

First Report of Fruit Anthracnose in Mango caused by *Colletotrichum gloeosporioides* in Southwestern Nigeria

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Abstract – Mango (*Mangifera indica* L.) fruit rot caused by anthracnose is the most economically important postharvest disease limiting shelf life and export of fresh mango fruits in Nigeria. This study; investigated the etiology, disease incidence and disease severity of mango fruit anthracnose in Southwestern Nigeria. The result of the investigation revealed that 96 isolates out of 231 fungi isolates recovered from symptomatic mango fruits were *Colletotrichum gloeosporioides* isolates based on their whitish orange colony, septated hyphae and capsule-like appearance and pathogenicity test conducted. Other 14 fungi species encountered, accounted for 135 isolates. *Colletotrichum gloeosporioides* was more frequently encountered in all the study areas. One-way ANOVA with Duncan Multiple Range Test conducted, revealed that Ogbomosho area had the highest disease incidence mean of 48% and severity mean of 37.87% while Ayetoro recorded the least disease incidence with mean incidence of 37.33% and severity mean of 30.93%. From the result of the investigation, it was evident that anthracnose disease caused by *Colletotrichum gloeosporioides* was prevalent in all the four study areas of Southwest, Nigeria. Sixty percent of mango trees surveyed were found infected with anthracnose and over 34% of fruits produced on those trees were found severely infected.

Keywords: *Colletotrichum gloeosporioides*, disease incidence, Fruit anthracnose, postharvest disease

1 INTRODUCTION

Mango a fruit plant rich in vitamin C is grown abundantly throughout Southwestern Nigeria. It is the most popular and commonly eaten fruit among millions of people in tropical areas and especially the developed countries [6]. Universally, mangoes are considered as one of the finest and most important fruits of the world [6]. The crop is grown in over 87 countries in the world [19], [11], [23]. Developing countries account for about 98 percent of total production while, developed countries account for 80 percent of world import trade [9]. Mangoes fruits contribute immensely to diet especially in the tropics and have been observed to be higher in vitamin C than citrus fruits. They can be dried, pickled, jellied, or cooked [5], [15]. Green mangoes are the tropical equivalent of green apples; tart, crisp, and somewhat dry, often eaten with salt. The large seed can be processed into flour, and the fat it contains can be extracted and substituted for cocoa butter [29]. In Nigeria, the total area dedicated to mango production is estimated to be 126,500 hectares with a production output of 734,000 metric tons in 2007 and this place the country in the ninth position of top mango producing countries of the world [10] and highest producer in Africa.

Despite her enviable ninth position in the world fresh mango production, Nigeria is not among the world's leading fresh mango exporters and not much study seem to be directed to this trend in order to establish the cause of the country's mango not making it to the world market despite the large expanse of land area dedicated to its production. Mango production is limited by some biotic factors in humid forest region of Nigeria despite its economic importance. Fruit anthracnose disease was commonly found associated with mango fruits produced in the humid forest region of Southwestern, Nigeria. The fruits rot so quickly after harvest due to this anthracnose rendering marketable fruits unattractive and worthless. It has been reported that anthracnose is presently the most common and most important field and postharvest disease of mango widely distributed in all mango-growing regions of the world [22], [3], [18]. This disease has made mango production non-attractive to both farmers and home gardeners in Southwest, Nigeria. This present research was initiated to investigate the etiology of mango fruit anthracnose, its occurrence and severity in mango-growing areas of the humid forest region of Southwestern, Nigeria.

2 MATERIALS AND METHODS

2.1 Study areas

Survey of fruit anthracnose of mango in Ayetoro, Ibadan, Ogbomosho and Lagos in the humid forest zone of Southern Nigeria was carried out in 2008, 2009 and 2010 during mango fruiting seasons. Ayetoro located in Yewa North Local Government Area of Ogun State is located in latitude 7° 15'N and longitude 3° 3'E with annual rainfall 1909.3 millimeter and daily temperatures of 21 to 31° Celsius during the rainy season with mean annual relative humidity of 81 per cent. Ibadan; a lowland rain forest zone situated at latitude 7° 23'N and longitude 3° 55'E, is 200 mm above sea level with annual rainfall of 1200 mm and mean daily temperature of between 24°Celsius (minimum) and 34°Celsius (maximum) lying between the humid forest and derived savannah agro-

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ecologies of Nigeria. Ogbomosho in Oyo state representing derived savannah vegetation lying between latitudes 8° 07'N and 8° 12'N; longitudes 4°04'E and 4°15'E and situated at about 600 millimeter above sea level with annual mean temperature of 26.2°Celsius and monthly temperature of 28°Celsius. Annual rainfall of this area is about 1247 millimeter with relative humidity ranging between 75 and 95 per cent. Lagos; situated in the southern coast of Nigeria lies between latitude 6° 27' 11"N and longitude 3° 23' 45"E. The climate in Lagos is tropical, hot and wet with coastal wetlands, sandy barrier islands, beaches, low-lying tidal flats and estuaries. The daily average temperature in Lagos is 27°Celsius and annual rainfall is 1532 millimeter with relative humidity of 84.7 per cent.

2.2 Fungi pathogens associated with mango fruit anthracnose

Samples of infected mango fruits were, collected from mango orchards and home gardens in Ayetoro, Ibadan, Ogbomosho and Lagos and taken to the laboratory. At the laboratory, portions of peeled epicarp and flesh of the infected fruits, were removed at the point of progression of disease symptom; cut into small pieces and then soaked in 70 per cent ethanol solution for 3 minutes, later, soaked into 1 percent Sodium hypochlorite (NaOCl) for another 3 minutes, then rinsed in two changes of sterile distill water. The parts were, dropped on sterile paper towels, allowed to dry before plating them onto Potato Dextrose Agar (PDA) and incubated for 5 days at room temperature. Isolated colonies were, sub-cultured into fresh plates until pure cultures were obtained. Pure cultures obtained were identified by visual examinations and viewing under stereo and compound electronic microscopes. They were then described and classified based on conidia and colony morphology as described by [16] and [8]. Isolation was carried out in both the Nigeria Agricultural Quarantine Service and in International Institute of Tropical Agriculture, plant pathology laboratories.

2.3 Pathogenicity Tests

2.3.1 Preparation of spore suspension:

Following [24] method, suspension of conidia were prepared by suspending mycelia scraped from 5 to 7 days old of *Aspergillus niger*, *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* the three most frequently recovered fungi in the study separately in 3 milliliter sterile distilled water and shaking vigorously for 3 minutes. The resulting suspension was filtered through 2-layer cheesecloth. The concentration of spore suspension was adjusted to 10^6 spores or conidia/millimeter using haemocytometer.

2.3.2 Inoculations

Fruit wounding and whole fruit techniques were adopted for fruit inoculations while spore spray method was used for leaf and panicle inoculations.

2.3.2.1 Fruit wounding technique

Following Sun, et al. (2008) method of wound inoculation. Thirty green matured mango fruits were, randomly collected, thoroughly washed and disinfected in 70 per cent ethanol and 1 per cent NaOCl. The disinfected fruits were, then rinsed in four changes of sterile distilled water and air before inoculation. The fruits were each, pierced with sterilized needle in three places; five fruits were each injected with 0.02 milliliter

containing 1×10^6 per milliliter spore suspension of respective fungal isolates, then sealed in moist plastic bags, and incubated for 5 days in a moist chamber. Control fruits were, inoculated with sterile distilled water. Anthracnose symptoms were, evaluated after 5 days.

2.3.2.2 Whole fruit technique:

Following a modified [28] method, thirty green matured mango fruits randomly selected were, washed and surface sterilized. The isolated pathogens on 0.5-centimeter agar discs were placed on the surface the sterilized healthy fruits and incubated in a moist chamber at room temperature. Control fruits were, inoculated with clear agar discs. Anthracnose symptoms were, evaluated after 5 days incubation.

2.3.2.3 Detached leaf technique

Detached new leaves free from anthracnose symptom, were collected, washed, and surface sterilized. The leaves were then, sprayed with the spore solution of the respective fungal isolates and placed on plastic trays lined on the inside with moist paper tissue, covered with moist paper towels and incubated in a moist chamber for 5 days at 28°Celsius.

2.3.2.4 Detached panicle technique

Newly developed panicles of about one to two weeks old about 5 to 7 cm long were, carefully cut still attached to stems with three to five leaves and brought to the laboratory. Each panicle was placed separately in measuring glasses half filled with water, then spore suspensions of the respective fungal isolates were separately sprayed all over the panicles. Control panicles were, sprayed with sterile distilled water. The panicles were then, covered with black polyethylene bags to reduce excessive loss of water and incubated for 5 days under humid condition at room temperature.

2.3.2.5 Re-isolation of isolated fungal pathogens

The causative organisms in the diseased parts were re-isolated on potato dextrose agar as described in isolation of pathogen. The characters of the re-isolated pathogens were, compared with their original isolates.

2.4 Assessment of disease occurrence and severity

A systematic field survey of fruit anthracnose was, carried out in the four selected mango-growing areas; to determine fruit anthracnose frequency and/or occurrence and severity. Following [14] method with slight modification, survey and sampling of mango fruits in the mango growing areas was carried out. Fifteen mango trees instead of ten in each sampling location were randomly selected. On each tree, five on-tree ripened mango fruits were picked, examined, and scored. Fruit anthracnose was assessed using the standards for the assessment of fruit anthracnose of mango proposed by [1]. Scale 1 to 5 was used, where scale 1 represents no fruit lesions, 2 represents 1 to 3 lesions, 3 represents 4 to 6 lesions, 4 represents 7 to 15 lesions and 5 where more than 30 per cent of fruit surface is covered with lesions. Disease incidence (percentage of diseased fruits), and disease severity (percentage of area affected on the fruit on average) was then obtained by the following formula.

$$DI (\%) = \frac{x}{N} \times 100$$

$$DS (\%) = \frac{\sum(a+b)}{N.Z} \times 100$$

Where;

$\sum(a+b)$ = Sum of infected fruits and their corresponding score scale

N = Total number of sampled fruits

Z = Highest score scale

x = Number of infected fruits

2.5 Statistical analysis

Data generated from the study were subjected to a one-way ANOVA and means were separated by Duncan Multiple range Test at 0.05 probability level using Statistical package for social sciences (SPSS) Version 14.0..

3 RESULTS

The first observable phenomenon in the study areas was unmanned bushy mango orchards. Anthracnose symptom was commonly found on the leaves, panicles and fruits on the trees. Symptom on the leaf was small dark brown spots that coalesce to form irregular lesions. The centers of old lesions dry up and fall out giving the leaf a perforated or tattered appearance. In most cases, symptoms were observed only on leaf edges while in some cases the mid-rib of the leaf was also affected. On panicles, minute dark spots were initially observed at stem-ends of the panicles; the spots coalesce and progress upward to the tip resulting in shriveled, blighted flowers and small fruits. All blossoms on some panicles were completely dried up resulting into no fruit setting. On immature fruits, the symptom was minute dark spots on the fruit surface, which sometimes coalesce to form irregular spots. On matured and ripen fruits, the observable symptom was dark brown necrotic and sunken lesion which in some cases was just one. This sunken spot continues to enlarge until much of the fruit surface become rotten. On some fruits, black irregular lesions of varying sizes are scattered all over the fruit surface and within few days, may coalesced to give rotten fruits while on some the symptom was tear-stain black lesions that run from the stem-end of the fruit to the basal end.

3.1 Isolation and identification of anthracnose pathogen

Inoculation of solidified Potato dextrose agar with small cut pieces of lesions from the symptomatic mango fruits and incubation at temperature that fluctuated between 28 and 30°C for 5 days produced mixed fungal growth, which was later sub-cultured to obtain pure cultures. Some of the pure cultures obtained, on Potato dextrose agar, had colonies that were whitish to dark grey with thick to sparse lawns of aerial mycelium when viewed from the top of Petri dishes (Fig.1a) and were greenish to orange or dark brown centre bordered by creamy surrounding when viewed from the reverse side of the Petri dish (Fig. 1b). When viewed under the microscope, conidia were observed to be hyaline; single celled and cylindrical with obtuse ends (Fig. 1c). The fungus was identified to be *Colletotrichum gloeosporioides*. Other fungi isolates recovered were *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, *Bipolaris hawaiiensis*, *Botryodiplodia theobromae*, *Cochliobolus nodulosus*,

Curvularia lunata, *Entyloma spp.*, *Fusarium dimerum*, *Fusarium longipes*, *Fusarium verticilloides*.

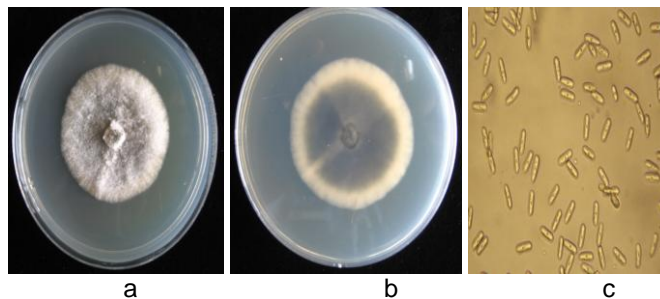


Fig. 1 *Colletotrichum gloeosporioides* the causal agent of mango fruit anthracnose disease (a) top view of colony in a Petri dish (b) reverse view and (c) Microscopic view.

3.2 Fungi Isolates recovered from symptomatic mango parts

Two hundred and thirty-one fungi isolates were recovered from infected mango fruits in the study (Table 1). Ninety-six isolates representing 41.55 per cent of the isolations, were identified to be *Colletotrichum gloeosporioides*, 28 isolates representing 12.12 per cent were identified to be *Botryodiplodia theobromae*, and 25 isolates representing 10.82 per cent were identified to be *Aspergillus niger*. Eighty-two isolates representing about 35.51 per cent comprise of 12 other fungi species encountered in the study.

TABLE 1.
FUNGI ISOLATES RECOVERED FROM MANGO FRUITS INFECTED WITH ANTHRACNOSE

Fungus recovered	No. of Isolates	% Isolation
<i>Alternaria alternata</i>	13	5.63e
<i>Alternaria tenuissima</i>	2	0.87j
<i>Aspergillus flavus</i>	25	10.82c
<i>Aspergillus fumigatus</i>	4	1.73i
<i>Aspergillus nidulans</i>	9	3.90g
<i>Aspergillus niger</i>	11	4.76f
<i>Bipolaris hawaiiensis</i>	13	5.63e
<i>Botryodiplodia theobromae</i>	28	12.12b
<i>Cochliobolus nodulosus</i>	1	0.43k
<i>Colletotrichum gloeosporioides</i>	96	41.55a
<i>Curvularia lunata</i>	8	3.46h
<i>Entyloma spp.</i>	2	0.87j
<i>Fusarium dimerum</i>	2	0.87j
<i>Fusarium longipes</i>	16	6.93d
<i>Fusarium verticilloides</i>	1	0.43k

Mean values followed by same letter are not significantly different within the column at 5 per cent probability by Duncan's multiple range tests.

3.2.1 Pathogenicity test

Pathogenicity tests carried out separately for the three highest occurring pathogens (*Colletotrichum gloeosporioides*, *Botryodiplodia theobromae* and *Aspergillus niger*) isolated from symptomatic mango fruits showed that only *Colletotrichum gloeosporioides* reproduced anthracnose

disease symptom typical of those observed on both healthy leaf and fruits of mango. *Botryodiplodia theobromae* showed rotting symptoms on fruits typical of stem end rot or soft brown rot only on wounded fruits but did not reproduce any symptoms on unwounded fruits, leaves and panicles. *Aspergillus niger* showed dusty charcoal symptoms at the points of wound on the fruit typical of black mould rot. Mixed inoculation of *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* isolates on fruits, produced mixed symptoms of lesions typical of anthracnose disease and soft brown rots. Cultures re-isolated from the inoculated fruits and leaves were similar to those of the original isolates used for the inoculations.

3.3 Occurrence and Severity of mango fruit anthracnose in the sampling areas

Table 2; shows the disease index, occurrence and severity of anthracnose disease on mango fruits sampled and assessed from 60 mango trees randomly selected in the four selected mango growing areas in the Southwestern, Nigeria. Thirty-six trees (60 percent) of the 60 trees had ripened fruits with anthracnose symptoms. The result of the assessment shows that, although the highest disease index of 1.89 and incidence of 48 per cent of mango fruit anthracnose occurred in Ogbomosho followed by Lagos 1.81 disease index and 46.67 percent incidence, they were not significantly different from each other at 5 per cent level of significance. In addition, there was no significant difference between anthracnose incidence in Ibadan and Ayetoro at 5 per cent level of significance. However, there were significant differences among the mango growing areas in fruit anthracnose severity with Ogbomosho recording highest severity of 37.87 per cent while Ayetoro recorded the least anthracnose severity of 30.93 per cent.

TABLE 2
OCCURRENCE AND SEVERITY OF MANGO FRUIT ANTHRACNOSE IN SOUTHWESTERN NIGERIA

Location	Disease Index	Disease Incidence (%)	Disease Severity (%)
Ayetoro	1.55 ^c	37.33 ^b	30.93 ^d
Ibadan	1.81 ^b	38.67 ^b	36.27 ^b
Ogbomosho	1.89 ^a	48.00 ^a	37.87 ^a
Lagos	1.69 ^d	46.67 ^a	33.87 ^c

Mean values in a column followed by same letter are not significantly different at 5 per cent probability by Duncan's multiple range tests.

4 DISCUSSIONS

Based on the results of this study carried out to determine; the fungi pathogen responsible for fruit anthracnose disease in mango that cause mango fruit rotting during ripening after harvest, 231 fungi isolates comprising of 15 different fungi species were recovered. Out of these 15 species, three species found associated with fruit rot of ripen mango, and were the most frequently encountered, included *Colletotrichum gloeosporioides* with 96 isolates, *Botryodiplodia theobromae*, 28 isolates and *Aspergillus niger* recording 25 isolates. This result is in consonance with several earlier reports of several workers including [20] and [21] that implicated these fungi species to be the fungi responsible for postharvest diseases of mango associated with fruit rotting during ripening.

Pathogenicity test conducted with the three most frequently encountered fungi pathogens from infected mango fruits indicated that only *Colletotrichum gloeosporioides* reproduced symptoms typical of the original anthracnose symptom observed on symptomatic mango fruits when re-isolated. This result supports the reports of several workers implicating *Colletotrichum gloeosporioides* as the casual agent of anthracnose of mango [26], [27], [22], [12]. On the other hand, *Botryodiplodia theobromae* and *Aspergillus niger* showed rotting symptoms typical of soft brown rot and black mould rot on wounded fruits respectively. This result agrees with [20] and [21] implication of the two fungi in causing stem-end and/or soft brown rot and *Aspergillus* rot (black mould) respectively. There were significant differences in the number of *Colletotrichum gloeosporioides* isolates recovered from the different study areas at 5 per cent level of significance; the areas sharing similar weather factors not withstanding. Highest number of 33 isolates occurred in Ibadan, followed by 24 isolates recovered from Ogbomosho. Twenty one isolates recovered from Ayetoro was slightly significant with the 18 isolates recovered from Lagos. Similarly, the incidence and severity of the disease though were significantly different among the mango growing areas; there was no area completely free from the malady of mango fruit anthracnose. This confirms [17] and [3] assertion that anthracnose disease, is found in all mango growing areas of the world. Based on the findings of this study, the export value of fresh mangoes and their nutritive values in the diet of people in many developing countries of the world, make mango one of the most important fruit crops under cultivation. In the Southwest zone of Nigeria, mango production is usually unattended and the fruits eaten as desserts. These unattended orchards are usually associated with bushy environments that are conducive for pathogen hibernation. Orchard sanitation particularly cleaning and pruning according to [13] are very useful to decrease the infection rate of fungi causing postharvest rotting of mango fruits. It is recommended that field sanitation involving the collection and incineration of fallen fruits and tree trash be adopted. In addition, mango fruiting in the zone usually coincides with rainy seasons and high humid conditions associated with high temperatures, which favours infection and colonization of the crop by fungal pathogens and which in turn predisposes mango production to serious fungi attack. It is therefore necessary to do all that are possible to keep the humidity level of the orchards as low as possible especially during the fruiting period. If orchards were free of bushes, there would be greater aeration within the orchards, which will in turn, reduce temperature and more sunlight penetration, which could reduce the relative humidity within the orchards. Adequate spacing of trees, pruning and removal of dead leaves and branches will go along way in reducing humidity and temperature in the orchards.

5 CONCLUSIONS

Although anthracnose was found prevalent in all the mango-growing areas surveyed, the occurrence and severity was probably more influenced by environmental conditions and cultural practices rather than climatic factors in the areas. Fruit yield was lowest in areas with highest anthracnose disease occurrence and severity an indication that anthracnose disease phenomenon has negative correlation with fruit yield. This phenomenon was largely influenced by environmental conditions and cultural practices.

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