Communication in Physical Sciences, 2022, 8(4): 573-580

Species- Specific- Pathogens of Selected Forest Trees in Tropical Secondary Moist Forest of Otuoke, Nigeria

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Received: 12 September 2022/Accepted 22 September 2022/Published online: 30 September 2022

Abstract: *This study was carried out to identify specific fungi and bacteria species associated with four economic forest trees:- Irvingia gabonensis, Nauclea diderrichii, Fagara macrophylla and Artocarpus altilis in Otuoke secondary forest. Leaf samples of selected trees with symptoms of diseases were randomly collected from three forest locations along the Otuoke axis making careful observations on the disease symptoms they showed. Collected samples were taken to the laboratory for mycological and bacteriological analyses. Results show that 4 fungi species were associated with Irvingia gabonensis, 3 with Nauclea diderrichii, 3 with Fagara macrophylla, and 4 fungi were associated with Artocarpus altilis. Percentage occurrence of bacteria species in plant samples was Pseudomonas aeruginosa (31%), Erwinia persicina(23%), Bacillus cereus (23%), and Agrobacterium spp. (23%). Plants leave appear most susceptible to pathogens' attack than other plants' parts. Identified fungi and bacteria were the cause of observable diseased symptoms in tree species. This is vital information for forest management and tree conservation, especially when considering plantation establishment of these species.*

Keywords: *Forest trees, secondary forest, specie specific pathogens, fungi, bacteria.*

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1.0 Introduction

Forest trees and plants in general are the primary producers on which all other members of an ecosystem depend (Leigh *et al.*, 2004). Because of this central importance to their hosts, plant pathogens drive many ecological and evolutionary processes in natural ecosystems. Disease-causing organisms can regulate host populations and/or modify their genetic composition, restrict host distribution at various spatial scales, promote or reduce community diversity, mediate plant-herbivore and plant-plant interactions, create canopy gaps, and reduce host growth or reproduction and thus affect the availability of food for man and animal (Benitez *et al.*, 2013). They may also drive the evolution of species and host defenses. In tropical forests, plant pathogens interact with a myriad of other organisms in soil that the potential to moderate plant disease and in turn affect community composition (Pyke *et al.*, 2001; Baltzer and Choi, 2008). Tree diseases are the leading cause of timber losses each year in the forest ecosystem (Worral and Parmeter, 1982). T The average total loss of timber due to disease-catastrophic epidemics that can wipe out entire tree equals the losses caused by all other stress agents combined (Worral and Parmeter, 1982). Diseases pose threat to tree survival and are no less problematic because they reduce growth significantly, degrade wood, destroy fruit and seed crops, and make

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landscape trees and ornamentals unsightly or hazardous (Baltzer and Choi, 2008).

Disease, as it pertains to trees, may be viewed as a sustained and progressive impairment of the structure or function of any part of a living tree. These diseases greatly impact natural or semi-natural ecosystems including forests, and affect the productivity of managed stands and threaten biodiversity. Most forest conservation effort that does not take cognizance of damage to forest trees occasioned by tree pathogens ends up without realizing the objectives.

Pathogens are parasitic microorganisms that cause disease, meaning they attack plants to obtain the energy and nutrients necessary for their metabolism to complete their life cycle resulting in harm to their host plant. Pathogenic (disease-causing) microorganisms include bacteria, viruses, nematodes, and most commonly, fungi (Engelbreecht *et al.*, 2007). Not all microorganisms are pathogenic; in fact, most microorganisms are obligate saprophytes meaning they can only feed on dead organic material. These microorganisms play an important role in decomposing dead plant material and recycling nutrients in the ecosystem. Other plant pathogens are facultative pathogens, meaning that they can live on dead plant material, but can also attack living plants and cause disease. Other pathogens are obligate pathogens that can only survive on a living host plant. Of course, pathogens continue to adapt induced plant defenses by hiding the chemical signals that alert plants to an attack or adding developing additional virulence factors (Hersh *et al.*, 2012).

All plant pathogens are infectious and transmissible, meaning they can spread from one host plant to infect another. Plant pathogens cause diseases, they are not diseases themselves. A diseases ase is resulting interaction between a susceptible host, a virulent pathogen, and an adequate environmental condition. Plant pathogens cannot attack and parasitize all plant species; instead, plant pathogens are host-specific. A host is a plant that can be infected and parasitized by a specific plant pathogen. Most plant pathogens have only one or a few suitable host species; however, some pathogens can attack hundreds of plant species (Baltzer and Choi, 2008). The mechanisms that determine which pathogens can attack which plants are very complicated and result from complex interactions and signals between the two organisms. Trees have evolved structural and chemical defenses such as thick bark, waxy leaf coatings, root secretions, and anti-microbial toxins that prevent infections. These “pre-formed” defenses are always in place and provide general protection from all microorganisms (Agrios, 2005). But certain plant pathogens have developed virulence factors that enable them to overcome general plant defenses (Swinfield *et al.*, 2012). Virulence factors such as enzymes that degrade plant tissues, special structures that can pierce plant cells, or specialized metabolic pathways that can neutralize host toxins, may allow a microorganism to become pathogenic. In response, plants have developed methods to detect pathogens that can overcome pre-formed defenses, and in response, they initiate powerful “induced” defenses (Gilbert, 2002).

2.0 Material and Methods

2.1 Study area

The study was carried out in the forest of Otuoke located in the Ogbia Local Government Area. The area lies within a latitude of 4^{o} 51’05.23” N, Longitude 6^{o}20’19.8”E. The study area has rainfall generally every month of the year with heavy downpours. The mean temperature is in the range of 25^{0}C to 31^{0}C and the hottest months are December to April. The relative humidity is high throughout the year and decreases slightly in the dry season (Seiyaboh et al, 2016; (Cheesbrough, 2006).

2.2 Collection of sample

Four forest trees were purposely selected from the forest for investigation. The selection was based on the observed economic use of selected species by local community dwellers. These are *Irvingia gabonensis, Artocarpus altilis, Fagara macropylla,* and *Nauclea diderrichii.* Physical observation and examination of tree trunks and leaves for signs of disease attack were carried out. Leaf samples of selected trees with symptoms of the disease were randomly collected from three forest locations along the Otuoke axis (Onuebum, Azikiel road, Otuaba). Observable symptoms such as chlorosis (yellowing of leaves), browning, curling, premature leaf drop, rots and other disease symptoms were documented. The samples upon collection in the morning were put in a new polyethylene bag and labeled according to their site of collection and taken to the laboratory for analysis.

2.3 *Laboratory analysis*

The microbiological analysis (mycological and bacteriological) of the plant samples was done following standard operating procedures (Barnett and Hunter, 1972). The diseased parts that were analyzed were chosen based on the physical disease symptoms such as browning, necrotic symptoms, curling, exudation (observed only on *Irvingia gabonensis*) and holes. The plant leaves showed more of such disease symptoms. The diseased parts of the plants were carefully cut out using a new sterile blade. Thereafter, they were soaked in formaldehyde to kill the commensal fungal and bacterial species on the surface of the plant parts. They were thereafter rinsed in distilled water to remove the Hypochloride solution from the plant parts. Plating of the samples (three plates per sample) was done using the direct plating method. 20ml of sterilized molten nutrient media cooled to 45^0c were poured into sterile Petri dishes, and were allowed to set. The plates were allowed to be set (solidify). Using sterilized forceps, the cut-out diseased samples were picked and placed on the surface of the nutrient media (nutrient agar and potato dextrose agar). Plating was done in triplicates. After plating, the nutrient agar plates were incubated for 24 hours at 37^0 c for 24 hours to obtain bacterial growth. The fungal plates, (potato dextrose agar) were incubated in a dark portion of the laboratory at ambient temperature for up to five days (120 hours).

2.4 *Identification of pathogens*

The fungal and bacterial isolates were identified by the aid of appropriate taxonomic keys used by (Fawole and Osho, 1995; Boerema *et al.*, 2004; (James and Natalie, 2013).

3.0 Results and Discussion

A systematic description of the isolated fungi was done from their macroscopic to microscopic properties. Four different fungal species were isolated from the leaves of *Irvingia gabonensis* in three different plates respectively. The isolated fungi are *Chaetomium funicola, Aspergillus candidus, Penicillium corylophilium, Aspergillus fumigates*. *Aspergillus fumigatus, Aspergillus candidus,* and *Penicillium corylophilum* were isolated from all three plates but one, while *Chaetomium funicola* was isolated from only one of the three plates.

Table 1 indicated that *Chaetomium funicola, Aspergillus candidus, Penicillium corylophilium* and *Aspergillus fumigates* are the most common fungi associated with *Irvingia gabonensis*.

Penicillium corylophilium; fusarium oxysporum and *Chaetomium funicola* were isolated from leaves of *Nauclea diderrichii* with *Penicillium corylophilum* and *Chaetomium funicola* isolated from two of the three plates, making it more common while *Fusarium oxysporum* was isolated from only one of the three plates implying it may not be a common fungus associated with the tree. Three fungi species *Aspergillus candidus; fusarium oxysporum* and *Penicillium* *corylophilium* were isolated from two of the three plates indicating its commonness.

The fungi isolated from *Artocarpus altilis* (Table 1) are *Aspergillus candidus, Penicillium corylophilium, Aspergillus niger* and *Alternaria alternata. Penicillium corylophilium* and *Aspergillus niger* are the two common fungi species associated with the tree. They were isolated from two of the three plates.

In this study, fungi species associated majorly with *Irvingia gabonensis* plant were *Chaetomium funicola, Aspergillus candidus,* and *Penicillium corylophilium,* while in *Nauclea diderrichii* plant, fungi species isolated were *Penicillium corylophilium, fusarium oxysporum* and *Chaetomium funicola*. Similarly, in *Fagara macrophylla* plant, the following fungi; *Aspergilus* of *candidus, fusarium oxysporum,* and *Penicillium corylophilium* were isolated, while in *Artocarpus altilis; Aspergilus candidus* and *Penicillium corylophilium* were isolated.

Table 1. Fungal Isolates from selected tree samples in a tropical moist forest, Otuoke Nigeria

Tree species	Fungi	Number isolated from 3 plates	Plants parts
Irvingia gabonensis	*Chaetomium funicola*	1	Leaf
	Penicillium corylophilum	2	Leaf
	Aspergillus candidus	2	Leaf
	Aspergillus fumigatus	2	leaf
Nauclea diiderrichii	*Penicillium corylophilum*	2	leaf
	Fusarium oxysporum	1	leaf
	Chaetomium funicola	2	leaf
Fagara macrophylla	*Aspergillus Candidus*	2	Leaf
	Fusarium oxysporum	1	Leaf
	Fusarium oxysporum	1	leaf
Artocarpus altilis	*Aspergillus candidus*	1	Leaf
	Penicillium corylophilum	2	Leaf
	Aspergillus niger	2	Leaf
	Alternaria alternate	1	leaf

Fungi species have been reported to affect tree species occurrence and distribution, especially in the regeneration layer. Soil-borne pathogens, in particular, are thought to be important in maintaining plant species diversity and distribution (James and Natalie, 2013). *Penicillium corylophilium* is a rapidly-growing fungus that produces a blue-green colony with a dark green reverse, within twelve days. It is commonly isolated worldwide but is found more frequently in warmer climates than in cool-temperate climates. It is isolated from soil, plants, foodstuffs, bees, and honeycombs. This fungus has an opportunistic nature which enters the plant through insect lesions or mechanical injuries. Once it gains entrance into the plant, it changes the colour of the plant parts (brownish or yellowish) depending on the associated pathogens present and initiates internal rotting of the plant. Furthermore, a moldy blue-green growth is mostly visible on the plant surface as a symptomic description of the pathogen's internal activity (Packer and Clay, 2000).

Fusarium oxysporum plays the role of a silent assassin - the pathogenic strains of this fungus can be dormant for 30 years before resuming virulence and infecting a plant. *Fusarium* *oxysporum* is famous for causing a condition called *Fusarium* wilt, which is lethal to plants and swift - by the time a plant shows any outward sign of infection, it is already too late, and the plant will die. Additionally, *Fusarium oxysporum* is not discriminating; it can cause disease in nearly every agriculturally important plant (Bahram *et al*., 2015). *Fusarium* role in native soil is predominantly harmless and acts as a beneficial plant endophyte or soil saprophyte but it is equally pathogenic to plants where it causes a wide range of diseases such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, damping-off, defoliation and plant death. When these infected plant-products are consumed by humans or animals, especially immune-compromised individuals, their toxins will be digested and result in several medical cases such as Onchomycosis.

Alternaria spp., have been reported to be the active causes of leaf spots, rots, blights and other diseases in foliage. They are opportunistic pathogens having numerous plant hosts. The onset of disease from *Alternaria spp*, occurs in the host's exposed leaves and when *Alternaria spp*, are in association with other fungi, it becomes more pathogenic to foliage (Prusky and Keen, 1993).

Chaetomium funicola is a facultative or heterotypic fungus that is responsible for subcutaneous lesions in plants. It is a dark-walled mold normally found in soil, air, cellulose and plant. This fungus is temperature tolerant and has been reported to cause toe infections in humans, and superficial and cutaneous lesions also in humans that ingest them from any food source (Stenlid *et al*., 2011).

Aspergillus species molds have a powdery texture but the colour of the surface of the mold differs from species to species and can be used to identify the type of *Aspergillus* growing coupled with the rate of growth. *Aspergillus fumigatus* is a known pathogen of humans more than any other species of *Aspergillus* displaying high genetic variability within geographic regions. It has a pathogenic relationship with trees where it attacks fruits and foods such as nuts. If ingested, it can cause diseases in immune-deficient humans as it produces aflatoxin which is both a toxin and a carcinogen.

Aspergillus niger is renowned to cause black mold in fruits with literature reporting a black mould in *Artocarpus altilis* (Breadfruit) on several occasions. *Aspergillus candidus* is a white-spored species of *Aspergillus*, and is a common contaminant of grain dust. The species is widely distributed in nature and grows on vegetation in the later stages of decay. It has been associated with a variety of diseases such as hypersensitivity diseases, and infectious diseases including aspergillosis, otomycosis, and onychomycosis (Gaston, 2000; Brasier,1983).

The percentage occurrence of bacterial species in plant samples (Fig. 1) show the following proportions: *Pseudomonas aeruginosa* (31%), *Erwinia persicina* (23%), *Bacillus cereus* (23%), and *Agrobacterium specie* (23%).

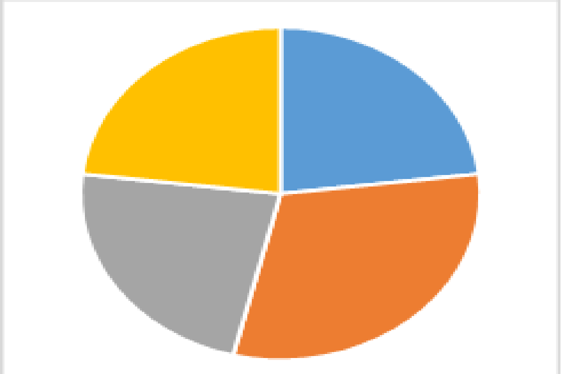

Fig.1.Percentage occurrence of bacterial species in plant samples

The following bacterial species; *Pseudomonas aeruginosa*, *Erwinia persicina*, *Bacillus cereus*, and *Agrobacterium species*, were found to be associated with the investigated tree species. Bacteria pathogens have been reported to alter forest tree abundance and distribution, at the individual, population and community levels (Brasier, 1983). Bacteria pathogens have been reported to cause various plant diseases (Gaston, 2000). *Pseudomonas aeruginosa* is a common Gram-negative, rod-shaped bacterium that can cause disease in plants such as rot, shoot-tip dieback, (which appears as dead, blackened tissue extending down some distance from the tip of the plant), necrosis (necrotic leaf spots) and developmental dystrophies such as galls. But, some plant-associated *Pseudomonas spp.* promote plant growth by suppressing pathogenic microorganisms, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance. It is renowned for its nutritional and ecological versatility (Stenlid *et al.*, 2011).

Erwinia persicina is a Gram-negative, rod-shaped, motile, oxidase-negative, facultatively anaerobic, and fermentative bacteria that mainly infect woody plants. It gets into plants through natural orifices (stomata) or wounds on the plants' surface and causes plant diseases such as chlorosis, sudden rots, bacterial wilt and necrosis in plants (Aschenbenner *et al*, 2002).

Bacillus cereus is an environmentally spore-forming organism, almost ubiquitously distributed in nature, whose reservoir is represented by soil, decaying organic matter, vegetation, fresh and marine waters, and the invertebrate gut, along with dirt, air, and stools. It can be used as a bio-control agent to promote plant growth because they produce antimicrobial compound such as lipopeptides, enzymes and antibiotics (Forestry Commission, 2012).

Agrobacterium species is a Gram-negative bacterium that uses horizontal gene transfer to cause tumors in plants. It causes crown-gall disease in plants characterized by a tumor-like growth or gall on the infected plant, often at the junction between the root and the shoot (Forestry Commission, 2012; Meentemeyer *et al.*, 2012). Biological control of diseases caused by *Agrobacterium species* has been achieved using non-pathogenic *Agrobacterium* strains which produce an antibiotic specifically inhibitory to *Agrobacterium* pathogens ((Forestry Commission, 2012; Meentemeyer *et al.*, 2012).

4.0 Conclusion

The result of this study has indicated a pathogenic relationship between the trees and the isolated microorganisms. The study also took into consideration the various locations where the tree samples were taken and the characteristic disease symptoms similar species of plants exhibited. This will aid subsequent research aimed at discovering tree pathogens and their impacts on the productivity of these trees. Though proximate analysis on these trees has deemed these trees suitable for several purposes, it is worth noting that the effects of the microbial relationships with these trees can threaten human health when their products/produce is consumed. They could affect the economy adversely especially when materials like timber lose their integrity for the purpose for which they are demanded.

The immunity of natural ecosystems to disease epidemics can be overcome mainly when virulent pathogens to which the trees have no resistance are introduced to the natural ecosystem. Introduced pathogens often lead to the elimination of entire species and result in permanent changes in the species composition of an ecosystem. Ineffective quarantine has often led to epidemics in indigenous and exotic tree populations.

The study revealed that fungi species associated with the trees: *Chaetomium funicola*, *Aspergilus candidus*, *Penicillium corylophilium*, *Aspergilus niger*, *Aspergilus fumigatus*, *Alternaria alternate*, *Fusarium oxysporum* and the the following bacterial species: *Pseudomonas aeruginosa*, *Erwinia persicina*, *Bacillus cereus*, and *Agrobacterium spp.* were the cause of the disease symptoms observed on these trees before sampling. The study suggests that identified fungi and bacteria pathogens are associated with these tree species in the study area.

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Consent for publication

Not Applicable

Availability of data and materials

The publisher has the right to make the data public

Competing interests

The authors declared no conflict of interest. This work was carried out in collaboration among all authors.

Funding

There is no source of external funding

Authors' contributions:

Both authors contributed equally to the development of the manuscript.