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CONTROL OF FUNGI CAUSING POST-HARVEST ROT IN WHITE YAM (*Dioscorea cayenensis*) USING SELECTED PLANT EXTRACTS

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ABSTRACT

Post-harvest yam tuber rot is a fungal disease affecting the storage of white yam [Dioscorea cayenensis subsp. rotundata (Poir.) J. Miège], and this poses a significant threat to global food security. There is a need to develop suitable measures to mitigate the threat and ensure the availability of rot-free tubers to consumers. This study aimed to isolate and identify the fungi responsible for post-harvest rot in white yam sourced from selected markets in Lagos, Nigeria, and the efficacy of various plant extracts in treating these fungi. Isolated fungal strains were subjected to pathogenicity tests to assess for virulence. The virulent strains were identified using molecular techniques. Ethyl acetate and n-hexane extracts of Pseudocedrela kotschyi, Parkia biglobosa, and Mezoneuron benthamianum were screened against the isolates using the agar well diffusion method. Six fungi were isolated from the diseased yam tubers, of which two were virulent and capable of inducing rot in healthy yam tubers after 14 days of inoculation. These were identified as Penicillium oxalicum strain LBCCN_YS_A3 (OQ550968) and Aspegillus niger strain LBCCN_YS_A8 (OQ550969). Of all plant extracts tested, P. biglobosa had the highest antifungal effects on the growth of the fungal strains. Therefore, this plant extract is a sustainable alternative to the inorganic fungicides for mitigating post-harvest spoilage of white yam tuber.

Keywords: antifungal, *Aspergillus niger*, post-harvest, *Parkia biglobosa,* pathogenicity

INTRODUCTION

Yams belong to the family Dioscoreaceae and are important staple and fallback foods in Africa, Pacific Islands, the Caribbean, Asia, and South American countries. The tuber has rough skin usually dark to light brown and is about 1.6 meters in height and weighs about 2-5 kg depending on size (Alinnor and Akalezi, 2010). The *Dioscorea rotundata* (white yam) is the most common and commonly grown variety of yam, which is endemic to Africa (Andres *et al*., 2017). According to a 2023 report from the National Root Crops Research Institute (NRCRI), Nigeria is the world's top producer of yam, accounting for 34 metric tonnes, or more than 70% of global yam production. Yams are high in carbohydrates, vitamin C, and other essential minerals. They are also a significant source of dietary fibre. According to available statistics, more than 25% of yam tubers produced in Nigeria are lost to disease during storage (Ano, 2019). Therefore, identifying the causative agent is essential before developing mitigation measures to guarantee the supply of tubers to consumers. Fungal pathogens such as *Lasiodiplodia theobromae*, A*spergillus flavus, Fusarium solani, Aspergillus niger, Trichoderma viride, Penicillium chrysogenum, Collectotrichum* spp, *Penicillium oxalicum*, F*usarium oxysporum, Geotrichum candidum, Penicillium digitatum, Rhizoctonia* spp, and *Rhizopus nodosus* have been implicated in rot diseases of stored yams in Nigeria (Okoro and Nwankiti, 2004; Okigbo *et al.,* 2015).

Several control measures, such as chemical and biological control, have been employed to combat rot pathogens in stored yams, including *Trichoderma harzianum* as a biocontrol agent (Gwa *et al.,* 2015). Several agrochemicals are extremely toxic and non-biodegradable (Gwa and Nwankiti, 2017), with various disadvantages. Given that plant extracts include a variety of bioactive substances, including phenols, saponins, flavonoids, glycosides, sterols, and alkaloids (Gwa *et al*., 2015), they may be a more effective way to manage plant diseases than toxic fungicides.

Pseudocedrela kotschyi commonly known as Dry-Zone cedar is a member of the family Meliaceae. *P. kotschyi* grows in the savannah of tropical Africa. Different plant parts of *P. kotschyi* are used to cure various ailments, including malaria, toothaches, abdominal pain, diarrhea, and epilepsy (Ahua *et al.,* 2007). *Candida albicans* is inhibited by petroleum ether, ethyl acetate, and methanol extracts of *P. kotschyi* leaves (Ayo *et al.,* 2010).

The African locust bean (*Parkia biglobosa)*, is a perennial deciduous tree of the Fabaceae family. It cures several illnesses, such as bronchitis, hypertension, and diarrhoea, by processing them into paste, decoction, and juice (Singh *et al.,* 2015).

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According to research by Akwaji *et al*. (2016), *P. biglobosa's* ethanolic and methanolic stem bark and leaf extracts inhibited *A. niger* and *Botryodiplodia theobromae* from growing. *Mezoneuron benthamianum* is a medicinal plant of the Fabaceae family and is native to many African countries. *M. benthamianum* is as cream and powder for wounds and dermal infections (Dickson *et al.,* 2011). The findings of the synergistic efficacy report indicate that *M. benthamianum* exhibits potent anti-candidal properties, highlighting its potential as a plant for further exploration in drug development as an anti-candidal agent (Fayemi *et al.,* 2012).

There have been several reports on the effectiveness of plant crude extracts using ethanol, methanol, and water (Fayemi *et al.,* 2012; Gwa and Nwankiti, 2017), whereas there is a scarcity of information on the potential of non-polar solvents and semi-polar solvents. The specific objectives of this study were to; phylogenetically characterize the fungi causing post-harvest yam tuber rot from some local markets in Lagos State, Nigeria, and investigate the effects of n-hexane and ethyl acetate extracts of *Pseudocedrela kotschyi, Parkia biglobosa* and *Mezoneuron benthamianum* on the post-harvest spoilage of yam.

MATERIALS AND METHODS

Collection of Samples

Diseased specimens of *Dioscorea cayenensis* subsp. *rotundata* (white yam) were collected from Iyana-Iba market (6.4611 ºN, 3.2043 ºE) and Okokomaiko market (6.4714ºN, 3.1879 ºE) within the Ojo Local Government Area, Lagos, Nigeria (Figure 1). The diseased specimens were inspected for rotted portions, stored in clean polypropylene ziplock bags, labelled and kept for further analysis. The stem of *Pseudocedrela kotschyi* (Schweinf.) Harms and the root of *Mezoneuron benthamianum* Baill. were collected from Bode Alagbo market (7.3776 ºN, 3.9471 ºE) in the Ibadan area of Oyo State, Nigeria, while *Parkia biglobosa* (Jacq.) R.Br. ex G.Don bark, on the other hand, was obtained from the Forestry Research Institute of Nigeria (FRIN) in Ibadan (7.3919 ºN, 3.8631 ºE) (Figure 1). These plant parts were identified at the Plant Taxonomy Unit, Department of Botany, Lagos State University, Nigeria.

Isolation of Fungi

These fungi were isolated using potato dextrose agar (PDA). The yam specimens with rot symptoms were washed several times under running water. The yam tubers were then cut open with a sterile kitchen knife to reveal the rotten and healthy parts. Small pieces of approx. 2 × 2 mm from yam specimens rotten at the transitional stage between the tubers' healthy and decaying sections were cut using a sterile scalpel (Gwa and Ekefan, 2018). First, they were surface sterilized by fully immersing them in a beaker filled with 5% sodium hypochlorite solution for a minute. The sections that were to be infected were then taken out and washed twice with sterile distilled water. The yam pieces were blotted with sterile filter paper to remove excess water before being inoculated onto the PDA medium that has solidified containing chloramphenicol in culture plates. The plates were incubated at room temperature (27±2 ºC) for 72 h. After being sub-cultured into freshly prepared media plates, the fungal colonies growing on the plates were kept in an incubator at room temperature (27±2 ºC). After three consecutive subculturing regimes, pure cultures were obtained.

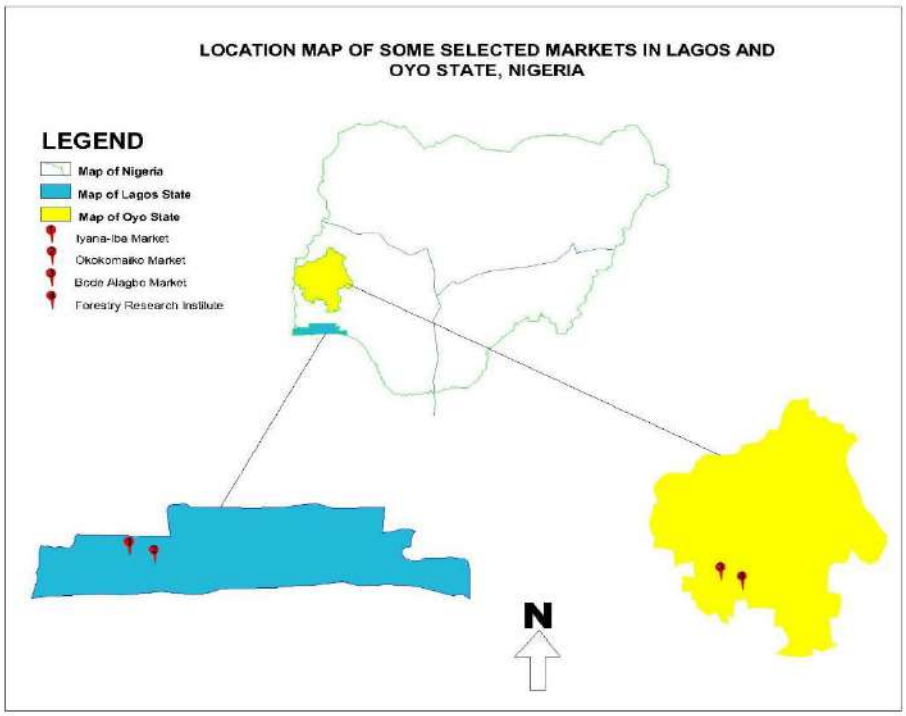

Figure 1: Map showing various collection sites in Lagos and Oyo States, Nigeria

Microscopic Characterization of Fungi
Microscopic and morphological characterization of fungi such as hyphae type, and asexual reproductive structure, were observed.

Pathogenicity Test
The pathogenicity test was carried out according to the method of Gwa and Ekefan (2017). Healthy yam tubers were surface sterilized for a few seconds using 5% sodium hypochlorite after being cleaned under running water and rinsed with sterile distilled water. The healthy yam tubers were pierced with a sterile 5 mm cork borer down to a depth of 4 mm, and the bored tissues were extracted. A 5 mm diameter disc from the pure cultures obtained was cut and replaced in the wells created. To prevent contamination from other pathogenic organisms, the wells were sealed using petroleum jelly, or Vaseline. Under aseptic conditions, the yam tubers that were inoculated were kept at room temperature (27±2 °C) in two replications. Following a 14-day incubation period, the tubers were allowed to facilitate the growth and maturation of the inoculum before being examined for signs of infection and disease development.

Molecular Identification of Fungi
The pathogenic species were further subjected to molecular analysis to confirm their identities. Extraction and PCR amplification of genomic DNA, DNA sequencing, and ITS region analysis were performed toward verifying the identification of the fungal pathogens following their microscopic and culture characterization.

Extraction of genomic DNA from pure cultures of fungi
Genomic DNA was isolated from 5-day-old mycelial cultures using the Quick DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No D6005, USA). The resulting ultra-pure DNA was stored at –80 ºC for subsequent analysis.

DNA sequencing
DNA sequencing was carried out at Inqaba Biotechnical Industrial (Pty) Ltd, Ibadan, Oyo State, Nigeria. The universal primer pairs ITS1-F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4-R (5'-TCCTCCGCTTATTGATATGC-3') were used for DNA amplification according to the method of White *et al*. (1990). The resulting amplicons were subjected to gel electrophoresis and extraction using the Zymoclean™ Gel DNA recovery kit (Zymo Research, catalog no. D4001). The extracted fragments were sequenced in both forward and reverse directions using Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit, and subsequently purified with the ZR-96 DNA sequencing clean-up kit™ (Zymo Research, catalog no. D4050). Sequencing was performed using the ABI 3500×l Genetic Analyzer (Applied Biosystems, Thermo-fisher Scientific), with each sample undergoing individual reactions to ensure accurate results. The resulting chromatograms (sense and antisense) were analyzed for quality using ChromasLite version 2.33 software (Hall, 2004) and subsequently edited using BioEdit Sequence Alignment Editor (Hall, 2004). The edited consensus sequences were then queried against the NCBI database using the Basic Local Alignment Search Tool (BLAST) for homology identification (Altschul *et al*., 1997). The obtained sequences were deposited in GenBank, and accession numbers were generated. Phylogenetic trees were constructed using the neighbor-joining algorithm (Saitou and Nei, 1987) with 1000 bootstraps consensus in the Mega 6.0 software package (Tamura *et al*., 2013) to elucidate the genetic relationships among the identified organisms.

Preparation of plant extracts
Using ethyl acetate and n-hexane as solvents, extraction from the fine powder of the three plant materials (70 g each) was done for 24 h. The samples were placed under vacuum and filtered through Whatman No. 1 filter paper. The filtrates were then concentrated in a vacuum rotary evaporator. The resultant extract was placed into pill vials and kept in a –20 ºC freezer. The filtrate was put in a weighed conical flask and evaporated to dryness. The dish and extract were weighed after cooling in the desiccator.

Anti-fungal activity of the plant extracts
The anti-fungal activity of the plant extracts was evaluated using the Holder and Boyce (1994) agar well diffusion method. A sterile cork borer with a diameter of 8.00 mm was used to create two wells in each plate. Then, concentrated extracts mixed with the growth media were incorporated into each well using a sterile micro-pipette and left for a few minutes to diffuse. 0.3 ml of the 10 mg ml^{-1} ethyl acetate extract of one plant was poured into one well, and 0.3 ml of the 10 mg ml^{-1} n-hexane extract of the same plant was poured into the second well of each plate using a sterile micro-pipette. The same procedure was repeated for the 20 mg ml^{-1} extracts of the same plant. From the margins of the 7-day-old pure culture, a 6 mm mycelial disc was removed and put in the center of the plate. Ketoconazole was used as the positive control with similar concentrations. The negative control was sterile distilled water. The plates were prepared in triplicate and incubated for five days at room temperature (27±2 ºC). The inhibitory zones in each plate were recorded in millimeters (mm).

Statistical Data Analysis
The data were put through analysis of variance, and the level of significance between values was indicated by separating the means using Duncan at the ($\alpha = 0.05$) probability level.

RESULTS

The fungi isolated from the yam tubers exhibiting rot symptoms were *Aspergillus niger, Aspergillus flavus, Lasiodiplodia theobromae, Aspergillus aculeatus, Penicillium oxalicum, and Fusarium* sp.

Pathogenicity Test

Six fungal species isolated were subjected to pathogenicity test, but only two, *Penicillium* sp. and *Aspergillus niger*, caused rot in healthy yam tubers following a 14-day inoculation period. Of these two, *Penicillium* sp. was more virulent than *A. niger*.

Molecular Identification of Fungi

Molecular analysis confirmed the identities of the two pathogenic species to be *P. oxalicum* and *A. niger*.

Phylogenetic Relatedness of Fungal Strains

The phylogenetic analysis revealed that *Aspergillus niger* LBCCN_YS_A8 (OQ550969) clustered with *A. niger* (MN100313), *A. niger* (MK534501), and *A. niger* (MN788109) respectively, and all with a relative percentage identity of 100 % (Figure 2). In the same manner, *A. niger* LBCCN_YS_A8 (OQ550969) clustered with *A. niger* (MK461090) and *A. niger* (MT588793) (Figure 2). Similarly, *Penicillium oxalicum* LBCCN_YS_A3 (OQ550968) exhibited similarities with *P. oxalicum* (OM252529), *P. oxalicum* (OM252530), and *P. oxalicum* (OM252528) respectively, with a percentage identity of 100 % (Figure 2).

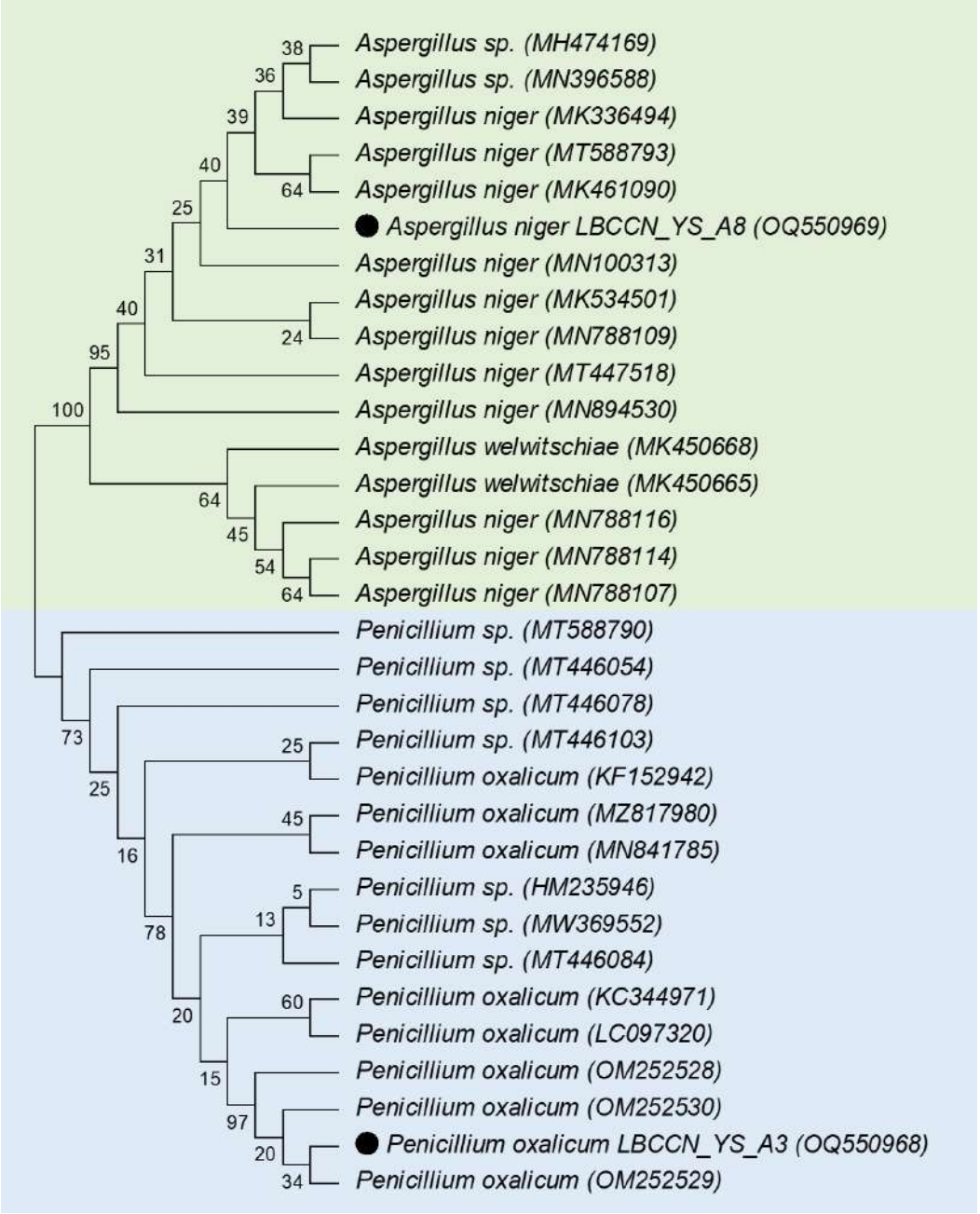

Figure 2: Phylogenetic tree showing the relationship between pathogenic fungi (*A. niger* (OQ550969) and *P. oxalicum* (OQ550968)) from *Dioscorea cayenensis* subsp. *rotundata* (White Yam) with other related fungal species

Efficacy of Different Concentrations of N-Hexane Plant Material Extracts on the Investigated Fungi Inhibition

As shown in Table 1, the n-hexane extract of *P. biglobosa* inhibited the growth of the tested fungi at a concentration of 10 mg ml^{-1} only. The n-hexane extracts of *P. kotschyi* inhibited the growth of *A. niger* only at 10 mg ml^{-1} and n-hexane extract of *M. benthamianum* inhibited the growth of *P. oxalicum* only at 20 mg ml^{-1}. The n-hexane extract of *M. benthamianum* had a negative effect on *A. niger* in all concentrations and on *P. oxalicum* at 10 mg ml^{-1} was sensitive to *P. oxalicum* at 20 mg ml^{-1}. The commercial fungicide (Ketoconazole), inhibited the growth of the tested fungi with the highest inhibitory value of (29.00±0.00) (Table 3), while the distilled water treatment showed no inhibition.

Efficacy of Different Concentrations of Ethyl Acetate Extracts of the Plant Materials on the Inhibition of the Tested Fungi

Table 1 shows that the ethyl acetate extract of *P. kotschyi* was insensitive to the tested fungi at concentrations of 10 and 20 mg ml^{-1} for *P. oxalicum* but inhibited the growth of *A. niger* at 20 mg/ml. The ethyl acetate of *M. benthamianum* was only effective for *P. oxalicum* at both concentrations (10 and 20 mg ml^{-1}). The commercial fungicide (Ketoconazole) used inhibited the growth of the tested fungi with the highest inhibitory value of 29.00 (Table 3), while the sterile distilled water did not show any inhibition.

Effects of Plant Extracts on *A. niger* Inhibition

For *A. niger*, inhibitory values recorded from both ethyl acetate and n-hexane extracts of *P. biglobosa, P. kotschyi, and M. benthamianum* were significantly ($p < 0.05$) less than the values from ketoconazole. The mean and standard deviation of the readings are shown in Table 2. From the results concerning n-hexane extracts, *P. biglobosa* at 10 mg/ml has the highest inhibitory effect with an inhibitory zone of 23.00±1.00. The minimum inhibitory effect was observed in *P. kotschyi* (10 mg/ml) with an inhibition zone of 19.67±1.53. For the ethyl acetate extracts, *P. biglobosa* at 20 mg ml^{-1} has the highest inhibitory effect, with an inhibition zone of 22.00±1.00 significantly lower than that of ketoconazole (26.00±0.00) at 10 mg ml^{-1} while the minimum inhibitory effect was recorded at 20 mg ml^{-1} for *P. kotschyi*.

Table 1: The efficacy of various plant material extracts using n-hexane and ethyl acetate on the inhibition of the tested fungi

Extracts	Organisms	n-hexane		Ethyl Acetate	
		10 mg ml^{-1}	20 mg ml^{-1}	10 mg ml^{-1}	20 mg ml^{-1}
P. biglobosa	*A.niger*	+	-	-	+
	P. oxalicum	+	-	-	+
P. kotschyi	*A. niger*	+	-	-	+
	P. oxalicum	-	-	-	-
M. benthamianum	*A. niger*	-	-	-	-
	P. oxalicum	-	+	+	+
Ketoconazole	*A. niger*	+	+	+	+
	P. oxalicum.	+	+	+	+
Distilled water	*A. niger*	-	-	-	-
	P. oxalicum.	-	-	-	-

Keys: + → Inhibition - → No inhibition

Table 2: The efficacy of Ketoconazole and various plant material extracts using n-hexane and ethyl acetate on the inhibition of *A. niger* five days post-inoculation

Extracts	n-hexane		Ethyl Acetate	
	10 mg ml^{-1}	20 mg ml^{-1}	10 mg ml^{-1}	20 mg ml^{-1}
P. biglobosa	$23.00±1.00^{b}$	$0.00±0.00^{d}$	$0.00±0.00^{e}$	$22.00±1.00^{c}$
P. kotschyi	$19.67±1.53^{c}$	$0.00±0.00^{d}$	$0.00±0.00^{e}$	$19.00±2.00^{d}$
M. benthamianum	$0.00±0.00^{d}$	$0.00±0.00^{d}$	$0.00±0.00^{e}$	$0.00±0.00^{e}$
Distilled Water	$0.00±0.00^{d}$	$0.00±0.00^{d}$	$0.00±0.00^{e}$	$0.00±0.00^{e}$
Ketoconazole	$26.00±0.00^{a}$	$24.00±2.00^{b}$	$26.00±0.00^{a}$	$24.33±2.08^{b}$

Note: ANOVA-1 reveals a significant ($p < 0.05$) variation in means and this is denoted by matching superscript alphabets.

Table 3: The efficacy of Ketoconazole and various plant material extracts using n-hexane and ethyl acetate on the inhibition of *P. oxalicum* five days post-inoculation

Extracts	n-hexane		Ethyl acetate	
	10 mg ml^{-1}	20 mg ml^{-1}	10 mg ml^{-1}	20 mg ml^{-1}
P. biglobosa	$18.00±2.65^{d}$	$0.00±0.00^{e}$	$0.00±0.00^{e}$	$19.50±1.50^{c}$
P. kotschyi	$0.00±0.00^{e}$	$0.00±0.00^{e}$	$0.00±0.00^{e}$	$0.00±0.00^{e}$
M. benthamianum	$0.00±0.00^{e}$	$25.00±1.0^{c}$	$19.67±1.53^{c}$	$11.67±1.53^{d}$
Distilled Water	$0.00±0.00^{e}$	$0.00±0.00^{e}$	$0.00±0.00^{e}$	$0.00±0.00^{a}$
Ketoconazole	$27.00±0.00^{b}$	$29.00±0.00^{a}$	$27.00±0.00^{b}$	$29.00±0.00^{a}$

Note: ANOVA-1 reveals a significant ($p < 0.05$) variation in means and this is denoted by matching superscript alphabets.

Effects of Plant Extracts on *P. oxalicum* Inhibition
Against *P. oxalicum*, the inhibitory values recorded from both extracts (ethyl acetate and n-hexane) of *P. biglobosa, P. kotschyi, and M. benthamianum* were significantly ($p < 0.05$) less than values obtained from Ketoconazole. The mean and standard deviation of the readings are shown in Table 3. From the results concerning n-hexane extracts, *M. benthamianum* at 20 mg ml^{-1} has the highest inhibitory effect with an inhibitory zone of 25.00±1.0. The minimum inhibitory effect was observed in *P. biglobosa* (10 mg/ml) with an inhibition zone of 18.00±2.65. For the ethyl acetate extract, *M. benthamianum at* 10 mg/ml showed the highest inhibitory effect (19.67±1.53), significantly lower than that of Ketoconazole (29.00±0.00), and the minimum inhibition was observed at 20 mg ml^{-1} of *M. benthamianum.*

DISCUSSION

This study indicated the specific fungal species responsible for white yam post-harvest rot in selected markets across Lagos State, Nigeria. The accurate taxonomy of every organism is necessary for future research (Adeniyi *et al*., 2018). Some fungal isolates cannot be taxonomically identified based on phenotypic characteristics. The classical method of fungal identification relies mainly on morphological characteristics that is not entirely reliable. However, the Internal Transcribed Spacer (ITS) portion of the DNA (rDNA) might be sequenced to distinguish them (White *et al*., 1990). Six fungal species which are *Aspergillus niger, Aspergillus flavus, Aspergillus aculeatus*, *Penicillium oxalicum*, *Lasiodiplodia theobromae,* and *Fusarium* sp. were isolated from the decaying white yam tubers. This finding agrees with Okigbo and Ikediugwu (2002) and Kamara *et al.* (2022).

The results of the pathogenicity test showed that *Penicillium oxalicum* and *Aspergillus niger* were the most virulent in causing rot in white yam tubers. The result obtained from the pathogenicity test corroborates the findings of Okigbo and Ikediugwu (2002) on yam tubers. This study showed that the plant materials used were composed of various antifungal components as they could inhibit the tested fungi from growing. This aligns with the reports of Ayo *et al*. (2010) and Fayemi *et al*. (2012) on *Candida* spp. and the report of Akwaji *et al.* (2016) on *Aspergillus niger* and *Botryodiplodia theobromae*. As a result, the plant extracts used have the potential to protect food crops from rot fungi. However, the efficacy of the extracts differed according to the type of plant.

Inference from this study revealed that the extracts of *P. biglobosa* inhibited the growth of the tested fungi more than other extracts screened for their inhibition against the various fungi responsible for post-harvest spoilage of yam. It was observed that the *P. biglobosa* and *P. kotschyi* n-hexane extracts at 10 mg/ml and their ethyl acetate extracts at 20 mg ml^{-1} inhibited the growth of *A. niger.* In contrast, the *M. benthamianum* extracts were insensitive at all concentrations. This result is consistent with the work of Bassey *et al.* (2013), who claimed that *A. niger* inhibition is mostly unaffected by the extract concentrations. However, the n-hexane extracts of *P. biglobosa* and *M. benthamianum* at 10 and 20 mg ml^{-1} respectively, inhibited the growth of *Penicillium oxalicum,* with *M. benthamianum* at 20 mg ml^{-1} having the highest inhibitory effects. The ethyl acetate extract of *M. benthamianum* inhibited the growth of *P. oxalicum* at all concentrations; ethyl acetate of *P. biglobosa* was effective against *P. oxalicum* at a concentration of 20 mg ml^{-1} only. Ketoconazole inhibited the growth of the tested organisms, while sterile distilled water did not show any inhibitory effects.

CONCLUSION

This study has shown that pathogenic fungi are the main cause of post-harvest rot in yam tubers. It has also shown the efficacy of plant extracts as antifungals (*P. biglobosa, P. kotschyi, and M. benthamianum*) against fungi associated with white yam tuber rot, which indicates the presence of antifungal components in the plant materials. Therefore, these plants might serve as a safer substitute for fungicides used in controlling fungi associated with post-harvest crop spoilage. Inference from this study also revealed the effectiveness of plant crude extracts obtained using non-polar and semi-polar solvents such as n-hexane and ethyl acetate.

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