ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH LEAF BLIGHT DISEASE OF MISTLETOE (*TAPINANTHUS* SP.)

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Abstract: Mistletoes (*Tapinanthus* sp.) are hemi-parasitic flowering plants that derive water and minerals from their host plants, often resulting in significant physiological impacts. This study investigated the fungal species associated with leaf blight in mistletoe leaves. Symptomatic and asymptomatic leaves were collected from *Terminalia catappa* host trees at Ahmadu Bello University, Zaria, Nigeria, and analyzed in the Mycology Laboratory, Department of Botany, Ahmadu Bello University, Zaria, using 39g of Potato Dextrose Agar (PDA) prepared in 1000 ml of distilled water. Six fungal species were isolated and identified: *Aspergillus* sp. *Gloeosporium* sp. *Rhizoctonia* sp. *Cunninghamella* sp. *Sclerotium* sp. and Phoma sp. Among these, *Aspergillus* sp. was the most frequently occurring species, appearing in 33.3% of symptomatic leaves and 55.5% of asymptomatic leaves. This study highlights the diversity of fungi associated with mistletoe leaf blight and underscores their potential ecological and pathological significance.

Keywords: Fungal pathogens, Isolation, Identification, leaf blight, mistletoe, parasitic plant

Introduction

Mistletoes (*Tapinanthus* sp.) are hemi-parasitic plants (Moreira, *et al*., 2019) commonly found in tropical and subtropical regions which belongs to the class Magnoliopsida, subclass Rosidae, order Santales (Judd *et al*., 2002). They depend on their host plants for water and minerals, which often leads to salt imbalances and physiological stress in the host (Milius, 2000). The two major families of mistletoe are Loranthaceae and Viscaceae. Most genera of African mistletoe belong to the family Loranthaceae (Dambele *et al*., 1994). It is the largest family of mistletoe having up to 73 genera and over 900 species (Judd *et a*l., 2002). The six major genera of Loranthaceae found in Nigeria are Tapinanthus, Agelanthus, Loranthus, Globometula, Phragmenthera and Englerina, with Tapinanthus being far more widespread in the Nigerian savanna (Bako *et al*., 2001). Mistletoes are parasitic plants that divert water and use far more water per unit carbon fixed during photosynthesis than their hosts (Scalon and Wright, 2015). All mistletoes produce a morphologically diverse structures (haustoria) which allow them to interface with their host (Calvin and Wilson, 2006). Trees can respond to mistletoes infection by producing sclerophyllous, yet less productive leaves (Scalon, *et al*., 2017). According to studies conducted over the past few years, the intensity of mistletoe damage on trees in countries increases from year to year (Varga , *et al*., 2012).

Seeds of most mistletoe are spread by birds that eat the fruits (Cowles, 1964) or by wind. Because of their easy dispersal and widespread tropism, mistletoes have become serious pests for commercial fruit and timber plantations. Mistletoes have economic importance as medicinal plants (Szurpnicka, *et al*., 2020), but their parasitic nature poses challenges to forestry and agriculture (Mudgal *et al*., 2022). Boussium *et al*. (2004) reported that mistletoe (*T. globiferus*) parasitized 126 species. Infection by mistletoe leads to water and nutrient stress of the host plant and deteriorates its healthy establishment and survival. Infection by mistletoes also triggers host plant responses, varying from mechanical to chemical mechanisms which are analogous to herbivory defences, and negatively impacts host plant growth and reproduction. (Muche *et al*., 2022).

Approximately 40 % of moderate and high level infestation rates increase the possibility of tree mortality in the absence of management applications (Zúñiga *et al*., 2024).

In contrast, ecologists may see mistletoe as a friend, in light of the wildlife habitat, biodiversity and nutrient cycling it p romotes (Griebel *et al*., 2017).

Leaf blight is a major fungal disease that affects mistletoe, causing necrotic lesions and reducing the plant's photosynthetic capacity. Although mistletoes have been studied extensively from botanical and ecological perspectives, the fungal communities associated with leaf blight remain poorly understood (Mayer, 2006).

This study aims to address this knowledge gap by isolating and identifying fungi associated with leaf blight in *Tapinanthus* sp. The findings could have implications for biological control strategies and the management of fungal diseases in economically significant host plants.

Materials and Methods

Study Area

The study was conducted in the Mycology Laboratory of the Department of Botany, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Sample collection

Symptomatic and asymptomatic mistletoe leaves were collected from *Terminalia catappa* trees within the university campus. The collected leaves were transported to the laboratory in sterile polyethylene bags for further analysis.

Preparation of PDA and Fungal Isolation

Potato Dextrose Agar (PDA) was prepared by dissolving 39 g of PDA in 1,000 ml of distilled water and sterilized at 121°C for 15 minutes. Chloramphenicol (500 mg) was added to inhibit bacterial growth (Deep and Emmanuel, 2015)

Symptomatic leaf tissues were surface-sterilized with 1% sodium hypochlorite for 3 minutes and then rinsed in three changes of sterilized distilled water, and plated on PDA. Plates were incubated at room temperature (25–28°C) for seven days (Okigbo and Osuinde, 2003) and pure cultures were obtained through sub-culturing.

Identification of Fungal pathogen

Fungal identification was based on macroscopic (colony morphology) and microscopic (conidial, spore, and hyphal structures) features. Slides were prepared using lactophenol cotton blue stain and examined under a light microscope at 10× and 40× magnification. Identifications were confirmed using standard fungal manuals.

Results

Six fungal species were isolated from symptomatic and asymptomatic mistletoe leaves: *Aspergillus* sp. (Plate II and III) *Rhizoctonia* sp. (Plate IV), *Cunninghamella* sp. (Plate V), *Gloeosporium* sp. (Plate VI) *Sclerotium* sp. (Plate VII) and *Phoma* sp. (Plate VIII). The percentage occurrence of each species is presented in Figure 1 and 2. *Aspergillus* sp. was the most frequently occurring fungus, found in both symptomatic (33.3%) and asymptomatic leaves (55.5%).

Plate 1. A – Mistletoe leaf blight with light brown lesions becoming necrotic used for isolation
B. Asymptomatic mistletoe leaf from same plant

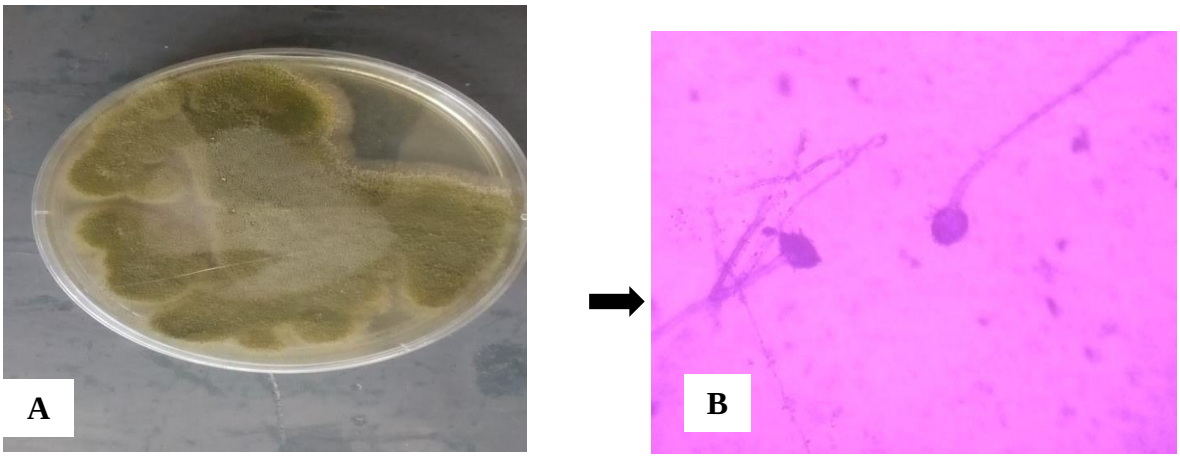

Plate II: Pure culture of *Aspergillus flavus*, A. Macroscopic view, B. Microscopic view of Aspergillus *flavus*

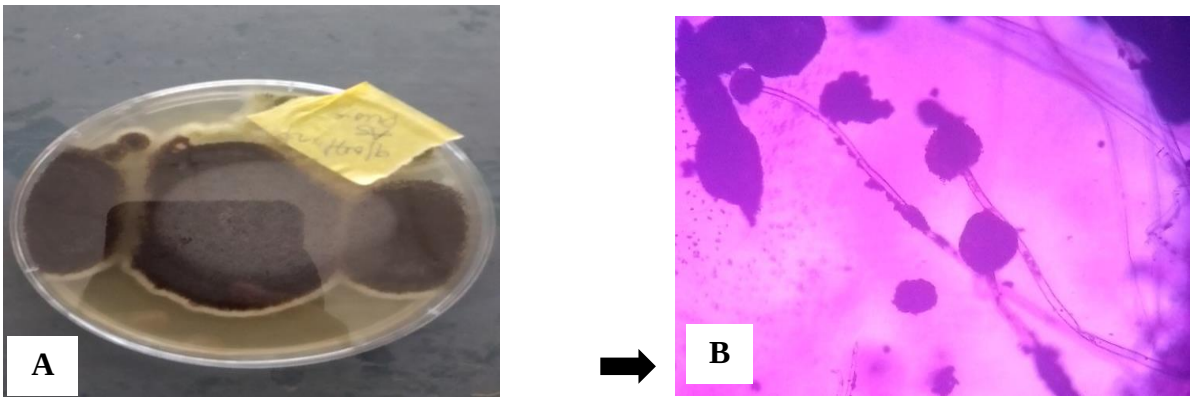

Plate III: Pure culture of *Aspergillus niger* A. Macroscopic view, B. Microscopic view of *Aspergillus niger*

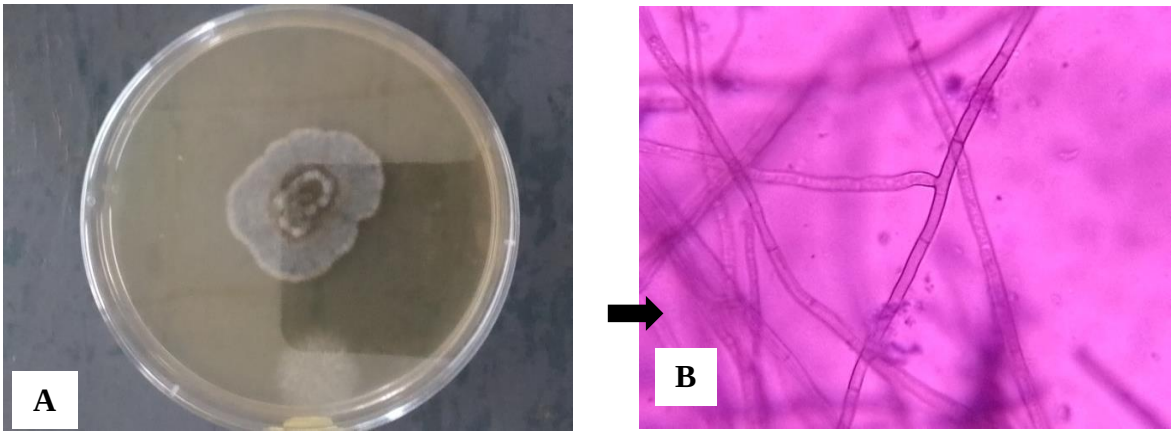

Plate IV: Pure culture of *Rhizoctonia* sp. A. Macroscopic view B. Microscopic view of Rhizoctonia sp.

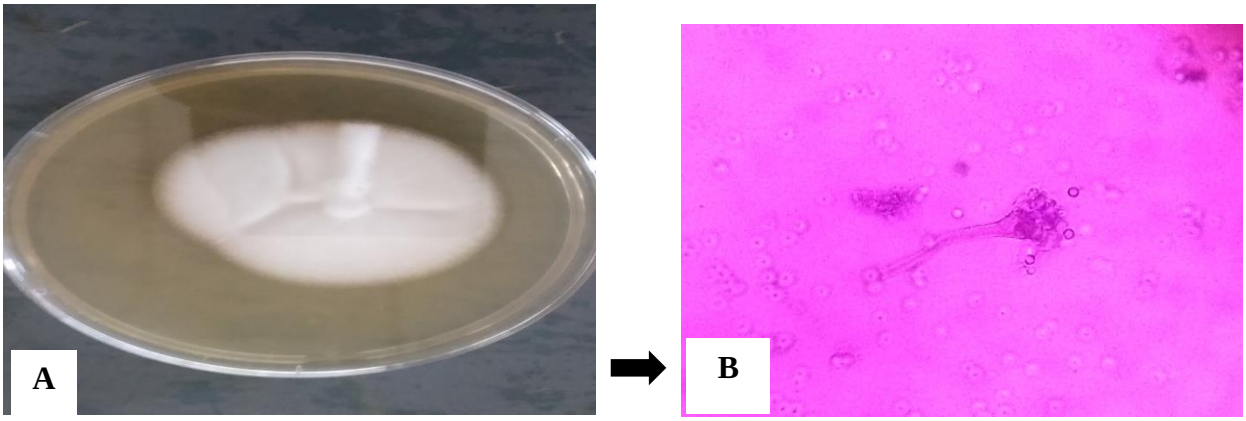

Plate V: Pure culture of *Cunninghamella* sp. A. Macroscopic view, B. Microscopic view of *Cunninghamella* sp.

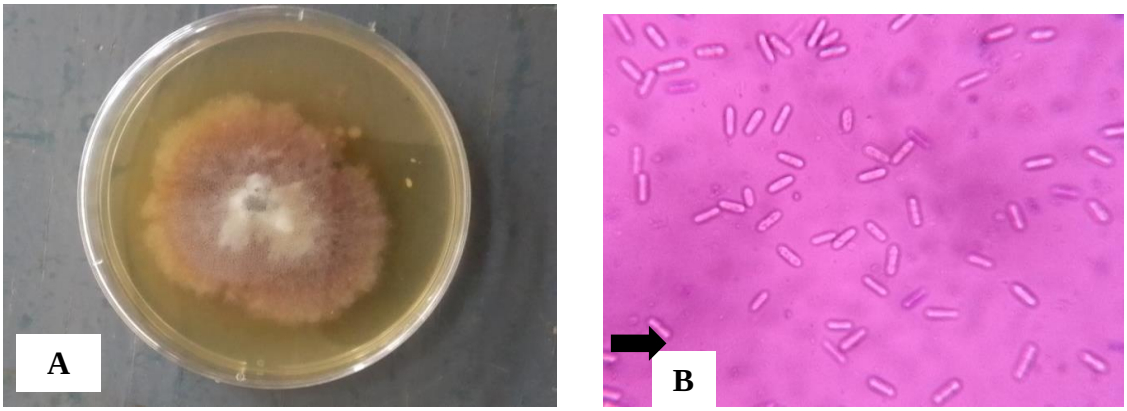

Plate VI: Pure culture of *Gloeosporium* sp. A. Macroscopic view B. Microscopic view of Gloeosporium sp.

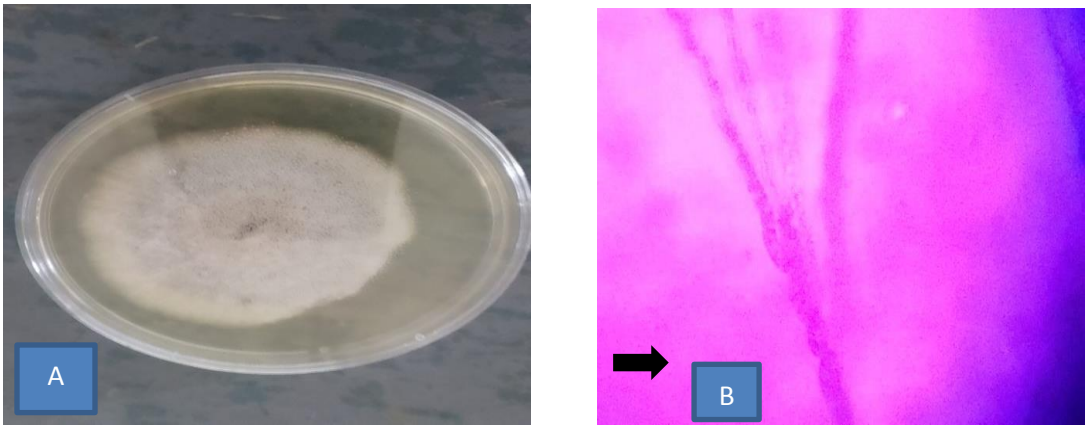

Plate VII: Pure culture of *Sclerotium* sp. A. Macroscopic view, B: Microscopic view of Sclerotium sp.

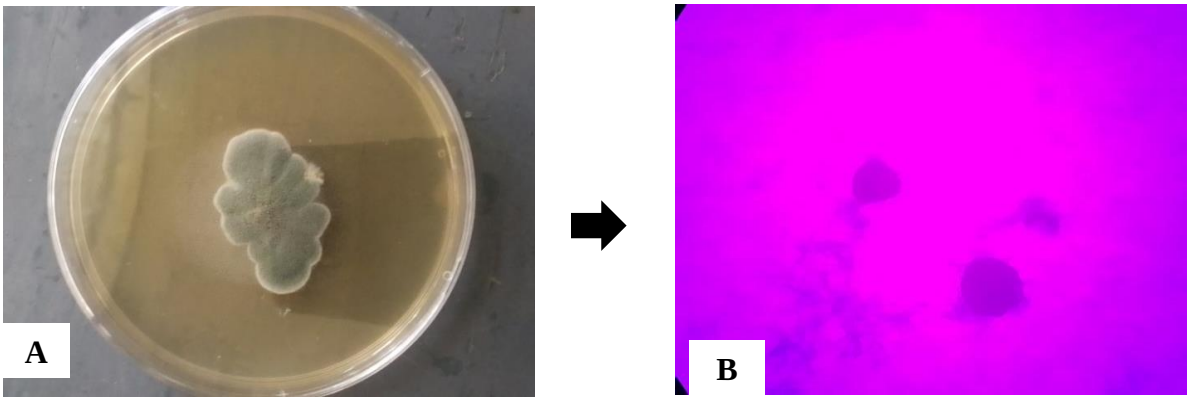

Plate VIII: Pure culture of *Phoma* sp. A. Macroscopic view b. Microscopic view of *Phoma* sp.

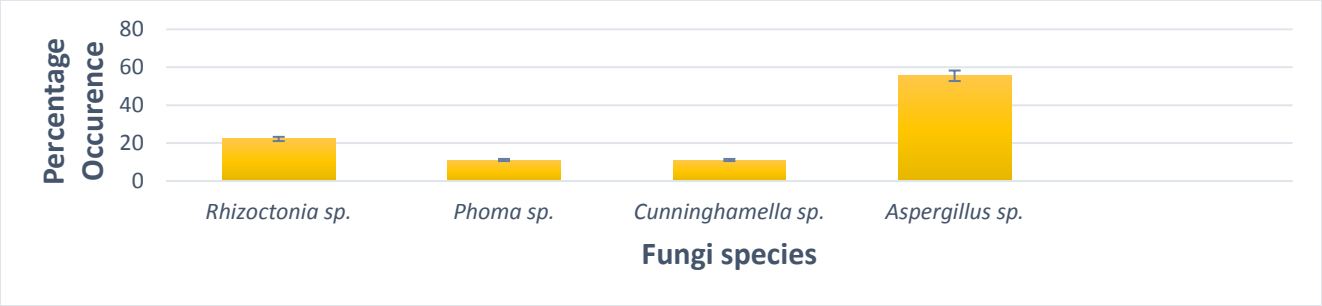

Fig 1: Percentage occurrence of Isolated Fungi Pathogens from symptomatic Mistletoe leave.

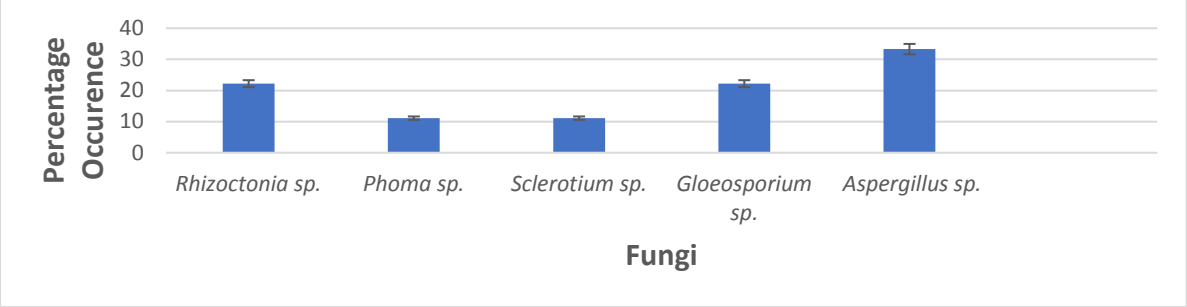

Fig 2: Percentage occurrence of Isolated Fungi Pathogens from asymptomatic mistletoe leave

Discussion

The study revealed that, nine fungi were isolated from infected leaves and nine from uninfected leaves which comprises 6 different fungi species, *Rhizoctonia* sp., *Cunninghamella* sp., *Phoma* sp., *Aspergillus* sp., *Sclerotium* sp. and *Gloeosporium* sp. Out of the 6 fungi species, *Aspergillus* sp. was found to be the most frequently encountered from both asymptomatic and symptomatic leaves consisting of 8 fungi isolates with 33.3% from symptomatic leaves and 55.5% from asymptomatic leaves. *Aspergillus* sp. is the predominant fungal pathogen associated with mistletoe leaf blight. *Aspergillus* sp. has been reported as one of the causal agent of blight on mistletoe leaves by (Kotan *et al*., 2012). *Aspergillus* is a genus of fungi that consists of several hundred species. Some which are significant human pathogens such as *A. flavus* which can cause aspergillosis. *A. niger* is also commonly known for producing black mold on fruits and vegetables (Samson *et al*., 2014). Latiffah, (2024) reported that the genus *Aspergillus* contains several species that are important plant pathogens. Pawłowicz *et al*., (2024) also reported four though different fungi as a saprotroph or parasite on the leaves and twigs of mistletoe in Poland. *Rhizoctonia* sp was also isolated in this study. Similarly, *Rhizoctonia solani* reported as a soil-borne fungal pathogen that causes disease in a wide range of plants worldwide (gonzalez *et al*., 2011). *Rhizoctonia solani* is an important plant pathogen that causes yield losses in legume crops worldwide, causing severe diseases such as blights, damping-off, and rots (Basbagci and Dolr, 2020). *Gloeosporium* sp isolated from this study was frequently isolated from blighted shoot and leaves of young plantation trees and nursery seedlings of *Eucalyptus nitens* and *E. globulus* in Australia (Yuan *et al*., 2000). Similarly, species of *Gloeosporium*, *G.* (*Colletotrichum*) *gloeosporioides*, was reported to cause anthracnose disease of vegetables and fruits, it lives saprophytically on crop residues, attacking the tissues at the crown and base of the stems of more developed plants (Cho *et al*., 2003). However, Schaible *et al*., (2015) isolated *Gloeosporium* sp as an endophyte of *Tsuga heterophylla* and that the volatile phase of this endophyte was active against a number of plant pathogenic fungi including *Phytophthora palmivora*, *Rhizoctonia solani*, *Ceratocystis ulmi*, *Botrytis cinerea*, and *Verticillium dahlia*. *Sclerotium* sp was one of the fungi isolated in this study. Similarly, Le et al., (2012) reported *Sclerotium rolfsii* as causative agent of white rot and one of the destructive pathogens of plants worldwide. *Phoma* sp isolated in this study has also been reported as a severe field pathogen of onion (Badillo-Vargas *et al*., 2008; Boerema *et al*., 2004)

The presence of fungi in asymptomatic leaves suggests that some maybe endophytic or latent pathogens. Other species, such as *Rhizoctonia* sp. and *Gloeosporium* sp. were also significant, causing necrotic lesions on leaves. These findings emphasize the need for further research on the ecological roles of these fungi.

Conclusion

The study isolated and identified *Aspergillus* sp. *Rhizoctonia* sp. *Sclerotium* sp. *Phoma* sp. *Gloeosporium*

sp. and *Cunninghamella* sp. from symptomatic and asymptomatic leaves from same mistletoe plants.

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