

# Bare the Black Mildew - Histomorphological Study of Fungi in Vicinity and Fungi in Tissue Biopsies of Mucormycosis

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## ABSTRACT

**Introduction:** The “Black fungus” epidemic hitting COVID - 19 patients has led to a scare among general public and inquisitiveness among medical fraternity about the probable source of this fungus. A wide range of fungi are seen in and around our vicinity especially growing on kitchen items like vegetables, fruits, other organic materials, etc. Hence, an attempt was made to study the morphology of these fungi and compare it with the morphology of fungi observed in tissue biopsies sent routinely. **Objectives:** 1. To study the morphology of fungi that grow in close vicinity especially food items. 2. To compare morphology of same with fungi that are identified in patient samples. **Methodology:** This is a cross-sectional study done in the Department of Pathology. Various fungi grown on vegetables, food, fruits and in the vicinity were included. Representative bits from these were fixed in 10% formalin and processed routinely. H and E sections were studied for morphology of the fungus under light microscopy. These were compared with the tissue biopsies of patients with fungal infection. **Results:** Thirty samples were processed (15 vegetables, 7 fruits, 5 organic materials, 3 plants). On morphological comparison of Group A with Group B, only aspergillus and candida appeared simila. Other details are discussed. **Conclusion:** Exposure to contaminated fruits, vegetables and other environmental materials maybe a risk for development of fungal infections in people with already existing co-morbidities/compromised immunity. **Novelty:** Attempt to explore common sources of fungi in our vicinity.

**KEY WORDS:** Black fungus, Fungi, Morphology of fungi, COVID19 pandemic, COVID, Fruits, Vegetables, Environment.

## Introduction

Mucormycosis, commonly known as the “Black fungus,” is a rare yet highly severe fungal infection attributed to a group of fungi called mucormycetes. It poses a significantly greater threat to those with compromised immunity. A concerning surge in mucormycosis cases has been noted in the context

of the COVID-19 pandemic, particularly among patients with pre-existing comorbidities. Fungi of diverse types are encountered in our surroundings, frequently inhabiting vegetables, fruits, and various organic materials<sup>[1]</sup>. Fungal spores and fragments represent a common category of airborne biological aerosols. The study aims to correlate microscopic morphology between fungi found in the environment (such as on edible items and in the surroundings) and those causing infections in COVID-19<sup>[2]</sup>.

## Objectives

To study the morphology of fungi that grow in close vicinity

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To compare morphology of same with fungi in patient tissue samples

### Methodology

This is a Cross Sectional Study done in the Department of Pathology in South India over a period of 2 months.

- Inclusion Criteria**

Fungi present on the edible things and in the surroundings.

Fungi identified in the tissue biopsies of Covid-19 patient.

- Exclusion Criteria**

Fungi on the organic materials that could not be processed and were friable.

Repeat sample from the patients who are already treated for fungal infection.

The samples were grouped into 2 categories (Group A and Group B). Group A consisted of fungi present on the edible things and in the surroundings. Group B comprised of all the Covid-19 patients with secondary complication of fungal infection isolated in tissue biopsy. All the Covid - 19 patients with secondary complication of fungal infection isolated in tissue biopsies were considered under Group B (Table 1 )

**Table 1: Sample categorization and sample size**

Group A	Group B
Fungi present on the edible things and in the surroundings	Tissue biopsies of COVID-19 patients with fungi
n = 30	n = 30

The materials with the fungal growth were fixed for 24 to 48 hours in 10% formalin. Representative bits were processed routinely using automated tissue processor (Leica). 4-5-micron thick paraffin sections were taken for the study. One/Two slides were made of 1-3 sections on each slide. Slides were stained with Haematoxylin and Eosin. Haematoxylin and Eosin-stained sections were studied microscopically for the

**Table 2: Fungi isolated under Group A**

Fungal Type (Group A)	n	%
Aspergillus	20	67%
Mixed (Aspergillus and Candida)	4	13%
Candida	3	10%
Unidentified	3	10%

**Table 3: Fungi isolated under Group B**

Fungal Type (Group B)	n	%
Mucor	24	79%
Mixed (Mucor, Aspergillus, candida)	5	17%
Aspergillus	1	4%

morphology of the fungi and were compared with tissue biopsies containing fungi. Thirty samples were processed. Eleven Fruits [38%], 7 Vegetables [24%], 5 Groceries [17%], 4 Plants [14%] and 3 Flowers [7%]. Morphology was compared with Group B. Majority of Group A had fruits and flowers were the least.

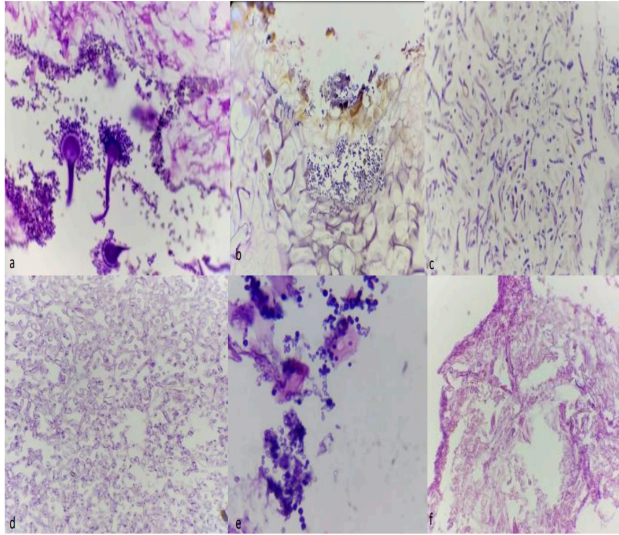
### Results

Based on the gross appearance of fungal growth on various vegetable mater Group A were categorized into black, white and mixed types. White fungi under GroupA consisted of Ginger, Jaggery, Lichi, Badam, Ridgegourd, Bay Leaf, Leaves, Cloves, Bittergourd and date. Onions, Lemons, Coconut and Beetle Leaf had black fungi. Bananas, Papaya Bread Loaf, Apple, Flowers and Tomato had mixed fungi. Microscopy of Fungal specimens from Group A showed aspergillus (Figure 1 - a), candida (Figure 1 - b), mixed type ie aspergillus and candida and one unidentified fungal elements (Figure 1 - c). Aspergillus owed to 67%, mixed type 13%, candida and unidentified type 10% each (Table 2 ). Microscopy of Fungal specimens from Group B showed mucor (Figure 1 - f), mixed type i.e., mucor, aspergillus and candida (Figure 1 - d) and one aspergillus (Figure 1 - e).

Mucor was 79%, mixed type 17%, Aspergillus 4% (Table 3). On Morphological comparison of Group A with Group B, only aspergillus and candida appeared similar (Figure 1).

### Discussion

Fungi are an important part of the natural environment and, therefore, have many roles in relation to



**Figure 1:** Stain: H and E, High Power a) Aspergillus - Group A b) Candida – Group A c) Mixed and unidentified – Group A d) Aspergillus – Group B e) Candida – Group B f) Mucor – Group B

food, few fungi are used in food production, some are food sources themselves, and others are agents of food spoilage. Fungi that contaminate food can also be harmful to human health. The harmful but noninfectious health consequences of mycotoxins have been well-characterized, but the extent to which fungi in food pose a risk for invasive infections is unknown. In our study we have tried to correlate the fungi present on such food items and compare them with those causing extensive infections during COVID19 pandemic. Specimens that come to laboratory from skin, tissue or normally sterile body fluids as well as any specimen from a patient strongly suspected of having fungal infections, should be examined microscopically for the presence of fungal element, this helps in early diagnosis and treatment of fungal infections especially during pandemics like COVID19.

Studies done by Kavya Vinaychandran et al<sup>[1]</sup>, Sohail A et al<sup>[3]</sup>, Kaitilin Benedict et al<sup>[4]</sup> show studied that aspergillus is the most common fungi isolated from vegetable materials. The results were in concordant with our findings. Some of the studies isolated *Rhizopus fusarium* species which are not found in present study. Environmental conditions and large sample size may facilitate the growth and isolated other fungus.

Among the different studies S. Mbajiuka et al<sup>[5]</sup>, Ge Song et al<sup>[6]</sup>, Prof. Saira Afzal et al<sup>[7]</sup> conducted

on isolation of fungi species in COVID 19 patients Aspergillus was the most common organism isolated followed by mucor. In contrast to present study which showed mucor as most isolated fungi followed by mixed type.

On comparing the morphology of both the groups, aspergillus and candida had same features. Morphology of the mucor could not be compared as it was not isolated among the vegetable matter.

## Conclusion

Most common fungal morphology in Group A was Aspergillus [67%]. Most common fungal morphology in Group B was Mucor [79%], followed by Aspergillus and Candida. The morphology of Aspergillus and Candida in both the groups were similar and they may be the probable sources of fungal infection. Fungi from damp indoor environments are known to be one of the main causes of degradation of indoor air quality and can pose a serious health hazard to occupants<sup>[2]</sup>. Hence it is important to determine the sources of these fungal spores and fragments in such environments<sup>[2]</sup>.

## Novelty

The novelty of this study lies in its attempt to explore and compare the morphology of fungi found in the vicinity, especially on food items, with the fungi identified in tissue biopsies of COVID-19 patients suffering from mucormycosis. Understanding the sources of fungal infections can have public health implications.

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