



Identification and Pathogenicity of Organisms Associated with Anthracnose Disease of Mango in Yola, Adamawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AH and FAJ designed the study and wrote the first draft of the manuscript. Authors MYJ and GAI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Some mango fruits marketed in Yola and environs show some anthracnose diseases symptoms.

Aims: Therefore, the aim of this study was to identify fungal organisms associated with Anthracnose disease of mango in Yola, Adamawa state, Northeastern Nigeria and to test their pathogenicity.

Study Design: Laboratory based controlled experiment.

Methodology: Naturally anthracnose infected mango fruits and leaves were purposely sampled from different home gardens, farms, and markets in Yola. The symptomatic plant parts were immediately taken to the laboratory for direct isolation, characterization, identification and pathogenicity testing of fungal isolates.

Results: A total of 19 fungal colonies were obtained from the anthracnose infected mango fruits and leaves. Based on similarity of morphological features (colony colour, texture presence of septate mycelia or not, spore shape and number of septa), fungal colonies were grouped into 3 species and were identified as *Colletotrichum gloeosporioides*, *Aspergillus niger* and *Rhizopus oryzae*. C.

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gloeosporioides had the highest percentage (66.7%) (77.8%) frequency of occurrence in infected fruits and leaves respectively, compared to *A. niger* which recorded 11.1% and 20.0% and *R. oryzae* with 22.2% and 10%. Pathogenicity test revealed only *C. gloeosporioides* was found pathogenic while the remaining two; *A. niger* and *R. oryzae* were non-pathogenic.

Conclusion: Findings of this study has indicated that *C. gloeosporioides* is the etiological agent of anthracnose of mango in the area.

Keywords: Disease; frequency of occurrence; fungal isolates; morphological characteristic; pathogenic isolates.

1. INTRODUCTION

Mango (*Mangifera indica* L.) is grown throughout the tropics and subtropics of the world being one of the most popular and commonly consumed fruit among millions of people in the tropics [1,2]. It is considered the king of fruits due to its wide ecological range, delicious state, excellent flavour, very high nutritive and medicinal values of great Religio-historical significance [3]. Production, trade and consumption of mango fruits have increased significantly both domestically and internationally [4]. According to [5], mango is commercially cultivated in more than 103 countries worldwide and production is increasing each year due to increasing consumer demand.

Mango production is seriously marred by anthracnose disease in all mango production areas. This wide spreading fungal disease of mangos presently recognized as the worldwide most important field and postharvest disease of mango [6,7]. The disease is capable of causing 30-60% yield losses across different countries of the world [8]. Anthracnose disease is characterized by black or dark brown sunken lesions on ripening fruits (postharvest phase) which directly affects quality of marketable fruit rendering it worthless, hence the most damaging and economically significant phase of the disease worldwide [9]. The initial infection (field phase) which usually starts on young twigs and leaves then spread to the flowers causing blossom blight and destroying the inflorescence and even preventing fruit set [10]. According to [11,10], mango anthracnose is caused by *Colletotrichum gloeosporioides* Penz. On the other hand, [6] reported *Botryodiplodia theobromae*, *Fusarium semitectum*, *Papulaspora* sp. and *Pestalotiopsis guepinii* being associated with anthracnose in Bangladesh. *C. gloeosporioides* as causal agent of anthracnose of mango was first reported in South Western Nigeria by [12].

However, not much has been done in the establishment of etiological agent of the disease in Northern part of the country. Presently in Yola, Adamawa State, Northeastern Nigeria, the disease has been observed in almost all mango growing areas, and has become a threat to both home gardeners and farmers, making mango production unattractive. It causes huge economic losses due to considerable reduction in fruit setting /production. Infected flowers get dried up and emerging small fruits aborted while the post-harvest phase of the disease appears as dark brown or black depressions of indefinite border that expand rapidly on ripening fruits thereby making them lose their economic value. Therefore, this study was focused on isolation, identification, and pathogenicity testing of organisms associated with Anthracnose disease of mango in Yola, Adamawa state, Nigeria. Such information about disease causal agent is fundamental to development of an effective disease control strategies.

2. MATERIALS AND METHODS

2.1 Sampling Sites

Purposeful sampling of anthracnose naturally infected mango fruits and leaves (Fig. 1) were carried out from three locations each and at each location, sampling was replicated three times. All locations are in Yola, Adamawa state Nigeria. Yola is located between latitude 9°11'N and 9°19' and longitude 12°12' and 12°31' E [13]. Anthracnose naturally infected mango fruits and leaves were collected from six different home gardens, farms, and market in Yola, Adamawa state, Nigeria. They include Ngurore, Bole, Kasuwan-Gwari, and Gerio.

2.2 Experimental Site

The experiment was conducted at the Pathology Laboratory of the Department of Crop Protection of Modibbo Adama University of Technology Yola.

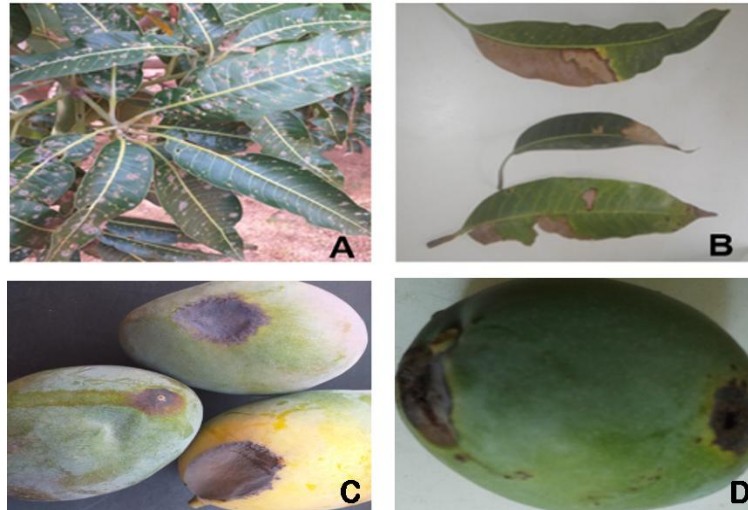


Fig. 1. Symptoms of anthracnose disease of mango; A and B on leaves showing irregular brown spots, while C and D is showing dark brown sunken lesions on fruit

2.3 Fungal Isolation

Potato dextrose agar (PDA) (Difco) was used for the isolation and it was prepared according to manufacturer's specification (at 39 g L⁻¹) autoclaved at 121°C for 15 min, then 0.1% of streptomycin was added to the sterilized media just before pouring into petri dishes to prevent bacterial growth [14]. Sampled infected plant parts were thoroughly washed with sterile distilled water. Fungi were isolated using direct isolation method described by [15], where 5 mm² pieces of symptomatic crown tissue were excised from the advancing lesion margins, sterilized with 70% ethanol for 2 min, then with 10% sodium hypochlorite for 1 minute and lastly rinsed in 3 changes of sterile distilled water. The cut sterilized tissues were placed between 2 pieces of sterile tissue papers to blot-dry [16]. Three-points plating was used, where 3 pieces of the sterilized lesion cut piece were placed on each petri dish containing the solidified PDA, sealed and incubated at 27 ± 2°C and 80-85% RH [15]. Fungal growth/colonies were looked out for after 4 days of incubation. Each visible colony was sub-cultured using fungal hyphal tips growing to obtain a pure culture and lastly, single spore isolation procedure was performed to further purify the colonies. Each experiment was replicated 3 times [17].

2.4 Morphological Characterization of the Isolates

All fungal colonies obtained were subjected to morphological characterization and identification.

Seven days old growing cultures were opened and viewed under the light microscope. Colony colour, texture, mycelia being septate or non-septate hyphae, spore shape and number of septa of 30 spores per each plate were studied using Nikon, ECLIPSE E200, light microscope. A plug of sporulating mycelia was taken from each plate, mixed with 2 drops of Lactophenol cotton blue "RM" stain, (Biolution Resources), covered with a slide cover, making sure air was expelled by flaming was used for determining spore shape and number of septa [18,19]. Colonies having same features were grouped together and identified as same species [20].

2.5 Pathogenicity Test

To prove pathogenicity of fungal isolates, freshly harvested healthy matured mango fruits were collected from kasuwan gwari market, thoroughly washed under running tap water then surface sterilized by washing with 10% sodium hypochlorite solution for 3 min then rinse in 3 changes of sterile distilled water to get rid of Chlorox solution on the surface of the fruits as described by [6]. The surface sterile fruits were blot dried. Suitable sized plastic containers were sterilized with 70% ethanol lined with moistened sterile tissue papers to maintain humidity. Before inoculation, small holes of about 2-3 mm were made on the fruits using sterilized 5 mm cock borer, then 50 µL of 10⁵ spore mL⁻¹ suspension of *C. gloeosporioides* spores earlier adjusted using hemocytometer was placed in the holes [21,22]. Inoculated mangoes were placed in the

sterile plastic container lined with moistened tissue papers to maintain high humidity and kept at temperature $27 \pm 2^\circ\text{C}$ for 7 days. Observation for a typical characteristic symptoms of anthracnose lesion on the artificially inoculated fruits was made after 7 days. Organisms were re-isolated from the developed lesions of the artificially inoculated fruits and compared with the previous isolated from the naturally infected fruits (samples).

For the leaf experiment, matured healthy leaves were detached from mango tree, surface sterilized as in fruits. Then sterilized leaves were scratched with a pin before being inoculated with $20 \mu\text{L}$ of 10^5 spore mL^{-1} of suspension. Sterile distilled water was inoculated onto the control experiments (mango fruits and leaves). All experiments were replicated 3 times and arranged in a completely randomized design (CRD), then kept at $27 \pm 2^\circ\text{C}$ in a moist chamber maintained under high humidity for 7 days. After the 7th day, typical anthracnose disease symptoms were looked out for and fungi were re-isolated from those experiment (mangoes) that displayed anthracnose symptoms only then compared with the original isolates from the samples [23,24].

2.6 Statistical Analysis

All experiments were in triplicates and data on frequency of occurrence of isolates from leaves and fruits at different locations were statistically analyzed using One-way ANOVA, using the statistical software SAS version 9.4 (SAS Institute, Cary, NC, USA). Least significant difference (LSD) was employed to compare significant means of isolates' occurrence and was expressed as mean percentage score.

3. RESULTS AND DISCUSSION

Total of 19 fungal colonies were isolated from the sampled anthracnose naturally infected mango fruits and leaves collected from 6 Locations in Yola Adamawa state (sampling site). There was highly significant difference between the frequency of occurrence of isolates in both mango fruit and leaves ($P < 0.001$). *Colletotrichum gloeosporioides* was isolated 13 times and was the most frequently isolated fungus, it was found in all samples from all locations (Table 1). Other fungal isolates with low frequency were *Aspergillus niger* and *Rhizopus oryzae*, they were isolated 3 times each. In a similar study reported [6], saprophytic fungi; *Aspergillus flavus*,

A. japonicas, *A. niger*, *A. parasiticus*, *Rhizopus arrhizus* were also isolated from anthracnose infected mango in Bangladesh.

3.1 Percentage Frequency of Occurrence of Fungal Isolates from Mango Fruit and Leaves

From the samples of infected plant parts, highly significant difference ($P < 0.0001$) exist between the mean percentage frequencies of occurrence of isolates from both fruits and leaves in a location. From the samples of mango fruits, *C. gloeosporioides*, *R. oryzae* and *A. niger* recorded 66.7, 22.2, and 11.1%, respectively. Similarly, *C. gloeosporioides*, *A. niger* and *R. oryzae* had 77.8%, 20.0% and 10.0% frequency of occurrence from the infected mango leaves. In both plant parts thus, mango fruits and leaves, *C. gloeosporioides* had the highest frequency occurrence (Fig. 2).

3.2 Morphological Description and Identification of Isolates

Based on similarities that exist among the morphological characteristics of the 19 fungal colonies; colony colour, texture and shape of spores, isolates were grouped in to three. All the three groups were subjected to microscopic studies and were identified as *C. gloeosporioides*, *A. niger* and *R. Oryzae*. Fig. 3 shows the morphological features; colony colour, texture and spore shape (A) *R. oryzae*. (B) *C. gloeosporioides*. (C) *A. niger*.

R. oryzae: Colonies grow very fast on PDA media to cover 90mm petri dish in 2-3 days with initially whitish spongy aerial mycelia that become brownish with maturity. They produce spores after 3-4 days of incubation. Their spores are dark brown, smooth, globose to sub-globose in shape with no septa [25]. The morphological characteristics of *R. oryzae* described in this study are in line with that of [26].

C. gloeosporioides: The organisms grow moderately on PDA media. They have whitish grey or white cottony textured mycelia that have irregular edges and concentric circles of black or brown spots at the center of petri dishes, with septate hyphae and have different levels of sporulation which starts at the center and grows outwardly. It grows slowly to covers 90 mm petri dish in 8-10 days. Spores were oblong-cylindrical with broaden ends at both sides. These morphological description of *C. gloeosporioides* are in agreement with the report of [27].

Table 1. Fungal colonies obtained from samples of anthracnose naturally infected mango fruits and leaves from 6 locations in Yola with their frequency of occurrence

Plant parts	Location	Fungal isolates		
		<i>C. gloeosporioides</i>	<i>A. niger</i>	<i>R. oryzae</i>
Fruits	Bole	2 ^a	1 ^b	0 ^c
	Ngurore	1 ^b	0 ^c	2 ^a
	Kasuwan-gwari	3 ^a	0 ^c	0 ^c
	Total freq. in Fruit	6	1	2
Leaves	Gerio	2 ^a	1 ^b	0 ^c
	Ngurore	3 ^a	1 ^b	0 ^c
	Bole	2 ^a	0 ^c	1 ^b
	Total freq. in Leaf	7	2	1
Total freq. (fruit & leaves)		13	3	3
				19

Values followed by same letter in same row are not significantly different from each other at ($P < 0.05$). Key: (Freq.) Frequency

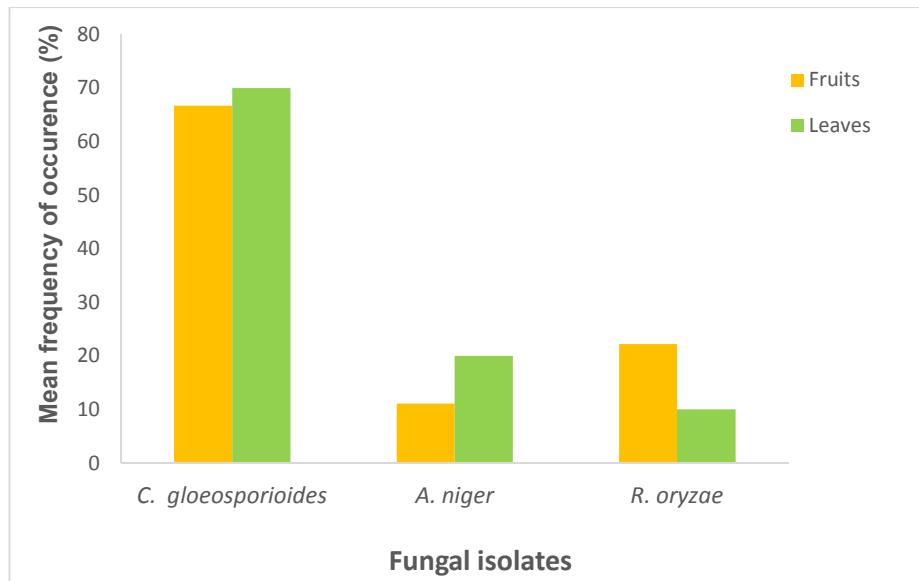


Fig. 2. Mean frequency of occurrence of fungal isolates from naturally infected mango fruits and leaves. n=19

A. niger grows very fast on PDA media. Initially, it has white mycelia that turn black after 2-3 days and produces radial fissure. They possess conidiophore that consists of vesicles, and phialide borne on metulae and produce profuse chains of globose spores. They mature to produce spores in 3-4 days. This is in agreement with the report of [28].

3.3 Pathogenicity Test

Results from the pathogenicity test shows two of the isolates; *R. oryzae* and *A. niger* isolates were not able to produce symptoms typical of anthracnose disease as in the initial sampled

infected mangoes. This suggests that *R. oryzae* and *A. niger* isolates were not the causal agent of anthracnose disease of mango both on field and postharvest (fruits, and leaves). However, *C. gloeosporioides* was able to produce the dark brown sunken lesions (anthracnose symptom) on both mango fruits and leaves, hence proved to be the etiological agent of the disease (Fig. 4). Accordingly, [12], *C. gloeosporioides* is the causal agent for Anthracnose of mango in South Western Nigeria. Similar report was made by [29] who reported that *C. gloeosporioides* is a common anthracnose pathogen on a variety of tropical crops such as mango, avocado and papaya.

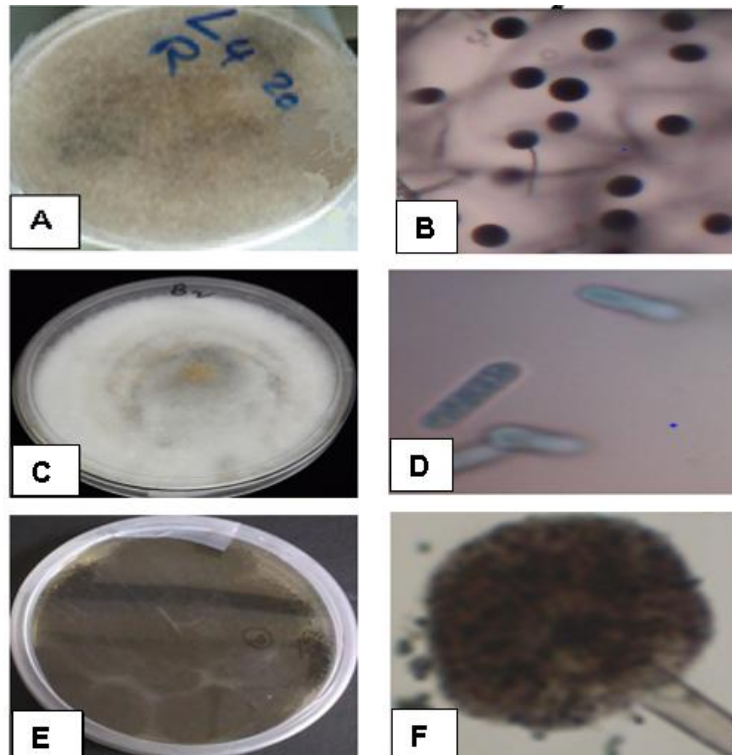


Fig. 3. Images of fungal isolates: (A) surface view of *Rhizopus oryzae* culture plate, (B) image of *Rhizopus oryzae* spores, (C) surface view of *Colletotrichum gloeosporioides* culture plate, (D) the spores of *Colletotrichum gloeosporioides*, (E) surface view of *Aspergillus niger* culture. While (F) *Aspergillus niger* spores, all at 40 x magnification

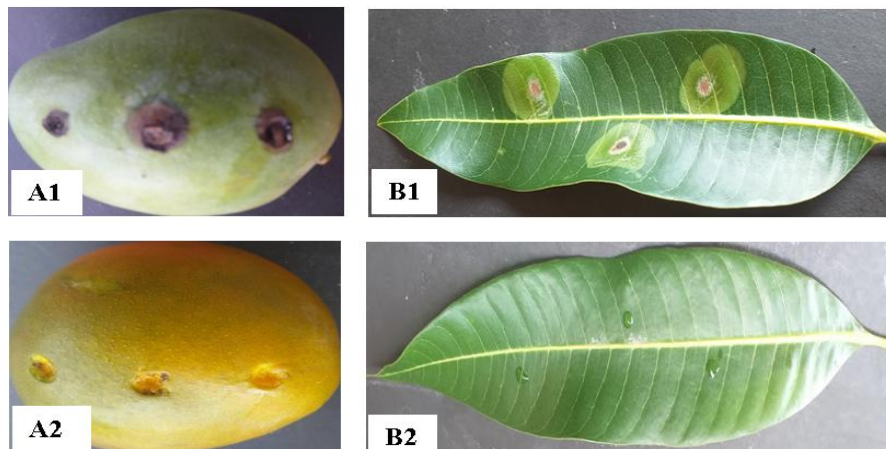


Fig. 4. Image of pathogenicity tests for *C. gloeosporioides* isolate (A1) isolate on mango fruit (B1) isolate on mango leaves while (A2) and (B2) were their respective controls after 7 days of incubation at room $27 \pm 2^\circ\text{C}$ and RH of 80 - 85%

4. CONCLUSION

The present study has revealed the etiological agents of anthracnose disease of mango which is threatening production and worthiness of the

fruits in Yola Adamawa state, Northeastern Nigeria. Fungal specie responsible for the disease both field and postharvest was found to be *C. gloeosporioides*. Finding of this study will serve as a fundamental and initial step for the

development of effective anthracnose control/management measure in the state.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Akem CN. Mango anthracnose disease: Present status and future research priorities. *Plant Pathology Journal*. 2006; 5(3):266-73.
2. Mukherjee SK, Litz RE. Introduction: Botany and importance. *The mango: Botany, production and uses*. 2009(Ed. 2): 1-8.
3. Lakshmi BKM, Reddy PN, and Prasad RD. Cross-infection potential of *Colletotrichum gloeosporioides* penz isolates causing anthracnose in subtropical fruit crops. *Trop. Agric Res*. 2011;22:183-193.
4. Ajila CM, Rao UP. Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* L. peel extract. *Food and Chemical Toxicology*. 2008;46(1):303-9.
5. FAO F. FAO Statistical Database, Food and Agriculture Organization of the United Nations; 2010. Available:faostat.fao.org
6. Islam F, Shamsi S, Bashar MA. Fungi associated with anthracnose of mango (*Mangifera indica* L.) fruits and their pathogenic potentiality. *Dhaka University Journal of Biological Sciences*. 2018; 27(1):93-100.
7. Rabari VP, Chudashama KS, Thaker VS. In vitro screening of 75 essential oils against *Colletotrichum gloeosporioides*: A causal agent of anthracnose disease of mango. *International Journal of Fruit Science*. 2018;18(1):1-3.
8. Chowdhury MN, Rahim MA. Integrated crop management to control anthracnose (*Colletotrichum gloeosporioides*) of mango. *Journal of Agriculture & Rural Development*. 2009;115-20.
9. Chala A, Getahun M, Alemayehu S, Tadesse M. Survey of mango anthracnose in southern ethiopia and in-vitro screening of some essential oils against *Colletotrichum gloeosporioides*. *International Journal of Fruit Science*. 2014; 14(2):157-73.
10. Ploetz RC. Anthracnose. In: *Compendium of tropical fruit diseases*, Ploetz (Ed.). APS Press, St Paul, MN, USA. 1994;35-36.
11. Fitzell RD, CM. Peak. The epidemiology of anthracnose disease of mango: Inoculum sources, spore production and dispersal. *Ann. Applied Biol*. 1984;104:53-59.
12. Onyeani CA, Osunaja SO, Oworu O, Olufemi S. First report of fruit anthracnose in mango caused by *Colletotrichum gloeosporioides* in south western Nigeria, "in term J. of Scientific and Technology Research. 2012;4:30-34.
13. Adebayo AA, Tukur AL. Adamawa State in maps. Paraclete Publishers; 1999.
14. Shi XX, Qiu HP, Wang JY, Zhang Z, Wang YL, Sun GC. A handy method to remove bacterial contamination from fungal cultures. *PloS One*. 2019;14(11).
15. Idris FM, Ibrahim AM, Forsido SF. Essential oils to control *Colletotrichum musae* in vitro and in vivo on banana fruits. *American-Eurasian Journal of Agricultural and Environmental Science*. 2015;15(3):291-302.
16. Ekhuemelo C, Yaaju MDMD. Identification and management of fungi associated with crown rot of banana in Makurdi, Benue State, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*. 2017; 13(2):50-55.
17. Bokhari NA, Siddiqui I, Siddique KPI, Rizwana H, and Soliman DAW. Management of anthracnose of banana by UV irradiation. *Journal of Animal and Plant Sciences*. 2013;23:1211-12114.
18. Ismail SI, Zulperi D, Norddin S, and Ahmad-Hamdani S. First report of *Neopestalotiopsis saprophytica* causing leaf spot of oil palm (*Elaeis guineensis*) in Malaysia. *Plant Disease*. 2017;101(10): 1821.
19. Agrios GN. *Plant pathology* 5th Edition: Elsevier Academic Press. Burlington, Ma. USA. 2005;79-103.
20. Zainab MB, Shinkafi SA. Isolation and identification of fungi responsible for leaf spots disease of mango (*Mangifera indica*)

- in Sokoto state, Nigeria. Bayero Journal of Pure and Applied Sciences. 2016;9(2): 166-173.
21. Abdalla AEM, Darwish SM, Ayad EHE, Hamahmy RM. Egyptian mango by products: Compositional quality of mango seed kernel, food chemistry. 2007;103: 1134–1140.
 22. Kumah P, Ampomah EO, Olympio NS, Moses E. Efficacy of four botanicals and two chemical fungicides in the control of crown rot disease of banana (*Musa spp. AAA*)'medium cavendish'. In II All Africa Horticulture Congress. 2012;1007:385-392.
 23. Sharma A, Verma KS. *In vitro* cross pathogenicity and management of *Colletotrichum gloeosporioides* causing anthracnose of mango. Annals of Plant Protection Sciences. 2007;15(1):186-188.
 24. Tovar-Pedraza JM, Mora-Aguilera JA, Nava-Díaz C, Lima NB, Michereff SJ, Sandoval-Islas JS, Leyva-Mir SG. Distribution and pathogenicity of *Colletotrichum* species associated with mango anthracnose in Mexico. Plant Disease. 2020;104(1):137-146.
 25. Ibeabuchi C, Olawuni I. Isolation and identification of spoilage fungi associated with rice (*Oryzae sativa*) millet (*Pennisetum Americamum*) and soybean. International Journal of Agriculture and Rural Development. 2011;14(2):614-8.
 26. Hartanti AT, Rahayu G, Hidayat I. *Rhizopus* Species from fresh tempeh collected from several regions in Indonesia. Hayati Journal of Biosciences. 2015;22(3):136-142.
 27. Phoulivong S, McKenzie EHC, and Hyde KD. Cross infection of *Colletotrichum species*; A case study with tropical fruits. Current Research in Environmental & Applied Mycology. 2012;2(2):99-111.
 28. Reddy KRN, Farhana NI, Wardah AR, Salleh B. Colonization rice grains in south Asia. Pakistan journal of biological sciences. 2010;13(16):794–801.
 29. Silva-Rojas HV, Ávila-Quezada GD. Phylogenetic and morphological identification of *Colletotrichum boninense*: a novel causal agent of anthracnose in avocado. Plant Pathology. 2011;60(5): 899-908.

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