# ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH THE RHIZOSPHERES OF SOME ECONOMIC TREES

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***Abstract***
*Fungi associated with the rhizospheres of some economic trees namely date palm (Phoenix dactylifera), pawpaw (Carica papaya), Sheabutter (Vitellaria paradoxa) and oil palm (Elaeis guinensis) trees were isolated and identified in this study in order to know their roles, whether pathogenic or saprophytic. The rhizospheres were collected at 5, 10, 15 and 30cm distances away from the trunk of the plants respectively for fungal isolation and identification using standard methods. Aspergillus niger, A. terreus, A. candidus, A. fumigatus, Fusarium proliferum, Pyrenochaeta romeroi and Alternaria alternata were isolated from the rhizosphere of date palm; Aspergillus niger, Candida albicans and Botrytis cinerea were isolated from the rhizosphere of pawpaw; Aspergillus niger, A. glaucus, A. cremonium killense and Cunninghamella bertholletiae were isolated from rhizosphere of shea butter while Aspergillus niger, Penicilium italicum, Mucor sp., Trichoderma sp. and Rhizopus stolonifera were isolated from the rhizosphere of oil palm trees respectively. Aspergillus niger was common in the rhizospheres of all the trees studied, justifying its attribute as a contaminant. Majority of the fungal isolates identified in this study are pathogenic (A. terreus, A. candidus, A. fumigatus, Fusarium proliferum, Pyrenochaeta romeroi, Alternaria alternata, Candida albicans, Botrytis cinerea, Penicilium italicum, Rhizopus stolonifera and Cunninghamella bertholletiae) while only Trichoderma sp. is beneficial as a biocontrol agent. Efforts should be geared towards suppressing the growth of the pathogens amongst the isolates while the proliferation of Trichoderma sp. should be encouraged where it existed, i.e. rhizosphere of oil palm tree.*

**Keywords**: Biocontrol agent, Contaminant, Pathogens, Rhizosphere and Trunk.

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## 1. INTRODUCTION

Economic plants are plants whose parts and products serve as medicine, food, shelter and raw materials for man and his industries. Date palm (*Phoenix dactylifera*), pawpaw (*Carica papaya*), shea butter (*Vitellaria paradoxa*) and oil palm (*Elaeis guinensis*) trees are some of the common examples of economic plants in the main campus of the University of Ilorin, Ilorin, Nigeria. Date-palm is a monocotyledonous angiosperm belonging to the family Arecaceae (Zaid and de Wet, 2002). The fruits can be eaten fresh, dried or in various processed forms as they are good source of some essential mineral elements as well as vitamins A, B and $B_2$ (Habib and Irahim, 2011). Pawpaw, a soft – wooded tree belongs to the family Caricaceae (Chay – Drove *et al*., 2000). It is cultivated mainly for its fruits, which apart from being eaten, have some medicinal importance. The leaves, seeds and latex (from the leaves, fruits and stem) are also important medicinally.

Shea butter tree belongs to the family Sapotaceae. It is cultivated for its fruits which are edible. Shea butter extracted from the nuts of the fruits is one of the most affordable and widely used vegetable fats. Shea nut cake is used as fodder for livestock and poultry. The leaves and young sprouts serve as forage while the sugary pulp of ripped fruits serve as food for humans, sheep and pigs. The tree is an excellent quality firewood that burns with a fierce heat while timber from the trees are strong, hard, heavy, durable, resilient and termite resistant. The roots and their bark have great medicinal values (Maranz and Wiesman, 2003). Oil-palm tree belongs to the family Arecaceae (Behrman *et al*., 2005). It is very important, all its parts being virtually useful.

Rhizosphere is the narrow region of soil immediately surrounding the plant roots (Marschner *et al.*, 2004). It is the region where the soil and plant roots make contact, thus, characterized by increased microbial activities. The rhizosphere microorganisms are important for plant health and nutrition in such a way that they strongly influence nutrient uptake by plants by either enhancing or decreasing nutrient availability (Marschner *et al.*, 2004). Also a large proportion of the root exudates such as sugars, organic acid anions or amino acids are easily degradable by microorganisms in the rhizosphere resulting in high microbial density and activity in the rhizosphere. The rhizosphere inhabiting microorganisms compete for water, nutrients and space with the plants and sometimes, improve their competitiveness by developing an intimate association (such as mycorrhiza) with the plant (Hartman *et al.*, 2009). An overwhelming number of studies have shown that many rhizosphere – associated microorganisms have profound effects on seed germination, seedling vigour, plant growth and development as well as nutrition, diseases and productivity of the host plant (David, 2014).

The aim of this study is to study the fungi associated with the rhizospheres of common economic trees in the main campus, University of Ilorin, Ilorin, Nigeria so as to know whether they are harmful or beneficial to their host plants while the objectives were to isolate fungi from the rhizosphere zones of date-palm, pawpaw, shea butter and oil-palm trees in the main campus, University of Ilorin, Ilorin, Nigeria as well as to identify the fungal isolates. The study is purely qualitative, not quantitative.

## 2. MATERIALS AND METHODS

### Collection of rhizospheres of date-palm, pawpaw, shea butter and oil-palm trees

The soil samples were collected with a sterilized hand trowel from 5, 10, 15 and 30cm distances from randomly chosen date palm, pawpaw, shea butter and oil palm trees at different locations within the Main Campus, University of Ilorin, Ilorin. The distances were measured with a plastic meter rule while the soil samples at each point for each economic tree were collected into separately labelled sterilized polythene bags and taken to the laboratory for microbial analyses.

### Isolation of fungi from the rhizospheres

Fungi were isolated from the rhizospheres (collected from each of the collection points for each of the economic trees in this study), using serial dilution techniques as described by Fawole and Oso (2007).

Serial dilution was done up to $10^{-6}$ and 0.1ml aliquot from $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$ dilutions were introduced on to the surfaces of sterile potato dextrose agar (PDA) plates using spread plate method (Fawole and Oso, 2007). The PDA plates were then incubated at room temperature for 72 hours inside an incubator.

### Purification of fungal isolates

Each fungal colony obtained was streaked on sterile PDA plates several times until a pure culture was obtained. The pure isolates obtained were then transferred onto sterile PDA slants and stored in the refrigerator at 4 to 8$^{o}$C prior to their identification (Fawole and Oso, 2007).

### Identification of the fungal isolates

The fungal isolates were identified using standard methods described by Fawole and Oso (2007). This was done by matching the microscopic and macroscopic features of each fungal isolate with those of known species described in relevant standard textbooks and journals.

## 3. RESULTS AND DISCUSSION

In this study, 7 fungal isolates were isolated and identified from the rhizosphere of date palm tree, namely *Alternaria alternata, Aspergillus niger, A. terreus, A. candidus, A. fumigatus, Fusarium proliferatum* and *Pyrenochaeta romeroi* while only 3 fungal isolates were identified from the rhizosphere of pawpaw namely *Aspergillus niger, Candida*

*albicans* and *Botrytis cinerea*. From rhizosphere of oil palm, 5 fungal isolates were identified namely *Aspergillus niger*, *Penicillium italicum*, *Mucor* sp., *Trichoderma* sp. and *Rhizospus stolonifer* while 4 fungal isolates were identified from the rhizosphere of shea butter tree namely *Aspergillus niger*, *A. glaucaus*, *Cunninghamella bertholletiae* and *Acremonium kiliense* (Table 1). *Aspergillus niger* is common to the rhizospheres of the economic trees in this study.

These results agreed with the results of previous studies. Sule and Oyeyiola (2012) had earlier reported that fungal species belonging to genera *Aspergillus*, *Rhizopus*, *Penicillium*, *Trichoderma*, *Mucor*, *Botrytis* spp. are significant members of rhizosphere mycoflora of popular crops such as cassava, exhibiting great beneficial rhizosphere effects on their respective host plants while some of them are pathogenic. Edien *et al.* (2016) studied the mycofloral of various plants including oil palm trees and isolated *Fusarium* sp., *Geotrichum* sp., *Verticillium* sp., *Penicillium* sp., *Trichoderma* sp., *Aspergillus* sp. and *Mucor* sp. from the rhizosphere of oil palm. Olahan *et al.* (2016) also isolated *Aspergillus niger*, *Rhizospus stolonifer*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Saccharomyces cerevisiae* and *Neurospora crazza* from the rhizosphere and non – rhizosphere zones of *Corchorus olitorius* (Jute).

*Alternaria alternata* is an opportunistic pathogenic causing leaf spots, rots and blights on many plant parts. *Aspergillus niger* is pathogenic and ubiquitous, growing very quickly. It is one of the most common species of the genus *Aspergillus* and causes black mould disease on fruits and vegetables; it is also a common contaminant of food (Sharma, 2012). *A. terreus* is pathogenic causing folia blight disease of date palm while *A. candidus* causes fruit spoilage of date palm. *A. fumigatus* is useful in recycling carbon and nitrogen from dead organisms. *Fusarium proliferatum* is pathogenic, causing Bayoud disease of date palm while *Pyrenochaeta romeroi* is a soil saprophyte.

*Botrytis cinerea* is also cosmopolitan and pathogenic, attacking more than 200 species of plants in the field, greenhouse and storage rooms (Holz *et al*., 2004). *Candida albicans* is the most pathogenic and prevalent species of the *Candida* genus (Williams *et al.*, 2013). *Acremonium kiliense* is an endophytic fungus (D'Amico *et al.*, 2008). *Cunninghamella bertholletiae* is also pathogenic, causing significant infections in some agricultural crops such as cotton (Panasenko, 2012). *Aspergillus glaucus* is non – pathogenic. Rosli *et al*. (2014) reported that even though *A. niger*, *Penicillium* sp. and *Mucor* sp. are not pathogenic to oil palm, *A. niger* is pathogenic to other crops. *Trichoderma* sp. has biocontrol potential against *Ganoderma boninense*, the pathogen of basal stem rot disease of oil palm tree, so its presence in the rhizosphere of oil palm trees is an advantage. Foody and Tong (2008) reported that *Rhizopus stolonifer* is a pathogen and also plays a key role in the carbon cycle, being decomposers.

**Table 1: Distribution of fungi in the rhizospheres of some economic trees**

| Economic Plants | | | |
|---|---|---|---|
| Date palm | **Pawpaw** | **Oil palm** | **Shea butter** |
| *Aspergillus niger* | *A. niger* | *A. niger* | *A. niger* |
| *Pyrenochaeta romeroi* | *Candida albicans* | *Penicillium italicum* | *A. glaucaus* |
| *A. terreus* | *Botrytis cinerea* | *Mucor* sp. | *Cunninghamella bertholletiae* |
| *A. candidus* | | *Trichoderma* sp. | *Acremonium kiliense* |
| *A. fumigatus* | | *Rhizospus stolonifer* | |
| *Alternaria alternata* | | | |
| *Fusarium proliferatum* | | | |

## 4. CONCLUSION

Mycoflora of rhizospheres of the economic trees studied are majorly pathogenic with only *Trichoderma* sp. being beneficial.

## 5. RECOMMENDATION

The economic trees studied should be given adequate protection against the pathogenic fungal isolates so as to derive maximum economic benefits from their cultivation.

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