



Thesis title: A study on fungal contamination of food products and their impact on human health

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Background

Filamentous fungi are a wide category of eucariotic microorganisms, characterized by the presence of long, tubular filaments called hyphae, which together form the mycelia. They are also known under the name of molds and can be found everywhere in the surrounding environment (soil, water, air), but they can cause infections mostly in immunosuppressed people.

In pathology, filamentous fungi are one of the most feared microorganisms, as they can cause a variety of infections (localized or invasive) and are associated with high mortality.

Nowadays, filamentous fungi represent a generous theme for research, rarely present in extensive studies, therefore this PhD thesis presents a few important aspects related to their presence in green leaf spices commercialized in Romania, their epidemiology when causing infections in humans and some modern methods of diagnosing such infections.

The thesis is built on two main parts. The first part analyses the state of the art, and assesses the maturity in terms of research on infections caused by filamentous fungi. Subsequently, the second part presents three significant studies, each with the purpose of bringing new knowledge in the domain of filamentous fungi.

Study number I - Fungal and Mycotoxin Contamination of Green Leaf Spices Commercialized in Romania: A Food Choice Perspective

Green leaf spices are predisposed to contamination with bacteria, yeasts, and molds. In healthy individuals, long-term exposure to molds can cause allergic reactions. A higher risk towards human health is caused by their secondary metabolites, mycotoxins.

This study aims to detect the fungal load of some of the most used green leaf spices commercialized in Romania, as well as the presence of three important mycotoxins (aflatoxin B1, ochratoxin A, and zearalenone), all with potential impact on human health. Furthermore, this study approaches the significance of environmental factors, such as humidity, and their impact on spice contamination, as well as the correlation between price and the fungal and mycotoxin contamination of these products.

Out of the 21 samples, six (28.57%) tested positive for different degrees of fungal contamination. The most common fungal contaminant was constituted from species from genus *Aspergillus* (*section Flavi* and *section Nigri*) followed by species from genus *Rhizopus*, *Absidia* and *Penicillium*.

On average, all spices suffered a certain degree of contamination with at least one mycotoxin. Aflatoxin B1 was detected over the admitted limit of 5 µg/kg in 95.24% of spices, ochratoxin A was over the limit of 15 µg/kg in all the green leaves and zearalenone was detected over the admitted limit of 50 µg/kg in 85.71% of the products. Overall, the average moisture was 11.25%, with a maximum of 14.99%.



Study number II - Filamentous Fungi Infections: Yet Another Victim of COVID-19?

In the past, fungal infections were mainly caused by species from the genus *Candida* spp., but lately, due to an increase in life expectancy and, along with it, the associated risk factors, more and more pathogens have emerged.

The purpose of this study is to evaluate the prevalence of filamentous fungi infections in a tertiary care hospital in Târgu Mureș, Romania, as well as evaluate the correlation between age, risk factors (underlying diseases) and their association with the presence of those fungal pathogens. Furthermore, the influence of the COVID-19 pandemic on the number of mold infections was studied.

A single-center retrospective observational study in which data was collected from the Bacteriology Compartment of the Medical Laboratory of the Clinical County hospital of Târgu Mureș was conducted between January 1st 2012 and November 1st 2022 in which over 20,000 samples were analysed.

Out of the 68 positive samples for filamentous fungi, 41 positive purulent discharges and 27 positive respiratory secretions were identified. From the purulent discharge samples, the one that tested positive for mold infections the most was the ear discharge (32.35%), while from the respiratory secretions, the orotracheal tubes were positive in 20.59% of cases. The most isolated fungus in both types of samples was *Aspergillus section Flavi*. Age distribution showed an increase in the number of cases proportionally with the age increase.

During the pandemic years, the number of positive samples increased from a number of approximately 4.75 cases of infection/year before the pandemic to a number of 10 cases/year during the pandemic. In COVID-19 patients, the most commonly isolated fungus from respiratory secretions was also *Aspergillus section Flavi*. Furthermore, in the positive sample group, a statistically significant correlation was found between the association of mold infection and the SARS-CoV-2 virus and an increase in mortality ($p < 0,0001$; OR=24,5).

Study number III - *Fusarium* spp. in Human Disease: Exploring the Boundaries between Commensalism and Pathogenesis

Fusarium is a large genus that exhibits global distribution and can be found everywhere in the surroundings, being characteristic of the northern temperate regions. In humans, *Fusarium* spp. causes a variety of infections, which are highly dependent upon the portal of entry and the immune status of the host. In immunocompetent people, it is the most common etiological agent of superficial infections such as keratitis and onychomycosis, while in immunosuppressed people, invasive infections are more common.

Diagnosing fusariosis can be challenging, but these infections can be detected through a rigorous clinical examination combined with laboratory findings in a patient who presents several risk factors.

The aim of this study is to present the common and complementary molecular diagnostic methods used in *Fusarium* spp. infections, which aside from the clinical findings may bring important information in the differentiation between colonization and infection when trying to reach a final diagnostic.

The study presents two particular cases of infection and colonisation with *Fusarium* spp., respectively, and the methods through which the strains were identified:

- macroscopical and microscopical examinations of the culture for identification to the level of genus;
- MALDI-TOF for species identification;
- molecular diagnosis used as both an alternative for classical methods of identification to the level of genus (amplification of the *Tri13* gene) as well as for the differentiation between two strains of *Fusarium* spp. (ERIC-PCR).