



ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH LEAF BLIGHT DISEASE OF MANGO (*Mangifera indica* L.) IN ZARIA, KADUNA STATE, NIGERIA

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ABSTRACT

Mango (*Mangifera indica* L.) is a tropical tree crop belonging to the family of Anacardiaceae. Blight disease include sudden and severe yellowing, browning, spotting, withering, or dying of leaves, flowers, fruit, stems, or the entire plant. This study was carried out to isolate and identify the fungi associated with leaf blight disease of *Mangifera indica*. Leaves were cut into small pieces, sterilized, rinsed in three (3) changes of sterile distilled water, placed in the Petri dishes containing the PDA, incubated at room temperature and observed for fungal growth. The positive plates were sub cultured to obtain pure cultures and the macroscopic and microscopic features of culture were examined. The fungi identified during the research work were: *Fusarium* sp., *Phoma* sp., and *Rhizoctonia* sp. As a pathogen, *Fusarium* infects above-ground and below-ground plant parts, either as the main or secondary pathogen. *Phoma* species and other related genera are known to contain a large number of pathogenic species associated with leaf, grain or stem diseases. *Rhizoctonia* sp. were found to be associated with leaf blight on the diseased mango leaves. The percentage abundance of the fungi isolated are: *Fusarium* sp. (40%), *Phoma* asp. (20%), and *Rhizoctonia* sp. (40%). It is recommended that molecular characterization should be carried out to determine the identification of fungi to species level and proper cultural practices, such as maintaining good hygiene, proper spacing, and removing infected leaves, can help manage the disease.

Keywords: Fungi, Identification, Isolation, *Fusarium* sp., Pathogen, *Phoma* sp., Percentage abundance, *Rhizoctonia* sp.

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INTRODUCTION

Mango (*Mangifera indica* L.) is a tropical tree crop belonging to the family of Anacardiaceae. Mango occupies relatively the same position in the tropics as enjoyed by the apple in temperate America and Europe [1]. The cultivars of mango commonly found in Nigeria are Edward, Early Gold, Local Alphonso, July Haden and Ogbomosho [2, 3]. The tree is cultivated in many tropical and sub-tropical regions of the world. Mango fruits vary in shape, size, color, texture and flavor [4]. It is a large branched, perennial tree, which attains great heights with symmetrical rounded canopy, approximately 30-40 feet tall. Flowers appear in large terminal inflorescence producing fruits. The skin of the fruit may be green, yellow, or red, depending upon the variety of the fruit [4, 5, 6].

Developing countries account for about 98% of the total world production [7]. Despite lack of encouragement to large scale production of tropical fruits, Nigeria occupies 8th position in world ranking of

mango producing countries as at 2002. This suggests the potential of tropical fruits in the country. Sokoto state occupies 10th position among the main mango producing states in the country which included Jigawa, Plateau, Yobe, Kebbi, Niger, Kaduna, Kano, Bauchi, Adamawa, Taraba, and FCT Abuja [7, 8]. Mango came to Nigeria in the 20th century through itinerant merchant missionaries and colonialist and is currently the integral part of indigenous cropping system [9]. The guinea and Sudan zones of Nigeria are credited with producing greater percentage of the fruit in the country.

Plant diseases are classified as infectious and non-infectious depending on the nature of a causative agent. The symptoms of the disease may depend on its cause, nature, and the location of the impact site [10]. Infectious plant diseases are mainly caused by pathogenic organisms such as fungi, bacteria, viruses, protozoa, as well as insects and parasitic plants [11]. Plant organs are damaged by the fungal mycelium, which leads to plant shrinkage, darkening, or compaction. Color changes usually occur due to

chloroplast dysfunction and low content of chlorophyll in the leaves, which manifests itself in the light color of some leaf areas (mosaic discoloration) or the entire leaf (chlorosis) [11, 12].

Blight, any of various plant diseases whose symptoms include sudden and severe yellowing, browning, spotting, withering, or dying of leaves, flowers, fruit, stems, or the entire plant. Most blights are caused by bacterial or fungal infestations, which usually attack the shoots and other young, rapidly growing tissues of a plant. Fungal and bacterial blights are most appropriate to occur under cool moist conditions, and most economically important plants are susceptible to one or more blights, including tomatoes, potatoes, mangoes, and apples, as well as many ornamental species. This study aims to isolate and identify the pathogens causing leaf blight on mango plant within Botanical garden, Ahmadu Bello University, Zaria.

MATERIALS AND METHODS

Study area

This study was carried out in the Mycology Laboratory, Department of Botany, Ahmadu Bello University, Zaria, Kaduna State, Nigeria (Lat: N11°09'087", Long: E007°39'26").

Collection of samples

Ten (10) infected mango leaves samples were collected from the Botanical Garden of Ahmadu Bello University, Zaria and taken to the laboratory for investigation.

Preparation of culture media

Work surface was sterilized using 70% ethanol and then 39 g of Potato Dextrose Agar (PDA) was weighed using a digital weighing balance and dissolved by stirring in a beaker using 1000 ml of distilled water that was measured using the measuring cylinder and was turned into a conical flask and covered with a foil paper. Two capsules of chloramphenicol at the rate 250 mg/ml were added to inhibit bacterial growth and was vigorously shaken. The solution was then heated in a water bath to ensure homogeneity of the medium and then placed in an autoclave at a temperature of 121°C for 15 minutes. The sterilized medium was then allowed to cool. The Petri

dishes and distilled water were sterilized in the autoclave to obtain distilled water and sterilized plates. After the medium has cooled off it was then poured into petri dishes (20 ml/plate) [13].

Culturing and sub-culturing for isolation of fungi

The leaf samples were cut into small pieces and sterilized using a bleach of 1% sodium hypochlorite (NaClO) then rinsed in three (3) changes of sterile distilled water and placed in the Petri dishes containing the PDA. Leaf tissues were then placed into the petri dishes and cultures were incubated at room temperature and observed for fungal growth for a period of 24 hours and then 7 days [14]. The fungal growth was later sub-cultured to obtain the pure cultures of the fungi by picking the portion of the fungal growth carefully with a sterilized wire loop into a fresh PDA. The plates were observed for growth over a period of 5-7 days [14].

Identification of fungal isolates

The macroscopic (growth, morphology, texture and color) and microscopic features of the fungal growth were observed. For the microscopic examination, lacto phenol cotton blue was dropped on a clean slide and a portion of the fungi was teased on it and covered with a slip carefully to avoid air bubbles. It was then mounted on a microscope, the reproductive and vegetative structures were observed and recorded. The identification was confirmed in the pests and diseases management section of the division of Agricultural College (DAC), Samaru, Zaria according to cultural characters described by [15, 16, 17].

Determination of percentage abundance of fungi

Percentage Abundance of Fungi = Number of times a fungus is encountered/Total number of fungi isolated × 100% [18].

RESULTS AND DISCUSSION

The infected leaves of the mango plant typically show a symptom of dark brown necrotic lesions on the surface of the leaves (blight). These lesions vary in size causing the leaves to wither and leading to a general decline in the health of the plant (Plate 1).



Plate I: Infected mango leaves exhibiting leaf blight disease symptoms

Percentage abundance of the fungi isolated is shown in Figure 1. Table 1 shows morphological and microscopic characteristics of the isolated fungi from the Infected mango leaves, while Classification of the fungi isolated from the sample is shown on Table 2

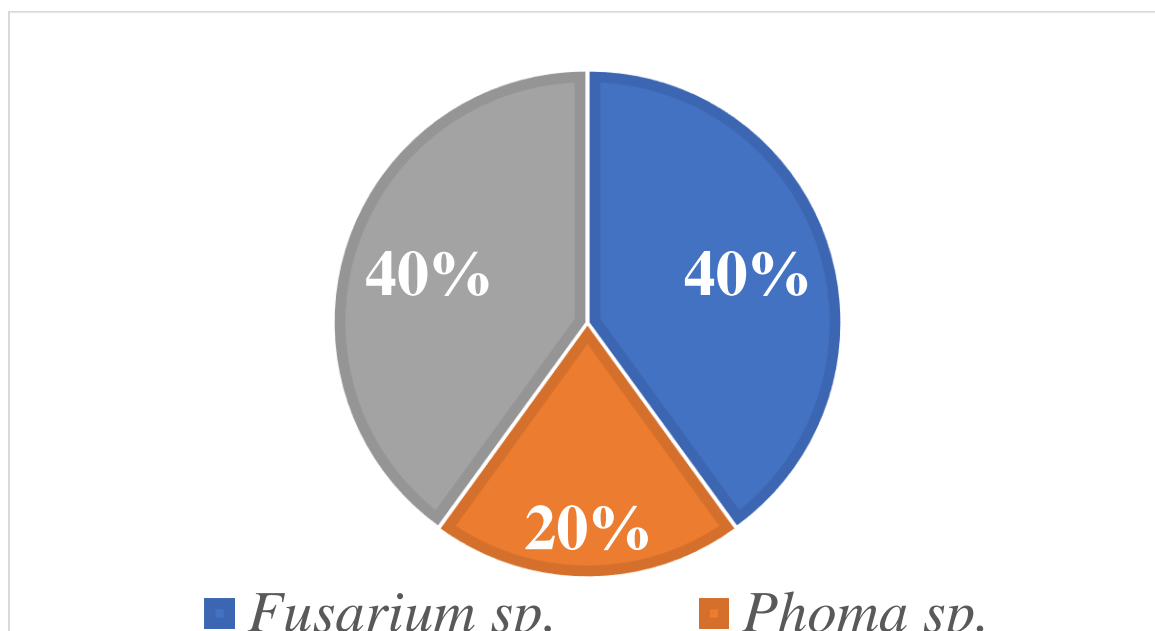


Figure 1: Percentage abundance of fungi associated with the diseased mango leaves

Table 1: Morphological and microscopic characteristics of isolated fungi from the infected mango leaves

Fungal species	Morphology	Reverse side of the plate	Topography	Microscopic Examination
<i>Rhizoctonia</i> sp.	Spongy black	Dark brown	Fluffy/Cottony Growth	It has septate hyphae (divided in to cells by cross walls). It reproduces asexually through production of conidia or sporangia with poorly developed sclerotia (Plate II).
<i>Phoma</i> sp.	Irregular brown	Brown	Flat cottony	It has oval conidia (asexual spores) singly arranged. It has conidiophores (conidia bearing structures). It lacks septation or cross walls and has sclerotia (compact masses of hyphal cells). (Plate IV).
<i>Fusarium</i> sp.	Fast-growing covering a significant portion of plate	Pale yellow and white	Cottony and velvety with flat topography	It has short conidiophores with hyaline conidia and thick-walled chlamydospores. Parasitic on higher plants or saprophytic on decaying plant material (Plate III).

Table 2: Classification of the fungi isolated from the infected mango leaves

Domain	Kingdom	Division	Class	Order	Family	Genus
Eukaryota	Fungi	Basidiomycota	Agaricomycetes	Centharellales	Ceratobasidiaceae	<i>Rhizoctonia</i>
Eukaryota	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	<i>Fusarium</i>
Eukaryota	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	<i>Phoma</i>

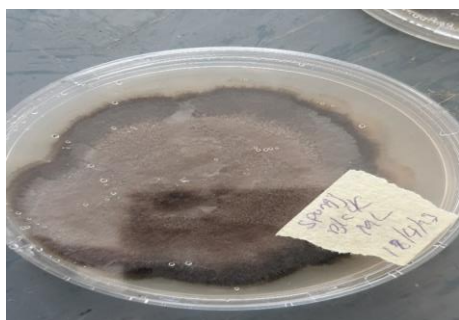


Plate II: A= Macroscopic Features; and B= Microscopic Features of *Rhizoctonia* sp.

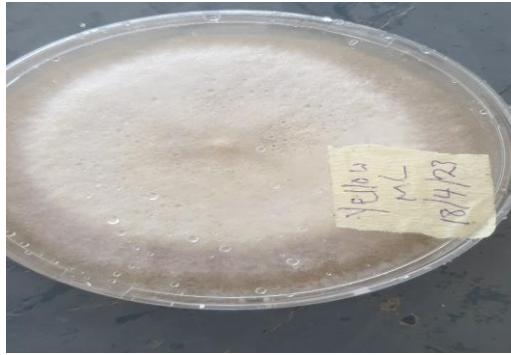


Plate III: A= Macroscopic Features; and B= Microscopic Features of *Fusarium* sp.

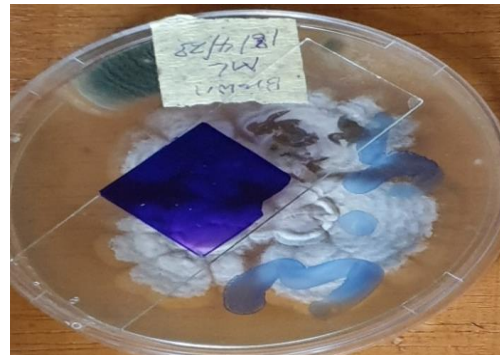


Plate IV: A= Macroscopic Features; and B= Microscopic Features of *Phoma* sp.

The result of this research work shows that *Rhizoctonia* sp., *Phoma* sp., and *Fusarium* sp. were isolated and identified from the mango leaves associated with leaf blight. A total of five (5) pure fungal colonies were isolated and identified at the end of this study: two pure colonies of *Rhizoctonia* sp., two pure colonies of *Fusarium* sp., and one colony of *Phoma* sp.

Many tropical crops are susceptible to infection by *Fusarium*, one of the most significant agricultural plant pathogens. The majority of *Fusarium* species are soil inhabitants, as saprophytes and pathogens. As a pathogen, *Fusarium* infects above-ground and below-ground plant parts, either as the main or secondary pathogen [19]. *Fusarium* species produce conidia which can disseminate through the air, rain splash, and irrigation water. Chlamydospores produced by some of the species can remain in the soil and plant debris for a long period of time [20] and become a source of inoculum. *Fusarium* sp. was isolated from a diseased mango leaf, this conform to the findings of Li [21], who also isolated *Fusarium* species from diseased leaf samples of mango in Sokoto State, Nigeria. The result is also similar to the findings of Long and Mo [22, 23] where they revealed that *Fusarium* is a large genus of filamentous fungi, widely distributed in soil, air and

plants. The species in this genus cause various diseases on host plants, such as wilts, rots and dieback. Furthermore, it was previously reported in Malaysia by Akhtar [24], that *Fusarium* species could cause leaf spots on mango, where *F. proliferatum*, *F. incarnatum* and *F. chlamydosporum* were found to be pathogenic to mango leaves.

This study found *Phoma* species to be associated with the leaf blight disease in mango leaves. These fungi can infect the leaves, resulting in symptoms like irregular brown spots, lesions, and eventually defoliation. Therefore, Leaf blight associated by *Phoma* spp. can impact mango trees' overall health and fruit production. Furthermore, *Phoma* species or fungi related to this genus (e.g. *Macrophoma*) have also been reported as causal agents of diseases on leaves, stems, roots and grains of maize [25, 26, 27]. *Phoma* species and other related genera are known to contain a large number of pathogenic species associated with leaf, grain or stem diseases. These have been shown to damage many important crops worldwide such as citrus lemon [28], sunflower [29, 30], oilseed rape [31, 32], tobacco [33], sorghum [34, 35], wheat [36, 37], rice [38], sugarcane [39] and coffee [40], potentially causing significant economic losses.

Rhizoctonia sp. were found to be associated with leaf blight on the diseased mango leaves, this is similar to the findings of [41 - 45] where *Rhizoctonia* and other species of fungi like *Trichoderma* are reported to be associated with root rot and diseases of mango seedling in different countries.

CONCLUSION

The fungi associated with leaf blight disease of *Mangifera indica* were isolated and identified as *Rhizoctonia* sp., *Phoma* sp., and *Fusarium* sp. in which all of the fungi are pathogens. The percentage abundance of the fungi associated with the leaf blight disease of *Mangifera indica* in the study are: *Rhizoctonia* sp. (40%), *Phoma* sp. (20%), and *Fusarium* sp. (40%). Molecular characterization should be carried out to determine the identification of fungi to species levels. Proper cultural practices, such as maintaining good hygiene, proper spacing, and removing infected leaves, can help manage the disease. Fungicides may also be used, if necessary, but integrated pest management strategies are generally recommended for sustainable disease control.

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