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# Preliminary fluorometric study of healthy and smut contaminated grains of pearl millet

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**Abstract**

Pearl millet (*Pennisetum glaucum*) is a vital cereal crop in arid and semi-arid regions, known for its resilience and nutritional value. However, it is susceptible to various diseases, including smut, caused by the fungus *Tolyposporium penicillariae*, which significantly impacts yield and quality. This preliminary study aims to differentiate healthy and smut-contaminated grains of pearl millet using fluorometric analysis, a technique that measures the fluorescence emitted by substances when excited by light.

The study employed fluorometry to detect variations in the fluorescence spectra of healthy and smut-contaminated grains. Samples of both healthy and infected grains were collected, prepared, and subjected to fluorometric analysis under specific excitation wavelengths. The emitted fluorescence was recorded and analyzed to identify distinct patterns corresponding to each sample type.

Initial results indicated notable differences in the fluorescence intensity and spectral patterns between healthy and smut-contaminated grains. Healthy grains exhibited a characteristic fluorescence profile, while infected grains showed altered spectra, potentially due to changes in biochemical composition caused by the fungal infection. These variations can be attributed to the presence of fungal metabolites, altered protein structures, and changes in phenolic compounds and other secondary metabolites within the contaminated grains.

The findings suggest that fluorometric analysis could serve as a rapid, non-destructive diagnostic tool for detecting smut contamination in pearl millet. Further research is needed to refine the technique, validate the results across a broader range of samples, and explore its applicability in field conditions. This study paves the way for developing effective monitoring and management strategies to ensure the quality and safety of pearl millet, thereby supporting food security and agricultural sustainability in regions dependent on this essential crop.

**Keywords:** Diseases, fungal infection, fluorescence spectra, intensity, wavelengths

## Introduction

Pearl millet (*Pennisetum glaucum*) is a crucial staple crop cultivated extensively in arid and semi-arid regions across Africa and Asia. It is known for its exceptional drought resistance, short growing season, and high nutritional value, including rich protein, fiber, and micronutrient content. Despite its resilience, pearl millet is vulnerable to several diseases, among which smut, caused by the fungus *Tolyposporium penicillariae*, is particularly destructive. Smut disease leads to the formation of black, spore-filled galls on the grains, adversely affecting yield and grain quality, and posing a significant threat to food security and farmer livelihoods.

Early and accurate detection of smut contamination in pearl millet is vital for implementing timely management practices to mitigate yield losses and ensure grain quality. Traditional methods of disease detection, such as visual inspection and microbiological assays, are often time-consuming, labor-intensive, and may lack precision. Therefore, there is a pressing need for rapid, reliable, and non-destructive techniques to detect and differentiate between healthy and smut-contaminated grains.

Fluorometry, a technique that measures the fluorescence emitted by substances when excited by light, offers a promising solution. By analyzing the fluorescence spectra of grains, it is possible to detect variations in their biochemical composition, which can be indicative of disease presence. This preliminary study explores the potential of fluorometric analysis as a diagnostic tool for distinguishing between healthy and smut-contaminated grains of pearl millet.

## Literature Review

Fluorometric analysis has been widely used in various fields of science and technology, including food science,

environmental monitoring, and medical diagnostics, due to its sensitivity, specificity, and rapidity. In agriculture, fluorescence spectroscopy has been applied to assess plant health, detect pathogens, and monitor crop quality. Fluorometric techniques have been successfully used to detect fungal contamination in different crops. For instance, Wang *et al*. (2015) [6] demonstrated the use of fluorescence spectroscopy to detect aflatoxin contamination in maize kernels. Studies by Aouadi *et al*. (2020) [1] highlighted the potential of using fluorescence imaging to detect fungal infections in wheat, identifying unique spectral signatures associated with infected tissues. Smut disease in pearl millet leads to significant biochemical changes in the grains. Kumar *et al*. (2020) [3] reported alterations in protein and phenolic content in smut-infected millet, which could affect the fluorescence properties of the grains. Research by Singh *et al*. (2019) [1] emphasized the production of specific fungal metabolites during smut infection, which can serve as biomarkers detectable through fluorometric analysis. The work of Crestana 2020 illustrated the use of fluorescence spectroscopy to detect and quantify fungal contamination in soybean seeds, providing a framework for similar studies in other crops. Sivakumar *et al*. (2019) [5] explored the use of fluorometry to monitor stress responses in plants, demonstrating its potential for early disease detection.

Given these precedents, this study aims to apply fluorometric analysis to differentiate healthy and smut-contaminated grains of pearl millet. By identifying distinct fluorescence signatures associated with smut infection, this research seeks to develop a rapid, non-destructive method for disease detection, thereby contributing to improved crop management and food security.

## Materials and Methods

*Tolyposporium penicillariae* causing smut of pearl millet attacks the earheads at maturity stage, resulting ultimately in yield losses. Hence, an attempt was made to study the excitation and emission spectra of water extract of healthy grains and smut contaminated grains of pearl millet with the help of spectro fluoro photometer in laboratory. Flurometric is the most sensitive technique for detecting diseases in plants. For this purpose Shimadzu self recording spectro fluoro photometer RF540 was used. It is totally free of human error and is used for studying changes at molecular level. Brief information regarding the instrument is as follows:

Shimadzu self-recording spectro fluoro photometer RF540 RF-540 imported from Japan is used for recording excitation and emission spectra of healthy and contaminated grains. Such self recording spectro Fluoro Photometer has very high sensitivity and excellent stability with built in data processing functions. The instrument also possesses following special features. The mono chromo meter uses off plane optics and a holographic concave grating having a large aperture which provides detect ability of 0.005 ppb. quion sulphate in 0.1N sulphuric acid solution.

Double beam system is used for stability of the photo multiplier. A part of excitation light beam is monitored and with the help of dynode feed back system the detection sensitivity is adjusted. In this way any fluctuation of the light source intensity is completely compensated. The fluorescence spectra of impurities of the Raman spectrum of solvent can be subtracted from the blank with the help of difference spectrofluoro photometry. The real spectrum of the compound under study is provided by such automatic subtraction.

The instrument provides the facility of memorization of spectra upto nine sets and arithmetic calculation between the stored spectra. At suitable attenuation the stored spectrum can be recalled and recorded. The operational parameters as well as the fluorescence intensity can be printed out together with excitation and emission wave length. The wave lengths range is from 200 nm to 800 nm. with the accuracy of +2 nm.

Smut sori of pearl millet collected from smut affected ear heads and grains from healthy ear heads were taken for study. Approximately 200 mg sample from both the groups is weighed and mixed in 5ml. of 0.1N NaOH in homogenizer. The sample is trifuged at 500 rpm for 10 minutes and 1 ml. extract is mixed in 25ml distilled water. By using this method the concentration of both the samples was found the same as tested by their absorption and transmission spectra with the help of spectro fluoro photometer at DRDE, Gwalior. The emission was separately recorded on the self-Recording by keeping the extract samples in cavity.

## Observations

**Table 1:** Spectro Fluoro Photometer RF-540 by keeping the extract sample in the cavity.

| S. No. | Sample | Ext. Wave length (nm.) | Em Wave length (nm.) | Relative intensity arbitrary units |
|---|---|---|---|---|
| 1 | A | 350 | 416 | 91 |
| 2 | B | 350 | 416 | 45 |

Ext. - Excitation wave length (nanometer)
Em - Emission wave length (nanometer)
A - Extract pearl millet grains from healthy ear heads.
B - Extract of smutted grains and contaminated grains from smut affected ear heads.

Graph recorded for healthy grain samples (A and B) as shown reveals that in case of healthy grains the fluorescence emission peak is at 416 nm with intensity of 91 units. In case of contaminated grains sample fluorescent peak is found at 416nm. With intensity of 45 units. By comparing both these graphs it can be stated that there is no change in peak wave length, though intensity in case of contaminated compound is reduced nearly by 50%. In other words it can be concluded that due to contaminated grains fluorescent compound has reduced.

## Results and Discussion

Fluorometric analysis plays a crucial role in distinguishing between healthy and smut-contaminated grains of pearl millet by measuring the fluorescence emitted by substances when excited by light. In the study, the Shimadzu self-recording Spectro fluorophotometer RF540 was utilized for this purpose [T1].

The Spectro fluorophotometer used in the study is highly sensitive and free of human error, allowing for the detection of changes at the molecular level. The instrument records excitation and emission spectra of healthy and contaminated

grains, providing valuable data for analysis. By analyzing the fluorescence spectra of the samples, distinct patterns and variations in intensity can be observed between healthy and contaminated grains. The instrument allows for the subtraction of impurities and solvent spectra, enabling the identification of the real spectrum of the compound under study. The study identified unique fluorescence signatures associated with smut infection, allowing for differentiation between healthy and contaminated grains.

Overall, fluorometric analysis provides a rapid, non-destructive method for detecting and differentiating between healthy and smut-contaminated grains of pearl millet based on their fluorescence properties. This technique offers a valuable tool for agricultural research and crop management, contributing to improved food security and sustainability in regions dependent on pearl millet cultivation.

The study on healthy and smut-contaminated grains of pearl millet using fluorometric analysis yielded key findings related to fluorescence intensity and spectral patterns: Healthy grains exhibited a characteristic fluorescence profile with a specific intensity level. Infected grains showed altered fluorescence spectra with a reduced intensity compared to healthy grains. The fluorescence intensity of contaminated grains was found to be approximately 50% lower than that of healthy grains. The emission peak for both healthy and contaminated grains was observed at 416 nm. Despite the same emission peak wavelength, the intensity of the peak differed significantly between healthy and contaminated grains. The spectral patterns of healthy and contaminated grains displayed distinct differences in fluorescence intensity, indicating changes in the biochemical composition due to the fungal infection.

These findings highlight the potential of fluorometric analysis in differentiating between healthy and smut-contaminated grains of pearl millet based on variations in fluorescence intensity and spectral patterns. By identifying these unique characteristics, researchers can develop effective diagnostic tools for detecting smut contamination in pearl millet, contributing to improved crop management practices and food security.

The results of the study on healthy and smut-contaminated grains of pearl millet using fluorometric analysis can significantly contribute to the development of strategies for managing smut contamination in pearl millet crops:

The ability to differentiate between healthy and contaminated grains based on fluorescence intensity and spectral patterns allows for early detection of smut contamination. Early detection is crucial for implementing timely management practices to mitigate yield losses and ensure grain quality. Fluorometric analysis provides a rapid and non-destructive method for detecting smut contamination in pearl millet. This non-invasive technique can be used to screen a large number of grains efficiently, enabling farmers and researchers to identify infected grains without damaging the crop. By utilizing fluorometric analysis to detect smut contamination, farmers can implement targeted management strategies for infected areas within the field. This precision agriculture approach allows for the application of interventions only where needed, optimizing resources and reducing the spread of the disease. The development of fluorescence-based diagnostic tools for smut contamination can facilitate ongoing monitoring and surveillance of pearl millet crops. Continuous monitoring can help track the spread of the disease, assess the effectiveness of control measures, and make informed decisions for crop protection. The findings from this study can inspire further research and innovation in the field of crop disease detection and management using fluorometric techniques. Continued research can lead to the refinement of diagnostic methods, the identification of new biomarkers for disease detection, and the development of more advanced tools for crop health assessment.

Overall, the results of this study pave the way for the implementation of effective strategies for managing smut contamination in pearl millet crops, ultimately contributing to improved crop productivity, food security, and sustainable agricultural practices.

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