

Original Research

**LABEL-FREE FLUORESCENCE-BASED OPTICAL BIOSENSOR FOR RAPID
DETECTION OF FUNGAL PATHOGENS**

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Abstract

*The aim of this study was to design a rapid, label-free method based on fluorescence spectroscopy for detection and identification of different fungal pathogens. Three species were investigated: *Aspergillus flavus*, *Fusarium verticillioides*, and *Alternaria alternata*. Fungal lysates were deposited onto a nitrocellulose membrane and analyzed under UV-A illumination, where differences in the intensity and morphology of fluorescent signals were observed. Additionally, fluorescence spectra revealed characteristic profiles with clearly distinct positions and intensities of emission maxima in the 410–460 nm range. *A. alternata* exhibited a maximum emission around 460 nm, while *A. flavus* and *F. verticillioides* showed peaks in the 410–430 nm range.*

The obtained results confirm that the combination of a membrane-based platform and optical detection enables reliable spectral discrimination of fungal pathogens without the need for labels. The proposed method represents a simple, fast, and potentially portable solution for monitoring food contamination and developing optical biosensors.

Keywords: Fungal pathogens, fluorescence spectroscopy, *Aspergillus flavus*, *Fusarium verticillioides*, *Alternaria alternata*.

INTRODUCTION

Fungal pathogenic cultures are a major source of food contamination due to crop infestation and production of hazardous toxins that pose a serious health threat to livestock and humans [1]. *Aspergillus flavus* (*A. flavus*) produces aflatoxins (AFs) that are considered

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as environmental carcinogens. Secondary metabolites of *Fusarium verticillioides* (*F. verticillioides*) include fumonisins, which cause severe animal and human diseases [2]. In addition to these genera, *Alternaria alternata* (*A. alternata*) is recognized as an important plant pathogen capable of producing a wide range of mycotoxins, including alternariol, tenuazonic acid, and altenuene, which have been associated with cytotoxic, mutagenic, and genotoxic effects [3,4]. These fungal species frequently contaminate cereals, fruits, vegetables, and stored agricultural products, leading not only to significant economic losses but also to reduced food quality and safety.

The occurrence of mycotoxigenic fungi is strongly influenced by environmental conditions such as temperature, humidity, and storage practices, which facilitate fungal growth and toxin biosynthesis. Due to the high stability of many mycotoxins during food processing, contamination remains a persistent global problem and represents a major challenge for the food and feed industry. Therefore, continuous monitoring and investigation of fungal growth characteristics and toxin production are essential for understanding contamination pathways and developing effective prevention strategies.

Early detection of plant pathogens is critical for global food security and minimizing crop losses. Traditional diagnostic methods, such as Polymerase Chain Reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), offer high sensitivity and specificity, but are poorly suited for field deployment. They require complex sample preparation, expensive laboratory equipment, and trained personnel, which can result in significant delays between sampling and diagnosis [5]. While conventional lateral flow assays (LFAs) provide rapid on-site testing, they rely heavily on antibodies. These biological reagents are expensive, susceptible to thermal degradation under field conditions, and often require complex conjugation steps.

The objective of this research was to develop a rapid, label-free screening test based on fluorescence measurements using an optical fiber, and to detect three different fungal species (*A. flavus*, *F. verticillioides* and *A. alternata*) based on their corresponding emission peak maxima.

MATERIALS AND METHODS

Sample preparation

Three fungal species, *A. flavus*, *F. verticillioides*, and *A. alternata* were grown at 25 °C. Fungal mycelium for each culture was collected after 7 days. Zymo Research (ZR) BashingBeads (Zymo Research Corporation, Irvine, CA, USA) were used as a lysing matrix and added into standard lysing tubes with 100 mg of collected wet weight fungal mycelium. Fungal samples were homogenized for three 60-second cycles (180 seconds total) with a handheld lysing device, MonoLyser (RotaPrep Inc., Tustin, CA, USA) at 50% power. After each cycle, the samples were cooled on ice for 2 minutes. The obtained fungal lysate was applied onto a Fusion 5 membrane and observed under UV-A light. Unlike traditional nitrocellulose, which is a microporous polymer film that relies purely on surface adsorption to bind proteins, Fusion 5 is a proprietary structured material containing glass fibers that allows

a single layer to act simultaneously as a sample pad, conjugate release pad, and analytical membrane without requiring multi-component assembly [6].

Fluorescence spectroscopy

A specific volume of the fungal extract was deposited onto the membrane surface and allowed to dry at room temperature. The fluorescence spectra of the samples were captured via an optical fiber cable at an excitation wavelength of 287 nm. Both excitation and emission slits were fixed at 5 nm, and the emission wavelengths were scanned from 305 to 500 nm with a data increment of 1 nm. The baseline fluorescence spectrum of the pristine membrane was recorded under identical conditions and subtracted from the sample spectra to eliminate background interference. Fluorescence spectra were normalized to the maximum intensity to enable comparison between samples.

RESULTS AND DISCUSSION

The macro-morphological evaluation of the isolated strains (Figure 1) revealed distinct phenotypic variations in color, texture, and growth patterns that are characteristic of each genus. These visual features—including the dark melanin pigmentation of *A. alternata*, the aerial pinkish mycelium of *F. verticillioides*, and the granular yellow-green spores of *A. flavus* are in full agreement with diagnostic criteria for these phytopathogenic species.

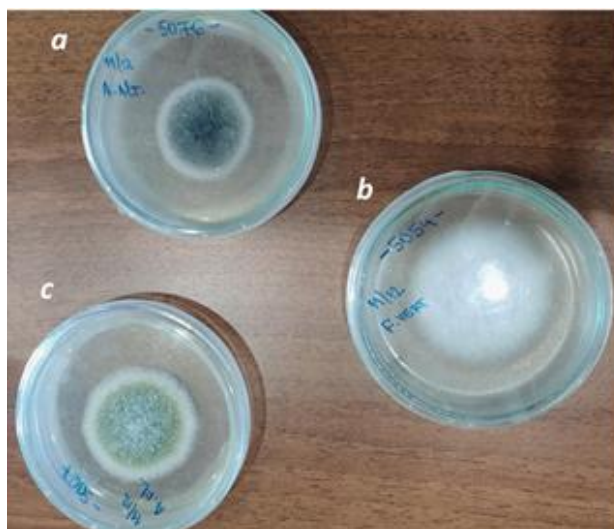


Figure 1 Macrophotographs of fungal colonies in petri dishes: a) *Alternaria alternata*; b) *Fusarium verticillioides*; and c) *Aspergillus flavus*

Figure 2a shows fungal lysates deposited on a nitrocellulose membrane under UV-A illumination, revealing clear differences in fluorescence intensity and spot morphology among the tested species. *Alternaria alternata* exhibited a more diffuse and broader fluorescent signal, while *A. flavus* and *F. verticillioides* formed more compact spots with lower signal spreading. Notably, *A. flavus* showed a darker central region within the fluorescent spot, possibly indicating uneven biomass distribution or local fluorescence quenching. Overall, these differences suggest species-specific variations in metabolite composition and optical properties, supporting label-free optical differentiation of fungal pathogens.

Figure 2b presents fluorescence emission spectra of fungal lysates under UV excitation, showing distinct spectral profiles for all three species. *A. alternata* displayed a broader emission and a red-shifted maximum around 460 nm with higher intensity in the 450–500 nm range. In contrast, *A. flavus* and *F. verticillioides* showed stronger peaks at 410–430 nm with a faster intensity decrease at higher wavelengths. These spectral differences reflect variations in fluorescent biomolecules and confirm that fluorescence spectroscopy can effectively distinguish fungal species based on their optical fingerprints, highlighting the potential of the label-free biosensor for rapid detection.

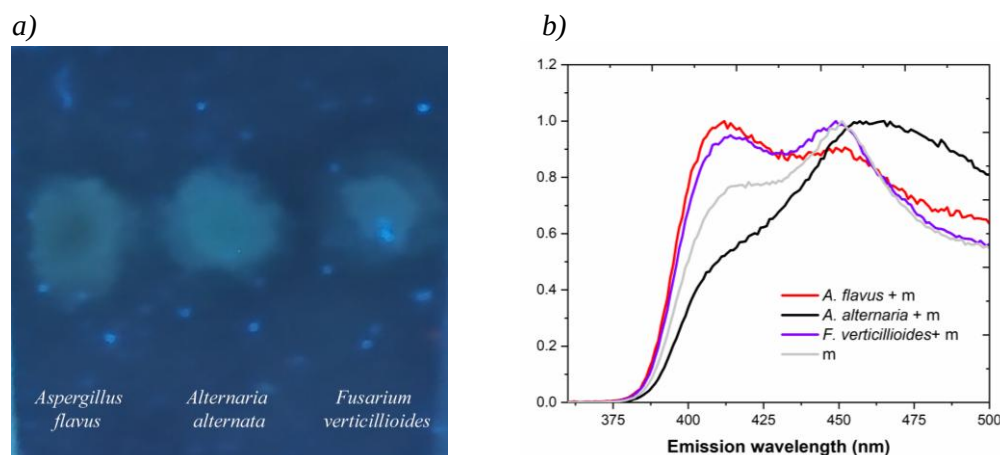


Figure 2 a) Fungal lysate on membrane under UV-A light; b) fluorescence emission spectra

CONCLUSION

This study shows that UV-A fluorescence imaging combined with optical spectroscopy enables fast, label-free differentiation of fungal pathogens. Distinct fluorescence patterns and emission spectra provide clear optical fingerprints for *A. alternata*, *A. flavus*, and *F. verticillioides*.

The results confirm species-specific spectral differences, particularly a red-shifted emission in *A. alternata* and lower-wavelength peaks in the other two species.

Overall, the method offers a simple and rapid approach with strong potential for portable biosensing and food safety applications.

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