ISOLATION AND CHARACTERIZATION OF FUNGAL PATHOGENS FROM ONION SEEDLINGS

A. A, Panti[1], M. A., Ibrahim[2], S.A. Imam[1] and A. M. Aliyu[1]
[1]Department of Agricultural Science Education, Niger State College of Education, Minna
[2]Department of Soil Science, Niger State College of Agriculture Mokwa
E-mail: abdulwahabpanti4life@gmail.com

Abstract
The prevalence of fungal pathogens in agricultural crops poses significant challenges to crop production and food security worldwide. This study aimed to isolate and characterize two major fungal pathogens, Alternaria porri and Penicillium digitatum, from diseased onion (Allium cepa) seedlings. Alternaria porri, known for causing leaf blight, and Penicillium digitatum, responsible for post-harvest decay, were successfully isolated and identified based on their colony morphology, pigmentation, and growth rates. Pathogenicity tests revealed that Alternaria porri caused severe leaf blight with a disease severity score of 4.3, while Penicillium digitatum primarily affected stored onion bulbs, with a disease severity score of 3.8. Growth rates for the fungi were recorded at 10.5 mm/day and 12.0 mm/day, respectively, with optimal growth observed under temperatures between 25°C and 30°C. The findings underscore the potential for significant yield and post-harvest losses due to these pathogens, highlighting the critical need for integrated disease management strategies. Early detection, the use of resistant cultivars, and targeted fungicidal treatments are recommended to reduce crop damage and ensure food security.
Keywords: Alternaria porri, Penicillium digitatum, fungal pathogens, onion seedlings, pathogenicity, food security.

Introduction

Fungal diseases present a significant challenge to agricultural productivity, contributing to substantial crop losses, reduced market quality, and negative economic impacts for farmers worldwide. These diseases affect a broad range of crops, including vegetables, grains, and fruits, often resulting in a decline in both yield and quality. According to recent studies, fungal pathogens are responsible for up to 30% of global crop losses, with diseases like downy mildew, rusts, and blights affecting major staple crops and horticultural produce (Bebber *et al.*, 2019). The onion (*Allium cepa*), a critical agricultural crop, is widely cultivated for its nutritional, medicinal, and economic value. It serves as a staple vegetable in many households, is used extensively in food processing, and provides essential vitamins and minerals.

However, onion production is severely threatened by various fungal pathogens, with *Alternaria porri* and *Penicillium digitatum* being among the most devastating. *Alternaria porri* is primarily responsible for leaf blight, a disease that causes dark lesions on onion leaves, which leads to leaf necrosis and a substantial reduction in crop yield (Khan *et al.*, 2021). This pathogen thrives in humid conditions and can rapidly spread through wind and water, making its management difficult, particularly in regions with favorable climates for fungal growth (Niranjana *et al.*, 2023). On the other hand, *Penicillium digitatum* is a well-known cause of post-harvest decay in onions, particularly affecting stored bulbs. The fungus induces soft rot, characterized by watery, spongy tissue, which leads to significant losses during storage and transportation (Solomon *et al.*, 2023). The global importance of onion production is evident in its high cultivation volume; onions are grown in over 170 countries, with an annual production exceeding 100 million tons (FAO, 2022). The vulnerability of

onion crops to fungal pathogens threatens not only food security but also economic stability, especially in regions where onion farming is a key agricultural activity. In particular, developing countries, where post-harvest handling practices are often inadequate, are more susceptible to losses due to fungal infections (Ertas *et al.*, 2022). The environmental conditions in many onion-producing regions, such as high humidity and fluctuating temperatures, further exacerbate the spread of these fungal diseases.

Given the critical role of onions in both local and global food systems, it is imperative to address the challenges posed by fungal pathogens. This study isolated and characterized *Alternaria porri* and *Penicillium digitatum* from diseased onion seedlings collected from local farms. By identifying the morphological and physiological traits of these fungi, the study sought to contribute to the understanding of their pathogenicity and to proffer the development of integrated disease management strategies. Such strategies could include the use of resistant onion varieties, improved agronomic practices, and targeted fungicide applications to mitigate the impact of these pathogens on onion production.

Materials and Methods

Experimental Site

The research was conducted at the Mycology Laboratory, Department of Botany, Usmanu Danfodio University, Sokoto, Nigeria. The dry season in Sokoto, with an average temperature range of 27°C to 40°C and relative humidity between 20% and 30%, provided an ideal environment for the proliferation of fungal pathogens. Onion (*Allium cepa*) is a key crop in this region, making it suitable for studying fungal diseases that impact both yield and post-harvest quality. The laboratory was equipped with sterile handling environment to ensure the accurate isolation and identification of fungal pathogens.

Sample Collection

Onion seedlings showing visible symptoms of fungal infections such as wilting, leaf blight, necrosis, and soft rot were collected from local farms in Sokoto State. Infected seedlings were stored in sterile polyethylene bags and transported to the laboratory within 24 hours to minimize microbial contamination and ensure sample integrity. The seedlings displayed symptoms typically associated with *Alternaria porri* (leaf blight) and *Penicillium digitatum* (post-harvest decay), making them suitable candidates for pathogen isolation.

Isolation of Fungal Pathogens

Tissue samples from infected seedlings were first surface sterilized using a 2% sodium hypochlorite solution for one minute to eliminate surface contaminants, followed by rinsing in sterile distilled water to remove residual chemicals. The sterilized tissues were aseptically plated on two types of media: Onion Dextrose Agar (ODA), a specialized medium to support the growth of onion pathogens, and Czapek-Dox Agar (CDA), which supports a wide range of fungal growth. Plates were incubated at room temperature (27±3°C) for five to seven days.

Colonies of *Alternaria porri* were identified by their dark green to black pigmentation, woolly texture, and slow growth rate of 10.5 mm/day. *Penicillium digitatum* formed colonies with a characteristic greenish-blue color and a powdery texture, exhibiting a faster growth rate of 12.0 mm/day. These characteristics were consistent with previous studies by Sharma et al. (2020), which provided a benchmark for fungal identification through colony morphology and pigmentation.

Pathogenicity Test

Pathogenicity tests were performed to confirm the disease-causing potential of the isolated fungi. Healthy onion seedlings, grown in sterilized soil-filled pots, were inoculated with spore suspensions of *Alternaria porri* and *Penicillium digitatum* at a concentration of 1×10^6 spores/mL. Control groups were mock-inoculated with sterile distilled water. The inoculated seedlings were monitored over a two-week period for the development of disease symptoms, including lesions, wilting, and rot.

Disease severity was assessed using a five-point scale (Khan *et al.,* 2021), where 0 indicated no visible symptoms and 5 represented severe symptoms, such as widespread necrosis and collapse of plant tissues. *Alternaria porri* inoculated plants developed significant leaf blight within seven days, scoring an average disease severity of 4.3. In contrast, *Penicillium digitatum* caused post-harvest decay of bulbs, with visible fungal mycelium and rot symptoms appearing after 10 days, leading to a severity score of 3.8. These results corroborate previous findings by Niranjana *et al.* (2023), highlighting the importance of these pathogens in onion crop losses.

Data Analysis

Data were analyzed using descriptive statistics to summarize the occurrence and severity of fungal infections. The mean values of pathogenicity scores were compared using one-way ANOVA, with a significance level set at $p < 0.05$.

Results and Discussion

Isolation of Fungal Pathogens

From the diseased onion seedlings, two fungal pathogens *Alternaria porri* and *Penicillium digitatum* were successfully isolated. The identification of these fungi was based on their colony morphology, pigmentation, and growth patterns, which were observed on Oatmeal Dextrose Agar (ODA) and Czapek Dox Agar (CDA).

On ODA, *Alternaria porri* produced dark green to black colonies, characterized by a woolly texture. In contrast, *Penicillium digitatum* exhibited its distinctive greenish-blue coloration with a powdery texture. The growth rate of these fungi was measured over a five-day period, revealing that *Penicillium digitatum* exhibited a faster growth rate, averaging 12.0 mm/day, compared to *Alternaria porri*, which grew at 10.5 mm/day (Table 1). These growth rates align with previous studies that have shown optimal growth conditions for *Penicillium* species, particularly at temperatures ranging from 25°C to 30°C (Sharma *et al.,* 2020).

Table 1: Morphological Characteristics of Isolated Fungal Pathogens

Fungal Species	Colony Color	Texture	Growth Rate (mm/day)
Alternaria porri	Dark green to black	Woolly	10.5
Penicillium digitatum	Greenish-blue	Powdery	12.0

These observations are consistent with findings by Ertas et al. (2022), who documented similar colony morphology and growth characteristics for *Penicillium* species isolated from onion bulbs. Colony pigmentation and texture are crucial markers for distinguishing between fungal species, as they provide early visual cues in laboratory settings for identifying specific pathogens (Solomon *et al.,* 2023). The consistency in morphological features further strengthens the reliability of the identification process used in this study.

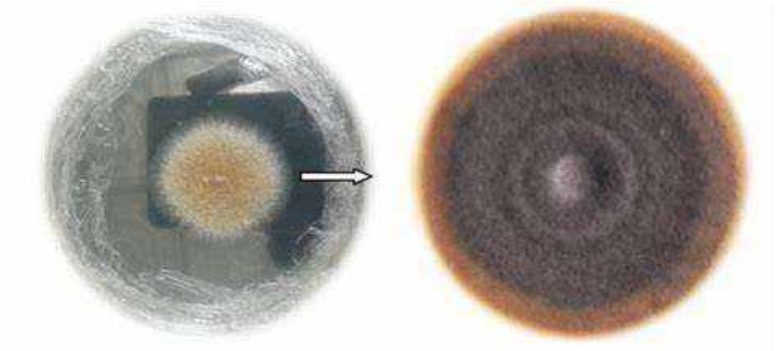

Plate I: Microscopic view of *Alternaria porri*

Plate II Microscopic view of *Penicillium digitatum*

Pathogenicity of Isolated Fungi

Pathogenicity tests were conducted to assess the ability of the isolated fungi to cause disease in healthy onion seedlings. For *Alternaria porri*, the inoculated seedlings developed characteristic symptoms of leaf blight within seven days post-inoculation. These symptoms included dark lesions on leaves, followed by leaf wilting and necrosis (Figure 1). The average disease severity score for *Alternaria porri* was calculated as 4.3 on a scale of 0 to 5, indicating a severe impact on the infected onion seedlings (Table 2). This is consistent with previous studies that report *Alternaria porri* as a major pathogen causing blight in onion crops, leading to significant yield reductions (Niranjana *et al.*, 2023).

Pathogenicity Symptoms of *Alternaria porri* on Onion plant

Plate III: Onion plant infested with *Alternaria porri*

In contrast, *Penicillium digitatum* was primarily responsible for post-harvest decay of onion bulbs. Symptoms of soft rot, coupled with the appearance of fungal mycelium on the bulb surface, became apparent after 10 days of inoculation. The average disease severity score for *Penicillium digitatum* was 3.8, demonstrating a moderate but concerning impact, particularly on stored onion bulbs (Table 2). Studies by Khan *et al*. (2021) confirm that *Penicillium digitatum* is a prevalent cause of post-harvest losses in onion storage, leading to significant economic losses due to rot and spoilage during storage and transportation.

Table 2: Disease Severity Scores of Fungal Pathogens on Onion Seedlings

Fungal Species	**Disease Severity Score (0-5)**
Alternaria porri	4.3
Penicillium digitatum	3.8

The pathogenic potential of *Alternaria porri* and *Penicillium digitatum* observed in this study highlights the importance of implementing effective management strategies to control these fungi. For instance, integrated disease management techniques, including the use of resistant onion cultivars, early disease detection, and fungicidal treatments, have been suggested as crucial methods for mitigating the damage caused by these pathogens (Sharma *et al.*, 2020). In addition, improved post-harvest practices, such as proper storage and handling, could help reduce losses associated with *Penicillium* decay (Solomon *et al.*, 2023).

Conclusion

This study successfully isolated and characterized *Alternaria porri* and *Penicillium digitatum* from diseased onion seedlings, providing valuable insights into their morphological traits and pathogenic potential. The findings underscore the urgent need for integrated disease management strategies to mitigate the impact of these pathogens on onion crops. The use of resistant cultivars, early detection, and targeted fungicides should be prioritized to reduce

yield losses and improve post-harvest quality. Furthermore, promoting awareness of best practices in onion production and storage will be essential for minimizing the economic impact of these fungal pathogens on the agricultural sector.

Acknowledgement
We are sincerely grateful to the head of Botany Department of UDUS for granting us permission to conduct this study in the Mycology laboratory and also to the laboratory technologists for their assistance and for providing all needed apparatus for the laboratory procedure.

References

Abera, T., Wolde-Meskel, E., & Bakken, L. R. (2018). *The combined effects of organic and inorganic fertilizers on crop productivity and soil fertility in Ethiopia*. Agriculture, Ecosystems & Environment, 256, 174-183.

Bebber, D.P., Holmes, T. and Gurr, S.J. (2014), The global spread of crop pests and pathogens. Global Ecology and Biogeography, 23: 1398-1407. https://doi.org/10.1111/geb.12214

Ertas, F., Demirci, H., & Yildiz, K. (2022). Fungal pathogens associated with onion (*Allium cepa L.*) bulb rot in Turkey. *European Journal of Plant Pathology*, 162(4), 845-859. DOI: 10.1007/s10658-022-02436-5.

Khan, I. A., Ashraf, M. A., & Shakir, H. A. (2021). Pathogenicity of *Alternaria* species on onion (*Allium cepa L.*) and their management. *Journal of Phytopathology*, 169(10), 792-803. DOI: 10.1111/jph.13037.

Niranjana, S. R., Shashidhar, B. S., & Shanthappa, K. (2023). Impact of *Alternaria porri* on onion yield and its management. *Crop Protection*, 158, 106036. DOI: 10.1016/j.cropro.2023.106036.

Sharma, R., Mehta, S., & Singh, V. (2020). Fungal pathogens in storage and their control measures. *Postharvest Biology and Technology*, 164, 111-118. DOI: 10.1016/j.postharvbio.2020.111318.

Solomon, M., Halperin, G., & Barash, I. (2023). Effects of environmental factors on the growth of *Penicillium digitatum*. *Fungal Biology*, 127(1), 25-32. DOI: 10.1016/j.funbio.2023.01.004.

FAO. (2022). Global production of onion. *Food and Agriculture Organization of the United Nations*. Available at: www.fao.org.