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Botanical Control of Some Fungal Rot Disease Causing Spoilage of Sweet Potato (*Ipeomea batata*) Tubers in Three Markets in Aba, Nigeria

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ABSTRACT

Production of sweet potato in Nigeria can be improved by increasing productivity and avoiding crop failures caused by storage rots. Pure fungal isolates were assessed for potency in causing tuber rot of sweet potato (*Ipeomea batata*) during storage using standard microbiological procedures. The isolates were *Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Fusarium oxysporum, Penicillium chrysogenum,* and *Rhizopus stolonifera*. Each fungus was inoculated into eighteen healthy potato tubers purchased from Afule market, Umungasi market and new market in Aba, Abia State and were left for 10 days after which extent of rot was determined by depth of rot caused. Control was evaluated using *aqueous* leaf extracts of *Azadirachta indica* (Neem) and *Piper guineense* (Uziza) against the fungi associated with spoilage of stored potato tubers. The fungitoxic effect of the plant extracts was determined by diameter of zone of inhibition (mm) at four concentrations of 100 mg/ml, 75mg/ml, 50 mg/ml and 25 mg/ml. The zone of inhibition for neem extract and that of Uziza ranged from 7.00-24.07 mm and from 14.03-18.17mm respectively. Zone of inhibition was significantly different at ($p<0.05$) for both plant extracts at the concentrations used. Extract of neem also showed significantly higher zones of inhibition than extract of uziza at all concentrations used. The highest zone of inhibition (24.07 mm) was observed against *R. stolonifer* while the lowest (6.33 mm) was against *A. niger, A. fumigatus* and *F. oxysporum*. Pathogenicity test of fungal isolates on healthy potato tubers revealed that *A. fumigatus* recorded highest diameter of rot (15.08 mm) while *Aspergillus niger* recorded the lowest (7.55 mm). The antifungal activities demonstrated by the plant extracts against the sweet potato rot fungi can be useful to identify phytochemicals in plant extracts that would act as fungicides in the control of potato rot fungi, hence, improve food security.

Keywords: Sweet potatoes, rot fungi, pathogenicity**,** Neem, Uziza, antifungal, food security.

Introduction

Sweet potato (*Ipomoea batatas* Lam) is the third most important root and tuber crop after cassava (*Manihot esculenta*) and yam (*Dioscorea rotundata*) within the sub-Saharan Africa. Sweet potato is a common root crop in Nigeria with enormous potential to be an efficient and economic source of food energy. The sweet potato tuber is rich in carbohydrate, lipid and low in fibre content (Onifade *et al*., 2004). The production of sweet potato in Nigeria can be improved by increasing productivity and avoiding crop failures caused by storage rots (Echerenwa and Umechuruba, 2004). As such, it is seen as one of the crops with strong potential to contribute to tropical food security (Maranzu, 2019).

However, production of this valuable tuber crop suffers from several constraints especially poor tropical storage conditions and hygiene as well as postharvest microbial deteriorations (Sokoto and Ibrahim, 2007; Nwanja *et al*., 2017; Maranzu, 2019). For these reasons fresh sweet potato tubers have been reported to store for about three weeks only after harvest if left untreated (Maranzu, 2019). A wide variety of microorganisms, particularly molds, have been implicated in tuber spoilage, relatively few are implicated as primary pathogens (Onifade *et al*., 2004). The use of chemicals has helped in control of rot but due to the identifiable problems such as chemical residuals, pollution, and development of resistance in target organisms makes them slow to

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adopt by farmers or the farmers have totally failed to adopt them because of cultural reasons.
Hence alternative control methods are being sort and employed. Presently, considerable efforts are directed at exploring the potentials of botanicals, hence, the significance of this study.

Materials and Methods

Collection of Sweet Potato Tubers (*Ipomea batata*) and Neem and Uziza

Eighteen healthy potato tubers used for pathogenicity test were purchased from New market, Afule market and Umungasi market all in Aba, Abia State Nigeria. Pure fungal isolates of *Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Fusarium oxysporum, Penicillium chrysogenum,* and *Rhizopus stolonifera* obtained from the laboratory of the Department of Biology/Microbiology of Abia State Polytechnic, Aba were used for this study. Fresh leaves of *Azadirachta indica* (Neem) and *Piper guineense* (Uziza) were sourced from Umungasi vegetable market at Aba-Owerri Road, Aba, Abia State. All the botanicals were authenticated by a botanist Dr Nwokocha N. J. of the Department of Biology/Microbiology, Abia State Polytechnic, Aba.

Extraction of Plant Materials

The plant materials *Azadirachta indica* leaves and *Piper guineense* leaves were properly washed separately with clean water, air dried at room temperature, and each ground into uniform powder using electric blender. Cold water extracts of the ground leaves were prepared by weighing 100g of each plant materials into different clean grease-free glass container. Then 500ml of water was added to each container containing *Piper guineense* leaves powder and another containing container *Azadirachta indica* leaves powder. The containers were shaken vigorously and allowed to stand for 24 hours. The extracts were filtered separately using muslin cloth and concentrated using a water bath at a temperature of 60ºC. Different concentrations of 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml of each extract were separately prepared for each plant leaf extract.

Pathogenicity Test

Eighteen (18) healthy sweet potato tubers were neatly washed using clean tap water to get rid of dirt. Five percent (5 %) sodium hypochlorite solution was used to further surface-sterilize the tubers for up to 2 minutes before the tubers were rinsed in four successive changes of sterile water to remove chemicals (Gwa *et al.*, 2018).

A five-day old culture of the fungal isolates identified was used as inoculums for pathogenicity tests. A sterile 5mm diameter cork borer was used to remove discs (1cm thick) from the “head” and “tail” regions of each potato tuber (Okigbo and Ikediugwu, 2000). Another 5mm sterile cork borer was used to cut plugs of mycelia discs from the five-day old cultures of the fungal isolates. These fungal plugs were put in the holes created in the tubers. A portion of the tuber flesh removed earlier was cut off to give way for inoculum size and the remaining tuber flesh was used to plug the remaining parts of the hole made in the potato tubers. Melted candle wax from a burning candle was used to seal the edges of the replaced potato discs to prevent any external influence on the positioned inocula.

Controls of each species were set up in which the sterile cork borer was used to remove 5 mm diameter tuber tissue. The disc was used to plug the hole without placing any fungal organisms in the hole, and its edges sealed with melted wax. The potato tubers were kept in the laboratory at room temperature for 10 days. The sweet potato tubers were assessed for rot development by cutting through the point of inoculation where rots developed. A transparent ruler was used to measure the depth of rots and recorded in millimeter (mm).

Antifungal Activity of Plant Extracts *in vitro* on Potato Rot Organisms (Agar Well Diffusion Method)

Each fungal species isolate was evenly streaked on each surface of prepared potato dextrose agar medium using sterilized inoculating loop to create agar lawn with the isolates and allowed to dry. A sterilized cork borer of 4 mm was used to create four wells in the agar media.

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In each plate, the various prepared concentrations (25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml) of the two aqueous leaf extracts, *Azadirachta indica* and *Piper guineense* were used to fill each well respectively using sterile micropipette. The Petri dishes were then incubated at 25°C for 24 hours. The zones of inhibition were measured using a transparent ruler and the results were recorded in millimeter (mm).

Results

The result of the pathogenicity test is presented in Table 1. The result revealed that the six fungi induced rot in healthy sweet potato tubers. This study has shown that the degree of pathogenicity caused by different fungi associated with storage rot of potato tubers differ significantly. The most virulent of these fungi was *A. flavus* (15.08 mm ± 1.15[a]) followed by *A. fumigatus* (14.03 mm ± 1.07[a]) and *R. stolonifera* (13.67 mm ± 0.56[a]) in Afule market while all the tested fungi isolates were lest virulent in potato tubers from Umungasi market.

The results of the Fungitoxic effect of leaf extracts of *Azadirachta indica* and *Piper guineense* (in mm) against the pathogenic fungi of potato tubers is shown in Figure 1. The aqueous extracts of *Azadirachta indica* and *Piper guineense* significantly (P = 0.05) inhibited the mycelial growth of these fungi. The mycelial growth decreased as the plant extracts inhibited the fungal growth in culture medium (Figure 1).

Table 1: Pathogenicity tests of some fungal pathogens showing rot development (mm) on healthy potato tuber after 10 days of incubation

Isolates	Market			F-ratio	P—value	Remark
	New	Umugasi	Afule			
A. niger	10.70 ± 1.55[b]	7.55 ± 0.54[c]	12.58 ± 1.67[a]	21.163	0.001	S
A. flavus	11.58 ± 1.68[b]	8.07 ± 1.11[c]	15.08 ± 1.15[a]	41.257	0.001	S
A. fumigatus	9.05 ± 1.13[b]	9.05 ± 1.13[b]	14.03 ± 1.07[a]	40.394	0.001	S
P. chrysogenum	11.57 ± 0.61[a]	11.53 ± 0.55[a]	11.60 ± 1.66[a]	0.006	0.994	NS
R. stolonifera	13.10 ± 1.34[a]	9.53 ± 0.58[b]	13.67 ± 0.56[a]	45.227	0.001	S
F. oxysporum	10.57 ± 1.71[a]	10.05 ± 1.11[a]	11.53 ± 1.70[a]	1.445	0.267	NS

NOTE: NS = Not significant

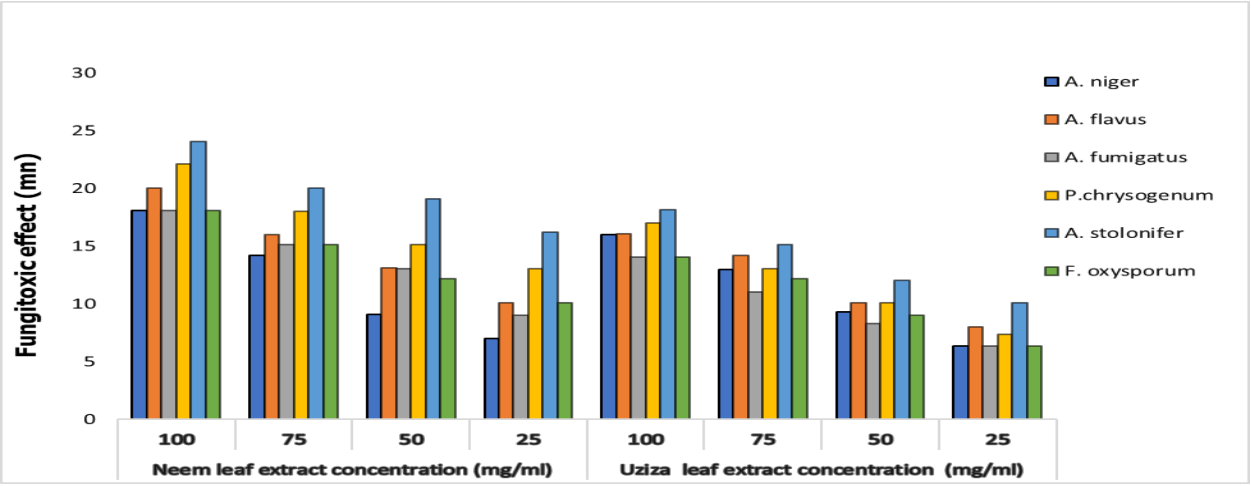

Figure 1: Fungitoxic effect of leaf extracts of *Azadirachta indica* and *Piper guineense* (mm) against the pathogenic fungi of potato tubers

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Discussion

Pathogenicity test showed that all the isolates induced rot on potato tubers 10 days after inoculation of the tubers with the test pathogens. Tubers that were not inoculated with rot causing fungi did not produce symptoms of rot. *Aspergillus flavus, A. fumigatus, Rhizopus stolonifer, Aspergillus niger* and *Fusarium oxysporum* are the most virulent fungi causing rot in the healthy potato tuber. However, the result of this of pathogenicity test is not in agreement with the study of (Agu *et al*, 2015). . The high incidence of storage rots of sweet potato tubers encountered in the study could also be attributed to handling procedures during harvest, transit, marketing, and storage places. Post harvest loss of root and tubers has been of serious problem to farmers and warring against food security.
The inhibitory efficacy of aqueous extracts of *Azadirachta indica* leaf (Neem leaf) and *Piper guineense* leaf (Uziza leaf) on sweet potato rot fungi measured in millimeter is shown in figure 1. The inhibitory efficacy of aqueous extracts of *A. indica* leaf (Neem leaf) and *P. guineense* leaf (Uziza leaf) on sweet potato rot fungi revealed appreciable efficacy of these indigenous plants extracts against rot fungi. The highest zone of inhibition 24 mm was recorded when *Azadirachta indica* extract was applied to *Rhizopus stolonifer* at 100 mg/ml concentration. While the least inhibition, 7mm of *Azadirachta indica* was applied to *Aspergilllus niger* at 25 mg/ml. The highest zone of inhibition was recorded when *Piper guineense* extract at 100 mg/ml was applied to *Rhizopus stolonifer* (18 mm) while the least inhibition was seen at 25mg/ml on *Aspergilllus niger, Aspergillus fumigatus* and *Fusarium oxysporum* (6 mm).

The highest zone of inhibition (18mm) was recorded when *Piper guineense* extract was administered at 100 mg/ml to *R. stolonifer* while the least inhibition (6 mm) was seen at 25 mg/ml on *A. niger,, A. fumigatus* and *F. oxysporum.* This is in line with the work by Gwa *et al.* (2018) on inhibition of *A. flavus,* and *F. oxysporum* using extract derived from *P. guineense*.. This also agrees with the report of Amadioha (2000), who reported high efficacy of *P. guineense* leaf extracts against post harvest rot fungi of tuber crops. The presence of antifungal substances in the different extracts which caused the inhibition of radial growth and spore germination *in vitro* agree with report of Amadioha (2000).

The result showed that increase in concentration of plant extracts resulted to decrease in rot indicating an increase in decay reduction. This study also confirmed and established the antifungal activity of these plant extracts, which are interestingly systemic in action and can be used or applied as postharvest tuber treatment against rot causing pathogens of potato.

In conclusion, this study has shown that the degree of rot caused by different fungi associated with storage rot of potato tubers differ significantly It is important to adopt disease control practices that will be affordable by the bulk of resource-poor farmers in our part of the world and it will ensure substantial contribution of the sweet potato to food supply and national economy.

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