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Control of Yam Rots with Botanicals: *Xylopia aethiopica* (*Uda*) and *Piper guineense* (*uziza*) Seeds Extract

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Abstract

The isolation and identification of fungi pathogens causing yam rot across markets in Awka and the control of this rot using botanicals was carried out using standard laboratory procedures. Randomized block sampling was used in the sample collection for this study. For the *in vitro* analysis, potatoes dextrose agar was used to prepare innocula for the 4 unhealthy yam tubers collected. Aqueous extract of seeds of *Xylopia aethiopica* and *Piper guineense* was used for growth inhibition treatment in this study. The results show the nature of fungal growth and total colony count of the fungi pathogens isolated. For the samples collected from Eke Awka and First Market, there was heavy growth of the identified fungi pathogens, while for the yam samples obtained from second Market and Amansea, there was moderate and scanty growths, respectively. There was inhibitory activity of seed extract of *Piper guineense* and *Xylopia aethiopica* on the fungi pathogens isolated from diseased yam across markets in Awka. The results showed that *X. aethiopica* (4.80±0.24) inhibited *Aspergillus terreus* more than *P. guineense* (4.53±0.21). *Fusarium oxzysporum* was inhibited more by *P. guineense* (5.74±0.26) than *X. aethiopica* (3.86±0.25). *Piper guineense* also had a higher inhibition rate on *Rhizopus stolonifer* (4.34±0.26) than *X. aethiopica* which had (3.75±0.22). The positive control (Grisovid) showed more inhibitory activity than the extracts in general. The result shows that all the fungi pathogens had a slow and consistent increase in pathogenicity from day 3 to the 6th day. This study has identified the fungi associated with spoilage of yam and the possible control of these pathogens using plant extracts. The study also revealed the fungi that caused these diseases and they included *A. terreus, R. stolonifer* and *F. oxysporum*. These fungi had fast growth and were spread within a short period of time on fresh yam; but with the use of *Piper guineense* and *X. aethiopica,* these fungi pathogens were significantly inhibited.

Keywords: Control; Yam; Rot, *Xylopia aethiopica; Piper guineense*

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Introduction

In terms of microorganisms, fungi and their associated secondary metabolites known as mycotoxins are of high concern in farm produce or storage facilities due to the production of mould, odours, the presence of microbial 'hot-spots', and the production of secondary metabolites which can lead to subsequent poisoning of food and animal feed, thus negatively impacting food safety [38]. There are a number of postharvest fungi that can attack and cause damage to foods such as yam and cocoyam, and they can be divided into two groups: field fungi and storage fungi [21]. Field fungi may modify the structure and quality of produce [6]. These cause damage to the tubers and corms before harvest and can generally be detected by routine assessment. Storage fungi are those that cause damage to grain during storage and usually do not occur at a serious level prior to harvest [22].

The mycoflora of stored grains predominantly consist of the ubiquitous mould genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor*, *Rhizopus* and *Penicillium* [20]. They are usually introduced into the stored produce as spores in minute quantities during handling and storage. Other microorganisms such as certain bacteria can also colonize the stored food materials. These bacteria mainly belong to the families *Pseudomonadaceae*, *Micrococcaceae*, *Lactobacillaceae* and *Bacillaceae* [19]. In Australia, Europe, and the US *Salmonella* spp., *Escherichia coli*, and *Bacillus cereus* are also present in wheat and flour at low levels but are prevalent in Africa and Asia Minor [3, 7, 29].

The presence of these bacteria and fungi and their adverse effects can be compounded further

by insect activity. Protection can be achieved by decreasing storage temperature and controlling moisture migration with aeration or by using ambient or refrigerated air [4, 8, 10, 13].

Yams are monocotyledonous plants belonging to the genus Dioscorea of the family Dioscoreaceae which constitute a multi-species of crops that are important for food, socio-cultural activities and income. *Dioscorea* species are important food crops in West Africa, and other tropical countries including East Africa, Central Africa, The Caribbean, South America, South East Asia and India [9].

The most important areas for the cultivation and usage of yam stretches from Ivory Coast through Ghana, Nigeria, Togo, Cameroon, Garbon, Central African Republic and the Western part of the Democratic Republic of Congo. According to [12] these regions produce about 93% of the World's annual yam production, estimated at 38.5 million metric tons and Nigeria alone accounts for about 26.4 million tons (70%) in the year 2000. There are about 600 species of *Dioscorea* with more than 10 species cultivated for food and 6 species for pharmaceutical use [9].

Only six species are economically important namely; white yam (*Dioscorea rotundata*), water yam (*Dioscorea alata*), aerial yam (*Dioscorea bulbifera*), yellow yam (*Dioscorea cayenensis*), trifoliate yam (*Dioscorea dumentorum*) and Chinese yam (*Dioscorea esculenta*) [27]. *Dioscorea rotundata* and *Dioscorea cayenensis* are indigenous to West Africa while *Dioscorea alata* and *Dioscorea esculenta* are native of Asia. According to Onwueme (1978) *Dioscorea alata* is one of the most important specie of yam in West Africa. Besides the cultivated yam species, there are a number of wild types which are also harvested as food [40]. The yam tuber is the only economical part of the crop, consumed roasted, fried, boiled, pounded or as flour for baking and steaming for swallowing with soup. In addition to its nutritional value, yam has considerable social and cultural significance, especially among the people of South-Eastern Nigeria [34]. According to [9, 26] yams are of a very high nutritional value where it is a major source of carbohydrate, minerals, phosphorus, calcium, iron and vitamins such as thiamine, riboflavin and vitamins B and C. Some species of yam have been used medically to treat diseases like *Diabetes mellitus*, to increase coronary flow and prevent high hyperchosteromia [39].

Materials and Methods

Study Area

This research was carried out in Awka metropolis; Awka lies between latitude ($7^{0}00$ and $7^{0}10^{1}$) E and ($6^{0}05$ and $6^{0}15^{1}$) N in Anambra state.

Source of Materials

Diseased yam (*Dioscorea rotundata*) samples were collected from four different markets; Eke Awka, First Market, Second Market, and Amansea all in Awka, Anambra State, Nigeria. They were placed in a sterile polythene bag and brought to the Department of Botany Laboratory Nnamdi Azikiwe University Awka for culturing, isolation, identification and cross innoculation.

Media Preparation

The medium used for the fungal isolation is Potatoes Dextrose Agar (PDA). Ten grams of the powder was dispensed into 100 ml of distilled water in a conical flask and then stopped tightly with cotton wool and foil, it was heated in water bath for about 2 hours until the agar is melted. The prepared medium was then sterilized using autoclave at 120°C and 30 psi [5, 16] for 15 minutes. Thereafter, it was allowed to cool and then dispensed into the Petri dishes.

Preparation of Sample Inocula

Inocula were prepared from four (4) unhealthy yam tubers. The unhealthy tubers were first washed in sterile water and then surface sterilized using 70% ethanol. A sterile kitchen knife was used to cut each of the samples so as to reveal the boundary zone between the rotten and healthy part of the tubers. Small bits were cut from the boundary zone of each tuber and transferred to sterile Petri dishes and later used for isolation of fungi pathogen.

Isolation of Test Fungi from Rotten Tubers

Isolation of fungi was done by agar dilution plate method. The method was used by [14]. The inoculum prepared from the diseased tubers was used for isolation of the fungi. Three pieces each of the four different samples of the tubers was placed in each Petri dish containing PDA media (making three plates of sample, giving a total of 12 plates). All plates were wrapped externally with masking tape and incubated at +/- 27°C for 72 hours and observed daily for growth of fungi. The number of colonies was counted and calculated using the formula below;

CfU (ml) = N/VxD

Where:

Cfu = Colony forming unit

N = Mean number of colonies

V = Volume of innoculum

D = Dilution factor.

Sub Culturing and Identification of Test Fungal Pathogens

Subcultures were prepared using inocula from different organism in the mixed cultures to obtain a pure culture; this was done by transferring from the colony edge of the mixed cultures to fresh sterile PDA plates with the aid of a scalpel. The plates were wrapped externally with masking tape and incubated for 72 hours. The resulted pure cultures were used for subsequent identification of fungi isolate. The identification was on the basis of their micro and macro- morphological characteristics using standard taxonomic key used previously by [32].

Pathogenicity Test of Fungi Pathogens on Fresh Yam Tubers

Fresh tubers were brought and the fungi colonies were inoculated in the fresh healthy samples. Healthy tubers weighing 3 grams each were weighed into four places and placed in sterile Petri-dishes. A sterile knife was used to created wounds in the sliced tuber samples and the isolated fungi were incubated into the wounds separately, labelled and incubated for 7 days. At the end of the 7^{th} day, the extent of the rot caused by the fungi was determined using the method as described by Kassim (1986).

Rot (%) = A-a/A x 100

Where:

A = Initial weight of tubers

a = Final weight of tubers after the removal of the rotten portion

Aqueous Extraction

The aqueous extracts of seeds of *Piper guineense* and *Xylopia aethiopica* were prepared by soaking the ground samples of the plant

in 100 ml of deionized water. The concentration of each extract was determined by adding 100 g, 150 g and 200 g in 100 ml of water. The experimental set-up was left for 24 h at room temperature and thereafter filtered using No. 1 Whatman filter paper. The extract was then concentrated to 50 ml of the original volume of the extract and stored in an air tight container in a refrigerator at 4^0C until when needed.

Determination of Antimicrobial Activity Using the Agar Well Diffusion Method

The agar diffusion method as described by [11] and [28] was adopted for the study. Standardised nutrient broth (PDA) culture of the test isolate containing approximately 10^7 cells/ml organisms was used. 0.1 ml of the broth culture was introduced into sterile Petri dishes and 15 ml of molten nutrient agar poured into the Petri dishes. The contents were thoroughly mixed and allowed to solidify. Three holes each measuring 5.0 mm in diameter were made in each of the solid agar plates using a sterile cork borer. 0.04 ml of the different concentration of plant extracts were transferred into the holes using a micropipette. Two Petri dishes containing a particular fungus were used for each concentration of the extracts. The plants were thereafter allowed to stand for 1 hour for pre-diffusion of the extracts and were subsequently incubated at 37^0C for 72 hours. After incubation, the plates were collected and the zones of growth inhibition were measured. The extent of inhibition was expressed in terms of the diameter of the inhibition zone as measured with a transparent metre rule. The effects of the extracts on fungi pathogens were compared with those of the standard commercial fungicide (Grisovid) as standard control respectively. Tests were carried out in triplicates.

Determination of Minimum Fungicidal Concentration (MFC)

The minimum fungicidal concentration was determined through sub-culturing of 10 µl content of microtitre plate well which is greater or equal to the lowest minimum inhibitory concentration on the sabouraud dextrose agar and incubated for 24 h. After 24 h incubation, the Petri dish was assessed for the presence of growth, and the minimum concentration of extracts or fractions with no visible growth was taken as minimum fungicidal concentration [1]. The experiment was done in triplicate.

Data Analysis

The data obtained was analyzed using the statistical package SPSS version 2023. Data obtained from the study was subjected to Analysis of Variance (ANOVA) at 5% significant level. Means are separated using Duncan Multiple Range Test.

Results

Nature of Fungal Growth in PDA and Total Fungi Count of Yam Samples

Table 1 shows the nature of fungal growth and total colony count of the fungi pathogens isolated from the diseased yam samples. For the samples collected from Eke Awka and First Market, there was heavy growth of the identified fungi pathogens, while for the yam samples obtained from Second Market and Amansea, there was moderate and scanty growths, respectively. Also, for the total fungal count, Eke Awka and First Market had the highest colony count ($160x10^2$ and $162x10^2$), respectively; while Amansea had the least at $120x10^2$.

Occurrence of Fungi Pathogens on Yam Samples from Different Locations in Awka

Based on the growth of the fungi on the cultured yam specimens, table two revealed the presence of *Fusarium oxysporum, Rhizopus stolonifer* and *Aspergillus* terreus. *Aspergillus* terreus had a total occurrence of 10 and a percentage occurrence of 34% while *Rhizopus stolonifer* had an occurrence of 9 with a percentage occurrence of 32%.

Table 1: Nature of fungal growth and total colony count.

Parameters	Eke Awka	First market	Second market	Amansea
Nature of fungal growth	Heavy	Heavy	Moderate	Scanty
Total fungal count ($x10^2$)	160.00	162.00	145.00	120.00

Table 2: Occurrence of Fungi Pathogens on Yam Samples.

Fungi Isolates	No of Occurrence	Percentage Occurrence
Fusarium oxyporum	10	34 %
Rhizopus stolonifer	09	32 %
Aspergillus terreus	10	34 %
Total	29	100 %

Table 3: Antifungal activities of *Piper guineense* and *Xylopia aethiopica* on fungi pathogens of yam samples.

	Zone of Inhibition (mm)		
Fungi Isolates	***P. guineense***	***X. aethiopica***	**Grisovid**
Aspergillus terreus	$4.53±0.24^b$	$4.80±0.24^b$	$8.89±0.20^c$
Fusarium oxysporum	$5.74±0.26^a$	$3.86±0.25^a$	$9.22±0.24^a$
Rhizopus stolonifer	$4.34±0.26^c$	$3.75±0.22^c$	$8.69±0.24^b$

Results show values of mean of triplicate analysis ± STD. Figures with different alphabets on the same column are significantly different (P<0.05).

Antifungal Activities of *Piper guineense* and *Xylopia aethiopica* Aqueous Extracts on Fungi Pathogens of Yam

Table 3 shows the inhibitory activity of seed extract of *Piper guineense* and *Xylopia aethiopica* on the fungi pathogens isolated from diseased yam across markets in Awka. The results showed that *X. aethiopica* (4.80±0.24 mm) inhibited *Aspergillus terreus* more than *P. guineense* (4.53±0.24 mm). *Fusarium oxzysporum* was inhibited more by *P. guineense* (5.74±0.26 mm) than *X. aethiopica* (3.86±0.25 mm). *Piper guineense* also had a higher inhibition rate on *Rhizopus stolonifer* (4.34±0.26 mm) than *X. aethiopica* which had (3.75±0.22 mm). The positive control (Grisovid) showed more inhibitory activity than the extracts in general.

Minimum Fungicidal Inhibition of *Piper guineense* and *Xylopia aethiopica*

The results revealed the minimum fungicidal concentration at which the *P. guineense* extract showed inhibitory activity against the *A. terreus, F. oxzysporum* and *R. stolonifer* to be at 25.0, 12.50 and 50.0 mg/ml. While MFC for *X. aethiopica* extract on the fungi pathogens were 12.50, 12.50 and 25.0 mg/ml respectively (Table 4). This means that any concentration below these MFCs (minimum fungicidal concentrations), the extract will show no inhibition.

Pathogenicity of Fungi Pathogens on Fresh Yam Samples

Table 5 shows the results of the pathogenicity test of fungi pathogens isolated from the diseased yam samples on the fresh samples. The results indicated that all the fungi pathogens had a slow and consistent increase in pathogenicity from day 3 to the 6th day, although *F. oxysporum* had a higher pathogenicity from day 3 to day 6 on the fresh yam samples when compared with other fungi pathogens

Table 4: Minimum Fungicidal Concentration of *Piper guineense* and *Xylopia aethiopica* Extract (mg/ml).

Fungi Isolates	*Piper guineense* (MFC)	*Xylopia aethiopica* (MFC)
Aspergillus terreus	25	12.50
Fusarium oxysporum	12.50	12.50
Rhizopus stolonifer	50	25.0

Table 5: Pathogenicity of fungi pathogens on fresh yam samples (cm).

Fungi Isolates	DAY 3	DAY 4	DAY 5	DAY 6
Aspergillus terreus	1.53±0.14[a]	1.74±0.14[a]	2.14±0.11[a]	2.90±0.10[a]
Fusarium oxysporum	2.64±0.18[b]	2.88±0.15[b]	3.12±0.18[b]	3.29±0.14[b]
Rhizopus stolonifer	1.42±0.11[a]	1.60±0.11[a]	2.00±0.10[a]	2.34±0.10[c]

Results show values of mean of triplicate analysis ± STD. Figures with different alphabets on the same column are significantly different (P<0.05).

and it showed significant difference in the pathogenicity on the fresh sample. The pathogenicity of the fungi isolates and the number of days were significantly different (P<0.05).

Discussion and Conclusion

Reports by researchers have shown that Aspergillus *terrius, Fusarium oxysporum* and *Rhizopus stolonifer* are seed-borne in most foods and fruits such as yam, coconuts, pear, maize etc. Reports also indicated that the aforementioned fungi reduced the nutritional and mineral compositions of these foods [31].

However, *Fusarium oxzysporum* and *Aspergillus terreus* had a total occurrence of 10 and a percentage occurrence of 34% each; while *Rhizopus stolonifer* had an occurrence of 9 with a percentage occurrence of 32%. The high occurrence of *Aspergillus* and *Fusarium* might be due to the report of its ability to grow faster and high pH tolerance, hence, this makes them an important cosmopolitan fungi associated with post-harvest decay and soft rot of different substrate [30]. These organisms are soil saprobes with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocelluloses and this is because of their ability to produce extracellular organic acids.

This result proves that fungitoxic compounds were present in *P. guineense* and *X. aethiopica,* since they were able to inhibit the growth of the test fungi. The finding is in consonance with the earlier reports of several researchers but on different fungal organisms [2, 25, 33, 37], hence the two plant extracts used have the potential application in the protection of mechanically injured yam tubers against rot fungi.

The present observations showed that *P. guineense* and *X. aethiopica* were highly effective against mycelia growth of almost all the test fungi with inhibition ranging from 3.75±0.22 to 5.74±0.26. This is similar to the results obtained by [34] on yam rot and that of [37] on post-harvest rot of yam, which reported a highly effective inhibition with *A. sativa and Azadirachta indica* respectively, but differs with the results of Okigbo *et al.* (2009) who reported a moderately effective inhibition by *A. sativa.* The commercial fungicides (Grisovid) showed a very significant effective inhibition on the radial mycelia growth of the fungi tested (8.89±0.00 for *A. terreus*), (9.22±0.24 for *F. oxysporum*) and (8.69±0.24 for *R. stolonifer*). There was a similar trend in the fungitoxic effect of the two plant extracts; *P. guineense* proved to be the most fungitoxic on *F. oxysporum* and *R. stolonifer. Xylopia aethiopica* seed extract had the highest inhibitory effect was on only *A. terreus* amongst all the test fungi. This agrees with the observations of [37] who stated a significant difference between mycelia growth value recorded on the various plant extract concentration. This suggests that there is difference in the solvent soluble antifungal element in the respective leaves extracts as reported by [15, 35].

The presence of bioactive substance have been reported to confer resistance to plants against bacterial, fungi and pest [36], this therefore explains the demonstration of antifungal activity by the plant extracts used in this study, hence the antifungal properties of these plant extracts is probably due to the presence of phytochemicals which are anti-microbial agents (Okwu and Joshia, 2006), that are inhibitory to the growth of these pathogens [23].

Conclusion

Dioscorea rotundata is one of the most popular foods in Nigeria, mostly used for making various delicacies. This study has identified the fungi associated with spoilage of yam and the possible control of these pathogens using plant extracts. The findings from the study therefore, revealed the fungi that caused the diseases which included: *A. terreus, R. stolonifer* and *F. oxysporum*. These fungi had fast growth and spread within a short period of time on fresh yam; but with the use of *Piper guineense* and *X. aethiopica,* these fungi pathogens were significantly inhibited.

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