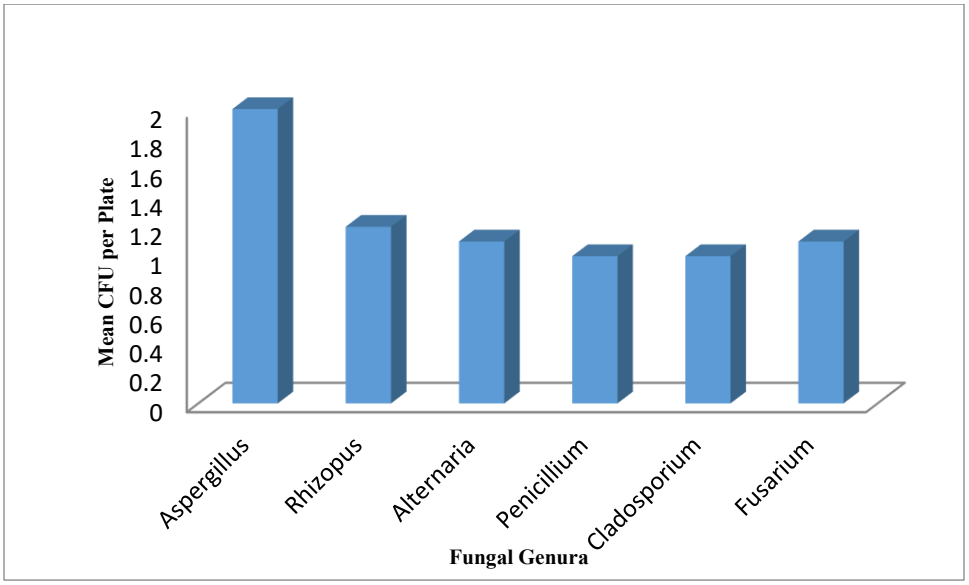

Figure 2. Mean colony-forming units (CFU) per plate of fungal genera isolated from air samples collected at the University of Swabi.

Site wise fungal spores: In present study total 17 sites were examined for the aerial fungal spores (Table 1). The maximum fungal colonies were counted from Old Canteen (back side) (25CFU) and minimum were counted from Old Gate 2 (8CFU).

Table 1. The detail of CFU from selected sites.

Selected sites	Number of Fungal colonies (CFU)
Pharmacy	17
Block D	11
English Department	20
Canteen (new campus)	19
VC Office (new campus)	20
Flag Area	16
Block A	13
Main Ground (old campus)	18
Block C	14
Girls Hostel	19
Road Right Side (new campus)	19
Road Left Side (new campus)	23
Old Main Gate (outside)	16
Old Gate 2	8
Boys Hostel	22
Rose Garden	12
Old Canteen (back side)	25
Total	292

Discussion

In this study, results revealed the identification and isolation of 7 fungal species collected from sampling sites of Swab University. These fungi include *Aspergillus flavus, Aspergillus niger, Cladosporium, Rhizopus oryzae, Alternaria alternate, Penicillium chrysogenum* and *Fusarium oxysporum.* The observations of this study also showed that the area selected for sampling had the most mold spores on the side of the old cafe (25), followed by the number of spores on the left side of the road (23). Therefore, this suggests that airborne mold spores are more likely to be in the air than on the ground and the higher number of mold spores recorded at the old campus may be related to human activity and other related environmental factors.

Many researchers examine about the potential risks to human health caused by toxins produced by the metabolic activity of certain species, such as *Aspergillus* or *Fusarium* and also about the relationship between these substances and air quality. Various research methods have been used in aeromycological studies; sampling methods can be weight or volume. Results coincide with the work of other researchers. Airborne fungal spores are more abundant in the air than in the soil and can cause various diseases in humans. The existence of some species may be due to their spores falling due to gravity after being released into the atmosphere.

The distribution of spores in different locations and different exposure times may explain their impact on human health. From this study, a total of 7 types of fungi were found in the air during the research period. However, it is important to note that many of the identified species are involved in numerous human diseases. Many of these diseases are real; because they affect the skin and subcutaneous tissue, some also cause systematic infections and even lethal (Hasnain, 2011). *Aspergillus niger* found to be more harmful to cause many disease in humans as compare to other Aspergillus species, but if large amounts of spores are inhaled, severe lung disease can be occured. *Aspergillus niger* also causes black mold on onions. Infection of shallot plants by *A. niger* can only occur systemically if conditions are suitable (Holdwaway, 2000). Many fungi produce antibiotics such as penicillin (Smith, 1990). Fungi, which are ubiquitous, pose significant public health risks indoors. Of particular concern are allergenic fungi, which often belong to the categories Ascomycota, Basidiomycota, or anamorphic fungi (Khan, 2012).

Conclusion

This study revealed higher concentrations of fungal spores on above-ground surfaces throughout the study period. This increases the risk of fungal infections and allergic reactions, which is vary greatly with the seasons. Although *Aspergillus* species have a wide distribution in all locations, understanding indoor microbial flora in environmental habitats promises ecological understanding and allergy prevention. Future research should investigate how socio-economic and hygienic factors influence internal fungal and microbial flora and clarify their relationships

with external factors. The organisms isolated from indoor library environments are known pathogens, particularly threatening people with weakened immune systems. This poses a risk to library staff and visitors. Implementing adequate preventative measures and regular environmental monitoring is essential to reduce the presence of mold in library indoor environments.

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