Isolation, characterization and identification of pathogenic fungal species associated with mango anthracnose in Northwest Nigeria

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Abstract

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Colletotrichum species have been found to be the causal agents of fruit and leave anthracnose of mango. It is the most essential postharvest disease restraining shelf life and export of fresh mango fruits in Nigeria. In the present study, four different fungi were isolated and identified, their pathogenicity confirmed both on leaves and fruits as *Colletotrichum gloeosporioides, Alternaria alternata, Botrytis cinerea* and *Curvularia* sp. All fungi were pathogenic to leaves and fruits and induced symptoms. However, *C. gloeosporioides* produced typical anthracnose symptom and highest lesion size at 7th day after inoculation using pinprick method of inoculation. Mycelial growth of *C. gloeosporioides* varied significantly with media. Maximum growth and spore production was observed on potato dextrose agar at 14th day, followed by oat meal agar. No mycelial growth of *C. gloeosporioides* on cornmeal and potato agar was observed. Highest lesion size 7 mm at 7th day after inoculation using the pinprick method was observed on *C. gloeosporioides*. Pathogenicity showed that tissue injury created by pinpricks supports the growth of *C. gloeosporioides.* From all the isolate only *Colletotrichum gloeosporioides* was identified to be the fungus responsible for anthracnose of mango in northwestern Nigeria.

Keywords: mango; anthracnose; fungal species; *Colletotrichum gloeosporioides*; pathogenicity

INTRODUCTION

Mango (*Mangifera indica* L.) is a member of the *Anacardiaceae* family, tropical crops full-grown profusely all through northern Nigeria. The crop is a host to a large number of pathogens like bacteria, fungi, and viruses (Diedhiou et al., 2007; Kumar et al., 2007). Studies from quite a lot of parts of the globe where mango is grown have revealed that anthracnose is the mainly devastating fungal disease which not only reduces mango fruit yield but also renders marketable fruits worthless. Anthracnose (*Colletotrichum gloeosporioides* Penz. Sacc.) is the most important field and post-harvest disease of mango in all mango-growing regions of the world (Ploetz and Prakash, 1997; Chowdhury et al., 2008; Sangeetha and Rawal, 2009), especially where high humidity prevails during the cropping season. The disease had been reported on mango fruits produced in the humid forest region of Nigeria (Onyeani et al., 2012). Mango fruit rots quickly after harvest rendering marketable fruits unattractive and worthless (Yusuf and Salau, 2007). Post-harvest infection is economically the most significant phase of the disease worldwide and is directly linked to the field phase as initial infection starts on young twigs and leaves spreading to the flowers, and causing blossom blight thereby destroying the inflorescences and preventing fruit set (Akem, 2006). In Nigeria, mango production and exportation are greatly limited due to post-harvest rot of fruits associated with anthracnose and over 30% of harvestable fruits are lost annually because of fruit abortions and abscission caused by anthracnose (Onyeani et al., 2012).

The aim of this study was to isolate, identify and ascertain pathogenicity of fungi associated with anthracnose of mango in northwestern Nigeria.

MATERIAL AND METHODS

Planting materials and survey locations

A total of 21 varieties from four orchards were used for this study. These orchards are: Institute for Agricultural Research (IAR), Zaria, (11° 04' 00"N; 07° 42' 00"E) in Sabon Gari Local Government Area (Northern Guinea Savannah), Niyya (10^0 20'58"N; 07^0 45'00 "E) in Kachia Local Government Area (Southern Guinea Savannah) both in Kaduna State; Dabiran (13^o 02' 11'N; 8^o 19' 04'E) in Daura Local Government Area of Katsina State (Sahel Savannah) and Yanbukar (11^0 49 'N; 8^0 51' E) in Wudil Local Government Area of Kano State (Sudan Savannah) (Fig. 1). Throughout the assessment, infected mango fruits and leaves samples were also collected from these orchards and taken to the laboratory for further studies. Ten mango varieties were assessed at Niyya, six assessed at IAR, Zaria, five varieties in Dabiran and five varieties in Yanbukar.

Isolation and identification of fungi

Samples of infected mango leaves and fruits collected from each orchard were brought to the Vegetable Pathology Laboratory in the Department of Crop Protection, Ahmadu Bello University, Zaria in sample bags for further studies. Small tissue (2 x 3 mm) of the infected portion were cut using a scalpel, disinfected with 1% Sodium hypochlorite (NaOCl) for three minutes, rinsed in three changes of sterile distilled water (SDW) and plated in 9 cm Petri dishes containing Potato Dextrose Agar amended with streptomycin sulfate (PDAs). The Petri dishes were incubated for five days at room temperature (28 ± 2°C), fungal mycelia were sub-cultured into freshly prepared PDAs to obtain pure cultures. The cultures were identified by cultural and microscopic examinations using manual identification (Barnett and Hunter, 2006). Pure cultures obtained were placed in labeled slant bottles and kept for future use. Specimens of isolated fungi were sent to International Mycological Institute (IMI), Egham, Surrey, U.K., for confirmation of identity.

Preparation of inoculum and inoculation

Isolated fungi (*Colletotrichum* sp., *Alternaria* sp., *Botrytis* sp., and *Curvularia* sp. were grown

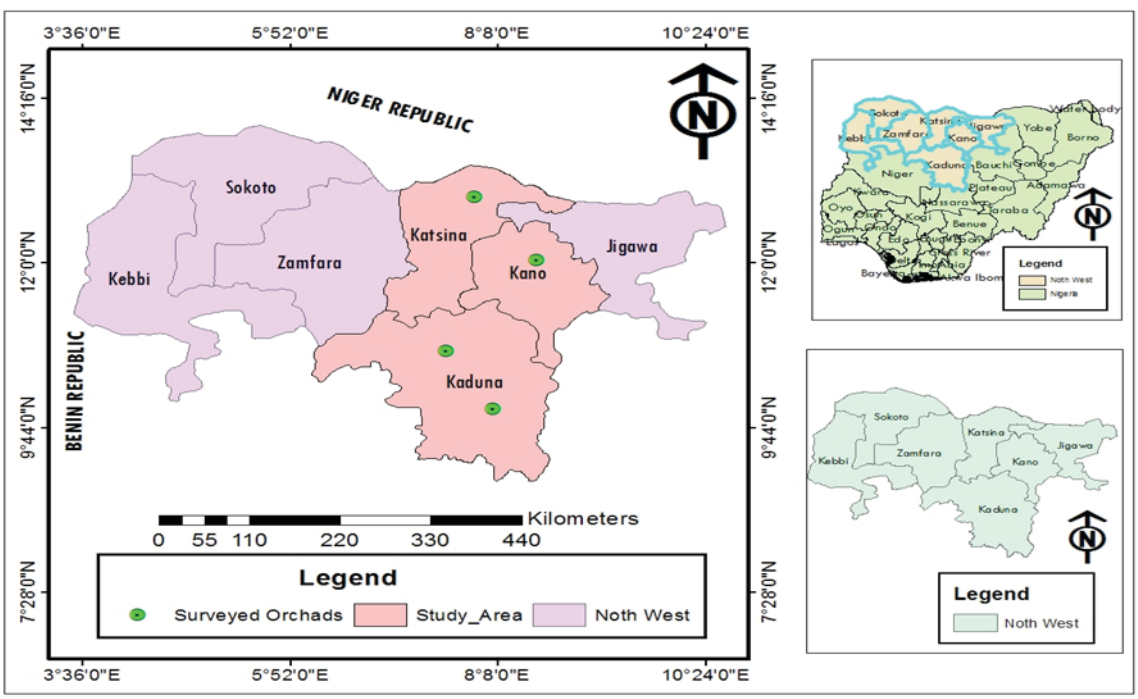

Fig. 1. Location of survey orchards in three states of North-Western Nigeria

on potato dextrose agar (PDA) in the laboratory for fourteen days for the organisms to sporulate. Ten milliliters of sterile distilled water (SDW) was added to each Petri dish and mycelial mat from the culture harvested using a sterile scalpel. The mycelia obtained was blended at low speed in an electric blender for 2 min, 200 ml of SDW was added in 500 ml conical flask and filtered using a double layer sterile muslin cloth. The number of conidia was determined using hemocytometer and conidia concentration adjusted to 10^6 conidia/ml.

Inoculation of fruits

Fruit wounding technique described by Sun et al. (2008) was used. Twenty green matured mango fruits (Temo) were collected from the IAR orchard, Zaria, washed thoroughly under running tap water, surface sterilized using 1% sodium hypochlorite (NaOCl) for 30 seconds and rinsed in three changes of SDW. Five fruits were used for each fungal isolate. The fruits were injured slightly with pinpricks using a sterilized needle. Spore suspension (0.5 ml/fruit) was sprayed over the fruits using a syringe. Fruits sprayed with SDW after pinprick injury served as control. Inoculated fruit surface was covered with moist cotton wool and incubated at room temperature (28±2°C) for five days. Disease symptoms were observed only to see whether the fungi are pathogenic or not, no data was taken, therefore, no analysis was done. Causative organisms were re-isolated and compared with the original culture to confirm Koch's postulates.

Inoculation of leaves

Mango stones sourced from Institute of Agricultural Research (IAR), Zaria, were surface sterilized, planted in 20 cm diameter plastic pots filled with heat-sterilized soil and arranged on a glasshouse bench. One stone per pot was planted and watered regularly. Seedlings were arranged in completely randomized design (CRD) repeated four times. At 12 weeks after sowing 8-10 leaves stage, the upper axial leaf surfaces of the mango seedlings were inoculated with the conidia of four fungi – *C. gloeosporioides, B. cineria, A. alternate* and *Curvularia* spp. using three inoculation methods infusion, pinpricking and droplet. The infusion method involved injecting 0.1 ml spore suspension of the inoculum into the midvein using a sterile hypodermic syringe, the inoculum concentration used was 10^6 spores/ml. For the Pinprick method, the mango leaf was injured by creating pricks with a sterile needle, 0.1 ml spore suspension was dropped on injured pricks. Droplet method involves dropping 0.1 ml spore suspension on an intact leaf surface using a micropipette. Four seedlings were inoculated in each of the inoculation methods. Seedlings inoculated with SDW served as control. High relative humidity around the plants was maintained by covering potted seedlings with polyethylene bags 24 hours before inoculation. Inoculated and control plants were labeled and covered for another 24 hours to maintain high humidity. After 24 hours the plants were watered and aerated for 20 minutes and by 48 hours the polyethylene bags were finally removed. These seedlings were observed daily for symptoms, data on lesion size was also taken daily for seven days after inoculation. Fungi were re-isolated from the leaves that showed symptoms and the cultures obtained were compared with the original culture to confirm Koch's postulates. Data on lesion size produced by each fungus were taken daily for 7 days.

Evaluation of media for the growth and sporulation of *Colletotrichum gloeosporioides*

Colletotrichum gloeosporioides isolated and identified was inoculated on four different media to identify the best media for its growth and sporulation. Potato agar (PA), cornmeal agar (CMA), oat meal agar (OMA) and potato dextrose agar (PDA) were tested. For media preparation, 200 g of peeled potato and 30 g each of oat flakes and corn were weighed separately and to each, 20 g of agar, were added in 1000 ml sterile distilled water (SDW). For PDA, 200 g of peeled potato, 20 g each of dextrose and agar were weighed and added to 1000 ml of SDW. These were autoclaved separately at 121°C under 15 *psi* for 20 min, and each media amended with streptomycin (0.6 µg/L) before pouring. Five mm diameter discs from 7-day old cultures of the isolated fungus were cut with a sterilized cork borer and placed in the center of 9 cm diameter Petri dishes containing 20 ml of each medium. Five Petri dishes were inoculated per media type and the Petri dishes were arranged in a Completely Randomized Design (CRD) on a laboratory bench. The inoculated media were incubated at room temperature (28±2°C) and observed daily for mycelia growth. Radial mycelia growth was measured at 48 hours interval for fourteen days after inoculation (DAI), by measuring the

diameter along two perpendicular lines drawn on the underside of the Petri dishes. The radial mycelia growth was taken in millimeter (mm). Sporulation of the fungus was determined at 14 days after inoculation. For each treatment, conidia suspension was prepared from the mycelial growth harvested using a sterile scalpel from each treatment. The harvested mycelial growth from each repetition was diluted in 100 ml SDW, blended and sieved using a double layer muslin cloth. Spore concentration was obtained by adding 10 ml of initial sieved inoculum to 90 ml of sterile distilled water and counting the spores under a light microscope with the aid of a hemocytometer. Five hemocytometer readings were taken per treatment.

Total number of spores/ml was calculated using the formula below:

$$\frac{n}{256} \times 4 \times 10^6$$

where: n = number of conidia counted in the chambers

256 = constant volume obtained from 16×16 squares of haemocytometer;

$4x10^6$ = constant.

Data analysis

Data on lesion size from each treatment taken were subjected to analysis of variance (ANOVA) means separated using Student-Newman-Keuls (SNK) test at 5% level of significance. Other data collected were subjected to analysis of variance (ANOVA) using SAS software version 9 (2002). Means were separated using Student-Newman-Keuls (SNK) test or LSD where suitable at 5% level of significance ($P<0.05$).

RESULTS

Isolation and identification of fungi

Fungi isolated from the infected mango leaves and fruits were identified as *Colletotrichum* sp., *Curvularia* sp., *Alternaria* sp. and *Botrytis* sp. These were confirmed by the International Mycological Institute (IMI) Egham, Surrey, U.K as *Colletotrichum gloeosporioides* (703252), *Alternaria alternata* (703251) and *Botrytis cinerea* (703254); *Curvularia* sp. (703253) was not identified to species level.

Morphology and cultural characteristic of *Colletotrichum gloeosporioides* on PDA

C. gloeosporioides on potato dextrose agar had cottony white with regular smooth margin. Microscopic examination of 14 day-old culture showed the presence of smooth septate conidia (Plate I).

Morphology and cultural characteristic of *Curvularia* sp. on PDA

Curvularia sp. on potato dextrose agar was grey in color, and black on the backside, presented irregular borders, cottony growth with concentric zones. Conidia formed were 4-celled, mostly immersed, slightly curved and forming smooth conidiophores, which appeared roughed when observed under a microscope (Plate II).

Morphology and cultural characteristic of *A. alternata* on PDA

On PDA *A. alternata* produced profuse mycelial growth. Initially, the mycelium was hyaline but later turned grey-brownish. In the early stage of growth,

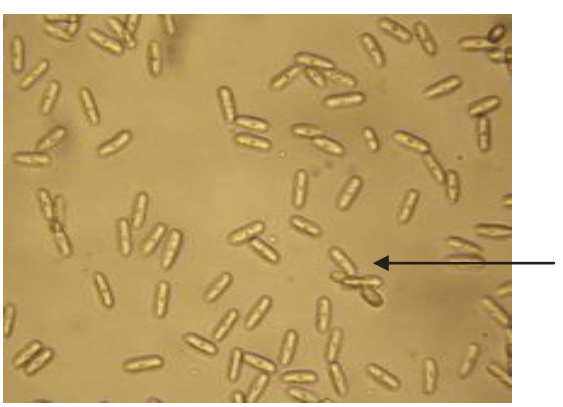

Plate I. Conidia of *Colletotrichum gloeosporioides* (×100)

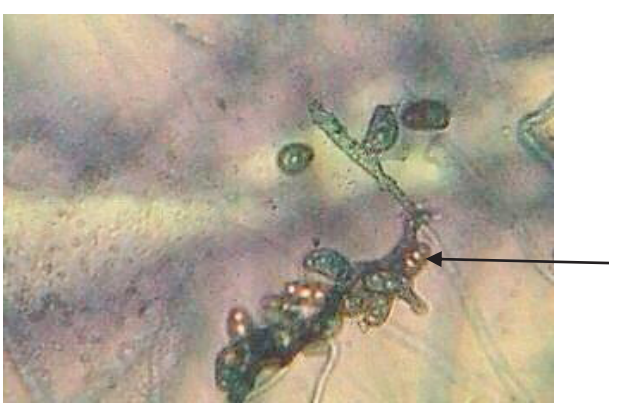

Plate II. Conidium of *Curvularia* sp. (×100)

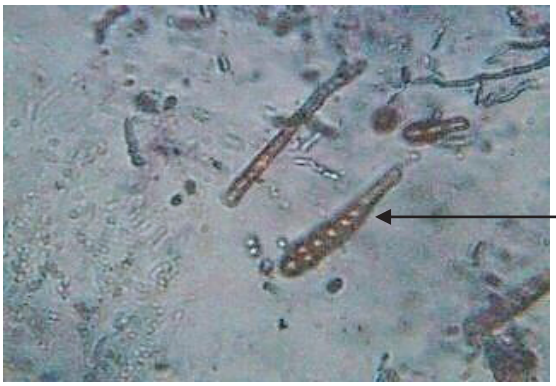

Plate III. Conidium of *Alternaria alternata* (×100)

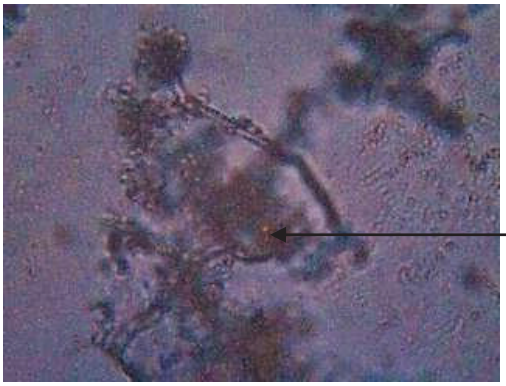

Plate IV. Conidia of *Botrytis cinerea* (×100)

hyphae were thin, narrow, but become thick as they grew old (Plate III).

Morphology and cultural characteristic of *Botrytis cinerea* on PDA

B. cinerea grown on PDA had thick and woolly mycelial growth. The cultures exhibited white to grey color, while the reverse side of the colonies was white to dark grey and orange in color. Microscopic examination of 14 day-old culture showed a mass of conidia (Plate IV).

Pathogenicity test on fruits of mango

Pathogenicity tests carried out on mango fruits with three fungi (*Colletotrichum gloeosporioides*, *Alternaria alternata,* and *Botrytis cinerea*) isolated from symptomatic fruits showed that only *Colletotrichum gloeosporioides* reproduced anthracnose symptom typical of those observed on collected diseased fruits. *Botrytis cinerea* inoculated resulted in rotting symptoms on fruits typical of fruit rot or soft rot. *Alternaria alternata* inoculated led to the production of brown to black spot symptoms on the fruit typical of black rot. Cultures re-isolated from the inoculated fruits were similar to those of the original isolates used for the inoculation.

Pathogenicity test on mango leaves

Leaves, inoculated with the four fungi using pinprick and infusion methods produced symptoms. However, no lesions were produced when leaves inoculated using droplet method. There was a significant difference between lesion sizes of the inoculated fungi at 7th day after inoculation (DAI). The lesions induced by *C. gloeosporioides* were higher than those caused by other fungi from 1st-7th DAI (Table 1). The least lesion size was recorded on *B. cinerea* and *Curvularia* sp. from 1st-7th DAI, respectively. The lesions induced by *C. gloeosporioides* were larger than those caused by other fungi in all the inoculation methods. In infusion method, C. *gloeosporioides* induced lesion size up to 4 mm at 7th DAI. The lesions were first noticed 24 h after inoculation. The fungus *C. gloeosporioides* induced lesion size of 2.93 mm at 7th DAI, while *Curvularia* sp. recorded the least 1.86 mm at 7th DAI, although they was no significant difference with the lesion size observed on *B. cinerea* (Table 1). Infusion method had the highest lesion size on all the tested fungi from 1st-7th DAI (Table 1). Highest lesion size of (4.50 mm) at 7th DAI was recorded using infusion method, followed inoculation method (4.19 mm) at 7th DAI. There was no lesion size observed in the droplet method of inoculation.

Effect of fungi and the different method of inoculation on lesion sizes

Significance differences ($P \leq 0.05$) exist among the fungi and the inoculation methods on the lesion size observed (Table 2a, 2b). At day I *C.gloeosporioides* had the highest lesion size on pinprick method (3.00 mm), and the least recorded on *A. alternata* (1.77 mm) while with infusion method *A. alternata* recorded the highest lesion size (2.01 mm) although no significant difference exists between the lesion size observed on *B. cinerea* (1.81 mm), no lesion was observed using droplet and the control methods at all the days of inoculation (Table 2a).

Table 1. Lesion size produced by the fungi using three inoculation methods

	Days of lesion size (mm)						
Fungi	1	2	3	4	5	6	7
C. gloeosporioides	1.00a	1.56a	2.00a	2.28a	2.50a	2.71a	2.93a
Alternaria alternata	0.94b	1.31b	1.68b	1.74b	1.76b	1.97b	2.00b
Botrytis cinerea	0.45c	0.44d	1.11d	1.55c	1.77b	1.81c	1.89c
Curvularia sp.	0.44c	0.89c	1.24c	1.42d	1.62c	1.79c	1.86c
SE±	0.004	0.019	0.018	0.011	0.010	0.027	0.022
Methods							
Infusion	1.65a	2.53a	3.32a	3.85a	4.07a	4.37a	4.50a
Pinprick	1.19b	1.67b	2.71b	3.14b	3.59b	3.91b	4.19b
Droplet	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c
Control	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c
SE±	0.004	0.019	0.018	0.011	0.010	0.027	0.022
Interaction							
Fungi × methods	**	**	**	**	**	**	**

Means with the same superscript in a column are not significantly different at 5% level of significance (P≤0.05) using Student-Newman-Keuls (SNK) test; ** = highly significance at 5%

The fungus *C. gloeosporioides* produced the highest lesion size in the pinprick method of inoculation at 2 DAI (4.00 mm) and the least (2.69 mm) observed on *A. alternata.* In infusion method of inoculation at 2 DAI *Curvularia* sp. had the highest lesion size (3.56 mm) (Table 2a).

Pinprick method significantly had the highest lesion size on *C. gloeosporioides* at 3 DAI (5.00 mm) and the least lesion size on this inoculation method (0.60 mm) was observed on the fungus *Curvularia* sp. In infusion inoculation method, *Curvularia* sp. recorded the higher lesion size (4.34 mm) at 3 DAI and the least lesion size on this method was recorded on *B. cinerea*, which is not significantly different with the lesion observed on *C. gloeosporioides* using the same method (Table 2a).

At 4 and 5 DAI, the fungus *Curvularia* sp. recorded the highest lesion size of (4.80 and 4.91 mm) with infusion method of inoculation respectively, no significant difference on lesion produced by other fungi using infusion method at 4 DAI (Table 2b). In pinprick method at 4 and 5 DAI *C. gloeosporioides* recorded the highest lesion size (5.50 and 6.00 mm) respectively, the least lesion size (0.88 and 1.59 mm) was observed on *Curvularia* sp. (Table 2b).

At 6 DAI, *Curvularia* sp. had the highest lesion size using infusion method of inoculation (5.36 mm), followed by *C. gloeosporioides* (4.25 mm) and the lowest lesion size was observed on *A. alternata*, although there was no significant difference observed with the lesion induced by *B. cinerea.* The fungus *C. gloeosporioides* recorded the highest lesion size (7.12 mm) using pinprick method of inoculation 7 DAI, the lowest lesion size (1.80 mm) was observed on the fungus *Curvularia* sp. (Table 2b).

Pathogenicity test conducted with all the four fungi (*Colletotrichum gloeosporioides, Alternaria alternata, Botrytis cinerea,* and *Curvularia* spp.) isolates from mango were pathogenic to mango leaves and able to induced symptom using pinpricking and infusion inoculation methods as indicated using arrows in (Plate V-VIII).

Effect of media type on growth and sporulation of *C. gloeosporioides*

Highly significant differences (P≤0.05) were recorded for mycelial growth of *C. gloeosporioides* on different media 14 DAI (Fig. 2). Among the four different nutrient media tested, highest growth (89.70 mm) was observed on PDA, followed by oat meal

Table 2a. Effect of fungi and the different method of inoculation on lesion size (mm) (1-3 DAI)

	Day 1					Day 2					Day 3				
	Methods					Methods					Methods				
Fungi	inf	pin	dro	cont	SE±	inf	pin	dro	cont	SE±	inf	pin	dro	cont	SE±
Cgl	1.00c	3.00a	0.00	0.00	0.008	2.25c	4.00a	0.00	0.00	0.040	3.00c	5.00a	0.00	0.00	0.037
Alt	2.01a	1.77b	0.00	0.00	0.008	2.55b	2.69b	0.00	0.00	0.040	3.26b	3.46b	0.00	0.00	0.037
Bot	1.81a	0.00c	0.00	0.00	0.008	1.78d	0.00	0.00	0.00	0.040	2.68c	1.78c	0.00	0.00	0.037
Cur	1.76b	0.00c	0.00	0.00	0.008	3.56a	0.00	0.00	0.00	0.040	4.34a	0.60d	0.00	0.00	0.037
SE±	0.008	0.008	0.008	0.008	0.008	0.040	0.040	0.040	0.040	0.040	0.037	0.037	0.037	0.037	0.037

Means with the same superscript in a column are not significantly different at 5% level of significance (P≤0.05) using Student-Newman-Keuls (SNK) test

Cgl = *C. gloeosporioides*; Alt = *Alternaria alternate*; Bot = *Botrytis cinerea*; Cur = *Curvularia* sp.; inf = infusion; pin = pinprick; dro = droplet; cont = control

Table 2b. Effect of fungi and the different method of inoculation on lesion size (mm) (4-6 DAI)

	Day 4					Day 5					Day 6				
	Methods					Methods					Methods				
Fungi	inf	pin	dro	cont	SE±	inf	pin	dro	cont	SE±	inf	pin	dro	cont	SE±
Cgl	3.62b	5.50a	0.00	0.00	0.088	4.00b	6.00a	0.00	0.00	0.021	4.25b	6.57a	0.00	0.00	0.056
Alt	3.40b	3.57b	0.00	0.00	0.088	3.43d	3.63b	0.00	0.00	0.021	3.90c	4.00b	0.00	0.00	0.056
Bot	3.57b	2.63c	0.00	0.00	0.088	3.94c	3.16c	0.00	0.00	0.021	3.97c	3.28c	0.00	0.00	0.056
Cur	4.80a	0.88d	0.00	0.00	0.088	4.91a	1.59d	0.00	0.00	0.021	5.36a	1.80d	0.00	0.00	0.056
SE±	0.088	0.088	0.088	0.088	0.088	0.021	0.021	0.021	0.021	0.021	0.056	0.056	0.056	0.056	0.056

Means with the same superscript in a column are not significantly different at 5% level of significance (P≤0.05) using Student-Newman-Keuls (SNK) test

Cgl = *C. gloeosporioides*; Alt = *Alternaria alternate*; Bot = *Botrytis cinerea*; Cur = *Curvularia* sp.; inf = infusion; pin = pinprick; dro = droplet; cont = control

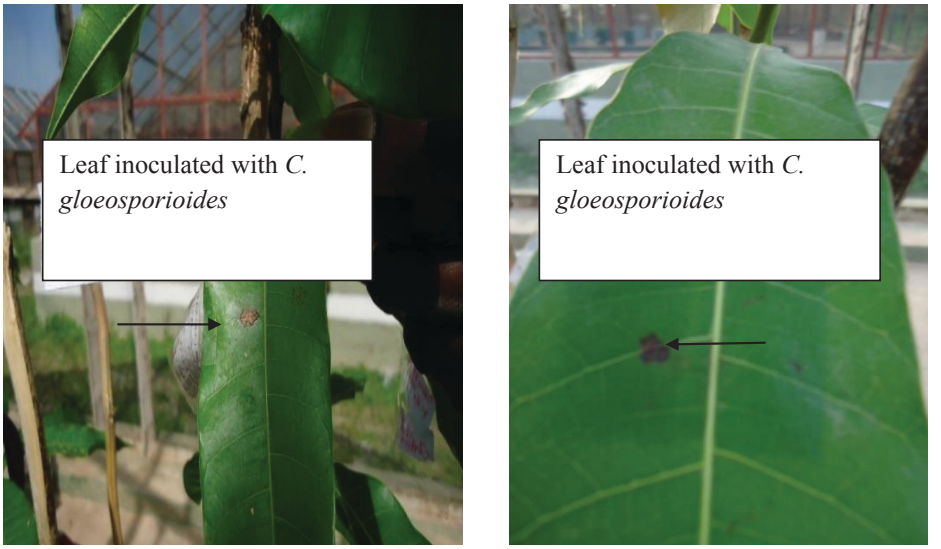

Plate V. Lesion induced by *C. gloeosporioides* using pinprick (a) and infusion (b) inoculation method

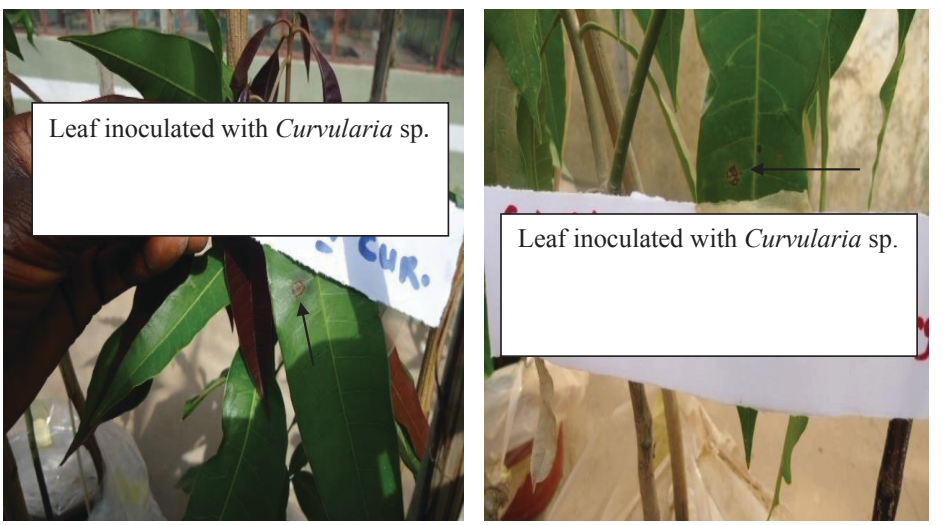

Plate VI. Lesion-induced by *Curvularia* sp. using infusion (c) and pinprick (d) inoculation method

agar (78.70 mm). The fungus significantly varied in growth on PDA from 2-14 DAI than in oat meal agar (OMA). The growth of the fungus significantly increased in the media that supported its growth. No growth was observed on corn meal agar (CMA) and potato agar (PA). Mycelial growth on PDA and OMA was fast, covering 9 cm diameter Petri dishes in 10 days. The mycelia were cottony white with smooth margin on PDA.

Fungus sporulation varied significantly with the media tested as a higher number of spores 3.21 x 10^6/ml were counted on PDA, followed by oat meal agar 2.90 x 10^6/ml. Corn meal agar and potato agar did not support the growth of *C. gloeosporioide*s, therefore, no spores were produced (Fig. 3).

DISCUSSION

There were other fungi associated with mango infected with anthracnose. Those isolated and identity

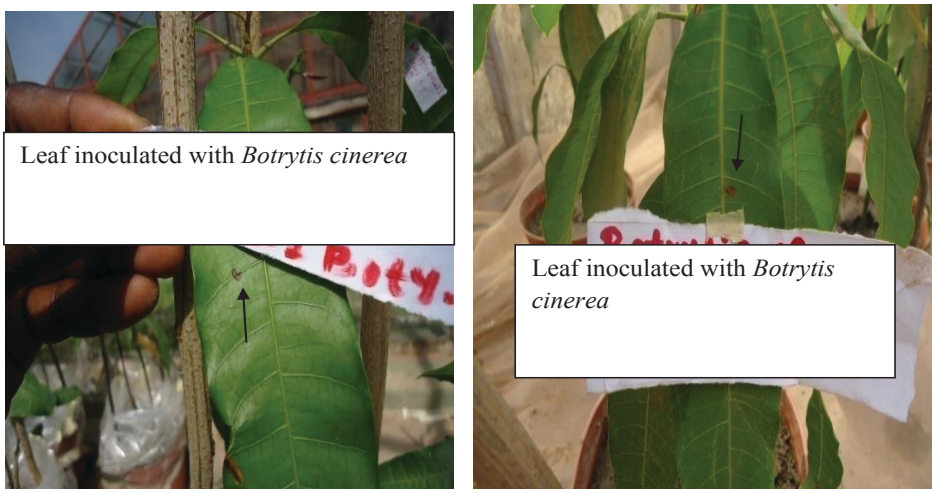

Plate VII. Lesion induced by *Botrytis cinerea* using infusion (e) and pinprick (f) inoculation method

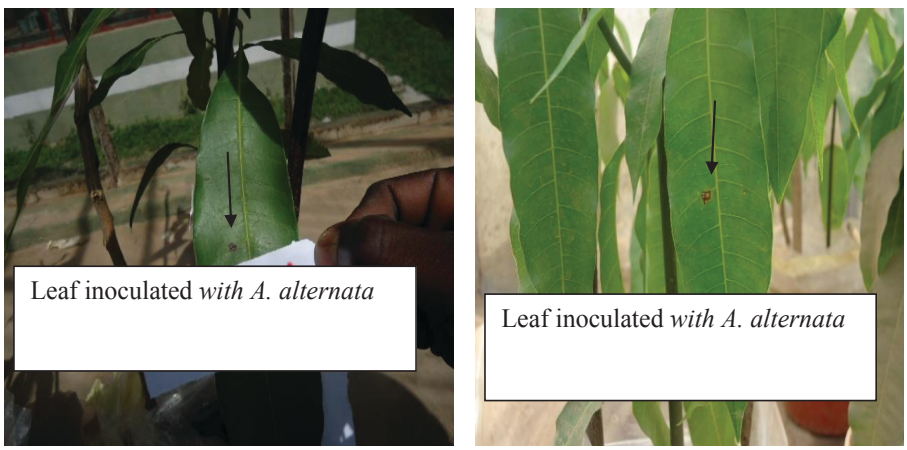

Plate VIII. Lesion-induced by *A. alternata* inoculated using infusion (g) and pinprick (h) inoculation method

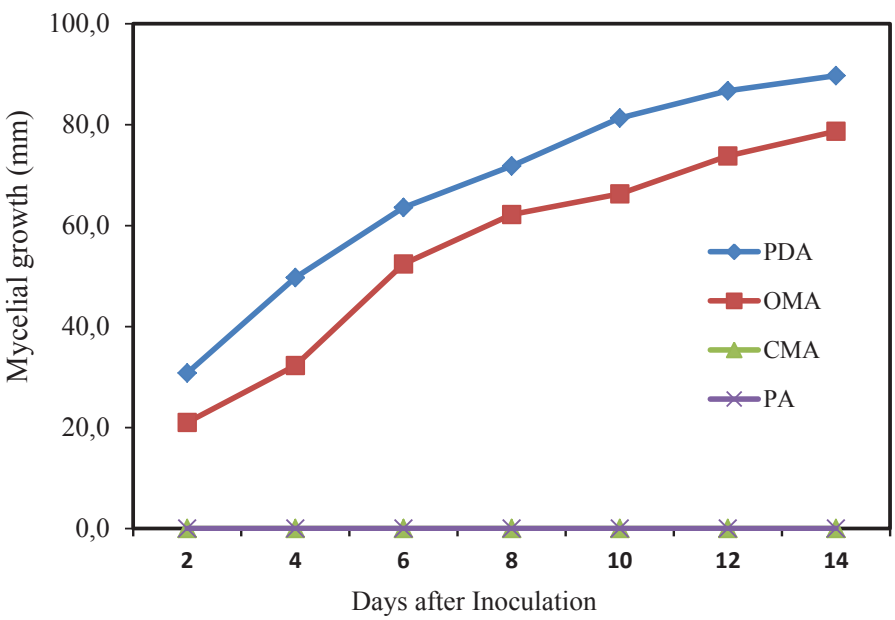

Fig. 2. The mycelial growth of *C. gloeosporioides* on four nutrient media 2-14 days after inoculation (DAI)

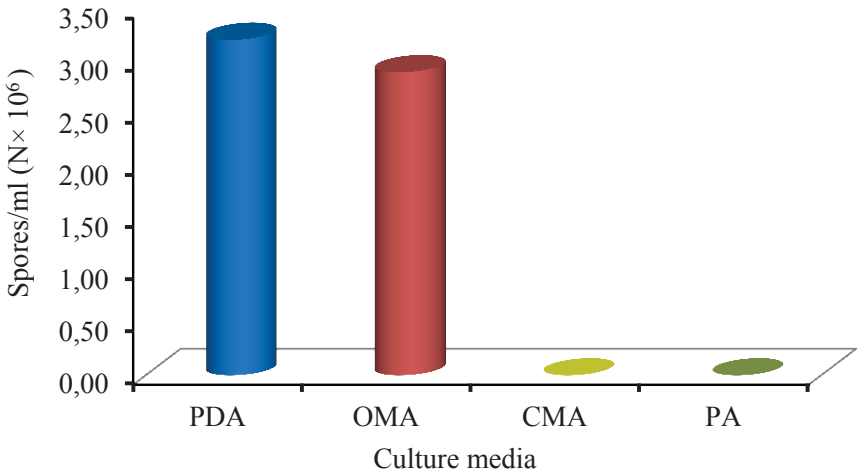

Fig. 3. Sporulation of *C. gloeosporioides* on four culture media (spores/ml) 14 days after inoculation

confirmed at International Mycological Institute, were *Colletotrichum gloeosporioides, Alternaria alternata,* and *Botrytis cinerea*. *A. alternata* and *B. cinerea* induced the soft brown rot and black rot respectively, while *C. gloeosporioides* incited typical anthracnose disease symptom. This is in conformity with findings of Onyeani et al. (2012) who recovered thirteen (13) fungal organisms from anthracnose infected mango fruits in south-west Nigeria, these include *Colletotrichum gloeosporioides, Alternaria alternata, Curvularia lunata*, *Botryodiplodia theobromae,* and *Alternaria tenuissima* which were pathogenic and caused other diseases on mango fruits. This result supports the reports of several workers implicating *Colletotrichum gloeosporioides* as the causal agent of anthracnose of mango (Wharton and Dieguez-Uribeondo, 2004; Than et al., 2008; Sageetha and Rawal, 2009; Jayasinghe and Fernando, 2009; Onyeani et al., 2012). The four fungi isolated from mango leaves were pathogenic to mango seedlings and been able to induce symptom when infusion and pinprick inoculation method was used, but only *C. gloeosporioides* produced small dark brown irregular spots lesions typical of anthracnose. *C. gloeosporioides* also recorded the highest lesion size using the pinprick method of inoculation among the other tested fungi. Datar (1995) found *A. alternata* and *C. gloeosporioides* as pathogenic to mango. Basak et al. (1996) reported *A. alternata*, *C. gloeosporioides, Fusarium oxysprum* and *Botrytis cinere*a as the causal agent of different fruits rots in the tropics. Effect of media type showed significant differences ($P \leq 0.05$) among the mycelial growth of *C. gloeosporioides*. Maximum mycelial growth was observed when cultured on PDA, followed by oat meal agar (OMA). Pandey et al. (2012) reported that PDA and OMA were most suitable over other tested media on the growth of *C. gloeosporioides*. Santoso et al. (1996) and Marikar (2009) reports showed that PDA and CWA (coconut water agar) was ideal for the growth of *C. gloeosporioides*. The result was also in agreement with Prema et al. (2011) who evaluated different nutrient media on the growth of *Colletotrichum musae*, the causal organism of banana anthracnose. Highest diameter (87.47 mm) was on PDA followed by OMA (81.65 mm), Richards's agar (80.00 mm) and Walkman's agar (78.37 mm). No growth of *C. gloeosporioides* cultured on corn meal and potato agar was observed. This disagreed with a report by Pandey et al. (2012) who observed mycelial growth on corn meal agar. Highest spore counts was recorded when the fungus was grown on PDA, followed by OMA. In another study, it is noticed that sporulation of *C. gloeosporioides* was maximum in PDA and oat medium (Mello et al., 2004). Marikar (2009) also reported the highest sporulation of *C. gloeosporioides* on PDA. Deshmukh et al. (2012) reported maximum spore production on PDA and Richards' agar medium on the growth and sporulation of *C. gloeosporioides.*

CONCLUSION AND RECOMMENDATIONS

While isolating the causal agent of anthracnose on mango, four different fungi were isolated and identified, their pathogenicity confirmed both on leaves and fruits. The fungi were provisionally identified at Department of Crop Protection, Ahmadu Bello University, Zaria, which were eventually confirmed by the International Mycological Institute (IMI), Surrey, U.K. as *Colletotrichum gloeosporioides, Botrytis cinerea, Curvularia* sp., and *Alternaria alternata*. All fungi were pathogenic to leaves and fruits and induced symptoms. However, *C. gloeosporioides* produced typical anthracnose symptom and highest lesion size at 7th DAI using pinprick method of inoculation. Mycelial growth of *C. gloeosporioides* varied significantly with media, maximum growth and spore production was observed on PDA at 14 DAI, followed by OMA. No mycelial growth of *C. gloeosporioides* on cornmeal and potato agar was observed. Four fungi were isolated, identified and their pathogenicity ascertained, but only *Colletotrichum gloeosporioides* was found to cause typical symptoms of mango anthracnose.

Highest lesion size 7 mm at 7 DAI using the pinprick method was observed on *C. gloeosporioides*. Pathogenicity showed that tissue injury created by pinpricks supports the growth of *C. gloeosporioides*. Therefore, farmers and orchard attendants are advised to avoid injuring/wounding of the fruits during harvest in order to minimize post-harvest losses. Based on these findings, potato dextrose agar (PDA) should be used for culturing of *C. gloeosporioides* in order to obtain maximum growth and sporulation.

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