Trop J Nat Prod Res, September 2024; 8(9): 8593-8598

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antifungal Potential of Selected Plant Extracts on Pathogenic Post-Harvest Fungi Causing Tuber Rot in *Ipomoea batatas* (L.) Lam. (Sweet Potato)

Tolulope S. Ewekeye*, Esther A. Fashina, Sikiru M. Muhammed, Abdulrazak O. Adebayo, Omobosola B. Fagbohunlu, Oyekunle O. Esan, Oyedamola A. Oke

Department of Botany, Faculty of Science, Lagos State University, Ojo, P.M.B. 0001, LASU Post Office, Ojo, Lagos, Nigeria

ARTICLE INFO

Article history:
Received 18 January 2024
Revised 29 May 2024
Accepted 25 July 2024
Published online 01 October 2024

Copyright: © 2024 Ewekeye *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Post-harvest deterioration limits the effective processing and utilization of *Ipomoea batatas* L. (sweet potato). This study identified the fungi associated with post-harvest tuber rot of *I. batatas* from selected markets in Lagos, Nigeria, and the antifungal efficacy of selected plant extracts in treating this disease. Sweet potato tubers collected from local markets in Iyana-Iba and Okokomaiko were observed for rot diseases. Isolation, identification, and characterization of fungi and pathogenicity tests were carried out on the specimens collected from the markets. Five fungi were isolated from the diseased tuber, they were identified as *Aspergillus aculeatus, Aspergillus flavus, Fusarium* sp., *Lasiodiplodia theobromae* and *Penicillium oxalicum.* The pathogenicity test revealed that only *A. aculeatus* and *P. oxalicum* induced rot in healthy sweet potato tubers 14 days after inoculation, with *P. oxalicum* being the most virulent. The molecular analysis confirmed the identity of the two virulent isolates as *Penicillium oxalicum* and *Aspergillus aculeatus*. The extracts of *Parkia biglobosa*, *Pseudocedrela kotschyi*, and *Mezoneuron benthamianum* exhibited antifungal potency on the fungi isolates. *P. kotschyi* against *A. aculeatus* at 20mg/ml had the highest inhibitory zone of 18.17±0.76 for n-hexane extract while against *P. oxalicum* at the same concentration, the highest inhibitory zone of 25.00±1.00 was recorded. Hence, the recommendation of the use of these plant extracts product as a substitute to synthetic antifungal agents for controlling the growth of fungi causing post-harvest tuber rot of *I batatas*.

Keywords: Antifungal, Molecular Analysis, *Parkia biglobosa*, Pathogenicity, Sweet potato.

Introduction

Ipomoea batatas L. (sweet potato), is a tuberous perennial plant. It is a member of the Convolvulaceae family. *I. batatas* is the 5th most important food crop in the tropics and ranks 7th globally in annual crop production. [1]

This crop is an important staple crop, mostly cultivated in northern Nigeria. The Nigerian population depends on *I. batatas* as an important crop for food security. [2] Sweet potatoes can become infected with various microbes at different phases, covering the phases of fieldwork, harvest, storage (if not well harvested and kept), and sales. Microbial food spoilage is one of the biotic factors that impact food insecurity, [3] the infection and degradation of sweet potatoes, significantly reduce their potential as a crop for nourishment and well-being. [4] Agu *et al.* [5] earlier attributed the post-harvest losses of sweet potatoes in the southeastern part of Nigeria to some fungal species - *Aspergillus fumigatus, A. niger* and *Rhizopus stolonifer*. Six fungal species including *A. flavus, Botryodiplodia theobromae, Fusarium solani* and *A. niger*, were also reported to be accountable for the post-harvest rot that occurs in sweet potato tubers in Nigeria. [6,7]

***Corresponding author. E mail: tolulope.ewekeye@lasu.edu.ng
Tel: +234-8028783539

Citation: Ewekeye, TS, Fashina, EA, Muhammed, SM, Adebayo, AO, Fagbohunlu, OB, Oyekunle O. Esan, OO, Oke, OA.. Antifungal Potential of Selected Plant Extracts on Pathogenic Post-Harvest Fungi Causing Tuber Rot in Ipomoea batatas (L.) Lam. (Sweet Potato). Trop J Nat Prod Res. 2024; 8(9): 8593 – 8598. https://doi.org/10.26538/tjnpr/v8i9.49

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Fungal pathogens have a detrimental economic impact on crops, therefore control measures are required. Fungicide treatment, gamma irradiation, and hydro-heating are techniques for controlling post-harvest pathogens. Although the effects of these methods on preventing rot and extending the shelf life of *I. batatas* roots have been stated to be intermediate, [8] there are certain limitations to the use of these methods. These include being inaccessible to farmers, detrimental to ecological renewability, toxic to humans, and the likelihood of causing antagonism in the intended microbes. [9] In light of these shortcomings, the emphasis is now on the use of extracts of plants as innovative fungicidal agents for safeguarding crops. [9] For instance, the result of the research conducted by Enyiukwu *et al.*, [10] revealed that the aqueous extracts of *Stachyterpheta jamaicensis*, *Cyathula prostrata*, and *Hyptis suaveolens* effectively reduced blight incidence and severity *in vivo* and enhance sweet potato yield by inhibiting spore germination and radial growth of pathogens *in vitro*.

Pseudocedrela kotschyi (Meliaceae) widely known as Dry-zone cedar. *P. kotschyi* is common within the savannah of tropical Africa. Various parts of *P. kotschyi* treat diverse ailments, such as malaria, stomach ache, diarrhoea, and toothache. [11] Petroleum ether, ethyl acetate, and methanol extracts of *P. kotschyi* leaf have been used to suppress the growth of *Candida albicans*. [12]

Parkia biglobosa is a perennial deciduous tree in the Fabaceae family commonly known as "African locust bean." Parkia plant materials are processed into paste, decoction, and juice to treat a range of illnesses, particularly diarrhoea, hypertension, as well as bronchitis. [13] A study showed that both aqueous and ethanolic extracts of leaves and stem of *P. biglobosa* successfully impeded the growth of *Aspergillus niger*, *Trichophyton mentagrophyte*, and *Candida albicans*. [14]

Mezoneuron benthamianum commonly known as “Jeniferan or Meyinro” in Yoruba, is of the Fabaceae family. It is a medicinal plant indigenous to many African countries. In Ghana, skin cream and powdered root of this plant are administered externally to heal wounds and dermal infections.[15] The synergistic efficacy report proposes that

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

M. benthamianum is a strong anti-candidal plant and could be used in the formation of drugs. [15]

Previous research reported the efficacy of crude plant extracts that were obtained with polar solvents such as ethanol, methanol, and water on some pathogenic fungi, [15,16] however there is a scarcity of information on the potential of non-polar solvents and semi-polar solvent extracts. Therefore, this research sought to phylogenetically characterize the fungi causing post-harvest rot of sweet potato tuber from selected markets in Lagos State, Nigeria, and also assess the effects of ethyl acetate and n-hexane extracts of *Pseudocedrela kotschyi*, *Parkia biglobosa* and *Mezoneuron benthamianum* on fungi isolated from rotten sweet potato.

Materials and Methods

Collection of samples

Infected *Ipomoea batatas* tubers were collected from Iyana-Iba market (6.4611ºN, 3.2043 ºE) and Okokomaiko market (6.4714ºN, 3.1879 ºE) within the Ojo Local Government Area, Lagos, Nigeria (Fig. 1). Samples were inspected for rotted portions, stored in clean polypropylene zip lock bags, labelled properly and transported to the laboratory of the Department of Botany, Lagos State University (LASU), Ojo for additional analysis. The stem of *Pseudocedrela kotschyi* (Voucher Number: LSH001178) and the root of *Mezoneuron benthamianum* (Voucher Number: LSH001179) were collected in January 2023 from Bode Alagbo market (7.3776 ºN, 3.9471 ºE) in Ibadan area of Oyo State, Nigeria (Fig. 1), while the bark of *Parkia biglobosa* (Voucher Number: LSH001180) was obtained in January 2023 from the Forestry Research Institute of Nigeria (FRIN), Ibadan (7.3919 ºN, 3.8631 ºE). These plant parts were identified and authenticated by Dr Omoteso Oluwa at the Department of Botany, LASU, Nigeria.

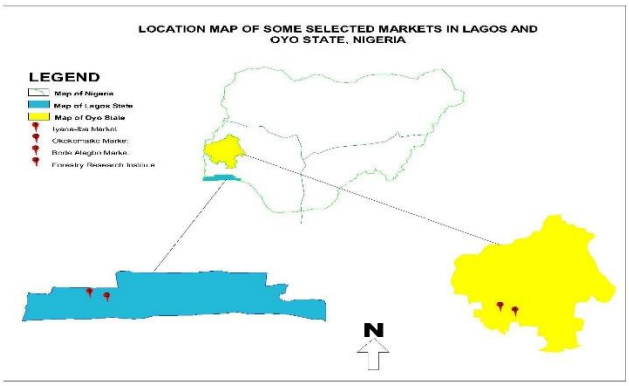

Figure 1: Map showing various collection sites in Lagos and Oyo States, Nigeria

Sample Preparation

The *I. batatas* tubers with disease symptoms were washed repeatedly under running water. The tubers were then cut open with a sterile kitchen knife (Stainless Steel Knife, VWR International, USA) to reveal the infested portion and the healthy parts. A small lesion of approximately 2x2 mm from the infested portion was cut with a sterile scalpel (Fist handle 3, Swann-Morton, UK). The sectioned specimens were surface sterilized first by immersing them fully within a beaker (Borosilicate Glass Beaker, 250ml, Pyrex, USA) containing 5% NaOCl solution (Sigma-Aldrich, St. Louis, MO, USA, reagent grade with a concentration of 4.00-4.99% w/v available chlorine and a percentage purity greater than 95%) for one minute; following sterilization, the sections to be inoculated were taken out and rinsed twice over with sterile distilled water. Afterward, the sectioned pieces' surfaces were dried in between two sterile filter papers (Whatman No. 1 filter paper, GE Healthcare, UK) to remove excess water.

Fungal Isolation

The use of Potato Dextrose Agar (PDA Powder, Thermo Fisher Scientific, USA) was employed to isolate these fungi. The prepared samples were aseptically transferred to 9 cm diameter Petri dishes (Sterile Polystyrene Petri dishes, 90mm, Greiner Bio-One, Germany) containing solidified sterile PDA. Chloramphenicol (Chloramphenicol Antibiotic Tablet, 25mg/ml, Sigma-Aldrich, USA) was added to the PDA to suppress the growth of bacteria. The plates were incubated for 72 hours (three days) at room temperature (27±2ºC) and checked daily for fungal growth.

Visible fungal colonies observed between days three and five were sub-cultured and incubated at room temperature (27±2 ºC). Pure cultures were obtained after three consecutive subcultures. Further research such as microscopic and molecular identification, pathogenicity testing and antifungal activity was conducted using the pure isolates.

Identification of Fungi isolates

Microscopic and morphological features of the fungal isolates were observed. The isolated fungi were identified with reference to Mathur and Kongsdal. [17]

Molecular Identification of Fungi

The pathogenic species were further subjected to molecular analysis to confirm their identities.

After the cultural and microscopic characterization of fungal pathogens, Extraction and PCR amplification of genomic DNA, DNA sequencing, and Internal Transcribed Spacer (ITS) region analysis were carried out to confirm their respective identities.

Extraction of Genomic DNA

Genomic DNA was extracted from five-day-old fungi with Quick DNA™ kit, following standard procedure. Two hundred microliters (200 µL) of phosphate buffer solution were used briefly to pick 60 mg of each fungal mycelia, which were subsequently dropped into 0.l mm and 0.l5 mm ZR BashingBead™ lysis tube. The tube was filled with 750 µL of BashingBead™ buffer and centrifuged at 10,000 rpm for 5 minutes. Then 400 µL aliquot of supernatant was dispensed into another tube containing a Zymo-Spin™ III-F filter, and this was centrifuged for one minute at 8000 rpm. After adding 500 µL of gDNA wash buffer and 200 µL of DNA pre-wash buffer, it was transferred to a fresh collection tube containing the Zymo-SpinTM IICR column. The tubes were centrifuged for one minute at 10,000 rpm respectively. To extract the DNA, 100 µL of DNA buffer was added after transferring the column matrix to a clean 1.5ml centrifuge tube. The column was then centrifuged for 30 seconds at 10,000 rpm and the DNA was stored at –80ºC.

Using universal primers that have been previously described, the Internal Transcribed Spacer (ITS) region of the rDNA was amplified via PCR. The PCR reaction mixture contained 50 µL of the total volume which included 30–50 ng of DNA, 100 mM of each primer, 0.05 U/µL Taq DNA polymerase, 4 mM $MgCl_2$, and 0.4 mM of each dNTP. A C1000 Touch thermal cycler (BioRad, USA) was used to carry out the amplification reaction. For thermal cycling conditions, a slightly modified version of Adeniyi *et al*.'s [18] method was used. For denaturation 35 cycles were done at 95 °C for 1 minute, and an annealing temperature of 57 °C for 1 minute with an initial extension and final extension at 72 °C for 2 minutes and 1 minute respectively after the initial denaturation at 95 °C for 15 minutes.

Electrophoresis was used to analyze the PCR amplicons. Two hours were spent running the agarose gel at 60 V after loading 7 µL of the samples and 5 uL of DNA ladders (1 kb) in each well (1% w/v with ethidium bromide) and NucleoSpin Gel were used to purify the remaining PCR products.

Analysis of DNA Sequence

Identification and sequencing of DNA was carried out at the Molecular lab of Inqaba Biotechnical Industrial Ltd in Ibadan, Oyo state, Nigeria using the universal primer pair ITS1 (Forward) and ITS4 (Reverse). [19] Amplicons were run and extracted from the gel. Nimagen Brilliant Dye™ Terminator Cycle Sequencing Kit was used to sequence the extracted fragments (forward and backward) while DNA sequencing clean-up kit was used to purify the sequences. The purified fragments were examined using an ABI 3500xl Genetic Analyzer.

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Chromatograms (sense and antisense) analysis from the sequencing was done using ChromasLite version 2.33 software to ensure high-quality sequences. BioEdit Sequence Alignment Editor [20] was used to modify the resulting chromatograms. Sequences consensus was searched for homology in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search tool (BLAST) to identify related organisms.[21] The sequences were deposited in the GenBank and assigned accession numbers. Phylogenetic trees were constructed with neighbour-joining techniques, [22] and 1000 bootstrap consensus using the Mega 6.0 software package.[23]

Pathogenicity Test

The method of Gwa and Ekefan, [24] was employed for pathogenicity testing. Ten healthy tubers, weighing between 80 and 100 grams were selected. They were then cleaned with flowing water to remove dirt. The samples were surface sterilized for 30 seconds using a 5% NaOCl solution, rinsed three times with sterile distilled water and air dried. [25] A 5 mm diameter, 4 mm deep sterile cork borer (Stainless Steel Cork Borer Set, Fisher Scientific, USA) was used to extract portions of the tissue of the samples. [26,27] A 5 mm diameter disc from the pure fungal cultures was extracted and replaced in the wells created in the healthy tubers as a result of the tissues initially extracted. To prevent contamination from other pathogenic organisms, petroleum jelly (Vaseline Petroleum Jelly, Unilever, Nigeria) was applied to fully seal the wells. The procedure was replicated twice for each fungal isolate and the samples were incubated at room temperature (27±2°C) for fourteen days to allow the five inocula to grow and mature before they were checked for signs of infection and the emergence of disease.

Preparation of plant extracts

Ethyl acetate (Fisher Scientific, Pittsburgh, PA, USA, HPCL grade, pure solvent with a percentage purity greater than 99%) and n-hexane (Fisher Scientific, Pittsburgh, PA, USA, ACS reagent grade, pure solvent with a percentage purity greater than 95%) were used as solvents for the extraction process.

Seventy grams of the fine powder of each plant material was measured into a sterilized conical flask and then 700 ml of each solvent (ethyl acetate or n-hexane) was carefully dispensed into the measured plant material and placed on a mechanical shaker for 24 hours. Whatman No. 1 filter paper was used to sieve the samples under a vacuum, and a vacuum rotary evaporator (R-300 Rotary Evaporator, Buchi, Switzerland) was used to concentrate the filtrates. The extracted product was then sealed in pill vials (Amber Pill Vials with Caps, 20ml, Fisher Scientific, USA) and stored in a freezer at -20 ºC. The filtrate was dispensed into a weighed conical flask (Borosilicate Glass Conical Flask, 500ml, DURAN Group, Germany) and evaporated to dryness. The conical flask and extract were weighed after cooling in the desiccator (Vacuum Desiccator with Stopcock, Kartell, Italy).

Anti-fungal Assay

The method of agar well diffusion of Adeyemi *et al*. [28] was employed to evaluate the plant extracts' antifungal efficacy. Two wells were made in each PDA-containing Petri plate using an 8.0 mm diameter sterile cork borer. Subsequently, concentrated extracts were incorporated into each well using a sterile micropipette (Adjustable Volume Micropipette, 2-20µl, Eppendorf, Germany), left for a few minutes to diffuse, and then sealed with the PDA. 0.3 ml of the 10 mg/ml ethyl acetate extract of one plant was poured into one well, and 0.3 ml of the 10 mg/ml n-hexane extract of the same plant was poured into the second well of each plate using a sterile micropipette. The same procedure was repeated for the 20 mg/ml extracts of the same. From the edges of the 7-day-old pure culture, a 6 mm mycelial disc was removed and put in the middle of the plate. Ketoconazole (KETORAL-200 Ketoconazole Tablets USP 200 mg) was used as the positive control and sterile distilled water as the negative control. The plates were incubated at room temperature (27±2 ºC) for five days. The assay was carried out in triplicate and the zones of inhibition were recorded for every plate.

Statistical Analysis

Analysis of Variance (ANOVA) was used to analyze the collected data, and Duncan's test at 0.05 probability level was used to separate the means to show the degree of significance between the values.

Results and Discussion

The following fungi were identified from the sweet potato tubers exhibiting rot symptoms after 14 days of inoculation: *Aspergillus flavus*, *Aspergillus aculeatus*, *Penicillium* sp., *Lasiodiplodia theobromae* and *Fusarium* sp. (Plates 1-5).

Pathogenicity Test

Five fungal species, which were *Aspergillus flavus*, *Aspergillus aculeatus*, *Penicillium* sp., *Lasiodiplodia theobromae* and *Fusarium* sp. were isolated, but only two, *Aspergillus niger* and *Penicillium* sp. were pathogenic and able to induce rot in healthy *Ipomoea batatas* (L.) after fourteen days of inoculation. The most virulent was *Penicillium* sp.

Molecular Identification of Fungi

Molecular analysis confirmed the identities of the two pathogenic species to be *Aspergillus aculeatus and Penicillium oxalicum*.

Phylogenetic relatedness of fungal strains

The phylogenetic analysis revealed that *Aspergillus aculeatus* LBCCN_PS_A2 (OQ550967) clustered with *A. aculeatus* (MH656795), *A. aculeatus* (JX501355), *A. aculeatus* (MT742816), *A. aculeatus* (MW260073) with a relative percentage identity of 99.65%, and *A. aculeatus* (OM714599) with a relative percentage identity of 99.48%. In the same manner, *A. aculeatus* LBCCN_YS_A8 (OQ550969) clustered with *A. aculeatus* (MK461090) and *A. aculeatus* (MT588793) (Fig. 2).

Additionally, the analysis further revealed that *Penicillium oxalicum* LBCCN_PS_A1 (OQ550966), *P. oxalicum* (OL616037) and *P. oxalicum* (OQ342861) are in the same cluster with a percentage identity of 98.81%.

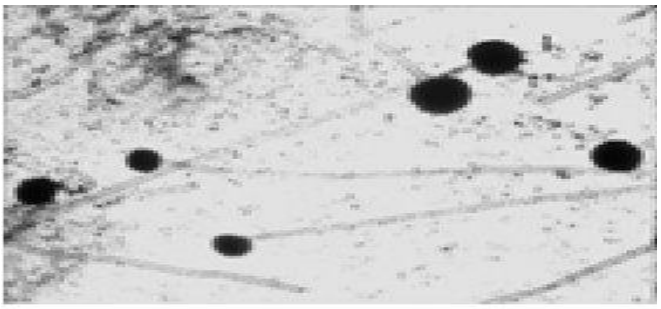

Plate 1: Photomicrograph of *Aspergillus aculeatus* (X100)

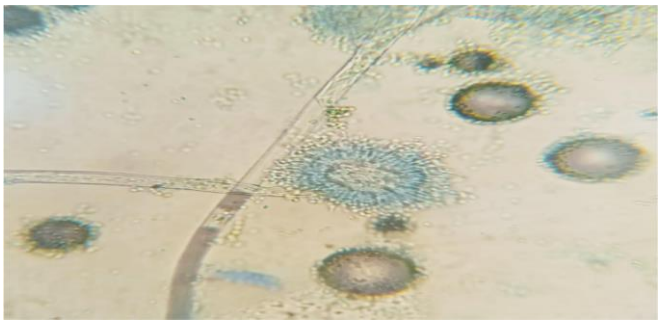

Plate 2: Photomicrograph of *Aspergillus flavus* (X100)

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

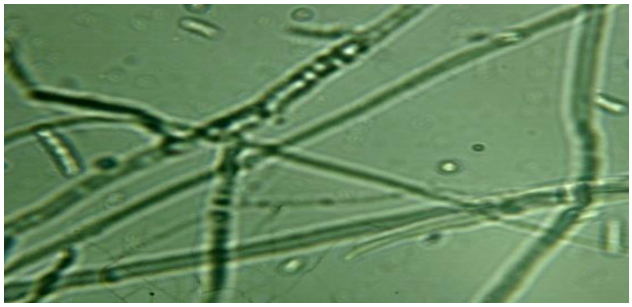

Plate 3: Photomicrograph of *Fusarium* sp. (X100)

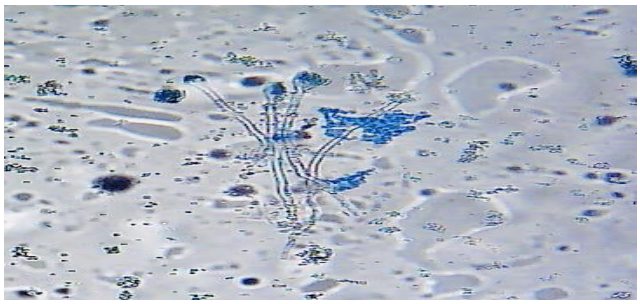

Plate 4: Photomicrograph of *Penicillium oxalicum* (X100)

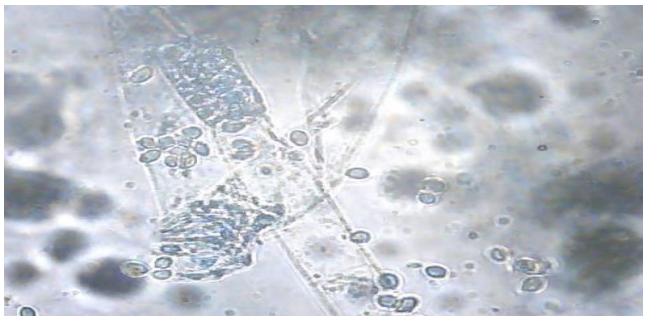

Plate 5: Photomicrograph of *Lasiodioplodia theobromine* (X100)

Antifungal activity of n-hexane extracts of P. biglobosa, P. kotschyi and M. benthamianum on test fungi

P. kotschyi n-hexane extracts inhibited the growth of the fungi under test only at a concentration of 20 mg/ml, whereas M. *benthamianum*'s n-hexane extract only inhibited *P. oxalicum's* growth at a concentration of 20 mg/ml. The n-hexane extract of M. *benthamianum* had a negative effect on *A. aculeatus* at a concentration of 10 mg/ml but was sensitive to it at 20 mg/ml (Table 2). n-hexane extract of *M. benthamianum* was effective for *P. oxalicum* at both concentrations. The commercial fungicide used (Ketoconazole) repressed the development of the tested fungi with the maximal inhibitory value of (28.33±1.53) (Table 3), while the negative control (Sterile distilled water) did not show any inhibition.

Efficacy of Different Concentrations of Ethyl Acetate Extracts to Inhibit Mycelia Growth of Tested Fungi

Table 1 shows the efficacy of the ethyl acetate extracts of all plants used. The ethyl acetate extracts were insensitive to the tested fungi at all concentrations. The commercial fungicide (Ketoconazole) used repressed the development of the tested fungi with the maximal inhibitory value of (28.33±1.53) (Table 3), while the negative control (Sterile distilled water) did not show any inhibition.

Antifungal potential of n-hexane and ethyl acetate plant extracts on A. aculeatus

For *A. aculeatus*, inhibitory values recorded from n-hexane extract of *P. biglobosa, P. kotschyi,* and *M. benthamianum* were notably lower compared to the values acquired from Ketoconazole. The mean and standard deviation of the readings are shown in Table 2. From the result, 20mg/ml of n-hexane extracts of *P. kotschyi* had the highest inhibitory effect with an inhibitory zone of 18.17±0.76 mm. The minimum inhibitory effect was observed in *M. benthamianum* (20 mg/ml) with an inhibition zone of 13.23±0.68 mm. The ethyl acetate extracts were insensitive to *A. aculeatus*. Ketoconazole has an inhibitory effect of 28.00±0.00 mm at 10 mg/ml while the minimum inhibitory effect was recorded at 20 mg/ml of ketoconazole.

Antifungal Potential of n-hexane and ethyl acetate Plant Extracts on the Inhibition of *P. oxalicum*

For *P. oxalicum*, the inhibitory values recorded from both n-hexane and ethyl acetate extracts of *P. biglobosa, P. kotschyi,* and *M. benthamianum* were noticeably less than the values acquired from Ketoconazole. The mean and standard deviation of the readings are shown in Table 3. From the results, 20 mg/ml of n-hexane extracts of *P. kotschyi* had the highest inhibitory effect with an inhibitory zone of 25.00±1.00. The minimum inhibitory effect was observed in *M. benthamianum* (20 mg/ml) with an inhibition zone of 12.00±1.00 mm. The ethyl acetate extract was insensitive to *P. oxalicum*. Ketoconazole has an inhibitory effect of 28.00±1.53 mm at 20 mg/ml while the minimum inhibitory effect (0.00±0.00) was recorded at 10 mg/ml of ketoconazole.

This study provided some of the data needed for the application of various concentrations of *Parkia biglobosa, Pseudocedrela kotschyi* and *Mezoneuron benthamianum* extracts as a useful bio-fungicide for *Ipomoea batatas* rot control method instead of synthetic fungicides. In this study, the pathogens linked to post-harvest decay of *I. batatas* were *A. flavus, A. aculeatus, P. oxalicum, Fusarium* sp. and *L. theobromae.* Usually, the method used for fungal identification relies mainly on morphological and microscopic characteristics, but this method is not completely reliable. They could, however, be better characterized through DNA (rDNA) sequencing of the ITS region. This method was used to further characterize two fungal isolates in this study.

The pathogenicity test carried out showed that *P. oxalicum* and *A. aculeatus* induced rot in the *I. batatas* tubers, with *P. oxalicum* being the most virulent. A related result was reported by Agu *et al.* [5] linking sweet potato post-harvest loss to similar fungi. Amienyo and Ataga, [6] also examined samples of sweet potatoes from various markets in Port Harcourt and identified 6 similar fungi as causative agents of post-harvest rot in sweet potatoes. In the study by Alum *et al*. [7] four of the most frequently isolated fungi from sweet potato rot in Ebonyi State were similarly obtained from this research. The fungi are: *Botryodplodia theobromae, A. flavus, F. solani*. and *P. expansum*. *Botryodiplodia theobromae* and *Fusarium* spp. have also been reported by Ray and Ravi as being among the most important fungi inducing the rot of *I. batatas* in storage. [8]

Investigation of the antifungal properties of *Parkia biglobosa, Pseudocedrela kotschyi*, and *Mezoneuron benthamianum* on the growth of isolates of *A. aculeatus and P. oxalicum in vitro* in this study revealed some inhibitory components in the crude extracts of these plants, which reduce the fungus mycelial growth. Deresa and Diriba, [16] in their review equally highlighted that the antifungal activities of botanicals mostly include inhibition of mycelial growth. Therefore, the plant extracts employed in this study can reduce post-harvest rot caused by fungi in *I. batatas*. However, the efficacy of the extracts used varied based on the extraction solvent, concentration and the tested fungus. Comparing the various plant extracts used in this study, *Pseudocedrela kotschyi* showed the greatest efficacy in preventing the fungi isolates, this is followed by *Parkia biglobosa*, and *Mezoneuron benthamianum.* The antifungal activities of these plant crude extracts, which can be applied as a post-harvest tuber treatment, were also verified and established by this study. Secondary metabolites produced by plants have been described to cause changes in the morphology and stop fungi from producing toxins. [16]

The antifungal efficacy of the n-hexane and ethyl acetate extracts of the plant materials differs depending on the concentrations and extraction solvents used. Only at concentrations of 10 mg/ml and 20 mg/ml did the n-hexane extract of *P. biglobosa* inhibit the growth of *P. oxalicum* as illustrated in Table 1.

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Efficacy of different concentrations of n-hexane and ethyl acetate plant material extracts on the suppression of the test fungi

		n-hexane		ethyl acetate	
Extracts	**Organism**	**10 mg/mL**	**20 mg/mL**	**10 mg/mL**	**20 mg/mL**
P. biglobosa	*A.niger*	-	-	-	-
	P. oxalicum	+	+	-	-
P. kotschyi	*A. niger*	-	+	-	-
	P. oxalicum	-	+	-	-
M. benthamianum	*A. niger*	-	+	-	-
	P. oxalicum	+	+	-	-
Ketoconazole	*A. niger*	+	+	+	+
	P. oxalicum.	+	+	+	+
Negative control	*A. niger*	-	-	-	-
	P. oxalicum.	-	-	-	-

Keys: + → Inhibition - → No inhibition

Table 2: Effects of n-hexane and ethyl acetate extracts of plants on mycelia growth inhibition of *Aspergillus aculeatus* after 5 days

	n-hexane		ethyl acetate	
Extracts	**10 mg/mL**	**20 mg/mL**	**10 mg/mL**	**20 mg/mL**
P. biglobosa	00.0 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
P. kotschyi	0.00 ± 0.00^{e}	18.17 ± 0.76^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
M. benthamianum	0.00 ± 0.00^{e}	13.23 ± 0.68^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Negative control	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Ketoconazole	28.00 ± 0.00^{a}	27.33 ± 0.58^{b}	28.00 ± 0.00^{a}	27.33 ± 1.53^{b}

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

Table 3: Effects of n-hexane and ethyl acetate extracts of plants on mycelia growth inhibition of *Penicillium oxalicum*, after 5 days

	n-hexane		ethyl acetate	
Extracts	**10 mg/mL**	**20 mg/mL**	**10 mg/mL**	**20 mg/mL**
P. biglobosa	22.00 ± 1.00^{c}	18.33 ± 3.06^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
P. kotschyi	0.00 ± 0.00^{f}	25.00 ± 1.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
M. benthamianum	19.67 ± 1.53^{d}	12.00 ± 1.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Sterile Distilled Water	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Ketoconazole	28.00 ± 0.00^{a}	28.33 ± 1.53^{a}	28.00 ± 0.00^{a}	28.00 ± 1.53^{a}

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

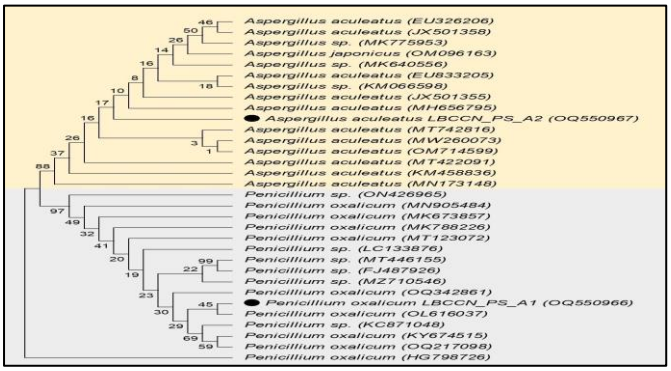

Fig. 2: Phylogenetic tree showing the relationship of *Aspergillus aculeatus* and *Penicillium oxalicum* from *Ipomoea batatas* (L.) Lam. (sweet potato) with other related fungal species. Using 30 additional reference sequences from GenBank and the partial 16S rDNA sequences of two fungi that were obtained for this study for phylogenetic analysis. The maximum likelihood method used in Mega 6.0 was applied to reconstruct the tree.

Conclusion

This study revealed that the n-hexane extract of *Parkia biglobosa*, *Pseudocedrela kotschyi*, and *Mezoneuron benthamianum* have the potential to protect or reduce the susceptibility of *Ipomoea batatas* (sweet potato) to rot fungi. Therefore, since fungicides are environmentally unfriendly and extremely costly, this affordable, harmless, and biodegradable plant materials might be utilized by farmers as a substitute method for managing the post-harvest rot disease of sweet potatoes. Further study is required to detect and purify the active principles accountable for the antifungal potential of the extracts.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Acknowledgments

The authors wish to thank Prof. Henry J. Hunjo for his role in assisting with the grammar check of the manuscript.

References

1. Waseem M, Saeed W, Khan MA. Sweet Potato (*Ipomoea batatas*): An Intervention Food in Management of Food and Nutritional Security in South Asia. In: Ismail, T., Akhtar, S.,
4. Sci. Food Saf. 2020; 19(2): 311-331.
5. Echerenwa MC, Umechuruba CI. Post-harvest fungal diseases of pawpaw (*Carica papaya* L.) fruits and seeds in Nigeria. Global Jnl Pure & Applied Sciences. 2004; 10(1): 69-73.
6. Agu KC, Nweke GU, Awah NS, Okeke BC, Mgbemena ICC, Okigbo RN, Ngenegbo UC. Fungi Associated with the Post-Harvest Loss of Sweet Potato. Int. J. Res. Stud. Biosci. (IJRSB). 2015; 3(9): 32-37.
7. Amienyo CA, Ataga AE. Use of indigenous plant extracts for the protection of mechanically injured sweet potato (*Ipomoea batatas* (L.) Lam) tubers. Acad. J. Sci. Res. Essay 2007; 2(5): 167-170.
8. Alum EA, Umeh SO, Amajor JU, Reuben-Kalu JI. Occurrence of post-harvest fungi affecting sweet potato (*Ipomoea batatas* (L) Lam) in Ebonyi State, southeastern Nigeria. Niger. Agric. J. 2019; 50(1): 101-109.
9. Ray RC, Ravi V. Post-harvest spoilage of sweet potato in tropics and control measures. Critical Rev. Food Sci. Nut. 2005; 45(7/8): 623-644.
10. Okigbo RN, Nmeka IA. Control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. Afr. J. Biotechnol. 2005; 4(8): 804-807.
11. Enyiukwu DN, Chukwu LA, Nwaogu AG, Bassey IN, Nwaneri JA. Antifungal Potentials of Aqueous Extracts of Selected Indigenous Flora Against Leaf and Stem Blight (*Alternaria bataticola*) Disease of Sweet Potato. Trop. J. Nat. Prod. Res. 2021; 5(8):1493-9. Doi.org/10.26538/tjnpr/v5i8.27
12. Alhassan AM, Ahmed QU, Malami I, Zakaria ZA. *Pseudocedrela kotschyi*: a review of ethnomedicinal uses, pharmacology and phytochemistry. Pharm. Biol. 2021; 59(1): 953-961.
13. Ayo RG, Audu OT, Ndukwe GI, Ogunshola AM. Antimicrobial activity of extracts of leaves of *Pseudocedrela kotschyi* (Schweinf.) Harms. Afr. J. Biotechnol. 2010; 9(45): 7733-7737.
14. Saleh MS, Jalil J, Zainalabidin S, Asmadi AY, Mustafa NH, Kamisah Y. Genus *Parkia*: Phytochemical, medicinal uses, and pharmacological properties. Int. J. Mol. Sci. 2021; 22(2): 618.
15. Entonu EE, Ngene AC, Odu CE, Ogaji AO, Nguhemen HW, Ochiheche EG, Coulthard OD, Egbere JO. Evaluation of antifungal effect of *Parkia biglobos*a and *Vitellaria paradoxa* against selected pathogenic fungi. MYCOPATH. 2023; 21(1).
16. Fayemi SO, Osho A, Atolani O. In vitro anticandidal and antioxidant potential of *Mezoneuron benthamianum*. J. Acute Dis. 2012; 1(2): 120-125.
17. Deresa EM, Diriba TF. Phytochemicals as alternative fungicides for controlling plant diseases: A comprehensive Lazarte, C.E. (eds) Neglected Plant Foods of South Asia. Springer, Cham. 2023; https://doi.org/10.1007/978-3-031-37077-9_15
2. Adeyonu AG, Ajala AO, Adigun GT, Ajiboye BO, Gbotosho OO. Determinants of sweet potato value addition among smallholder farming households in Kwara State, Nigeria. Agro-Science. 2016; 15(1): 17-22.
3. Odeyemi OA, Alegbeleye OO, Strateva M, Stratev D. Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. Compr. Rev. Food review of their efficacy, commercial representatives, advantages, challenges for adoption, and possible solutions. Heliyon. 2023; 9(3). e13810. https://doi.org/10.1016/j.heliyon.2023.e13810
18. Mathur SB, Kongsdal O. Common laboratory seed health testing methods for detecting fungi. ISTA, Basseradorf. Switzerland; 2003; Pp 427.
19. Adeniyi M, Titilawo Y, Oluduro A, Odeyemi O, Nakin M, Okoh AI. Molecular identification of some wild Nigerian mushrooms using internal transcribed hspacer: polymerase chain reaction. AMB Express. 2018; 8(1): 1-9.
20. White TJ, Bruns T, Lee SJWT, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A Guide to Methods and Applications. 1990; 18(1): 315-322.
21. Ermawati D, Panjono P, Bintara S, Hartatik T. Diversity of partial sequence leptin gene (Exon 3) in crossbred cattle compared to GenBank database. Biodiversitas J. Biol. Divers. 2022; 23(11).
22. Altschul SF, Madden TL, ShaÈffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 1997; 25: 3389-3402.
23. Lima AC, Araujo E, Stefanes MA, Rozante LCS. A flexible variant of the Neighbor-Joining for building phylogenetic trees. In International Conference on Computational Science and Computational Intelligence (CSCI). 2022; 578-583.
24. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 2013; 30(12): 2725-2729.
25. Gwa VI, Ekefan EJ. Fungicidal effect of some plant extracts against tuber dry rot of white yam (*Dioscorea rotundata* Poir) caused by *Aspergillus niger*. Int. J. Hort. Agric. 2018; 3(3): 1-7.
26. Lui LH, Kushalappa AC. Response surface models to predict potato tuber infection by *Fusarium sambucinum* from duration of wetness and temperature, and dry rot lesion expansion from storage time and temperature. Int. J. Food Microbiol. 2002; 76(1/2): 19-25.
27. Jia JY, Li XH, Zhang W, Zhou YY, Yan JY. First report of *Botryosphaeria dothidea* associated with stem canker on *Rosa chinensis* in China. Plant Dis. 2019; 103(12): 3280.
28. Peters RD, Arsenault WJ, Sturz AV. Tuber response of six potato cultivars to inoculation with *Phytophthora erythroseptica*, the causal agent of pink rot. Can. J. Plant Pathol. 2004; 26(1): 63-69.
29. Adeyemi AI, Vincent OI, Olujenyo OM. Phytochemical screening and antifungal activity of *Chromolaena odorata* extracts against isolate of *Phytophthora megakarya* using agar-well diffusion method. Asian J. Med. Biol. Res. 2018; 4(1):7-13.

© 2024 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License