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Research article

Isolation, characterization and pathogenicity of fungal pathogens from indigenous postharvest fruits in Akwa Ibom State, Nigeria

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Abstract
The study investigated fungal pathogens associated with spoilage of five indigenous fruits (*Persia americana*, *Citrus sinensis*, *Carica papaya*, *Annona muricata* and *Solanum lycopersicum*) in Akwa Ibom State, Nigeria. Diseased fruit samples were purchased from nine markets located in three senatorial districts of the State. The phenotypic and genotypic identifications of the fungal pathogens isolated from spoilt fruit samples were carried out using standard cultural, morphological and molecular methods, respectively. The phylogenetic relationship among the fungal species was also constructed using neighbor-joining phylogenetic tree generated based on modified Rogers' genetic distance matrix. The fourteen fungal genera identified (and classified into twenty-two species) were *Talaromyces*, *Lasiodiplodia*, *Trichoderma*, *Penicillium*, *Pichia*, *Rhizopus*, *Aspergillus*, *Fusarium*, *Moniliella*, *Mucor*, *Geotrichum*, *Candida*, *Absidia* and *Purpureocillium*. Pathogenicity tests revealed that the fungal isolates were able to cause rots with a range of severity. The most rapid rots were caused by *Rhizopus oligosporous*, *Pichia kudriavzevii* and *Aspergillus niger* within 24 h, while *Aspergillus aculeatus* and *Moniliella suaveolens* were slower in initiating rots with earliest being after 48 h. The identification of fungi related with fruit rots and data coming from pathogenicity test are crucial information in order to plan and to apply control strategies during postharvest storage.

Keywords
Fungal pathogens, fungal rot, diseased fruits, phylogenetic relationship, molecular identification

Introduction

The relationship among fruits, microorganism and humans have been long and interesting and may have developed before recorded history (Willey et al., 2008; Balali et al., 2020). Nutrients needed for

growth, repair and control of body processes are usually obtained from fruits since they possess minerals like calcium, potassium, magnesium, as well as dietary fiber, vitamins and sugar (Hawksworth, 2004; Yahia et al., 2019). It is normal for these fruits to be consumed raw as this is the best way of obtaining their valuable nutrients. The fruits could be used for making of juices, wine, marmalades, jams, and salads (Isitua and Ibeh, 2010; Awe, 2011). There are also medicinal properties attributed to some fruits. According to Nakamura and Miyoshi (2006), eating *Carica papaya* L. may reduce the risk of some types of cancer, while Eno et al. (2000) demonstrated that the fruit juice of *C. papaya* can decrease the blood pressure in the study carried out with mice. *Citrus sinensis* L. when taken as an infusion has shown the ability to lower fevers, stop headaches and stabilize heart palpitations. The juice from *C. sinensis* hastens removal of metabolic waste from the body. Vitamin C, a major component found in *C. sinensis* helps to boost the body immune system thus helping the body to fight infections (Etebu and Nwauzoma, 2014). Fruits like *Persia americana* P. Mill, *C. sinensis* and *C. papaya* contain several biologically active compounds known for antisickling properties (Iweala et al., 2010; Naiho et al., 2015; Nurain et al., 2016). They are consumed both as food as well as integrative medicine (Naiho et al., 2015; Nurain et al., 2016). These fruit crops represent an important economic incoming for local farmers and consequently, the fruit spoilage may contribute to poverty in those communities.

The postharvest diseases usually originate from infections that occurred during and after harvest (Tripathi et al., 2021). Often these infections occur through surface wounds created by mechanical or insect injury. Secondary fungal pathogens are those fungi which may not invade a healthy tissue but can cause spoilage after the tissue has been damaged by some physical or physiological causes. Entrance of fungal pathogens does not depend on the size of the wounds, even a microscopic size wound may be colonized. Microorganisms can affect the fruit quality as well as human health (Adeoye et al., 2009; Kiaya, 2014). This is facilitated by washing healthy fruit with sewage contaminated groundwater (Adegoke and Stenstrom, 2019; Nzima et al., 2020). The main fungal pathogens involved in post-harvest diseases are *Botrytis cinerea* Pears., *Penicillium* spp., *Monilinia* spp., *Rhizopus stolonifer* (Ehrenb.) Vuill., *Aspergillus parasiticus* Speare, *Fusarium* spp., *Candida tropicalis* (Castell.) Berkhout (Nweke and Ibiam, 2012; Spolti et al., 2012; Sutton et al., 2014).

Earlier study classified *B. cinerea* as well as *Magnaporthe oryzae* B.C. Couch, the most aggressive plant pathogens able to cause gray mold disease for horticultural crops (Dean et al., 2012). Their contamination may be aided by irrigation with sewage contaminated water (Adegoke and Stenstrom, 2019; Nzima et al., 2020) Fungal infections make difficult the cultivation of the crop, decrease the quality of yield and increase problems during storage (Kebede et al., 2021), with economic impact (Dean et al., 2012; Drenth and Guest, 2016). In addition to causing quality deterioration and economic losses, some postharvest fungi also pose threat to human health, due to the production and the release of mycotoxins (Li et al., 2015; Sanzani et al., 2016).

Huge economic loss can be ascribed specifically to *R. stolonifer*, which is an opportunistic fungal pathogen isolated and identified in fruit samples by Agbabiaka et al. (2015). It had earlier been reported that *R. stolonifer* is a soft rot pathogen of tomato in Nigeria, able to colonize damaged fruits (Mahovic et al., 2009). Some strains of *Geotrichum candidum* Link were reported to possess proteolytic, peptidolytic and lipolytic activities, and it is often use in food industry (Kamilari et al., 2023). *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. causes damage to both postharvest fruit and food crops (Sandlin and Ferrin, 1992; Denman et al., 2000; Muniz et al., 2011). It plays significant

role in root rot of *Brachychiton populneus* (Schott & Endl.) R.Br. seedlings (Sandlin and Ferrin, 1992), leaf necrosis, and stem cankers on Proteas (*Protea magnifica* Link) (Denman et al., 2000), gummosis of cashew in Brazil (Muniz et al., 2011). Aim of this study was to isolate and characterize fungal pathogens associated to fruit rots, collected in local fruit market in Uyo (Akwa Ibom State, Nigeria).

Materials and Methods

Fruit samples

Diseased samples of *C. papaya*, *P. americana*, *Solanum lycopersicum* L., *Annona muricata* L. and *C. sinensis* were randomly purchased from nine local fruit markets located in each Senatorial Districts of Akwa Ibom State (Nigeria). The 225 fruit samples, stored in sterile plastic bags were transported to Microbiology Laboratory for mycological analyses

Isolation and morphological characterization of fungal pathogens

Media were prepared according to manufacturer's specifications. For growth, both potato dextrose agar (PDA) and potato dextrose broth (PDB) were used. Following sterilization, media were cooled to 50 °C and 1000 mg L^{-1} chloramphenicol and 160 mg L^{-1} gentamycin were added, respectively, to inhibit potential bacterial contamination. All media and glassware were sterilized using an autoclave at 121 °C, at about 1 bar for 15 min. Isolation and identification of fungal pathogens were done based on morphological characteristics. Fungal isolates were obtained from diseased fruits by first washing with sterile distilled water and surface decontamination with 10% hypochlorite solution for 5 min (Vose, 1980).

Sterile scalpel was used to cut 3 × 3 mm sections of the fruits, moving from healthy portions to the diseased portions where the fungi were likely to be more active, and aseptically plating on PDA medium and incubating at room temperature (28 °C ± 2 °C). The fungal isolates were repeatedly transferred into new PDA plates in order to obtain pure cultures, which were submitted to a preliminary morphological identification by microscope examination according to the protocol proposed by Samson et al. (1988) and Barnett and Hunter (1987).

Molecular characterization

ZymoBIOMICS™ DNA Miniprep Kit (Zymoresearch, USA) was used for fungal DNA extraction and purification, in accordance with the manufacturer's instruction. After the final purification and elution, the quality of the extracted DNA was determined using Nanodrop spectrophotometer (Thermo Fisher Scientific, USA).

PCR amplification of the extracted DNA was carried out in a total of 25 µl mixture containing 12.5 µl of Mastermix 2x (DreamTag MM ThermoFisher, USA), 5 µM of each forward and reverse primers (Inqaba Biotechnology, South Africa) and 10 ng μl^{-1} template DNA. The forward and reverse primers were ITS4 and ITS5 (White et al., 1990; Vongphachanh et al., 2016). The amplification program consisted in initial denaturation and enzyme activation at 94 °C for 5 min; followed by 36 cycles at 94 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 45 s; and final extension at 72 °C for 7 min and hold temperature at 4 °C. Gel electrophoresis using 1.5% agarose gel in tris-boric acetate was used to separate the base pairs. The expected base pair of the amplicons was around 650 bp. The PCR products was purified using ethanol precipitation method as described by Al-Saad

and Al-Zaalan (2019). The purified PCR products were sequenced by Inqaba Biotechnology (South Africa) and carried out Blast analysis to determine the identity of the fungal isolates. Sequencing was done for some fungi representative of phenotypic characteristics.

The evolutionary distances were computed using the Maximum Composite Likelihood method and were in the units of the number of base substitutions per site. Empirical base frequencies ranged from 0.27030 to 0.29889 where G/C ratio was ~0.93345. All positions containing gaps and missing data were eliminated and there was transition/transversion ratio of 2.00000. The optimal tree with the branch length that ranged from 0.00006 to 0.34833 was taken and the analysis involved 20 nucleotide sequences. Neighbor-Joining phylogenetic tree of 20 isolates and similar described strains were used. This neighbor-joining tree was generated based on modified Rogers' genetic distance matrix using Mega 7 software, version 7.0.63 (Tamura et al., 2021).

Novel sequences were deposited in the GenBank database of the National Center for Biotechnology Information (NCBI) along with their distinct identifiers and accession numbers in line with standard approach (Adegoke et al., 2022) (Supplementary Table S1).

Pathogenicity tests

The procedure described by Agrios (2005) was followed. Ninety healthy fruits were surface-sterilized with 70% ethyl alcohol and wound created using a sterile scalpel. Disc of pathogens listed in Tables 2 to 5 previously grown on PDA, identified by morphological and molecular tools, were aseptically transferred into fruit of *P. americana*, *C. sinensis*, *C. papaya*, *A. muricata* and *S. lycopersicum* and sealed with vaseline to prevent the entry of contaminant microorganisms. The inoculated fruits were placed in sterile polyethylene bags with moist cotton wool placed inside to create a conducive environmental condition for the pathogens. The set up was incubated at room temperature of 28 °C ± 2 °C for five days. The incubated fruits were observed daily to monitor any symptom of rot.

Results

The mycological approach allowed to isolate and identify according to morphological criteria 510 fungal pathogens from 225 natural infected fruits. The isolated pathogens belonging to 14 genera (*Talaromyces*, *Lasiodiplodia*, *Trichoderma*, *Penicillium*, *Pichia*, *Rhizopus*, *Aspergillus*, *Fusarium*, *Moniliella*, *Mucor*, *Geotrichum*, *Candida*, *Absidia* and *Purpureocillium*), classified into 22 species (Table 1) able to cause symptoms of rots on *P. americana*, *C. sinensis*, *C. papaya*, *A. muricata* and *S. lycopersicum* (Fig. 1).

Eighteen different identified fungal isolates with varied percentages of occurrences per sample were obtained from the diseased *P. americana* fruit samples with *R. oligosporus* having the highest occurrence (Table 1). Only one *Talaromyces verruculosus* strain DIV-20 (accession number PP249671) was found in 113 samples of *P. americana*, to give an approximate frequency of 0.9 (i.e., 1/113*100). From the diseased *C. sinensis* fruit samples, 19 fungal species were isolated with *L. theobromae* strain DIV-19 (PP249672) having the highest percentage of occurrence. Seventeen of the fungal species were isolated from the diseased *C. papaya* fruit samples with the highest frequency of occurrence shown to be *P. citrinum* strain DIV-17 (PP249674) and *A. nomius* strain DIV-04 (PP249686). From diseased *A. muricata,* 18 fungal species were isolates and *L. theobromae* showing the highest percentage occurrence. From the diseased *S. lycopersicum* fruits, 14 different fungal species were isolated with the highest and lowest % occurrence observed in *G. candidum* and *L.*

theobromae, respectively (Table 1). Supplementary Figure S2 shows the morphology of the main fungal isolates.

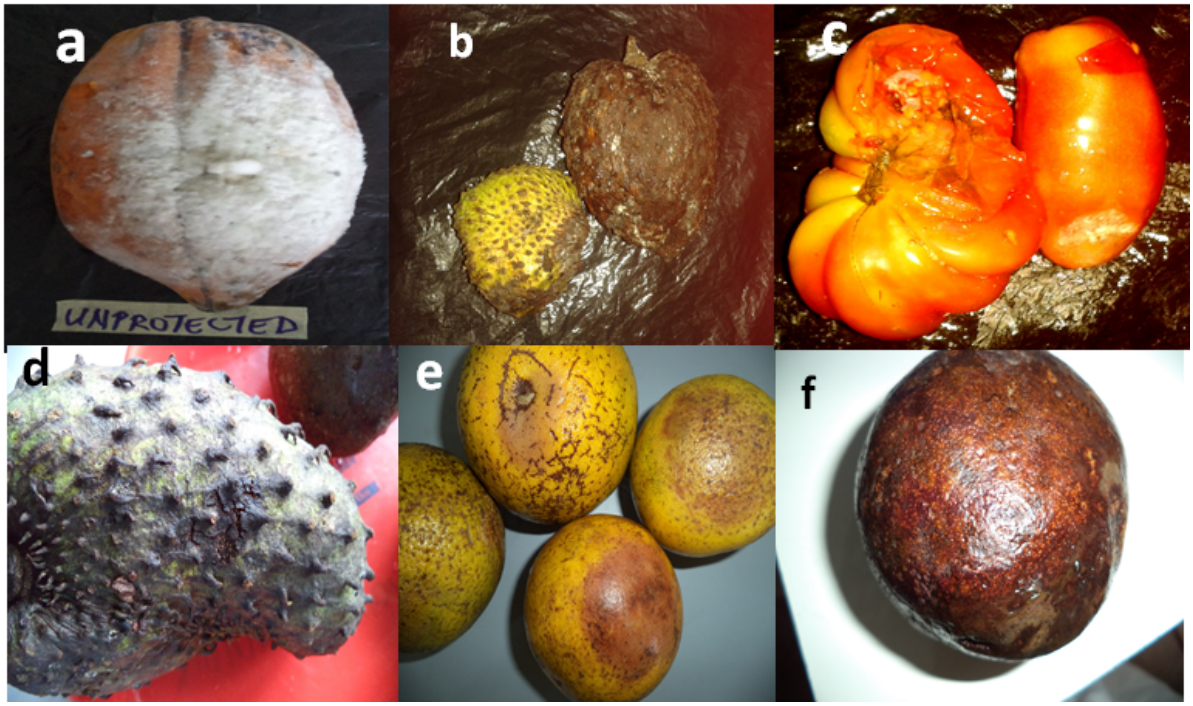

Fig. 1 – Symptoms of rot recorded on (a) *C. papaya,* (b) *A. muricata*, (c) *S. lycopersicum*, (d) *A. muricata* (e) *C. sinensis*, (f) *P. americana*.

Molecular characterization

The DNA extracted from the fungal isolates was of good quality and integrity (Supplementary Fig. S1a) and the results of PCR amplification of the fungal isolates allowed to yield a specific band around 650 bp (Supplementary Fig. S1b).

The following nucleotide sequencing of specific amplicon allowed to generally corroborate the identification of fungal pathogens carried out according to morphological criteria. There were some exceptions represented by *Aspergillus,* which is a complex genus difficult to be identified only by morphological tools. The nucleotide sequences were used also to analyze the phylogenetic relationship among the fungal isolates. There were more similar clusters among organisms of the same genus and the sequences of *Lasiodiplodia*, *Penicillium* and *Aspergillus* showed this expected close relationship (Supplementary Fig. S3). *Candida tropicalis strain* DIV-08 with the accession number PP256043 appears distantly related from all the other fungal isolates.

Pathogenicity of the fungal pathogens on post- harvest fresh healthy fruits

The results of the pathogenicity tests indicated that the selected fungal isolates (*T. verruculosus* strain DIV-20*, T. verruculosus* strain, DIV-2*, L. theobromae* strain DIV-1*, T. koningiopsis* strain DIV-18*, G. candidum* strain DIV-9 and the other isolates) were evidently pathogenic, owing to their capabilities to produce similar spoilage signs on fresh healthy fruits of *P. americana* within 5 Dots

Table 1 - Fungal pathogens and % occurrence in the disease fruits.

Fungal Isolates	Fruits					Total (%)
	P. americana N. (%)	*C. sinensis* N. (%)	*C. papaya* N. (%)	*A. muricata* N. (%)	*S. lycopersicum* N. (%)	
Talaromyces verruculosus (Peyronel) Samson, N. Yolmaz, Frisvad & Seifert	1 (0.9)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	2(0.4)
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	13 (11.5)	8 (9.2)	9 (8.8)	20 (16.8)	1 (1.1)	51 (10.0)
Trichoderma koningiopsis Samuels, Carm. Suárez & H.C. Evans	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Penicillium citrinum Thom	4 (3.5)	4 (4.6)	12 (11.8)	7 (5.9)	3 (3.4)	30 (5.9)
Pichia kudriavzevii Boidin, Pignal & Besson	6 (5.3)	5 (5.7)	1 (0.9)	5 (4.2)	5 (5.6)	22 (4.3)
Aspergillus niger Tiegh.	5 (4.2)	4 (4.6)	10 (9.8)	4 (3.7)	6 (6.7)	29 (5.7)
Fusarium culmorum (Wm.G. Sm.) Sacc.	7 (6.2)	6 (6.9)	5 (4.9)	4 (3.7)	4 (4.5)	26 (5.1)
Fusarium solani (Mart.) Sacc.	6 (5.3)	4 (4.6)	4 (3.9)	5 (4.2)	3 (3.4)	22 (4.3)
Aspergillus carbonarius (Bainier) Thom	5 (4.2)	6 (6.9)	2 (2.0)	6 (5.0)	2 (2.2)	21 (4.1)
Mucor racemosus Fresen	5 (5.2)	7 (8.0)	6 (5.9)	5 (4.2)	2 (2.2)	25 (4.9)
Geotrichum candidum Link	10(8.8)	6 (6.9)	9 (8.8)	8 (6.7)	18 (20.2)	51 (10.0)
Candida tropicalis (Casttell) Berkhout	6 (5.3)	4 (4.6)	3 (2.9)	7 (5.9)	14 (15.7)	34 (6.6)
Absidia corymbifera (Cohn) Sacc. & Trotter	5 (4.2)	2 (2.3)	4 (3.9)	3 (2.5)	12 (13.5)	26 (5.1)
Candida utilis Ladder & Kreger-van Rij	5 (4.2)	5 (5.7)	3 (2.9)	5 (4.2)	0 (0.0)	18 (3.5)
Aspergillus aculeatus Iizuka	5 (4.2)	6 (6.9)	5 (4.9)	14 (11.8)	0 (0.0)	30 (9.5)
Aspergillus nomius Kurtzman, B.W. Horn & Hesselt	8 (7.1)	5 (5.7)	12 (11.8)	9 (7.6)	5 (5.6)	39 (7.6)
Candida pseudotropicalis (Castell.) Basgal	4 (3.5)	0 (0.0)	2 (2.0)	4 (3.7)	11 (12.4)	21 (4.1)
Rhizopus microsporus var. *oligosporus* (Saito) Schipper & Stalpers	17 (15.0)	5 (5.7)	10 (9.8)	4 (3.7)	3 (3.4)	39 (7.6)
Aspergillus parasiticus Speare	0 (0.0)	5 (5.7)	0 (0.0)	5 (5.4)	0 (0.0)	10 (2.0)
Aspergillus sclerotiorum G.A. Huber	0 (0.0)	3 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.6)
Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken Hywelm-Jones & Samson	0 (0.0)	0 (0.0)	5 (4.9)	4 (3.7)	0 (0.0)	9 (1.8)
Moniliella suaveolens (Lindner) Arx	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Total	113 (100)	87 (100)	102 (100)	119 (100)	89 (100)	510 (100)

Table 2 - Pathogenicity of the fungal isolates on *P. americana* fruit samples.

Fungal Isolates	Macroscopic observations					Inference
	Day 1	Day 2	Day 3	Day 4	Day 5	
T. verruculosus	No spoilage	No spoilage	Small white mycelia growth at point of inoculation	White mycelia growth on fruits. Fruits soft at point of inoculation	Whole fruits soft & darkened. More mycelia growth	Spoilage started on 3rd day
L. theobromae	No spoilage	No spoilage	White mycelia growth appears on fruits	White mycelia growth spread out from point of inoculation	Fruits turned black & soft from point of inoculation outwards	Spoilage started on 3rd day
T. koningiopsis	No spoilage	No spoilage	Brownish discolouration at inoculation site	Internal damage & discolouration	Internal damage & discolouration	Spoilage started on 3rd day
R. oligosprous	Fruits firm except at site of inoculation	Soft areas increase but no exudates	White mycelia growth at inoculation site	Black spores appeared on the white mycelia. Fruits all soft	Fruits totally soft with much mycelia growth & black spores	Spoilage started on 1st day
G. candidum	No spoilage. Both fruits are firm	Fruits getting soft by ripening process	Mucoid yeast-like growth at point of inoculation	Mucoid growth increases. Inoculation area very soft	Mucoid growth slight offensive odour. Fruits very soft	Spoilage started on 3rd day
C. tropicalis	No spoilage, fruits are firm	Small patches of white mat-like growths observed.	Mat-like growth increasing in area	Mat-like growth spreading all over the fruits. Fruits soften	Increased mat-like growth spreading all over the fruits. Fruits soften	Spoilage started on 3rd day
A. aculeatus	No spoilage	No spoilage	No spoilage	No spoilage	Little white growth with brown spores. Inoculation site soft	Spoilage started on 5th day
A. nomius	No spoilage	No spoilage	Moderate cottony white mycelial growth at inoculation	Extensive white cottony growth taking over the whole fruits	Whole fruits having cottony white growth (in tufts). Fruit soft & spongy	Spoilage started on 3rd day
Uninoculated fruit	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage

Table 3 - Pathogenicity of the fungal isolates on *C. sinensis* fruit samples.

Fungal Isolates	Macroscopic observations					Inference
	Day 1	Day 2	Day 3	Day 4	Day 5	
T. verruculosus	No spoilage	No spoilage	Point of inoculation looks water-soaked	Water-soaked area increasing & fruits softening	Whole fruit looking brownish & watersoaked	Spoilage started on 3rd day
L. theobromae	No spoilage	Presence of watersoaked appearance at site of inoculation	Increased watersoaked appearance & discolouring	Extensive brown discolouration & offensive odour	Extensive rot & offensive odour	Spoilage started on 2nd day
A. niger	No spoilage	No spoilage	Water-soaked appearance around inoculation sites	Water-soaked, white mycelia growth & black spores	Much rottenness. Black spores on white mycelia	Spoilage started on 3rd day
F. culmorum	No spoilage	No spoilage	Water-soaked appearance at inoculation site	Cottony white growths at site of inoculation	Inoculation sites turn brownish with white cottony growths	Spoilage started on 3rd day
M. suareolens	No spoilage	No spoilage	No spoilage	No spoilage	Massive discolouring with white dry mycelia growth	Spoilage started on 5th day
M. racemosus	No spoilage	Water-soaked appearance at inoculation site	Increased watersoaked appearance	Small amounts of white mycelia	Small amounts of white mycelia	Spoilage started on 2nd day
G. candidum	No spoilage	No spoilage	Water-soaked appearance at inoculation site	Larger water-soaked areas, exudates & offensive odour	Increased water-soaked area, exudates & offensive odour	Spoilage started on 3rd day
A. aculeatus	No spoilage	Water-soaked appearance at site of inoculation	Increased watersoaked appearance at inoculation site	White mycelia with brown spores on inoculation site	White mycelia growth with brown spores & offensive odour	Spoilage started on 2nd day
Uninoculated fruit	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage, just ripening	No spoilage

Table 4 - Pathogenicity of the fungal isolates on *C. papaya* fruit samples.

Fungal isolates	Macroscopic observations					Inference
	Day 1	Day 2	Day 3	Day 4	Day 5	
L. theobromae	No spoilage	No spoilage	No spoilage	Water-soaked appearance mycelia growth	Extensive white mat-like growth from inoculation site	Spoilage started on 4th day
F. culmorum	No spoilage	Small white mycelia at point of inoculation	Increased white mycelia growth with fruit softened	Massive fungal growth on fruits. Whole fruit softened	Whole fruit covered with fungal growth Whole fruit softened.	Spoilage started on 2nd day
R. oligosprous	No spoilage	Fruits firm fruits at inoculation site which appeared water-soaked	Water-soaked & soft areas around inoculation site	Little white floccose mycelia seen	Little mycelial growth with presence of black spores	Spoilage started on 2nd day
A. niger	No spoilage	No spoilage	Little white mycelial growth seen	Little white mycelial growth with no spores	Extensive mycelial growth & black spores, rot & exudates	Spoilage started on 3rd day
M. racemosus	No spoilage	White cottony mycelia growing out of inoculation site	Increased white cottony mycelia & presence of grey-black spores	Cottony mycelia, grey-black spores & fruits softness	Cottony mycelia, grey-black spores & fruits softness on whole fruits	Spoilage started on 2nd day
G. candidum	No spoilage	No spoilage	Mycelia &water-soaked appearance at inoculation site	Increased mycelia & water-soaked appearance	Extensive mycelia & water-soaked appearance	Spoilage started on 3rd day
A. nominus	No spoilage	Moderate cottony white mycelia at inoculation site	Extensive cottony growth on whole fruits	White cottony growth developed white spores	Whole fruits having cottony growth with yellow-green spores	Spoilage started on 2nd day
P. lilacinum	No spoilage	Slight softness at inoculation site	Slight softness at inoculation site	Presence of white felt-like mycelia at inoculation site	Spread of mycelia with whole fruits becoming soft to touch	Spoilage started on 2nd day
Uninoculated fruit	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage

Table 5 - Pathogenicity of the fungal isolates on *A. muricata* fruit samples.

Fungal isolates	Macroscopic Observations					Inferences
	Day 1	Day 2	Day 3	Day 4	Day 5	
L. theobromae	No spoilage	No spoilage	Fruits turning brown at point of inoculations	Increased brown points & white mycelial growth	Extensive white mycelial growth & browning	Spoilage started on 3rd day
R. oligosprous	No spoilage	Little white mycelial growth at inoculation site	Increased mycelial growth & presence of black spores	Increased black spores & white mycelial growth	Extensive black spores on fruits & softened fruits	Spoilage started on 2nd day
A. parasiticus	No spoilage	No spoilage	No spoilage	Presence of white mat-like mycelia & browning	Extensive white mat-like mycelia & brownish area	Spoilage started on 3rd day
A. corymbifera	No spoilage	No spoilage	Site of inoculation becoming soft	Small white mycelia at inoculation site	Increased growth of fluffy white mycelia	Spoilage started on 3rd day
C. utilis	No spoilage	No spoilage	Softening at inoculation site & fungal growth	Increased softening & fungal growth	Extensive damage at site of inoculation	Spoilage started on 3rd day
A. aculeatus	No spoilage	No spoilage	Presence of white mycelia & softening	Increased softening & white mycelia	Extensive softening & white mycelia	Spoilage started on 3rd day
A. nomius	No spoilage	No spoilage	Site of inoculation darkened	Increased darkening & fungal growth	Extensive darkening & fungal growth	Spoilage started on 3rd day
P. lilacinum	No spoilage	No spoilage	No spoilage	Slight mycelial growth & discolouration	Extensive mycelial growth & darkening	Spoilage started on 4th day
Uninoculated fruits	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage

Table 6 - Pathogenicity of the fungal isolates on *S. lycopersicum* fruit samples.

Fungal isolates	Macroscopic Observations					Inference
	Day 1	Day 2	Day 3	Day 4	Day 5	
P. kudriavzevii	Swelling at site of inoculation	Softening at site of inoculation soft	White yeast-like growth with watery exudates from site of inoculation	Increased yeastlike growth & fruit collapse	White yeast-like growth with total fruit collapse	Spoilage started on 1st day
A. niger	Water-soaked appearance at site of inoculation	Heavy black spores at site of inoculation & white mycelia	Heavy black spores. Inoculation site become sunken & water soaked	Heavy black spores & fruit collapse	Total fruits collapse, watery exudates, heavy black spores	Spoilage started on 1st day
F. culmorum	No spoilage	Softening at inoculation site	Softening & white mycelia growth at inoculation sites	Increased white mycelia growth & fruit collapse	Extensive white mycelia growth & total fruit collapse	Spoilage started on 2nd day
G. candidum	No spoilage	No spoilage. Fruits still firm	Dirty mucoid growth on the fruits. Sunken spots	Increased mucoid growth sunken spots	Total fruit collapse & exudates	Spoilage started on 2nd day
M. racemosus	Water-soaked appearance at inoculation site	White mycelia from point of inoculation	White fluffy mycelia increased	White fluffy mycelia with black spores	White fluffy mycelia abundant. Black spores abundant	Spoilage started on 1st day
C. tropicalis	No spoilage	No spoilage	Water-soaked appearance at inoculation site	Water-soaked appearance & watery exudates	Total fruit collapse & watery exudates	Spoilage started on 3rd day
C. utilis	No spoilage	No spoilage	White mycelial growth on fruits & water soaked-appearance	White mycelia growth & soft inoculation site	Total fruit collapse, watery milky exudates	Spoilage started on 3rd day
Rhizopus spp.	Water-soaked appearance	White mycelia seen	White fluffy mycelia	Increased white fluffy mycelia & black spores	Extensive fluffy white mycelia with black spores	Spoilage started on 1st day
Uninoculated fruits	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage	No spoilage

Per Inch (dpi) at 28 ± 2 °C. *Rhizopus oligosporus* and *A. aculeatus* elicited spoilage on healthy *P. americana* fruit within 24 h after inoculation and 5 dpi, respectively (Table 2).

In the case of the fresh healthy *C. sinensis* fruit samples artificially inoculated with *L. theobromae, M. racemosus* and *A. aculeatus,* spoilage was recorded after two days of post inoculation, displaying water-soaked appearance at the inoculation site. For fruit samples inoculated with *T. verruculosus*, *F. culmorum* and *G. candidum*, spoilage started on the third day after inoculation while fruit samples inoculated with *M. suaveolens* did not initiate spoilage until the fifth day (Table 3).

For the healthy *C. papaya* fruits, when inoculated with *F. culmorum*, *R. oligosprous*, *M. racemosus* and *A. nominus*, spoilage was observed from the second day after inoculation while *A. niger* and *G. candidum* elicited spoilage from the third day. When inoculated with *L. theobromae* and *P. lilacinum*, spoilage of *C. papaya* fruit samples started 3 and 4 days after inoculation, respectively (Table 4). *Annona muricata* inoculated with *L. theobromae*, *A. conymbifera*, *C. utilis*, *A. aculeatus* and *A. nominus* started to spoil on the third day after inoculation while those inoculated with *P. lilacinum* started to spoil on the fourth day (Table 5).

In the case of the fresh healthy *S. lycopersicum* fruit samples, when inoculated with *P. kudriavzevii*, *A. niger*, *F. culmorum*, *G. candidum*, *C. tropicalis*, *C. utilis*, *Rhizopus* sp. and *M. racemosus*, spoilage was observed 5 days post inoculation. For the fruits inoculated *P. kudriavzevii*, *A. niger*, *Rhizopus* sp. and *M. racemosus*, spoilage was elicited within 24 h. Spoilage was observed 48 h post inoculation for fruits when inoculated with *F. culmorum* and *G. candidum,* while those inoculated with *C. tropicalis* and *C. utilis* showed spoilage about 72 h after inoculation (Table 6).

Discussion

The roles of fungal pathogens in the rot of fruits have been established in several previous research (Moss, 2008; Whitehead et al., 2015; Triest and Hendrickx, 2016; Zeilinger et al., 2016) and has been shown in this study carried out in Akwa Ibom State, Nigeria. The morphological approach can be a preliminary approach, but the use of molecular analysis, sequencing of the ITS region of fungi proved to be very useful. During the research, fungal samples initially classified as *A. flavus* were molecularly identified as *A. nomius* strain DIV-4. Those identified as *A. ochraceous* were molecularly classified as *A. sclerotiorum* and *A. tamari*. In particular, *A. nomius* are often misidentified as *A. flavus* (Tam et al., 2014). The indeterminate nature of the sclerotia would have been a vital divergence between the *A. nomius* and *A. flavus* but not all strains of either *A. nomius* or *A. flavus* produce sclerotia. Only molecular characterization can give a clear distinction between *A. nomius*, *A. flavus* and *A. tamari*.

The prevalent isolated fungi, including *T. verruculosus* strain DIV-20, *T. verruculosus* strain DIV-2, *L. theobromae* strain DIV-19, *L. theobromae* strain DIV-1, *T. koningiopsis* strain DIV-18, *P. citrinum* strain DIV-17, *P. kudriavzevii* strain DIV-16, *A. niger* strain DIV-14, *G. candidum* strain DIV-9 and the other isolates (Table 1, Supplementary Table S1), were in accordance with the list of fungal species reported by Udoh et al. (2015). They were isolated in completely different environmental conditions, demonstrating the ability of fungi to cause spoilage of fruits (Egbuta et al., 2016). The inoculum source might start from farm through to washing with sewage contaminated groundwater or surface water containing fungal pathogens (Adegoke and Stenstrom, 2019; Nzima et al., 2020) or cross contamination from currency notes paid while going through supply chains (Adegoke and Okoh, 2011).

The deterioration observed in our studies revealed that in a period of 5 days of storage, even the weakest fungi would have done meaningful damage to the post-harvested fruits, causing economic losses (Dean et al., 2012; Drenth and Guest, 2016). In addition, some postharvest fungi also constitute serious threat to human health, because of mycotoxigenic potentials of some fungal genera, including those isolated in our study like *Penicillium* and *Fusarium*, with high toxicity to consuming humans and animals (Li et al., 2015; Sanzani et al., 2016). This can be exemplified by the *Fusarium* species which are primary plant pathogens characterized by being septate, fusiform to crescent shaped macroconidia, and may contain microconidia (Leslie and Summerell, 2006). They are proven phytopathogens as well as aetiological agents of diseases and infections in humans and animals (Nucci and Anaissie, 2007). Like many pathogens, their contamination of these fruits is made easier, when contamination waters are used to wash the fruit (Adegoke and Stenstrom, 2019); the error that comes with severe human health risks. The health risk may include toxin productions. *Fusarium* species produce several types of toxins that affect both humans and animals (Jimenez-Garcia et al., 2018). Naturally, most postharvest pathogenic fungi are known for necrotrophic attributes, in which their secreted enzymes or toxins cause cytocidal while the fungi then live saprophytically on the dead cells (Tian et al., 2016).

This study established the array of fungal pathogens associated with post-harvest fruits rots in Akwa Ibom State. the data coming from this study allow to start to plan specific control strategies against the main post-harvest fungi starting from the field.

Authors' contributions

Divine-Anthony O: Conceived and designed the experiments, performed the experiments, analyzed and interpreted the data, contributed reagents, materials, analysis of data and contributed to the writing of the manuscript first draft. **Adegoke AA:** redrafted the manuscript, analyzed and interpreted the data, did extensive editing/revision of the manuscript, contributed reagents, material s and analysis of data. **Oduoye OT:** performed the experiment, analyzed and interpreted the data, contributed reagents, materials and analysis of data. **Akpor OB:** analyzed and interpreted the data and wrote the manuscript first draft

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