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EXPLORATION OF FUNGI ASSOCIATED WITH DIFFERENT PARTS OF *Pinus caribaea* Morelet (CARRIBEAN PINE) GROWN AS ORNAMENTALS IN ZARIA AREA, KADUNA STATE NIGERIA

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ABSTRACT

Pinus is a tree planted as an ornamental plant in Zaria Area. The aim of the research was to identify fungi from *Pinus* plant tissues grown as ornamental and rhizosphere in Zaria Area. Sampling was carried out at the Faculty of Veterinary Medicine, Area H Quarters, Botanical garden and Savanna Forestry Research Institute, Ahmadu Bello University, Zaria. Needles, stems, roots and rhizosphere soil were collected and analyzed respectively using standard mycological techniques. Ten genera of fungi were isolated with different percentage occurrences, *Rhizoctonia* sp had the highest percentage occurrence of 27.66% followed by *Fusarium* sp. 25.33%, *Aspergillus* sp. 23.40%, *Penicillium* sp. 10.64% while *Rhizopus* sp., *Pestalotia* sp., *Dothistroma* sp., *Aspergillus flavus*, *Fusarium solani* species complex, and *Microsphaeropsis ovaliceae* had the lowest percentage occurrence of 2.13% each. There was a diversity of fungi genera isolated from different parts of the plant *Rhizoctonia* sp. and *Fusarium* sp were isolated from all parts of the plant (needles, stem, root and rhizosphere soil), *Dothistroma* sp., *Aspergillus flavus*, *Fusarium solani* species complex, *Microsphaeropsis ovaliceae* were isolated from needles only, *Aspergillus* sp. was isolated from needles, stem and rhizosphere soil, *Penicillium* sp. was present in root and rhizosphere soil only, *Pestalotia* sp. and *Rhizopus* sp. were present in rhizosphere soil only.

Keywords: Pinus, Ornamental, Rhizosphere, Isolation, Identification, Fungal Diversity

INTRODUCTION

The pine tree is an evergreen, coniferous, tall tree which grows 3-80 m in height, Pine is the largest and the most durable of all conifers. Pines live for a long time usually between 50-1000 years, some species can live longer than that. pines are planted as ornamentals in parks and large garden. *Pinus* is a genus of approximately 111 living trees and shrubs (Gernandt *et al*., 2005). Vizcaino-Palomar *et al*. (2014) reported *Pinus* to be from the family Pinaceae which has about 120 species that can be found worldwide due to its ability to withstand any type of soil.

Pines are commercially grown and harvested for Christmas trees, needles of pine are used for making decorative articles e.g. baskets, trays, landscape design projects, as houseplants, cut flowers, and specimen displays, they are plants which can be grown indoors or outdoors (Daughtery and Benson, 2005). *Pinus* trees are widely distributed worldwide and are a source of potential medicinal compounds (Li *et al*., 2025) with various pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects. Pine woods exhibit good physical performance, and many studies have explored their primary applications as timber (Suri *et al*., 2022; Park *et al*., 2024). *Pinus* species are well known for their adaptability and ecological importance across temperate and tropical ecosystems. However, their survival and performance are deeply influenced by the fungi they host. Fungal associations with *Pinus* include beneficial symbionts, as well as pathogenic species responsible for diseases like root rot and needle blight. Exploring these fungal relationships is essential for understanding *Pinus* ecology, improving plantation management, and developing strategies for disease control.

MATERIALS AND METHODS

Study Area

The research was conducted in Zaria which is located between latitude 11º 11ˈN and longitude 007º 73ˈE in the Northern Guinea Savanna zone of Nigeria. The area is characterized by a distinct wet and dry season with an elevation of 613 m above sea level. Zaria has an average temperature, range of 15.3 ℃ - 36.25 ℃ and an average rainfall of 1050 mm annually.

Sample Collection

Sampling was carried out from three different locations in Zaria Area as shown in figure 1. The needles and stems showing disease symptoms as well as roots and rhizosphere soil were collected and taken to the mycology laboratory of the Department of Botany Ahmadu Bello University Zaria for further analysis. A total number of 15 samples were collected.

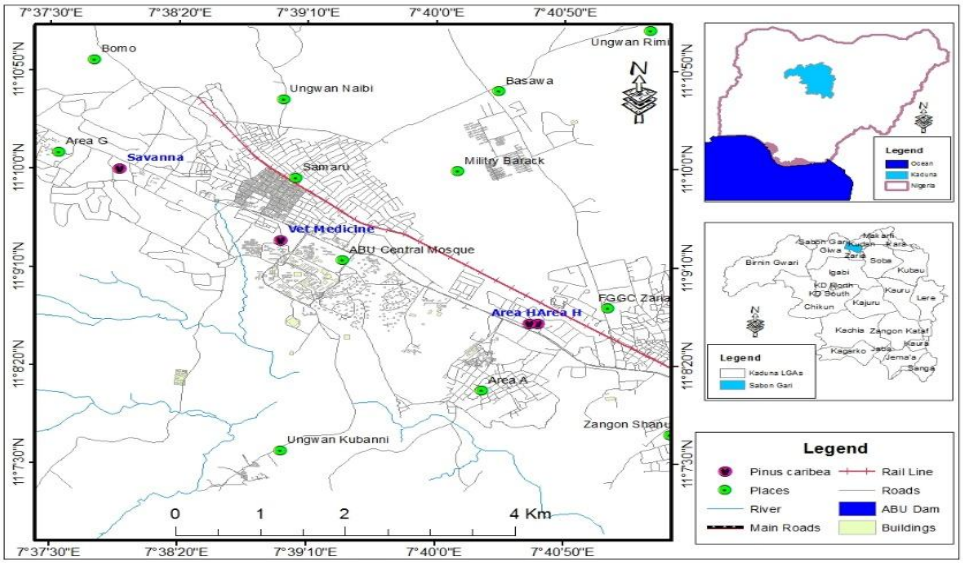


Figure 1: Map of Study Area Showing Locations of *Pinus caribaea* in Zaria Area
Source: GIS Lab Department of Geography ABU Zaria Using ArcGIS 10.3 Software

Isolation of Fungi

Media Preparation

Potato Dextrose Agar and filtered ground Pine Needle Agar were used for the growth of fungi where 39 g of Potato Dextrose Agar was dissolved in 1 L of sterile distilled water, the mixture was heated until a homogeneous mixture was obtained, and the mixture was autoclaved before pouring into a petri dish and allowed to solidify. Plant tissues were cut into small segments, surfaced sterilized using sodium hypochlorite for 3 minutes and rinsed in 3 changes of sterile distilled water. Sterilized tissues were placed on solidified media, rhizosphere soil was serially diluted, streaked on media and incubated at 27-33℃ for 3-7 days. (standard mycological techniques were used for isolation of fungi). Isolation was carried out after 7 days. Filtered ground pine needle agar. Green needles of pine were ground into fine powder. 80 g of fine powder was added to 1L of distilled water and boiled on a magnetic stirrer for 10 mins. The mixture was filtered through Whatman number 1 filter paper. Twenty (20) g of agar was added to solidify the solution. Isolation from diseased plant samples to confirm the presence of fungi was carried out. Plant samples were chopped up using a flame-sterilized scalpel. The samples were surface-sterilized in 1 % sodium hypochlorite for 3 minutes and rinsed properly with sterile distilled water in 3 changes for 3 minutes (each rinse was after 1 minute). The sterilized samples were plated on Pine needle agar each sample was duplicated, for soil samples serial dilution was done before it was streaked on the culture media. The Petri dishes were incubated at room temperature and were observed for a period of three to seven (3-7) days for fungi colonies. The Petri dishes with mixed colonies were sub-cultured to get pure cultures which were used for identification (Schulz *et al*., 1993; Bonello and Blodgett, 2003; Rao *et al*., 2004).

Data Analysis

Descriptive statistics was used to calculate the percentage occurrence of fungi in the sampling areas,

$$\text{Percentage occurrence} = \frac{\text{frequency of occurrence of fungi}}{\text{Total number of fungi}} \times 100$$

RESULTS AND DISCUSSION

Eight (8) different symptoms were observed on *P. caribaea*. four (4) different symptoms were observed on needles and necrotic needle tip, 1 symptom each on stem, branch, root and soil.

Necrotic spot and short needles were observed in vet medicine, stem, branch with pinkish fruiting body, soil and root were observed in Area H, the most prevalent symptoms were Needle blight and Needle spot as the symptom were observed at Vet. medicine and Savanna as shown in Table 1. Different fungi were isolated of which *Rhizoctonia* sp. (27.66%) was the highest isolated, *Fusarium* sp. (25.53%), *Aspergillus* sp. (23.40%), *Penicillium* sp. (10.64%), *Aspergillus flavus*, *Dothistroma* sp., *Fusarium solani species complex*, *microsphaeropsis ovalicea*, *Pestalotia* sp., and *Rhizopus* sp. (2.13%) were the least isolated as shown in Figure 2

Table 1: Disease Symptoms on Different Parts of *P. caribaea* and Fungal Species Isolated

Disease symptoms on different tree parts	Fungal species isolated
Needles	*Fusarium* sp., *Rhizoctonia* sp., *Aspergillus* sp., *Dothistroma* sp., *Fusarium solani species complex*
Needle blight	
Needle spot	*Rhizoctonia* sp., *Fusarium* sp., *Aspergillus* sp., *Aspergillus flavus*
Needle necrotic tip	*Fusarium* sp., *Rhizoctonia* sp., *Aspergillus* sp.
Short needles	*Aspergillus* sp., *Microsphaeropsis ovalicea*
Stem	
Stem	*Fusarium* sp., *Rhizoctonia* sp., *Aspergillus* sp., *Penicillium* sp.
Branch	
Pinus branch with pinkish fruiting body	*Fusarium* sp., *Rhizoctonia* sp., *Penicillium* sp.
Root	

Disease symptoms on different tree parts	**Fungal species isolated**
Root	*Fusarium* sp., *Rhizoctonia* sp., *Penicillium* sp.
Soil	
Rhizosphere Soil	*Fusarium* sp., *Rhizoctonia* sp., *Aspergillus* sp., *Penicillium* sp.

Needle spot was observed on *P. Caribaea*, Micro and macro conidia of *Fusarium* sp., Conidiophore of *Aspergillus* sp., branched septate mycelia of *Rhizoctonia* sp. and *Aspergillus flavus* were isolated from needle spot symptom as detailed in Plate I (a- d)

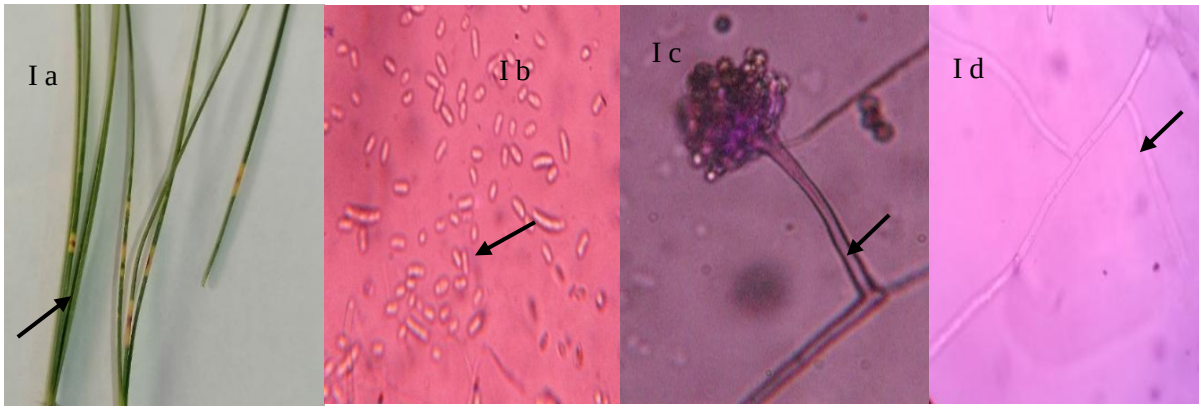
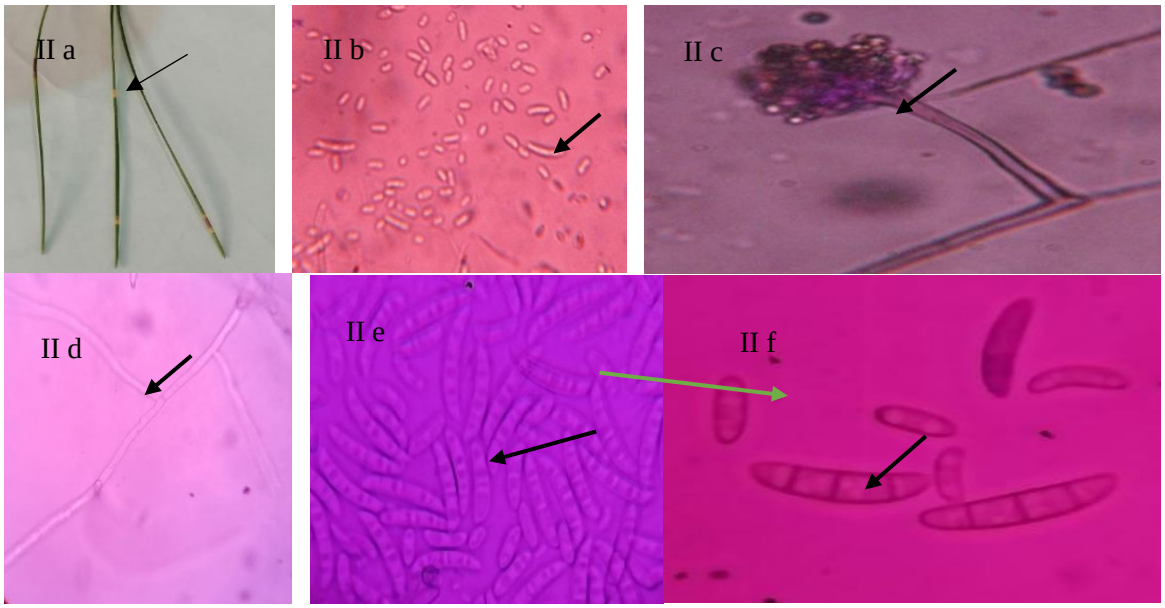


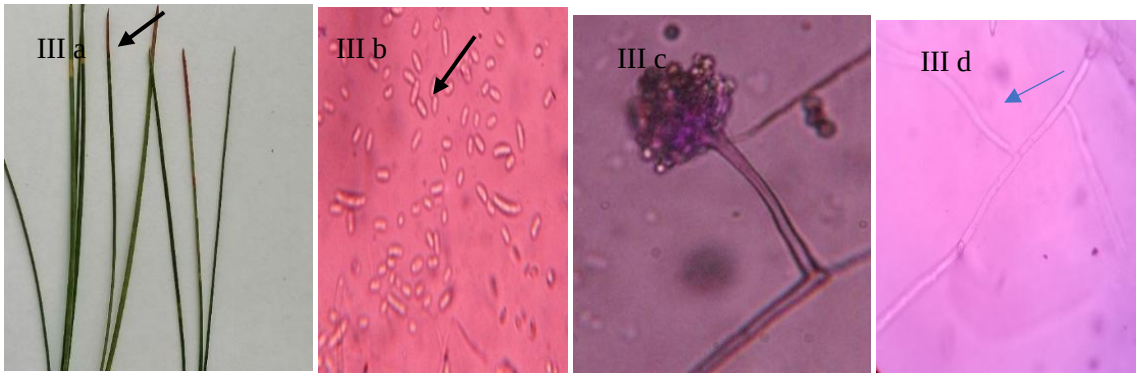
Plate I: a. Needle Spot (Arrow), b. Micro and Macro Conidia of *Fusarium* sp., c. Conidiophore of *Aspergillus* sp. (Arrow), d. Branched Septate Mycelia of *Rhizoctonia* sp. (Arrow)

Needle blight symptom was observed, Micro and macro conidia of *Fusarium* sp., Conidiophore of *Aspergillus* sp., Branched septate mycelia of *Rhizoctonia* sp., Septate conidia of *Dothistroma* sp. and *Fusarium solani* species complex were isolated as represented in Plate II (a- e).



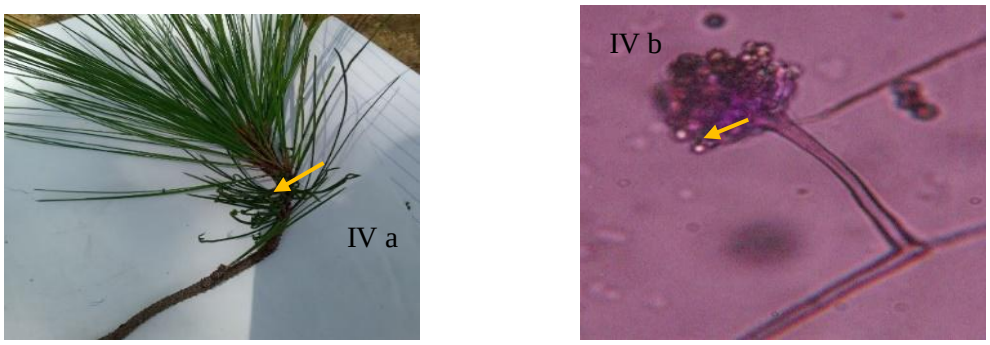
Plates II: a. *Pinus* Needle Blight (Arrow), b. Micro and Macro Conidia of *Fusarium*, c. Conidiophore of *Aspergillus* sp. d. Branched Septate Mycelia of *Rhizoctonia* sp., e. Septate Conidia of *Dothistroma* sp.of *Aspergillus* sp

Needle necrotic tip was observed on needles of *P. Caribaea*, Micro and macro conidia of *Fusarium* sp., Conidiophore of *Aspergillus* sp. and Branched septate mycelia of *Rhizoctonia* sp. were isolated as detailed in Plate III (a- d).



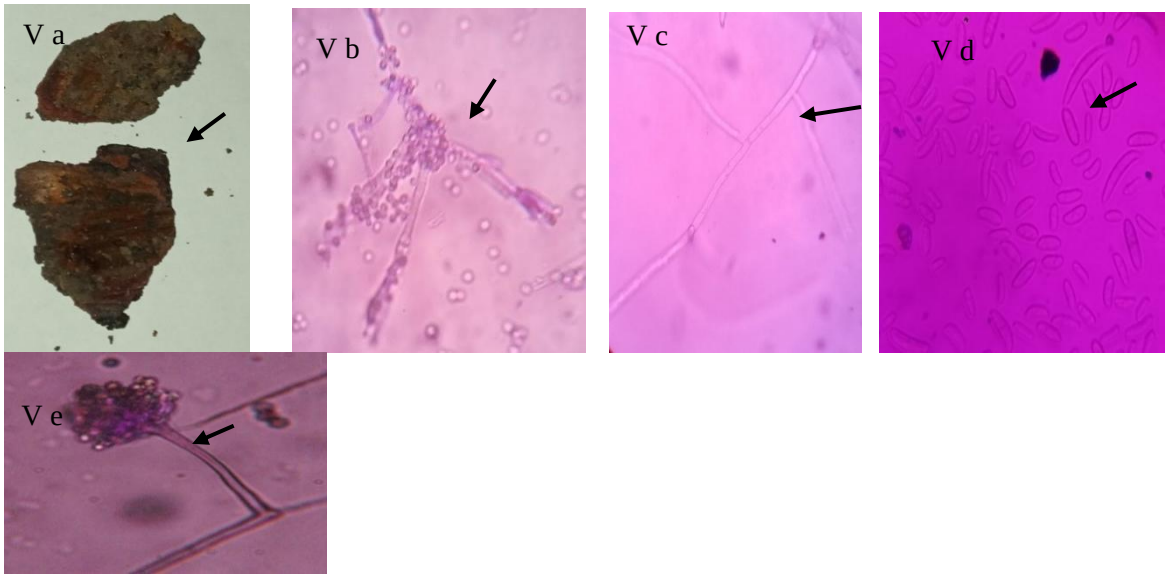
Plates III: a. Necrotic of *Pinus*, b. Micro and Macro Conidia of *Fusarium* sp., c. Conidiophore of *Aspergillus* sp. d. Branched Septate Mycelia of *Rhizoctonia* sp

Short needles were observed on some branches of *P. Caribaea*, Conidiophore of *Aspergillus* were isolated as represented in Plate IV (a- b)



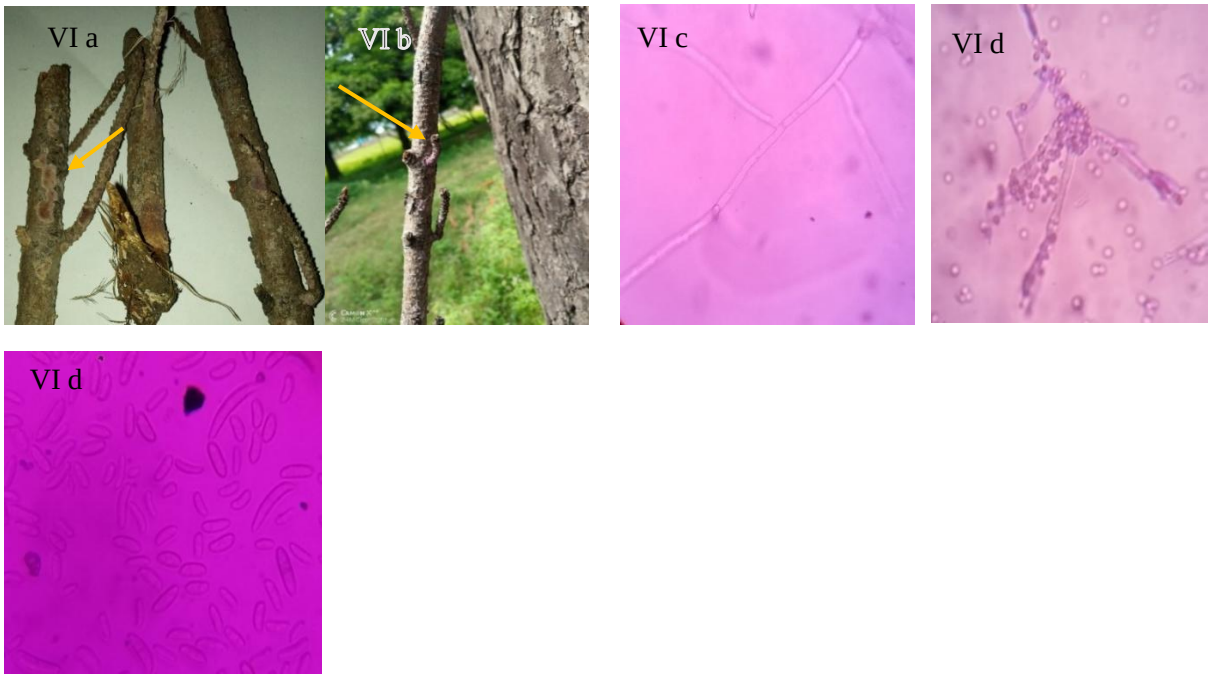
Plates IV: a. Needles of *Pinus.*, b. Conidiophore of *Aspergillus* sp

Stem bark of wilted of *P. Caribaea* tree was collected, Branched conidiophore of *Penicillium* sp., branched septate mycelia of *Rhizoctonia* sp., Micro and macroconidia of *Fusarium* sp. and Conidiophore of *Aspergillus* sp. were isolated from the stem of a wilted *P. Caribaea* tree as represented in Plate V (a- e).



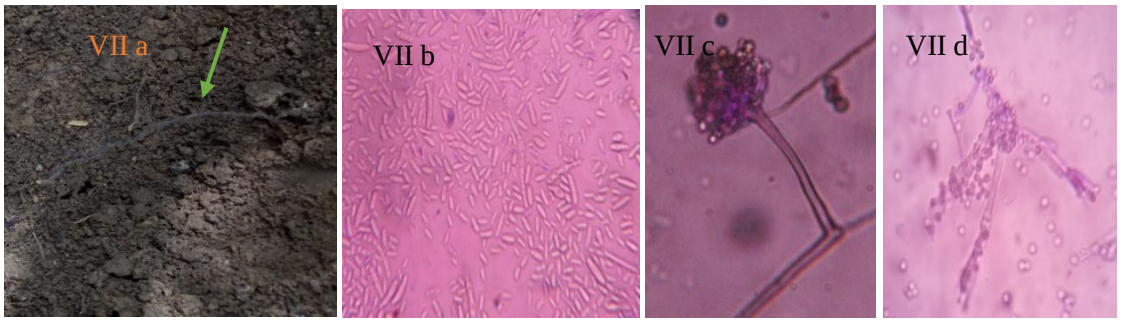
Plates V: a. Stem Bark of Wilted *P. Caribaea* tree, b. Branched Conidiophore of *Penicillium* sp., c. Branched Septate Mycelia of *Rhizoctonia* sp., d. Micro and Macroconidia *Fusarium* sp., e. Conidiophore of *Aspergillus* sp

On field, a *P. Caribaea* stem with fruiting body was observed, branched conidiophore of *Penicillium* sp., branched septate mycelia of *Rhizoctonia* sp. and micro and macroconidia of *Fusarium* sp. were isolated from the stem with fruiting body on it as detailed in Plate VI (a- d).



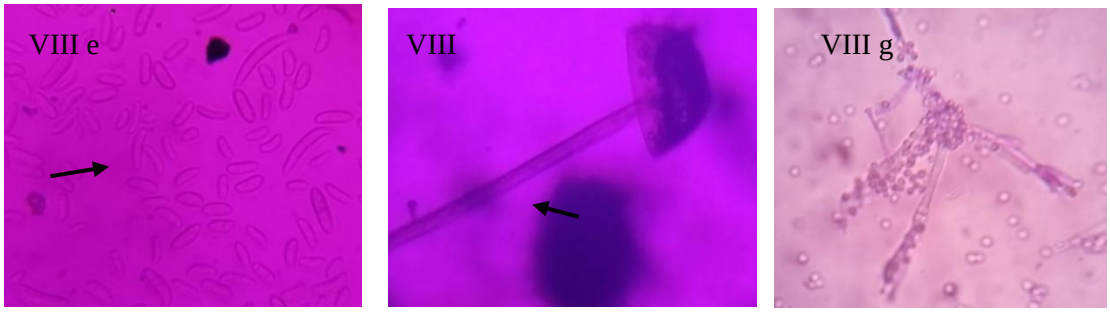
Plates VI: a. Stem of *Pinus* Plant with Fruiting Body (Arrow). b. Branched Septate Mycelia of *Rhizoctonia* sp., c. Branched Conidiophore of *Penicillium* sp., d: Micro and Macroconidia of *Fusarium* sp

P. Caribaea root was collected, Conidiophore of *Aspergillus* sp., Micro and macroconidia of *Fusarium* sp. and Branched conidiophore of *Penicillium* sp. were isolated from the root as represented in Plate VII (a- d).



Plates VII: a. Root of Wilted *P. caribaea*, b. Micro and Macroconidia of *Fusarium* sp. c. Conidiophore of *Aspergillus* sp. d. Branched Conidiophore of *Penicillium* sp

Rhizosphere soil of wilted *P. Caribaea* tree was collected, branched septate mycelia of *Rhizoctonia* sp., Septate conidia of *Pestalotia* sp., Conidiophore of *Aspergillus* sp., Micro and macroconidia of *Fusarium* sp., Branched conidiophore of *Penicillium* sp. and Sporangiophore of *Rhizopus* sp. were isolated from the rhizosphere soil as represented in Plate VIII (a- g).



Plates VIII: a. Rhizosphere Soil (Arrow), b. Branched Septate Mycelia of *Rhizoctonia* sp., c. Septate Conidia of *Pestalotia* sp., d. Conidiophore of *Aspergillus* sp., e. Micro and Macro Conidia of *Fusarium* sp, f. Sporangiophore of *Rhizopus* sp., g. Branched Conidiophore of *Penicillium* sp

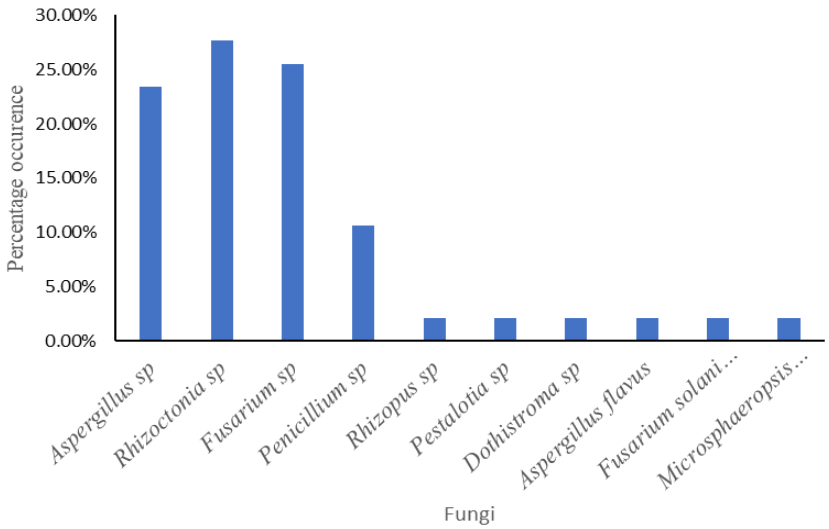


Figure 2: Percentage Occurrence of Fungi on *Pinus caribaea*

The present work shows that *Pinus caribaea* is planted as ornamental plants in Zaria area due to their aesthetic value. *P. caribaea* attain their types of leaves and needles, branching pattern as well as evergreen coloration. *P. caribaea* are exotic species but very much adapted, this tallied with the findings of Aksenova, (1999); Crisp and Cook, (2005) who reported *Pinus*, and *Casuarina* are planted as ornamental plants due to their shape of needles. As reported by Jones *et al*. (2014) who added that *Fusarium* sp and other fungi are importance pathogens on conifers. Study reports *Dothistroma* sp. on *Pinus* associated with needle necrotic tip, this is similar to the findings of Watt *et al*. (2009) and Prihatini *et al*. (2015) who reported the isolation *D. septosporum* on *Pinus* and *Casuarina*. Needle blight was the most prevalent symptom observed on *P. caribaea*. Needle blight have been reported to be among the most devastating diseases of *Pinus* sp all over the world (Karadzic, 1998a; Barnes *et al*., 2004; Bulman *et al*., 2008; Jankovsky *et al*., 2004). This shows that needle blight symptom in this study is an indication that *P. caribaea* can be infected by this disease to the best of my knowledge needle blight have not been reported on *P. caribaea* in this area. Needle blight also known as *Dothistroma* needle blight (DNB), is caused by *Dothistroma* sp and it has been reported to occur in almost every country around the world when there is susceptible host and conducive environmental conditions for growth (Barnes *et al*., 2004; 2011). *Dothistroma* is the anamorph of *Mycospharella*. *Pinus* is not the only host of *Dothistroma*, the exact number of hosts is yet to be ascertained (Bednarova *et al*., 2006; Watt *et al*., 2009; Drekhan *et al*., 2014). *Dothistroma* is tolerant to almost all kind of environmental conditions. It has a worldwide distribution both in Asia, Europe and Africa (Doroguine, 1911; Gibson 1972; Fabre *et al*., 2012; EPPO, 2015). *Dothistroma* sp have been reported to cause DNB disease in Kenya and South Africa (Barnes *et al*., 2004). This study confirms the presence of *Dothistroma* sp. to be the cause of DNB in *Pinus* within Zaria, this have been reported in Kenya and South Africa to be the countries in Africa that have *Dothistroma* infection on *Pinus* sp. To the best of my knowledge, this is the first report of *Dothistroma* on *Pinus caribaea* in Nigeria.

Fusarium sp. was isolated from different parts of plants, study reports *Fusarium solani* species complex from *P. caribaea*. However, Aoki *et al*. (2013) reported the occurrence of *Fusarium* sp. as pathogens which are harmful to conifers, cause damaging to seedlings, root rot, damping off disease and pitch canker. In this study *Fusarium* sp. were isolated from diseased *Pinus caribaea* and this is similar to the findings of Jones *et al*. (2014) who reported *Fusarium solani* species complex as agents of disease of coniferous trees.

Pestalotia can survive in different forms as saprophytes, endophytes and parasites. It can be found on different parts of plants. *Pestalotia* sp. causes a disease known as Pestalotiopsis. Guba, (1961) reported members of *Pestalotia* sp. as saprophytes on most plants. Peace, (1962) reported *Pestalotia* sp. to be of little economic concern in Malaysia, the result in this study tally with the findings of Browne, (1968) who reported *Pestalotia* to be the cause of serious

disease of *Pinus caribaea*, even though Shaw and Toes, (1977) reported other fungi like *Pestalotia* sp., *Aspergillus* sp., *Rhizoctonia* sp. and *Penicillium* sp. overgrow fast and makes it difficult to get the actual pathogen. In conifers, *Pestalotia* have the ability of causing diseases like cankers, dieback, needle spots, needle blight, needle tip necrosis and chlorosis, even though very little studies have actually carried out pathogenicity but a lot of studies isolated *Pestalotia* on pine and other conifers. Studies carried out by Hu *et al*. (2007) in China and in Europe and North America, they isolated *Pestalotia* from different parts of *Pinus*.

This study showed that different parts of *Pinus* can be infected by *Rhizoctonia*, this is in conformity with the findings of Sharma *et al*. (1984) where they isolated *Rhizoctonia* from different parts of *Pinus* plants showing needle blight, needle spot, needle necrotic tip and stem.

Aspergillus sp were isolated from all parts of *Pinus* including the rhizosphere soil this is similar to the study of Horn and Dorner, (1998); Diener *et al*., (1987) who also isolated *Aspergillus* from all parts of plants including the rhizosphere soil. According to Keredzic, (1989b) saprobes do not cause diseases.

The *Penicillium* isolated from this study was from wilted *Pinus* plant which have been infected by other pathogens. Rapper and Thom, (1949) reported *Penicillium* sp to be saprophyte which helps in decomposing dead or decay matter. Peterson, (2000) also reported *Penicillium* as a saprophyte found on all parts of dead decay matter.

Rhizoctonia sp., *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Pestalotia* sp., *Dothistroma* sp., *Aspergillus flavus, Fusarium solani* species complex, *Microsphaeropsis ovaliceae* were isolated from leaves, root, stem, soil showing different disease symptoms and diseases, these pathogens might have been transmitted from other plants since conifers are propagated through cuttings, wind might have blown spores of these pathogens. *Rhizoctonia* sp., *Fusarium* sp., *Pestalotia* sp., *Dothistroma* sp., *Fusarium solani* species complex, have been reported to be pathogenic while *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Aspergillus flavus* have been reported to be saprophytic and do not cause disease. *Pinus* is not the only host of *Dothistroma*, the exact number of hosts is yet to be ascertained (Bednarova *et al*., 2006; Watt *et al*., 2009; Drekhan *et al*., 2014).*Dothistroma* sp. have been reported to be a dangerous and invasive pathogen, it causes needle and shoot diseases which can lead to dieback and eventual wilting of plant (Santini *et al*., 2013). *Dothistroma* have a wide distribution as reported in Europe (Fabre *et al*., 2012; Doroguine 1911), in Africa, Gibson, (1972) reported *Dothistroma*. Distribution of *Dothistroma* have been reported by EPPO, (2015). DNB has been recorded in Ethiopia, Kenya, Malawi, South Africa, Swaziland, Tanzania, Uganda and Zambia. Barnes *et* al., (2004) also reported the presence of this Fungus in South Africa and Kenya. To the best of my knowledge this is the first report of *Dothistroma* on *Pinus* in Nigeria.

Fusarium sp. is among the cause of Needle spot on conifers. Studies by Doidge, (1950); Darvas, (1976); reported *Fusarium* to be associated with root rot. it is similar to the findings of Sutherland van Eeden, (1980) where he isolated *Fusarium* from bark, needles with spots, stunted needles and also on wilted *Pinus* plants. Aoki *et al*. (2013); Jones *et al.* (2014) reported *Fusarium* sp. to be harmful pathogen on conifers. In this study, *Fusarium solani* species complex was isolated, thus confirming the association of *Fusarium* with *Pinus* sp.

Pestalotia can be pathogenic or saprophytic, Guba, (1961); Peace, (1962) in Malaysia reported members of *Pestalotia* sp. as saprophytes on conifers, this study is similar to the findings of Browne, (1968); Philips and Burdekin, (1992); Guo *et al*. (2004); Ganley and New combe (2006); Promputha *et al*. (2007); Huang *et al*. (2008) who reported *Pestalotia* to be the cause of serious disease of *Pinus Caribaea*, even though reported *Phoma* sp. and *Pestalotia* sp. as important fungi which causes decline in Pine species, *Pestalotia* have been reported to be a secondary pathogens but it causes infection in association with other pathogens.

In conifers, *Pestalotia* have the ability of causing diseases like cankers, dieback, needle spots, Needleblight, needle tip necrosis and chlorosis, even though in this study *Pestalotia* was isolated from root and soil. Hu *et al*. (2007) in China and in Europe and North America, they isolated *Pestalotia* from different parts of *Pinus*.

In this study *Rhizoctonia* was isolated from different parts of *Pinus*, this is in conformity with the findings of Sharma *et al*. (1984) where they isolated *Rhizoctonia* from different parts of *Pinus* plants exhibiting different symptoms. Also, in South Africa, Darvas, (1976); Darvas *et al*. (1978) isolated *Rhizoctonia* sp. from *Pinus* seedlings and nursery, Vaartaja and Cram, (1956); Perrin and Sampangi, (1986) reported isolation of *Rhizoctonia* sp. in other parts of the world. *Rhizoctonia* have a wide distribution all over the world.

Aspergillus is highly cosmopolitan. It produces millions of spores. It is mainly saprophytic. Diener *et al*. (1987); Horn and Dorner, (1998) reported *Aspergillus flavus* are found in the soil as contaminants and are also in the air throughout the world. *A. flavus* was isolated from other parts of *Pinus*. From this study, *Aspergillus* sp were isolated from all parts of *Pinus* including the rhizosphere soil.

According to Keredzic, (1989b) saprobes do not cause diseases but in this study, *A. flavus* was isolated from needles showing needle spot symptoms and this isolation was confirmed by CABI. This raise the question of if saprophytes are able to cause diseases when environmental conditions are favorable, also Rapper and Thom, (1949) reported *Penicillium* sp. to be saprophyte which helps in decomposing dead or decay matter. Peterson, (2000) also reported *Penicillium* as a saprophyte found on all parts of dead decay matter. The *Penicillium* isolated from this study is from wilted *Pinus* plant which have been infected by other pathogens.

CONCLUSION

This study demonstrated that *Pinus caribaea* grown as ornamental plants in Zaria hosts a diverse range of fungi across its needles, stems, roots, and rhizosphere soil. Both pathogenic and saprophytic fungi were identified, with *Rhizoctonia* sp. and *Fusarium* sp. being the most prevalent across all plant parts. The occurrence of *Dothistroma* sp., associated with needle blight, is particularly significant as it suggests a potential emerging threat to *Pinus* in the study area. Overall, the findings highlight the importance of monitoring fungal associations in ornamental plants to support effective disease management and sustain their ecological and aesthetic value.

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