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Fungal Pathogens Associated with Mango Diseases in Usmanu Danfodiyo University, Sokoto State, Nigeria.

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ABSTRACT

Identification of fungal pathogens associated with mango disease in Usmanu Danfodiyo University, Sokoto was carried out in selected Areas of the University, with the aim to Isolate and identify the fungi associated with fruits, leaves and stem, and genotypically characterize the most significantly occurred fungal isolates by PCR assays. Mango trees were selected from four different locations; there are about eight different symptoms associated with the diseases of mango trees in the study site. These include; gum exudation, bark splitting, discoloration and darkening of the bark, wilting of leaves, root decay, whitish substance on and around the root surfaces and wilting of branches. Based on morphological identification, 448 fungal isolates were tentatively identified into 7 genera and 11 species, namely *Alternaria alternata*, *Aspergillus flavus, A. fumigatus, A. niger*, *Colletotrichum gloeosporioides, Fusarium semitectum, Papulaspora* sp., *Penicillium* sp., *Pestalotiopsis guepinii* and *Rhizopus stolonifer* among others. The extracted DNA from the most frequent selected isolates of fungal species was successfully amplified with universal primer ITS1 and ITS4 primer, sequencing confirmed the identification of 7 species of fungi from gel amplicons with 99-100% similarities index. The present study showed that diverse groups of fungal genera were associated with leaf spot of mango. The F*. verticillioides* and L*. theobromae* were found to be associated with anthracnose of mango, implying that other fungal species may act in synergy with *C. gloeosporioides* to cause fungal disease of mango in the study areas. The study recommends awareness creation on good orchard management practices, regular monitoring of the orchards by the management and establishing a link with an expert for advice.

INTRODUCTION

Mango (*Mangifera indica* L.) is the most popular and commonly eaten fruit among millions of people in tropical areas; it is a family member of the *Anacardiaceae*, one of the crops grown abundantly throughout the Nigerian tropics. The cultivars of mango commonly found in Nigeria are Edward, Early Gold, Local Alphonso, July Haden and Ogbomosho (Okibo 2001). The crop is a host to a large number of pathogens such as bacteria, fungi and viruses (Kumar *et al*., 2007). Fungal pathogens are frequently encountered on leafs, stem and rotting mango fruits, and are the major agents of rot after harvest (Diedhiou *et al.*, 2007). Mango tree vegetation in Usmanu Danfodiyo University, Sokoto is under threat by identified and unidentified pathogens. Diseases has rendered its production non attractive to both farmers and gardeners in the university. So far many works were made by researchers on mango fruits collected from different parts of the state, but little or no attention is paid to foliar and stems diseases of the tree.

Studies from several parts of the world where mango is grown have shown that fungi are the most devastating disease which does not only reduce mango fruit yield but also render marketable fruits worthless. In Nigeria, mango production and exportation is greatly limited due to post harvest rot of fruits associated with fungi and over 30% of harvestable fruits are lost annually because of fruit abortions and abscission caused by fungi (Onyeani *et al.* 2012).Like any other crops, mango is also susceptible to diseases caused mainly by Ascomycetes and mitosporic fungi. One of the major diseases of mango is leaf spot, caused by the fungi from the genera *Colletotrichum*, *Alternaria*, *Cercospora*, *Curvularia*, *Cladosporium*, *Ascochyta* and *Botryodiplodia* (Agrios, 2005). Symptoms of leaf spot can vary depending on the fungal pathogen. The spot vary in size and shape but commonly begins with pinhead point's lesion and spread forming circular or irregular lesion with dry, brown or black raised centre. The infection of pathogen will cause chlorosis and necrosis on the leaf surface and thus reduce photosynthetic areas, which affects carbohydrate production as well as nutrient transportation to plant organs (Agrios, 2005). Consequently, the infection will reduce plant growth and fruit yield.

As many fungal genera can cause leaf spot disease, identification of the causal pathogen is important to initiate preventive or curative measures. For that reason, accurate identification of fungal pathogens is necessary to determine appropriate disease control measures as well as to improve disease management. The most prevalent technique used to identify plant pathogens is by observing morphological characters. Morphological characteristic commonly used for identification of fungi, include macroscopic and microscopic characteristics such as colony colour and texture, pigmentation, growth diameter, the shape of conidia, arrangement of spore or conidia, conidiophore, presence of resistant structure such as chlamydospore and presence of fruiting bodies such as pycnidia and acervuli (Watanabe, 2002; Barnett and Hunter, 2006).

In Sokoto state, fungal species associated with mango leaf is not well-documented and its Pathogenicity has not been reported. Therefore, identification and characterization of fungi causing fruits, leaf and stem disease of mango based on morphological and molecular approaches are important in order to protect the plant from further damage as the yield can be affected.

In the last few decades, molecular approaches employing the Polymerase Chain Reaction (PCR) has been found to provide sensitive means of characterizing and classifying plant pathogenic fungal species (Etebu and Osborn, 2012). In this study, molecular approach was adopted in a view to using region of DNA sequence best suitable for accurate identification of the fungal pathogens associated with mango diseases.

MATERIALS AND METHODS

Study Area

The research was carried out at Usmanu Danfodiyo University Sokoto permanent site in Wamako Local Government Area of Sokoto State which is at the extreme of north western block of Nigeria between latitude 11.6^0N-13.9^0N and longitude 3.7^0E-6.9^0E (Bello, 2002). The area falls under Sudan savanna zone characterized by two distinct seasons (wet and dry) of varying duration and intensity (Anon, 2014). The rainy season is often 3-4 month (usually from May to September) with the highest rainfall in August, the mean annual rainfall 500-750mm with, relatively high temperature though it varies with season (Anon, 2014).

Sampling Technique

Purposive sampling was employed to sample out the diseased trees in the study area as the whole area was divided into four (4) locations; each location was further sub-divided into four (4) blocks to serve as a replicate. The locations were:

Location 1: This extends from Kofar Mata through Female hostel, behind Convocation Arena, and the Male hostel in the premises of Abdullahi Fodiyo Library Complex

Location 2: Behind the Department of Bursary, Usman Danfodiyo University, Sokoto.

Location 3: This extended from the University premises down to the Student hall of resident area.

Location 4: The University staff quarters

Survey

Surveys were carried out in July to December, 2021 in Usmanu Danfodiyo University Sokoto permanent site to determine the frequency of occurrence of fungal disease of mango. At each site, 10 randomly selected and 5 year old mangoes were inspected. The frequency of occurrence was taken as the number of mango trees affected by the disease expressed as percentage of the total number of mango trees at a location. A visual assessment technique was used with which many plantations can be evaluated in a relatively short time (Derso 1999). Analysis of variance was used to compare the mean occurrence of the disease at different locations.

Sample Collection

Mango fruits, leaves and stems showing symptoms of disease were collected in a series of sampling in mango farm in the University and several residential areas in the University from July to December. The samples were randomly collected and brought back to the mycology laboratory for fungal isolation. Fungal isolates were given a code based on their location. Several typical symptoms of mango diseases were observed such as dark brown, yellow, grey, red or black spots. Some spots are raised, shiny and others had dropped out leaving ragged holes and some were marked with light and dark concentric halos. Numerous spots develop yellow, reddish brown to black colour, increased in size and merge into large, angular to irregular dead areas.

Fungal Collection and Isolation

Segments of tissues of mango fruits, leaves and stem with symptoms of disease were sectioned and subjected to disinfestations at the Mycology Laboratory of the UDUS, followed by washing and rinsing using sterile distilled water. Then, pieces of tissue (0.1 cm) were transferred to a Petri dish containing Potato Dextrose Agar (PDA) medium and incubated at 25 °C for five days (Alfenas et al., 2016).

Five days after the cultivation of the fungi, 8 mm diameter mycelium discs were inoculated in the center of a Petri dish containing PDA culture medium. The fungi were incubated for 8 days of cultivation, with a daily assessment of the growth diameter of the fungi by means of measurement using a digital caliper. The isolates that showed greater development were referred for molecular characterization by PCR analysis. The experimental design was completely randomized, containing 4 replicates for each isolate. The experimental unit consisted of a Petri dish.

Molecular characterization of the isolates

DNA extraction was performed using the method described by (Kuramae-Izioka, 1997). After DNA purification, the fungi samples were subjected to amplification by the Polymerase Chain Reaction (PCR) of the internal transcript spacer region (ITS) of the ITS1 rDNA, 5.8S-ITS2, according to Mirhendi et al., (2006). The total reaction volume was 25 µL containing the genomic DNA, 0.2 µM of the forward ITS1 primer (5'-TCCGTAGGTGAACCTGCGG-3') and the reverse ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3') Invitrogen TM, 0.4 mM of the dNTP mix; 4 mM MgCl2; and 1.0 U of Platinum Taq DNA Polymerase High Fidelity, in the appropriate enzyme buffer (InvitrogenTM). The amplification conditions were an initial 5 min at 94 °C, followed by 25 cycles of 30 seconds at 94 °C, 45 seconds at 55 °C and 1 minute at 72 °C, with a final extension of 7 minutes at 72 °C, in a Bioneer MyGenie™ 96 thermal cycler.

The amplified fragments were purified using PCR Purification Kit/Ludwig Company. The purified DNA was sequenced using ABI-Prism 3500 Genetic Analyzer (Applied Biosystems). Nucleotide sequences were initially edited using the Bioedit version 7.2.5 software (Hall, 1999). After editing, processing was carried out, which was based on comparing the DNA sequences obtained with those found on the Nucleotide BLAST (National Center for Biotechnology Information Site). With the information provided by BLAST, the most probable species for the fungal isolates were obtained.

Statistical Analysis

The results obtained in this study were subjected to analysis of variance and comparison of means, both at the significance level of α=0.05, using the computational applications Statistic (Statsoft., 2004) and SPSS (IBM Corp. Released, 2020).

RESULT

Symptoms Associated with the Death of Mango Trees

Result on the observed symptoms responsible for the disease of the mango trees in the study site is presented in Table 1.The most frequent visible symptoms associated with various factors responsible for mango tree diseases in the study site, were about 8 different symptoms reported with varying degree of occurrence; Whitish substance on the root was the most common mentioned symptom with 19%, wilting of leaves 14% followed by discoloration and darkening of the bark 13% and gum-exudation 11%. Bark splitting and wilting of the branches/truck and root as well as appearance was consecutively mentioned by 7.4% and 5.6% respectively.

Table 1: Visible Symptoms Associated with the Diseased Mango Trees

Symptoms	Frequency	Percentage (%)
Gum exudation	18	11.11
Bark splitting	12	7.41
Discoloration/darkening of the bark.	21	13.00
Wilted leaves	23	14.00
Root decay	11	6.80
Whitish substance on the root.	32	19.80
Wilted root	16	9.90
Wilted branches and trunk	9	5.60
None	20	12.35

Source: field survey, 2021.

The survey showed that the incidence of Mango plant diseases was highest at Location 2 (72%) and less at location 131% (Table 2).

Table 2: Frequency of Occurrence (%) of Fungal Diseases of Mango in UDUS, Sokoto Nigeria

Location	Frequency of Occurrence (%)
Kofar Mata through Female hostel, Location 1	31.4[d]
Behind Department of Bursary Location 2	72.3[a]
University premises down to Student resident area Location 3	44.6[c]
The University staff quarters Location 4	62.4[b]

Values within the same column followed by the same letter are not significantly different at $P > 0.05$ by Duncan's Multiple Range Test

A total of eleven fungi were isolated from the infected fruits of *Mangifera indica.* The isolated fungi were *Alternaria alternata*, *Aspergillus flavus, A.fumigatus, A. niger*, *Colletotrichum gloeosporioides, Fusarium semitectum, Papulaspora* sp.,*Penicillium* sp., *Pestalotiopsis guepinii* and *Rhizopus stolonifer* (Table 3).

Table 3: Frequency of Fungi Associated with Diseased Mango

Name of fungi	% frequency of fungi from different locations.				
	Location 1	Location 2	Location 3	Location 4	Mean
Alternaria alternate	3	21	9	13	11.50
Aspergillus flavus	5	19	11	15	12.50
A. fumigates	4	16	19	17	14.00
A. niger	11	28	21	26	21.50
Colletotrichum gloeosporioides	7	17	13	19	14.00
Fusarium semitectum	3	11	8	13	8.75
Fusarium verticillioides	7	16	9	11	10.75
Rhizopus stolonifer	8	14	7	12	10.25
Penicillium sp.	2	8	6	6	5.50
Papulaspora sp.	0	4	2	5	2.75
Pestalotiopsis guepinii	1	3	1	3	2.00
TOTAL	51	157	100	140	448

The extracted DNA from selected isolates of fungal specieswas successfully amplified with universal primer ITS1 and ITS4 primer. The size of the amplicon of PCR product was 1000bp (Figure 1). The analysis of the nucleotide sequences of ITS1 region of the isolates produced from their rDNA data showed 7 identified isolates.

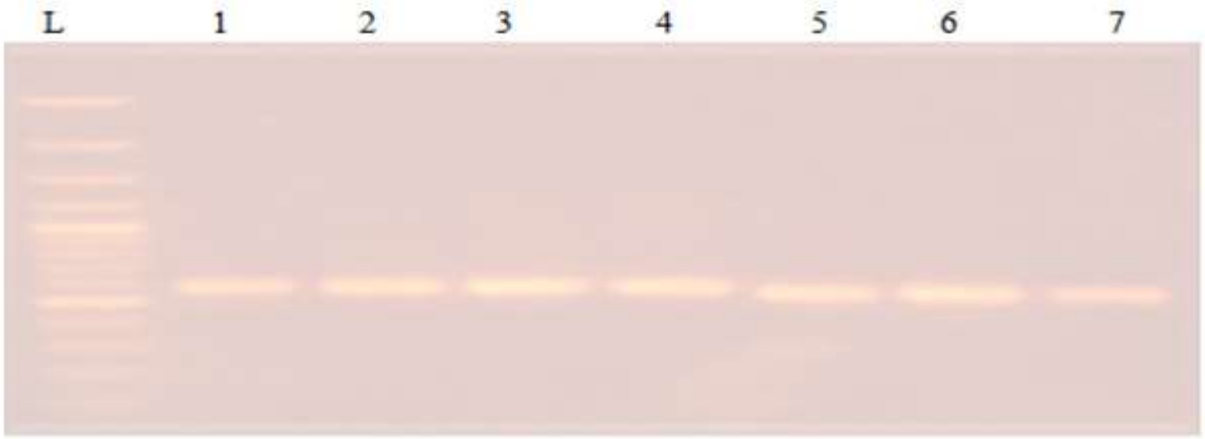

Figure 1: PCR Agarose gel electrophoresis of genes amplified using ITS1 and ITS4 from DNA extracted of7 fungal Isolates.

Thus, DNA from the isolates, which showed greater growth in PDA, were extracted with good quality, and the PCR methodology applied with the universal primers generated fragments of approximately 550 base pairs. With the determination of the sequences of the amplified fragments and their respective editions, and submitting the data to the analysis by BLAST, it was found that the 7 isolates (Table 4) were*Alternaria alternate, Aspergillus flavus, A. fumigates, A. niger, Colletotrichum gloeosporioides, Fusarium verticillioides, Rhizopus stolonifer* respectively.

Table 4: Molecular identification by comparing the sequences obtained from the amplification of the genomic region of the ITS of the rDNA using BLAST in the NCBI

Amplicon No.	ITS 1 and ITS 4	SIMILARITY	SEQUENCE (NT)
1	*Alternaria alternate*	100%	551
2	*Aspergillus flavus*	99%	641
3	*Aspergillus fumigates*	99%	860
4	*Aspergillus niger*	100%	891
5	*Colletotrichum gloeosporioides*	99%	446
6	*Fusarium verticillioides*	99%	441
7	*Rhizopus stolonifer*	98%	398

DISCUSSION

Eleven fungal species were isolated from the infected fruits, leaves and fruits of three varieties of *Mangifera indica.* The isolated fungi were *Alternaria alternata*, *Aspergillus flavus, A. fumigatus, A. niger*, *Colletotrichum gloeosporioides, Fusarium semitectum, F. verticolor, Papulaspora* sp.*, Penicillium* sp.*, Pestalotiopsis guepinii* and *Rhizopus stolonifer.* All these isolates were identified as responsible for the disease of mangoes in Usmanu Danfodiyo University, Sokoto.

This result is also consistent with reports by several workers implicating *C. gloeosporioides* as the causal agent of diseases in mango (Than *et al.*, 2008; Kim *et al*., 2008; Sangeetha and Rawal, 2009; Jayasinghe and Fernando, 2009). This study identified *F. verticillioides* and *L. theobromae* tobe associated with anthracnose of mango, implying that other fungal species may act in synergy with *C. gloeosporioides* to cause fungal disease of mango. Several workers including Johnson (2008), had implicated other fungal species in earlier reports, to be responsible for postharvest diseases of mango associated with fruit rotting during ripening, worldwide. Okereke *et al*. (2010) also reported the isolation of these fungal species from infected mangoes in their study.

Maqsood *et al*. (2014) identified *C. gloeosporioides, Lasiodiplodia theobromae, Alternaria alternata, Aspergillus niger* and *Dothiorella domonicana* from Sindhri mango fruits, in which *C. gloeosporioides* was found to be the most prevalent in Pakistan. Rajmane and Korekar (2016) reported that *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Botryodiplodia theobromae, C. gloeosporioides, Penicillium chrysogenum* and *R. stolonifer* associated with the spoilage of mango fruit in India. Abdullah *et al.* (2016) found that *Alternaria alternata, A. aculeatus, Aspergillus flavus, A. japonicas, A. niger, A. parasiticus, Eurotium amstelodami, Mucor circinelloides, Penicillium*

viridicatum, Rhizopus arrhizus, Trichoderma koningii, T. harzianum and *Verticillium tenerum* were associated with post harvest rot disease of mango in Yemen. All the fungi associated with mango as pathogens deteriorate fruits.

In this study, a fragment was amplified by PCR from fungal isolates with universal primer specific for distinguishing fungal species. The nucleotide sequences of the ITS1 region of species, implying that all the *Colletotrichum* species isolated were *C. gloeosporioides* and not *C. acutatum.* This result agrees with Nigeria Plant Quarantine Service (2002) which listed *C. gloeosporioides* as the causal agent of mango fruit anthracnose in Nigeria although there were no representation of pathogen isolation and identification in their study. For the identification of all the 7 fungal isolates that belonged to the 4genera, pathogenicity test that confirm the pathogenesis of *C. gloeosporioides* implied that, *C. gloeosporioides* was the pathogen responsible for anthracnose inmango, in Nigeria and not *C. acutatum* as listed by CAB International (2007).

CONCLUSION

Fungal diseases of mango have become a menace to many garden and orchards in the study area, sometimes resulting in complete loss of the crop in some locations. This identification method clearly identified the 7 most frequent fungal species isolated from mango fruits, leaves and stems in Usmanu Danfodiyo University, Sokoto. In addition, A. *niger* and *C. gloeosporioides* were found to be the fungus responsible for anthracnose of mango in the study area.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

REFERENCES

Abdullah Q, A Mahmoud and A Al-Harethi (2016). Isolation and identification of fungal postharvest rot of some fruits in Yemen. PSM Microbiology 1(1): 36-44.

Agrios, G.N (2005). Plant Pathology. Fifth (5th) edition. Pp. 27-557.

Barnett, H.L. and Hunter, B.B. (1998). Illustrated Genera of Imperfect Fungi. APS Press Pp.163-193.

CAB International (2007). Pests excluded from Phytosanitary measure assessments because they already occur in Nigeria. Retrieved December 13, 2008, from CABI Crop Protection Compendium software.

Derso E. (1999): Occurrence, prevalence and control methods of phaeoramularia leaf and fruit spot disease of citrus in Ethiopia. Fruits, 54: 225–232.

Diedhiou, P.M., Mbaye, N. Drame, A. & Samb, P.I. (2007). Alteration of post-harvest diseases of mango *Mangifera indica* through production practices and climatic factors. *African Journal of Biotechnology,* 6(9): 1087 -1094.

Etebu, E. & Osborn, A. M. (2012). Molecular approach to plant fungal identification in agricultural soils. *Nigerian Journal Plant Protection,* 26: 18 -34.

Hall, T.A. (1999). BioEdit: A User-friendly biological sequence alignment editor and analysis program for window 95/98/NT, *Nucl. Acids Symp. Ser.* 41: 95 – 98.

Jayasinghe, C.K. & Fernando, T.H.P.S. (2009). First Report of *Colletotrichum acutatum* on *Mangifera indica* in Sri Lanka. Cey. *Jour. Sci. (Bio. Sci.)* 38(1): 31 – 34.

Johnson, G.I. (2008). Status of mango post harvest disease management R and D: Options and solutions for the Australian mango industry. *Horticulture Australia final report for project MG08017. Horticulture Australia* p.130

Kim, J.K., Park, S., Choi, W., Lee, Y. & Kim, H.T. (2008). Characterization of *Colletotrichum* Isolates Causing Anthracnose of Pepper in Korea. *Plant Pathol. Jour.* 24(1): 17 – 23.

Kumar, A.S., Reddy, N.P.E., Reddy, K.H. & Devi, M.C. (2007). Evaluation of Fungicidal Resistance among *Colletotrichum gloeosporioides* Isolates Causing Mango Anthracnose in Agri Export Zone of Andhra Pradesh, India. *Plant Patho. Bull.* 16: 157 – 160.

Maqsood A, A Rehman, I Ahmad, M Nafees, I Ashraf, R Qureshi, M Jamil, M Rafay and T Hussain (2014). Physiological attributes of fungi associated with stem end rot of mango (*Mangifera indica* L.) varieties in postharvest fruit losses. Pak. J. Bot. 46(5): 1915-1920.

Nasiru A. M and Idris M (2021). Study of the Causes of Death of Mango Stands in Some Fadama Lands of Sokoto Metropolis, Nigeria. *Cross Current Int J Agri Vet Sci, 3*(3), 15-21.

Nasiru, A. M. Salau, I. A and M. Yakubu, (2015), Fungi Associated With Spoilage of *Citrus Sinensis* in Fruits and Vegetables Market, Sokoto, Nigeria. *Global Advanced Research Journal of Agricultural Science* 4(12): 919-922.

Nigeria Plant Quarantine Service (2001). Check-List of Plant Pests in Nigeria. NPQS Publ. Ibadan Univ. Printry, Ibadan, Nigeria.

Okereke, V.C., Godwin-Egein, M.I. & Arinze, A.E. (2010). Assessment of Postharvest Rot of mango at Different Stages of market in Port Harcourt, Nigeria. *Int'l Jour. Curr. Res.,*11:006 – 010.

Okibo R.N. (2001): Occurrence, pathogenicity and survival of *Macrophoma mangiferae* in leaves branches and stems of mango (*Mangifera indica* L.). Plant Protect. Sci., 37: 138–144.

Rajmane SD and SL Korekar (2016). Isolation and identification of fungi associated with spoilage of mango fruit, India. International Journal of Scientific Research 5(8): 2277-8179.

Sangeetha, C.G. & Rawal, R.D. (2009). Temperature Requirement of Different Isolates of *Colletotrichum gloeosporioides* Isolated from Mango. *American-Eurasian Journal of Scientific Resources* 4(1): 20 – 25.

Than, P.P., Prihastuti, H., Phoulivong, S., Taylor, P.W.J. & Hyde, K.D. (2008). Chilli anthracnose disease caused by *Colletotrichum* species. Jour. of Zhejiang Univ. Sc. Vol. 9 No. 10, pp. 764 – 778.

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