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Survey and Identification of Fungi Associated with *Eucalyptus camaldulensis* in Modibbo Adama Universiy, Yola

Authors: Audu Sanusi Kiri[1]*, Ahmad Idi[2],Yakubu Saifullah Mohammed[1] and Onuche Victor Ikani[1]

[1]Department of Plant Science, Faculty of Life Sciences, Modibbo Adama University (MAU), Yola, Nigeria

[2]Department of Biotechnology, Faculty of Life Sciences, Modibbo Adama University (MAU), Yola, Nigeria

Corresponding Author: sanusikiri15@gmail.com

ABSTRACT

Eucalyptus species are valuable for timber, pulp, and essential oil production. A laboratory study was conducted which shows the percentage frequency of the fungal isolates in Modibbo Adama University and was calculated based on the total number of isolates obtained from the four different locations. The results showed varying levels of abundance for the four identified fungal species: *Rhizopus nigricans* was isolated in 2 out of 14 samples, resulting in a percentage frequency of 14.2%. *Aspergillus flavus* had the second-highest occurrence, being isolated in 5 out of 14 samples, with a percentage frequency of 35%. *Penicillium notatum* was the least frequent, isolated in just 1 out of 14 samples, giving a percentage frequency of 7.1%. *Aspergillus brasiliensis* was the most abundant species, isolated in 6 out of 14 samples, accounting for 42.8% of the total isolates. The results of this study highlight the prevalence of fungal pathogens associated with *Eucalyptus camaldulensis* in the Modibbo Adama University area. *Aspergillus brasiliensis* and *Aspergillus flavus* were the most commonly found fungi, indicating that these species may play a significant role in the degradation of Eucalyptus leaves and other tissues. *Rhizopus nigicans* and *Penicillium notatum* were less frequent but still present, suggesting their contribution to the overall fungal biodiversity within the study area. This research demonstrates the need for further investigation into the pathogenicity of these fungi, their potential impact on Eucalyptus trees, and the development of management strategies to mitigate fungal infections.

Keywords: Survey, Identication, Fungi, *Eucalyptus camaldulensis.*

INTRODUCTION

Eucalyptus trees, native to Australia but widely cultivated in countries like Nigeria, are valuable for their rapid growth and diverse economic uses, including timber, pulp, and essential oils, which have antimicrobial properties (Smith and Read, 2019). In Nigeria, these trees play a key role in afforestation, combating desertification, and improving soil stability, making them important for environmental conservation and local economies (Cordeiro *et al.*, 2020; Crisp *et al.*, 2020;). However, Eucalyptus plantations in Nigeria face significant challenges from fungal pathogens. Common diseases, such as leaf spot, stem canker, and root rot, severely damage the trees, leading to reduced growth, lower timber quality, and even tree death. These fungal infections diminish the trees' photosynthetic ability, impair nutrient transport, and weaken their structural integrity, resulting in substantial economic losses (Pautasso *et al.*, 2015). Effective management strategies, including integrated pest management, are crucial to mitigate the impact of these diseases (Babtola and Abubakar 2011).

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Fungi, an important group of organisms, can be beneficial or harmful to plants. Some fungi, like mycorrhizal species, form symbiotic relationships with plants, improving nutrient and water uptake, enhancing growth, and increasing resistance to environmental stresses (Smith and Read, 2019). Other fungi live inside plant tissues as endophytes, promoting plant growth and protecting against pathogens (Pautasso *et al*., 2015). On the harmful side, pathogenic fungi can cause significant damage to plants. For instance, *Phytophthora* species lead to root rot, causing plant decline, while *Ceratocystis* species cause stem cankers, weakening the plant and reducing productivity. Fungi like *Corynespora* and *Mycosphaerella* cause leaf spots, which can reduce photosynthesis and harm plant health (Wingfield*et al*., 2009; Roux and Wingfield, 2009; Plett and Martin, 2011).

Understanding the interactions between fungi and Eucalyptus trees is critical for managing plantations and ensuring their sustainability. Fungi play essential roles in ecosystems, such as nutrient cycling, and can impact both plant health and growth, making their study crucial for improving the management and productivity of Eucalyptus plantations in Nigeria and elsewhere (Smith and Read, 2020; Brundrett, 2021).

MATERIALS AND METHODS

Study Area

This research was conducted at Modibbo Adama University, Yola. Adamawa State, Nigeria, located at coordinates 9.349883, 12.500545, near Girei. Yola, situated in the northeastern part of Adamawa State, experiences a tropical savannah climate, with clear distinctions between the wet and dry seasons.

Source of Sample

Samples for this study were collected from four different Eucalyptus plantation locations within the University. The selected species for sampling was *Eucalyptus camaldulensis*, which is commonly planted in the region. The criteria for selecting the sample trees included their health status and specific location within the plantation, as outlined by Smith *et al.* (2020).

Sample Collection Procedures

Leaf samples were collected from *Eucalyptus* trees showing symptoms of disease to assess fungal diversity. The samples were gathered from four locations within the University viz: the Admin Block, Landscape Unit, Primary School Gate, and Kabiru Umar Male Hostel. Symptomatic leaves were carefully collected, placed in sterile containers, and conveyed to the Plant Science Department laboratory for fungal isolation.

Media Preparation

The media was prepared following the manufacturer's guidelines. Twenty grams of Potato Dextrose Agar (PDA) was dissolved in 500 mL of sterile distilled water, then autoclaved at 121°C for 15 minutes under 15 psi pressure. After cooling, 250 mg of broad-spectrum antibiotic Chloramphenicol was added to the molten PDA, and the mixture was gently shaken to ensure uniform distribution. The prepared media was then poured into 9 cm diameter Petri dishes for use.

Sample Preparation

The modified method of Carnegie and Peg (2020) was used for sample preparation. The samples were first washed under running water to remove debris, then surface sterilized with a sodium hypochlorite solution for 30 seconds, followed by rinsing in three changes of sterile distilled water. After sterilization, the

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samples were placed on the prepared media for further processing.

Isolation and Identification

A portion of the samples was carefully transferred onto the surface of the prepared media using sterilize forceps and incubated at 25-28°C for 5 days to facilitate fungal growth.

Sub-Culturing

Using a sterile inoculating loop, a small portion of fungal hyphae from the edge of the growing colony on the original culture plate was transferred to the center of a fresh PDA plate. The inoculated plates were sealed with adhesive tape, placed upside down to maintain humidity, and incubated at 25°C for 2 days. The plates were checked daily for fungal growth. After the incubation period, each plate was examined, revealing the growth of a single fungal colony.

Microscopic Examination

After obtaining a pure culture, a slide mount was prepared by first dipping the slide in ethanol to avoid contamination. Using a sterile inoculating loop, a small portion of fungal hyphae or spores from the edge of the growing colony was transferred to the center of the slide. The sample was then stained with lactophenol cotton blue, and its structural features were examined under a microscope to confirm the identity of the isolated organism.

Identification of Fungi

The microscopic features observed were compared with those in an identification atlas to identify the specific type of fungus isolated.

Calculation of Percentage Frequency

The method of Sharma *et al.* (2022) was used to calculate the percentage frequency of occurrence of the isolate. This was done by counting the total number of collected samples and the number of samples containing the specific fungal isolate, then multiplying by 100.

$$\text{Percentage Frequency} = \frac{(\text{Total number of samples})}{\text{Number of occurrences of the isolate}} \text{x } 100$$

RESULTS

Fungi were identified based on colony appearance, morphology, and cellular characteristics (Table 1). The percentage frequency of the fungal isolates was calculated from samples collected across four locations. The results revealed varying abundance levels for the four identified species (Table 2). *Rhizopus nigricans* (plate I) was isolated in 2 out of 14 samples, with a frequency of 14.2%. *Aspergillus flavus* (IV) was found in 5 out of 14 samples, accounting for 35%. *Penicillium notatum* (III) was the least frequent, isolated in 1 out of 14 samples (7.1%). *Aspergillus brasiliensis* (plate II) was the most abundant, isolated in 6 out of 14 samples (42.8%). Overall, *Aspergillus brasiliensis* and *Aspergillus flavus* (plate II) were the dominant species, while *Rhizopus nigricans* and *Penicillium notatum* had lower frequencies.

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Table 1: Morphological Characteristics of the Fungal Isolates.

Isolate Code	Macroscopy	Microscopy	Probable Identity
Kabiru umar	White fluffy mycelia mat with black sporangia on futher incubation	Non septet mycelia with sporangia laden with spores	*Rhizopus nigricans*
Admin block	Greenish conidia with deep brown seclerotia	Branched and septet hyphae colourless conidiospores with thick mycelial mat globose conidia and thick walled conidiophore	*Aspergilus flavus*
Mau Landscape	White colonies which becomes blue green to yellow on further incubation	Branched conidiospores produced from tip of philiades	*Penicillium notatum*
Primary school gate	Green fluffy mycelia mat with black sporangia	Spores radiating from hyphal end	*Aspergilus brasiliensis*


Plate I: *Rhizopus nigricans*

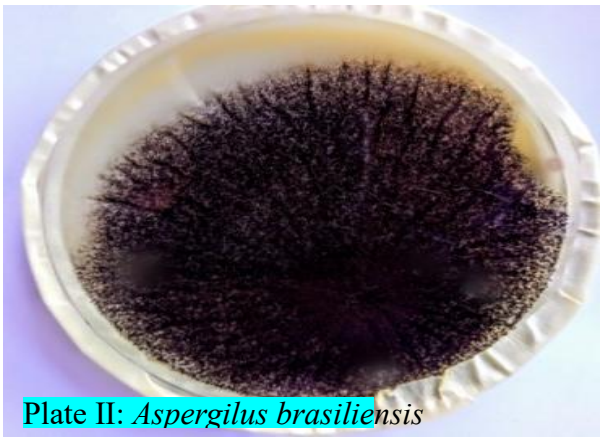
Plate II: *Aspergilus brasiliensis*


Plate III: *Penicillium notatum*


Plate IV: *Aspergillus flavus*

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Table 2: Distribution of fungi isolates based on sample location.

Isolate	Kabiru umar hostel	Admin block	Land scape	Primary school gate	Total
Rhizopus nigricans	01	_	_	01	02
Aspergilus flavus	02	01	02	_	05
Penecillium spp	_	01	_	_	01
Aspergillus brasiliensis	02	01	01	02	06
Total	05	03	03	03	14

Table 3: Determination of percentage frequency of occurrence.

Fungi	Number Isolated	Frequency
Rhizopus nigricans	02	14.3%
Aspergilus flavus	05	35.7%
Penicillium notatum	01	7.1%
Braciliensis	06	42.9%
Total	14	100%

DISCUSSION

The study on fungal species associated with *Eucalyptus camaldulensis* in the area revealed notable diversity and dominance. *Aspergillus brasiliensis* was the most prevalent, with a frequency of 42.9%, indicating its strong adaptation to the local environment. This aligns with findings by Smith *et al*. (2020), which emphasized the resilience of *Aspergillus* species in tropical and subtropical climates. Similarly, *Aspergillus flavus*, at 35.7%, was also widely present and is of concern due to its potential to produce aflatoxins, posing risks to agriculture and ecology. Its widespread occurrence suggests it plays a significant role in the fungal community of Eucalyptus trees in savannah vegetation (Glen *et al*., 2021). The study also aligns with findings from several studies that identify *Aspergillus* species as dominant fungi in warm, humid environments. *A. flavus* is commonly found in soil, air, and decomposing organic materials, and is known for producing aflatoxins, which pose significant risks to human and animal health. Studies in tropical and subtropical regions have consistently reported *A. flavus* as one of the most frequently isolated species in environmental samples (Frisvad *et al*., 2011; Klich, 2007). Similarly, *Aspergillus brasiliensis*, a species within the *A. niger* group, is commonly detected in air and food environments. Houbraken et al. (2020) found it to be widespread in both clinical and environmental samples.

In contrast, *Rhizopus nigricans* was less common, with a frequency of 14.3%, yet its presence in areas with decaying organic matter, like Kabiru Umar Hostel and Primary School Gate, suggests it thrives in such conditions (Barker *et al*., 2021). Finally, *Penicillium notatum*, with the lowest frequency of 7.1%,

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was found only in the Admin Block. This may point to specific local environmental factors such as humidity or temperature, which supported its growth (Gladieux*et al*., 2020). The low frequency of *Penicillium* suggests it faces competition from more dominant species like *Aspergillus brasiliensis* in most locations. This species is typically found in decaying organic matter and is commonly isolated from food items like bread and fruits. Richardson (2009) notes that while *Rhizopus* species are widespread in soil and decaying vegetation, they are generally less abundant in air samples compared to *Aspergillus* and *Penicillium* species. *Penicillium notatum* was the least frequently isolated species (7.1%), which is consistent with other reports. While *Penicillium* species are commonly found in various environments, their distribution is influenced by factors such as temperature, humidity, and available substrates. Samson et al. (2010) highlighted that while *Penicillium* species are frequently found in indoor environments, species like *P. notatum* are less commonly isolated compared to others like *P. chrysogenum*.

CONCLUSION

In summary, the percentage frequency analysis revealed that Aspergillus brasiliensis and Aspergillus flavus were the most prevalent fungi isolated from Eucalyptus trees, demonstrating their adaptability and important role in the fungal community at Modibbo Adama University. The lower occurrence of Rhizopus nigricans and Penicillium notatum suggests these species may have more specific environmental needs or face greater competition from other fungi. Overall, the findings highlight the fungal diversity associated with Eucalyptus camaldulensis and the ecological importance of fungal colonization in the area. Future research could examine the impact of these fungi on tree health, their ecological interactions, and the development of strategies to manage fungal infections.

We therefore recommend Based on the findings of this study that**,** Additional studies should focus on the pathogenic nature of the isolated fungi, particularly *Aspergillus brasiliensis* and *Aspergillus flavus*, which were the most frequently isolated fungi. Investigating their specific roles in causing diseases in Eucalyptus trees will provide better insights into effective management strategies. Implementing preventive measures such as regular monitoring and the use of fungicides could help reduce the impact of fungal infections on Eucalyptus plantations, especially

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