Original Research Article

INHIBITORY EFFICACY OF LEMON-GRASS (*CYMBOPOGON CITRATUS*)EXTRACTS ON SOME SELECTED FUNGAL ROOT PATHOGENS

ABSTRACT

The organisms *Aspergillus niger, Fusarium moniliforme, Botryodiplodiatheobromae, Rhizopus spp.,* and yeasts were isolated from rotten cassava tubers. A pathogenicity test was carried out on healthy cassava tubers using the previously isolated organisms. *Aspergillus niger, Fusarium moniliforme, and Botryodiplodiatheobromae*were found to be pathogenic against cassava. The inhibitory efficacy of leaf extracts of *Cymbopogon citratus*(lemongrass) on the fungal root pathogens isolated was investigated *in vitro* using the agar-well diffusion method. The extraction solvents used were ethanol and water, which caused inhibition of the three pathogens with varying diameters. Twenty-four millimetres was the highest zone diameter of inhibition recorded, and 11 millimetres was the lowest zone diameter of inhibition. The minimum inhibitory concentration (MIC) ranged from 25–1000 mg/ml, while the minimum fungicidal concentration (MFC) ranged from 50–200 mg/ml, depending on isolates and extracting solvent. Ethanolic extracts showed greater antifungal activity than aqueous extracts.

Keywords: Lemmon-grass, Aspergillus*niger, Fusarium moniliforme, Botryodiplodiatheobromae, Rhizopus spp,* cassava tubers

1. INTRODUCTION

Chemical control of plant pathogens, parasites, and pests essentially involves the use of synthetic pesticides that could lead to phytotoxicity of the plant, environmental pollution, and pathogen resistance [1, 2, 3]. Unsafe use of pesticides may result in deleterious effects on humans and animals. It is therefore imperative to develop naturally occurring fungicides, nematicides, and pesticides that may be less toxic to humans and animals but effective against pests, nematodes, fungi, and other pathogens of various crops [4].

In recent years, much attention has been given to the use of non-chemical systems for seed treatment against seed-borne pathogens. Plant extracts in this case have played a significant role in inhibiting plant pests and improving seed quality [5]. Plant extracts are products that are made out of plants in the form of decoctions, infusions, and powders [6, 7, 8]. Medical plants have been recognised for their characteristics and chemical attributes in nature.

Leaf extracts of *Cymhopogoncitratus* (lemon grass) have inhibitory effects on fungal pathogens. Nilanonth et al. [9] reported that the lemongrass extracts significantly reduced the incidence of *Aspergillus niger*, *Fusarium moniliforme,* and *Botryodiplodiatheobromae*that occurred in yam bean seeds.

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The fungal infection may possibly cause non-life-threatening mucocutaneous candidatures or a life-threatening candidemia (*Candida* bloodstream infection) through the dissemination of single or clustered cells from the fungal biofilm in conjunction with its inherent drug-resistance capacity [10, 11, 12]. The *Candida* infection affects people with immune-compromised conditions, including patients with cancer who have undergone chemotherapy and/or radiotherapy [13, 14, 15, 16].

2. MATERIALS AND METHODS

2.1 Collection of Materials

Fresh leaves of *Cyrnbopogoncitratus*plants were collected from Lodu Ndume, Umuahia, Abia State, and identified by the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The rotten cassava tubers used were gotten from the National Root Crops Research Institute (NRCRI). The media used were prepared in the Microbiology Laboratory, NRCRI, Umudike, Abia State.

2.2 Preparation of Plant Extracts

Fresh leaves of *Cyinbopogoncitratus* were washed with distilled water and air-dried for two weeks. The dried leaves were then ground thoroughly using an electric blender. Fourty gram of powdered leaves were placed in two conical flasks each and extracted using 400 ml of ethanol and 400 ml of distilled water, respectively, for the two flasks. The mixtures were stirred thoroughly and corked. These were allowed to stand for 24 hours, after which they were stirred thoroughly again and filtered using Whatsmann filter paper (9cm). The different extracts (ethanol and water) were concentrated by evaporation in an oven at 50 ^{o}C.

2.3 Isolation and Identification of Test Organisms

The test organisms were isolated from three rotten cassava tubers using the method described by Choonharuangdej*et al*. (2021). The surface of each tuber was sterilized with cotton wool soaked in ethanol. Then a flamed kitchen knife was used to cut open the tubers to reveal the inside. Rotted tubers were aseptically cut out of the area between the rot and the healthy portion (the most probable area of pathogen activity) and put in separate conical flasks containing 20 ml of distilled water each. The husks were corked and shaken vigorously to release the samples into the liquid in order to form inocula to be used in culture.

Sabouraud Dextrose Agar (SDA) plates were prepared, and one percent streptomycin solution was added to each medium before pouring into petri dishes to prevent bacterial growth. Loopfuls of the inoculums were placed on three sterile SDA plates, respectively, using the streak plate technique and incubated aerobically at 20°C for 3-5 days. The plates were examined daily for growth. On establishment of growth, the fungus culture plates were observed separately for the presence of distinct colonies. From such colonies, inoculum was aseptically subcultured on fresh

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SDA plates and incubated as described earlier. When growths were seen, they were examined for uniform colonies as a mark of purity. The pure cultures were used for characterization.

2.4 Characterization and Identification

The characterization of the fungi isolates was done using a combination of cultural morphology and structural features. The colonies on the plate were examined and their features recorded. Then a slide amount of each isolate was made on a microscope slide and stained with 2 drops of lactophenol cotton blue each to reveal their structures. On viewing with the microscope, the observed features were recorded.

The recorded characteristics were matched against those in standard manuals, such as the illustrated Genera of Imperfect Fungi [17].

2.5 Pathogenecity Test

Each obtained isolate was tested for its ability to cause disease in a healthy cassava tuber. The method described by Choonharuangdej*et al.* (2021) was used. The surface of healthy cassava tubers was cleaned with alcohol, and a flamed cork borer (5mm) was used to drill holes in the tubers. Then, carefully, with a smaller cork borer (3mm), the test isolates were collected from the culture plates and directly inserted into the drilled holes. The holes were closed using the same tissue that was removed from the drill. A control was set up using normal saline as the inoculum. The cassava tubers were labelled accordingly and incubated at ambient temperature for 5-7 days. They were observed for signs of spoilage, including softening, cracks, discoloration, moisture exudates, and mould growth. On observation of such signs, the cassava tubers were cut open along the line of inoculation to reveal the inside. The extent of disease (rot) was determined by measurement of rot length. Observations were recorded. The organisms that caused rot with a length above 10mm were considered to be pathogenic.

2.6 Antimicrobial Assay

This was determined using agar-well diffusion techniques [18]. Each pathogenic test isolate was inoculated onto two sterile SDA plates, respectively, and spread evenly over the surface. A flamed cork borer of 5 mm diameter was used to drill holes in the agar on the plate. A micropipette was used to carefully dispense 0.1 ml of each extract into the holes or on different plates, and the plates were allowed to stand for 5 minutes for the extracts to permeate into the medium. The plates were sealed, incubated at room temperature for 3-5 days, and observed daily. The presence of a dark zone around the well on the plates gave a positive test for antimicrobial activity. The extent of antimicrobial activity was determined by the diameter of the zone of inhibition, measured with a transparent metre rule. A triplicate assay was done for each extract on each plate against each isolate.

2.7 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration is the concentration giving the least inhibitory activity, below which there is no further inhibition. The MICs of the different extracts were obtained by

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dissolving 2g of each extract in 5 ml of normal saline to obtain an initial concentration of 400 mg/ml, which was serially diluted to 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.3 mg/ml. The different concentrations were dispensed into agar wells for each isolate as described earlier. The plates were labelled accordingly and incubated at room temperature for four days. The result of each concentration was recorded.

2.8 Minimum Fungicidal Concentration (MFC)

The minimum fungicidal concentration was determined by sub-culturing on SDA plates the MIC test dilutions, which inhibited the growth of the test organisms and were further incubated. The lowest concentration that yielded no fungal growth at all was taken as the minimum fungicidal concentration, as there was probably a cidal activity in the extracts.

2. RESULTS

The macroscopic and microscopic characteristic features of the isolates recorded were matched against standards in the illustrated genera of imperfect fungi and thus named.

The results in Table 2 show the occurrence of fungus isolates in rotten cassava tubers. Results obtained from the three rotten cassava tubers showed that five fungi species were isolated, including *Aspergillus, Fusarium, Rhizopus, Botryodiplodia,* and yeasts. *Aspergillus* and *Botryodiplodia*werepresent in all the samples, *Fusarium* and *Rhizopus* werepresent in two of the three samples; and yeasts were present in only one of the three samples.

The results of the pathogenicity of the fungi isolates on healthy cassava tubers showed that *Aspergillus spp.* have strong pathogenicity on cassava with rot lengths of 16–21 mm, *Botryodiplodia*caused rot of lengths of about 14–16 mm, and *Fusarium caused rot of lengths of* about 13–17 mm. Yeasts and *Rhizopus* were not so effective as the rot caused was below 10 mm. They were therefore considered non-pathogenic.

The diameter zones of inhibition of the antifungal activity of *Cymbopogon citratus* extracts against the established root rot pathogens showed that there were variations with significant differences between the water extract and the ethanol extract. The diameter zones of inhibition of water extracts were 12.67 mm-1.52 mm *(Aspergillus), 18.33 mm-12.52* mm *(Fusarium),* and 12.00mm± 1.00 *(Botryodiplodia).* The ethanol extracts had corresponding diameter zones of inhibition of 17.33 ±1.16mm, 23.33± l.16mm and I8.33±1.52mm for the three organisms, respectively.

The minimum inhibitory concentration test carried out showed variations in the lowest concentrations, which caused inhibition of the pathogens. Against *Aspergillus,* the least concentration of water extract to effect inhibition was l00mg/ml while the least of ethanol extract concentration was 50mg/mi. The corresponding values for *Fusarium* were 50mg/ml (water) and 25mg/ml (ethanol) and for *Botryadiphdia*were l00mg/ml (water) and 50mg/m] (ethanol). There were therefore significant variations (P<0.05) in the activity of the extracts of *Cymbopogon citratus* against the isolated fungi pathogens of cassava root rot.

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Results of the minimum fungicidal concentration test showed that the lowest concentrations to allow no further growth of the fungi are relatively higher than the concentrations in the MIC results. Against *Aspergillus,* 200mg/ml (water extract) and l00mg/ml (ethanol extract) were the least concentrations to possibly kill the fungi. For *Fusarium* and *Botiyodiplodia,* the minimum fungicidal concentrations are l00mg/ml (water). 50mg/ml (ethanol), 200mg/ml (water), and 100 mg/ml, respectively.

Table 1: Characterization of fungal isolates.

Macroscopic appearance	Microscopic appearance	Fungal organism confirmed
Fast growing, pure white, thick cottony mycelium which later turned brownish grey. Reverse is pale	Non-septate and rhizoid sporangiophores and sporangia present	*Rhizopus*spp.
Powdery colonies with blue-black centers. Reverse is black	Septate hyphae, conidiophores present	*Aspergillus*spp.
Fast growing, bright coloured white cottony mycelium	Hyaline microconidia. Chlamydoconidia present	*Fusarium*spp.
Grayish sepia colonies, fluffy with abundant aerial mycelium. Reverse is black	Septate, hyphae, simple hyaline, conidiophores present	*Botryodiplodiatheobromae*
Moist, white and cream colonies, smelling like bread	Round to oval in shape, asexual reproduction by budding	*Yeasts*

Table 2: Occurrence of fungi isolates in rotten cassava tubers.

Sample	*Aspergillus*spp.	*Fusarium spp.*	*Botryodiplodiatheobromae*	Rhizopus	Yeasts
1	+	+	+	+	-
2	+	-	+	+	-
3	+	+	+	-	+
Total	3	3	3	3	3
Number of positive	3	2	3	2	1

Result shows growth of the organisms in three different cassava tubers.

% occurrence = $\frac{number of positive}{total number}$ x $\frac{100}{1}$

Key: + = positive growth
- = negative growth

Table 3: Pathogenicity testing of test isolates on healthy cassava tubers.

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Isolate	A(mm)	B(mm)	C(mm)	Mean(mm)	Remarks
Control	3	3	4	3.33	N P
Aspergillus	18	16	21	18.33	Pathogenic
Botryodiplodia	16	14	16	15.33	Pathogenic
Fusarium	13	17	14	14.67	Pathogenic
Rhizopus	7	6	8	7.00	N P
Yeasts	2	4	4	3.33	N P

Values show result of triplicate analysis.

Key: Rot length ≥ 10mm = pathogenic

N P = non-pathogenic (<10mm).

Table 4: Diameter zones of inhibition (mm) produced by *Cymbopogon citrates* extracts against the test pathogens.

Pathogenic	Water extract (mm)	Ethanol extract (mm)
Aspergillus spp.	12.67 ± 1.52	17.33 ± 1.16
Fusarium spp.	18.33 ± 2.52	23.33 ± 1.16
Botryodiplodiatheobromae	12.00 ± 1.00	18.33 ± 1.52

The values in the table are the mean ± standard deviation of three separate replications of the experiment.

Table 5: Minimum Inhibitory Concentration of *Cymbopogon citrates* extracts against test pathogens.

Organism	Water extracts	Ethanol extracts
Aspergillus spp.	100mg/ml	50mg/ml
Fusarium spp.	50mg/ml	25mg/ml
Botryodiplodiatheobromae	100mg/ml	50mg/ml

Table 6: Minimum Inhibitory Concentration of *Cymbopogon citrates* extracts against test pathogens.

Organism	Water extracts	Ethanol extracts
Aspergillus spp.	200mg/ml	100mg/ml
Fusarium spp.	100mg/ml	50mg/ml
Botryodiplodiatheobromae	200mg/ml	100mg/ml

3.DISCUSSION

The results obtained in the pathogenically test can led out revealed that all test organisms isolated caused deterioration (rol) on healthy cassava tubers but to varying lengths, in general, any rot length less than 10mm was considered non-pathogenic. Deterioration caused by *Aspergillums, Futurism*and *Batriodiplodia*were above 10mm which confirms that they is actual causative agents of rot in the cassava root tubers. The other isolates which did not cause disease were considered to be either transient organisms or saprophytes which constitute secondary flora.

The efficacy of *Cymbopogon citratus* was tested against the pathogenic organisms using two solvents (ethanol and water). The extracts have effect on the pathogens but to varying degrees. The ethanol extracts showed higher activity against the organisms than the water extracts u iththe ethanol extract effecting one diameter of inhibition 17.33± 1.16, 23.33±2.16 and 18.33±1.12 on the three organisms *(Aspergillums, Fusarium and Bolryodiplodia)* respectively compared to the extract which had zone diameters of inhibition 12.67±1.52, 18.33±2.52 and 12.00±1.00 for the three organisms respectively. Major phytochemical constituents of *Cymbopogon citratus* like phenols and alkaloids are more soluble in ethanol than in water [17], Alkaloids are known to be toxic hut are used widely in medicines due to their pharmacological activities [18, 19, 20]. While phenols forms the major constituents of disinfectants and antibiotics and are strong against microorganisms. This probably explains the relative higher activity of ethanol extract than the wale extract. Findings in this present study confirmed works of Choonharuangdej et al. [17]where leaf extracts of *Cymbopogon citratus* reduced the incidence of *Aspergillusnigcr. Aspergillusflavns, Botryodiplodiathvobronute*and *Fitsariummoniliforme*that occurred in yam bean seeds (in vitro and in vivo).

The minimum inhibitory concentration results showed that the activity of water extract of the test plant was least at 50mg'ml while that of ethanol extract was least at 25mg/ml. These values are important in assessing the dose when used in ethnomedical formulas. To achieve a better or near cidal activity of the plant extract using different solvents, very high concentrations are required as is shown by the minimum fungicidal concentration values.

4. Conclusion

In conclusion, the fungi disease pathogens of cassava root rot include species of *Aspergillus, fusarium* and *Botriyodiplodia.* The extracts of *Cymbopogon citiatus* plant using different solvents demonstrated activity against the pathogens to varying extents with the ethanol extract being more potent than the water extract the activity of the extracts as attributed to their phytochemical constituents.

Consent

It is not applicable

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