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Control of Yam Spoilage Fungi Using *Xylopia aethiopica* and *Monodora myristica* Seed Extracts

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ABSTRACT

Background and Objective: Yam plays an important role in the food security and livelihoods of at least 60 million people, especially in West Africa. Effects of seed extracts of *Xylopia aethiopica* and *Monodora myristica* on yams were investigated using the cold extractions method. **Materials and Methods:** Two healthy and infested yam tubers were purchased from EKE-Awka Market, in Awka. Isolation, identification of causal organisms and phytochemical analysis were carried out using different standard methods. The experiment was set up in a completely randomized design while the data collected was analyzed using ANOVA. **Results:** Fungi isolated from the deteriorated yam tubers were: *Fusarium oxysporum* (11.11%), *Aspergillus flavus* (33.33) and *Aspergillus niger* (66.67%). *Aspergillus niger* (66.67) was the most pathogenic while the least pathogenic was *Fusarium oxysporium* (13%). Aqueous extracts of the two plants inhibited the growth of the fungal pathogens moderately while the most fungitoxic was the ethanoic extraction of *Xylopia aethiopica* which inhibited the growth of almost all the fungal pathogens studied. Extracts of *X. aethiopica* recorded the highest percentage inhibition of A. niger (19.71%) followed by *F. oxysporum* (10.24%) while *A. flavus* had the least (10.21%). **Conclusion:** The study concluded that pathogens (fungi) responsible for yam spoilage can be controlled or inhibited from growing by using plant extracts of *Xylopia aethiopica* and *Monodora myristica*. This can provide an alternative way of reducing and controlling rot by farmers.

KEYWORDS

Antifungal, biocontrol, *Aspergillus niger*, botanicals, phytopathology, *Fusarium oxysporum*, *Dioscorea*

INTRODUCTION

Yam (*Dioscorea*) is a monocotyledonous angiosperm that belongs to the order Dioscoreales, family Dioscoreaceae and genus *Dioscorea*. It is a common food in most tropical and subtropical countries[1]. Yam plays a very crucial role in the food economy of the country as it provides a source of energy to consumers. It's on record that about 20 million tons of yam are produced globally in a year with Nigeria contributing to 75% of the total world production[2]. Of the over 50 species of *Dioscorea* in Nigeria, only six are consumed as food in Nigeria and other African countries.

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Yam can thrive and produce maximally in an environment with water scarcity. Root and tuber crops such as yam, cassava and cocoyam contribute to the income of most families and also employ processing along the value chain. Yam is highly nutritious with relatively higher nutritive value compared to other root and tuber crops. Comparatively, its protein content (3-5%) is higher than that of cassava (1-2%).

Yam in storage and the field is prone to rot due to the maceration action of phytopathogenic microorganisms such as bacteria, fungi and viruses. Unfavorable environmental conditions can also predispose yam to rot or trigger microbial infections[3]. According to Okigbo *et al.*[4] the major micro-organisms responsible for yam diseases are *Apergillus flavus, Aspergillus niger, Fusarium oxysporum, Penicillium chrysogenum, Rhizoctonia* species*, Trichoderma viride* and *Rhizopus nodosus*. Soft rot is a major factor limiting the post-harvest storage of yam tubers and losses can be very high. Yam are subjected to several diseases and these disease-causing agents reduce the quantity and quality of the yam by making them unmarketable and unappealing to the consumers. Yam rots usually start in the soil and progress in storage, which occurs when infected do not have any sign of external symptoms, the incidence of rotting varies with the species and with the varieties within each species of yam.

Spoilage of yam tubers as a result of post-harvest diseases can be controlled through curing using plant extract or by chemical or biological method[2]. The use of chemicals is largely discouraged because they are cost-effective and due to a dearth of expertise in the proper and safe handling of chemicals. On this note, attention is now directed towards other cultural methods of controlling rot such push fallowing, crop rotation, shifting cultivation and healthy and disease-free planting materials[4]. However, the use of chemicals helps in yield increase and reduction in the degree of storage losses; on the other hand, it may induce pathogen resistance to pesticides and environmental pollution. Comparatively, the biological method is preferred to other methods of control because it is selective with little or no side effects and is cheap. Resistance to biological control is not common and biological control agents are self-propagating and self-perpetuating[5,6].

Most plants rich in phytochemicals with antimicrobial activities have shown promising potential for the control of both human and crop diseases[7]. *Monodora myristica* (calabash nutmeg) of the family Annonaceae is a species of calabash nutmeg. The edible seeds produce a nutmeg flavoured oil which is used in some parts of Africa for cooking. The seed is rich in essential pharmacological compounds such as alkaloids, flavonoids and vitamins A and E as well as important lipids.

Xylopia aethopica of the family Annonaceae is a slim, tall, aromatic, evergreen tree that grows to 15-30 m high and 60-70 cm in diameter[8]. It is known to naturally grow in the Savannah Region of Africa, particularly in Ghana, Nigeria, Cameroon, Ethiopia and Senegal. The fruit has repulsive properties which have been used in various forms to cure rheumatism. The trees are widely distributed in the humid forest zones especially along rivers in the drier area of the of the region[8]. The mixture of its fruit with *Capsicum* peppers and kolanuts is used as weevil repellent. The seeds have cosmetic, repulsive and stimulant applications. *Xylopica aethopica* is a good source of firewood. The importance of these natural plants is enormous because they are readily available with simple preparation procedures. They are also effective against phytopathogens and have little or no toxicity to humans[9].

This work is aimed at the isolation and identification of fungi associated with post-harvest yam rot and to establish the pathogenicity of fungal organisms associated with the pathogenicity of fungal organisms associated with rots of white yam varieties as well as to determine the potentiality of aqueous and ethanoic extracts of some plants on the identified pathogens.

MATERIALS AND METHODS

Study area: This study was conducted from June, 2023 to October, 2023 in Awka, Anambra State. Awka, the capital City of Anambra State is located in the Southeastern Region of Nigeria. It's situated between Latitude 60 5' and 60 15' N and Longitudes 70 0' and 70 5' E.

Source of materials: Two tubers of yam with symptoms of rot and five healthy yam tubers were purchased from Eke Awka Market in Anambra State and taken to the Department of Botany Laboratory Nnamdi Azikiwe University Awka for culturing, isolation and identification. The two local plants (500 g each): *Monodora myrisitica* (seeds) and *Xylopia aethopica* (seeds) used in the experiment were also purchased from Eke Awka Market, Anambra State.

Isolation of fungi from cocoyam with symptoms of rot: The infected yam tubers were surface sterilized with one percent (1%) sodium hypo chloride (NaOCl) solution for 1 min to remove surface contaminants after washing off soil and root debris or extraneous materials from the sample. This was followed by three successive rinses in distilled water. The infected yam tubers were cut into smaller pieces from the area between the healthy part and the dead part of the tuber. The pieces were surface sterilized by putting them in a beaker containing ethanol before being separately inoculated on PDA media contained in Petri dishes. The Petri dishes were labeled accordingly and then placed in an incubator for 4 days for the associated fungi to culture.

Sub-culturing and identification of pathogens: Pure cultures were obtained by sub-culturing each fungus that appeared by using an inoculating loop onto the PDA medium and the Petri dishes were incubated for 4 days. The inoculating loop used was sterilized by placing it in a beaker containing ethanol and then flaming to red hot after each streak, to avoid the yield of mixed cultures. The fungi were identified using morphological, cultural and microscopic characteristics of fruiting structure and the spores produced.

Pathogenicity test: The healthy yam tubers were washed with tap water and distilled water and sterilized with 70% ethanol to remove surface contaminants. With the aid of a 4 mm cork borer, part of the tuber was removed. A disc of 5 days old purified cultures of the isolated microorganisms was used to cork the holes created in the tubers and the disc of the tuber in the cork borer was replaced. After the inoculation, vaseline was applied at the point of inoculation. This was done for all the pure cultures isolated from the yam sample.

Preparation of seeds of *Monodora myristica* and *Xylopia aethiopica:* Fresh and healthy seeds of *Monodora myristica* and *Xylopia aethiopica* were bought from Eke Awka Market in Anambra State, seeds were spread and shade dried for 10 days at room temperature and the dried seeds were grinded with an electric blender (Silver Crest model:SC1589) to obtain a fine powder sample, before taken to the laboratory for extraction.

Aqueous extraction: The aqueous extract of the seed of the two plants was prepared by weighing 50 g of the fine powder on a weighing balance (IndiaMART, Delhi, India) and then transferred to a beaker. The 500 mL of hot water was then added to the powder in the beaker and was kept on a laboratory oscillator (mode:Kj201BS, Wincom Company Ltd, China) for 15 min. The extract was filtered through cotton wool twice and again through Whatman filter paper. The filtrate was then transferred into a plastic bottle, labeled and kept in a refrigerator (Hisense) at 4°C. Required concentrations; 50 and 75% and absolute concentration were made by dilution with distilled water.

Ethanolic extraction: The ethanolic extracts of the two plants were prepared by soaking the grinded samples of the plants in 100 mL of ethanol. The concentration of each extract was determined by adding 100, 150, 200 and 250 g in 100 mL of ethanol. The experimental set-up was left for 24 hrs at room temperature and thereafter filtered using Whatman No. 1 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°C until when needed.

Effect of plant extracts on fungal growth: Inhibitory effects of the aqueous and ethanol plant extracts on the mycelia growth of the test fungi were carried out according to the method of Ezeonu *et al.*[10]. One milliliter of each plant extract concentration (50, 75 and 100%) was dispensed into each Petri dishes with 9 mL of molten PDA. This gave rise to PDA-extract mixture with corresponding 5.0, 7.5 and 10% extract concentrations. After solidification of the agar-extract mixture, inoculation was done at the center with a 4 mm diameter mycelial dish obtained from pure cultures of the individual test fungi. The negative control setup consists of a blank agar plate (no extract) inoculated with the test fungi as described above. This was taken to be 0%.

The inoculated plates were incubated at 28±2°C for 5 days and examined daily for fungal growth and inhibitory potential. The inhibitory effect was calculated based on percentage inhibition calculated according to the method described by Anukwuorji *et al.*[11]:

$$\text{Inhibition (\%)} = \frac{R_1 - R_2}{R2} \times 100$$

Where:

R_1 = Farthest radial distance of pathogen in the control plate

R_2 = Farthest radial distance of pathogen in extract-incorporated agar plates

Phytochemical screening: The phytochemical tests were carried out to determine the presence of phytochemical constituents. This was carried out using the methods described by Bisso *et al.*[12]. Qualitative phytochemical screening of the extracts was conducted on the two plant samples and determine the presence of these phytochemicals: Hydrogen cyanide, alkaloids, flavonoids, saponins, sterols, tannins and phenols. A quantitative phytochemical test of the extracts was conducted to determine the percent quantitative contents of the above phytochemicals.

Statistical analysis: The experimental design used was a Completely Randomized Design (CRD) with three replicates. Data collected were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan letters at 0.05 probability level indicating the level of significance between values.

RESULTS

Percentage frequency of the isolated fungi: The fungi frequently isolated from this research work were *A. niger, A.flavus* and *A. oxysporium* with 66.67, 33.33 and 11.11% frequencies of occurrence, respectively (Table 1).

Pathogenicity test: The pathogenicity test showed that *Aspergillus niger* was very pathogenic with a rot incidence of 30 cm while *Aspergillus flavus* was less pathogenic with a 17 cm rot incidence while tubers inoculated with *Fusarum oxysporum* recorded the least rot incidence of 13 cm (Table 2).

Table 1: Frequency of occurrence of the isolated fungi

Isolates	Frequency (%)
Aspergillus niger	66.67
Aspergillus flavus	33.33
Fusarium oxysporum	11.11

Table 2: Pathogenicity of the isolated fungi

Isolates	Pathogenicity (cm)
Aspergillus niger	30
Aspergillus flavus	17
Fusarium oxysporum	13

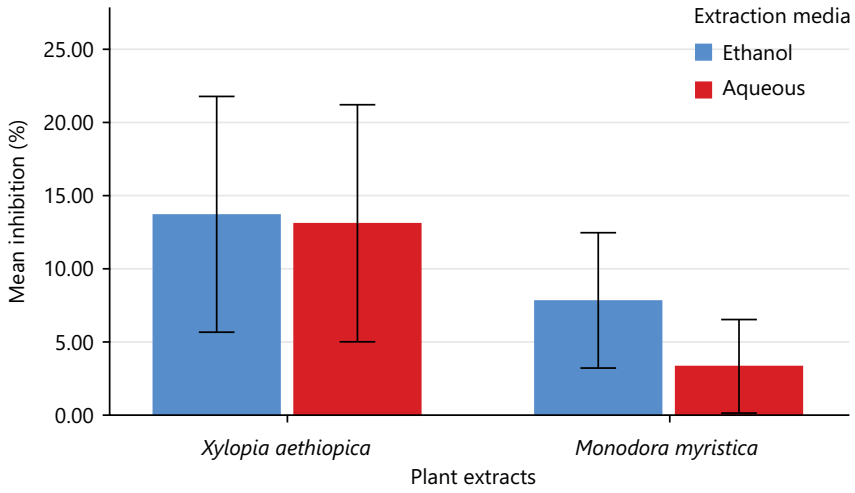

Fig. 1: Mean percentage inhibition of plant extracts at various concentrations

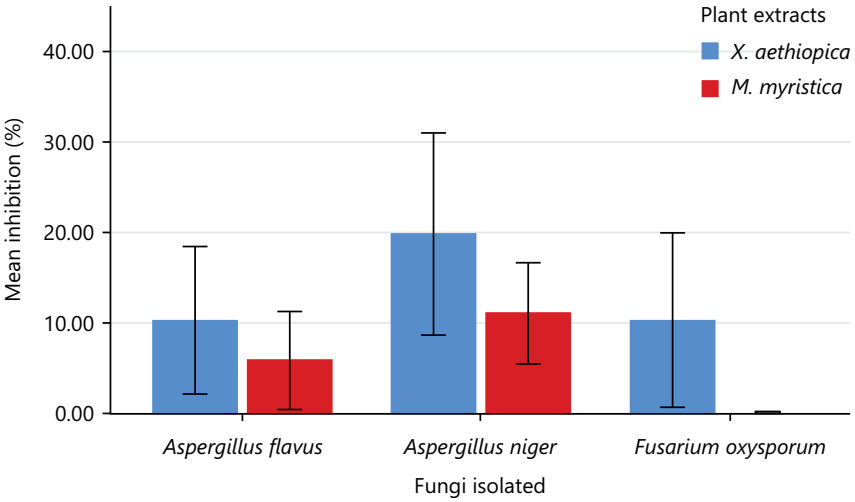

Fig. 2: Effects of plant extracts on the inhibition of various fungi isolates

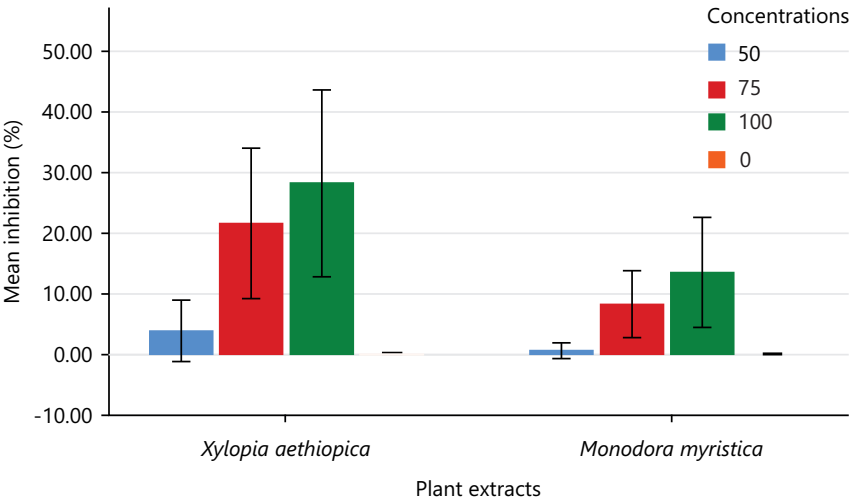

Fig. 3: Effects of plant extracts on the inhibition of fungi pathogens

Table 3: Phytochemical analysis of *Xulopa aethiopica* and *Monodora myristica*

Phytochemical component		Observation	Inference	
			X. aethiopica	*M. myristica*
Tannin	Ferric chlorides test	Greenish-black	+	+
Alkaloid	Mayer's and Wagner's test	Reddish brown precipitate	+	+
Saponin	Emulsion test	Presence of emulsion	+	+
Steroids	Salkowski test	Red colour at interface	-	-
Flavonoid	Ammonium test	Yellow colour	+	+
	Ammonium chloride test	Yellow colour		
Phenols	Ferric-chloride test	Greenish-brown precipitated	+	+

+: Positive and -: Negative

Table 4: Mean percentage inhibition of the plant extracts in the various extraction medium

Extraction media	Plant extract	Mean percentage inhibition ±SD
Ethanol	*X. aethiopica*	13.69±19.672
	M. myristica	7.82±11.283
	Total	10.75±16.138
Aqueous	*X. aethiopica*	13.09±19.814
	M. myristica	3.36±7.800
	Total	8.22±15.687

Result of preliminary phytochemical analysis on the test plants: Table 3 shows the preliminary phytochemical analysis of *X.aethiopica* and *M.myristica*. The results showed that various phytochemical components such as tanin, alkaloid, saponin, flavonoids and phenol were present in both plants while steriods were absent in both plants.

Effects of extraction media and plant extracts on the inhibition of fungi: The result in Fig. 1 revealed that ethanolic extract of *X. aethiopica* recorded a higher percentage inhibition of fungi (13.69%) than *M. myristica* (7.82%). The results further showed that aqueous extract of *X. aethiopica* caused higher inhibition of fungi (13.09%) than *M. myristica* (3.36%). There was no significant difference in the percentage inhibition of fungi between extraction media ($p>0.05$) but there was a significant difference between the plant extracts.

Effects of plant extracts on the inhibition of various fungi isolates: The result in Fig. 2 revealed that yams treated with an extract of *X. aethiopica* recorded the highest percentage inhibition of *A. niger* (19.71%) followed by *F. oxysporum* (10.24%) while *A. flavus* had the least (10.21%). The result further showed that extract of *M. myristica* caused the highest percentage inhibition of *A. niger* (10.97%) followed by *A. flavus* (5.79%) while *F. oxysporum* had least (0.00%). There was a significant ($p<0.05$) difference in the occurrence of the fungi isolates and percentage inhibition between the two plant extracts ($p<0.05$).

Effects of various concentrations of *X. aethiopica* and *M. myristica* on the Inhibition of fungi: The result in Fig. 3 revealed that yams treated with 100% extract of *X. aethiopica* recorded highest inhibition of fungi (28.14%) followed by 75 (21.54%) while the control (0%) had the least (0.13%). The result further showed that 100% extract of *M. myristica* caused the highest percentage inhibition of fungi (13.48%) followed by 75 (8.26%) while the control (0%) had the least (0.00%). There was a significant difference in the inhibition of the fungi isolates at the various concentrations of the *X. aethiopica* and *M. myristica* ($p<0.05$).

Table 4 shows the percentage mean inhibition of the plant extracts in various extraction medium (ethanol and aqueous). The ethanol extraction of *X. aethiopica* (13.69±19.672) shows more significant ($p<0.05$) inhibition than the aqueous extraction of *X. aethiopica* (13.09±19.814). This difference is also seen in the ethanol extraction of *M. myristica* (7.82±11.283) and aqueous extraction of *M. myristica* (3.36±7.800).

DISCUSSION

Fungi isolates found in the yam tubers with symptoms of rot in this study were *Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporium*. These fungi may have penetrated the yam tubers through wounds sustained during harvesting or other post-harvest operations. The inference of this study also proved that the fungi isolated were not mere opportunistic saprophytes but pathogenic as seen from the pathogenicity test. *Aspergillus niger* was observed to be the most pathogenic while *Aspergillus flavus* was the least even though *Fusarium oxysporum* was the least frequently occurred.

The findings of this work were in tandem with the reports of Okigbo and Ogbonnaya[1] on the anti-fungal effects of two tropical plant extracts (*Occimum gratissium* and *Afromamum melegueta)* on post-harvest yam. The antifungal effect of these plant extracts in the inhibition of different pathogens of crop plants has been reported by Okigbo and Ogbonnaya[1]. The result of the phytochemical analysis of *Xylopia aethiopica* and *Monodora myristica* revealed the presence of various phytochemical components such as tannin, alkaloids, saponin, flavonoids and phenols, respectively while steroids were absent.

Results of the pathogenicity test showed that *A.niger, F. oxysporum* and *A. flavus* were responsible for the post-harvest spoilage of yam tubers. *Aspergillus niger* was the most pathogenic while *Aspergillus flavus* was the least virulent. Ethanoic and aqueous extracts of *Xylopia aethiopica* have a relatively high inhibitory effect on the test fungi organisms; the inhibition being highest with *A.niger*. The control experiment depicted an uninhibited growth of the fungi pathogens. This agreed with the findings of Okigbo *et al*.[4] and Anukwuorji *et al*.[13] on yam storage rot caused by several.

Microorganisms responsible for rot in tubers in storage are mostly fungi and bacteria[1-11]. Some of the fungi identified in this work have also been reported by some researchers. These fungi penetrate intact tubers either through natural openings or wounds that occur during harvesting and post-harvest operations. Tubers, which are already attacked by rotting pathogens when harvested, get spoiled to a greater extent in storage. It was also clear that *Aspergillus* and *Fusarum* could be regarded as post-harvest pathogens of yams. Some biological control measures have been used to control white yam rot with *Bacillus subtilis* and *Trichoderma viride*[9]. This research showed that there is a high advantage of ethanol over water extract in inhibiting the fungal pathogens isolated from the rotten yam tubers. *Xylopia aethiopica* inhibited the growth of the fungal pathogens more effectively than *Monodora myristica*. This work revealed that fungi toxic compounds were present in *X. aethiopica* and *M. monodora* seeds since they were able to suppress the growth of the microorganisms tested.

CONCLUSION

Pathogens (fungi) responsible for yam spoilage can be controlled or inhibited from growing by using plant extracts of *Xylopia aethiopica* and *Monodora myristica*. This can provide an alternative way of reducing and controlling rot by farmers. Since the entire test organisms showed sensitivity to the two plant extracts, it is recommended that the extracts of *X. aethiopica* and *M. myristica* be used to control causative pathogens in yam tuber rot.

SIGNIFICANCE STATEMENT

Yam is an important staple crop in most countries in sub-Saharan Africa with Nigeria alone producing over 40% of the global yam production. It's on record that over 40% of yam cultivated across the globe annually is lost to rot. The use of chemicals in the control of these microorganisms is common but not without challenges. Chemicals are expensive, toxic and not readily available. This research work discovers an antifungal of plant origin capable of controlling the rot of yam. This study has shown that *Xylopia aethiopica* and *Monodora myristica* seed extracts have the potential to control yam rot. On this note, *Xylopia aethiopica* and *Monodora myristica* seed extracts are recommended for the control of yam rot since they have proven to be effective antifungal and are known to be eco-friendly; this is an advantage over the conventional synthetic/chemical fungicides.

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