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In Vitro Evaluation of Two Plant Extracts for the Control of Post-Harvest Fungi Rot of Sweet Potato Tuber (*Ipomoea Batatas* L. Lam) in Makurdi, Nigeria

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Abstract:
The study investigated the pathogens causing postharvest deterioration of sweet-potato tubers in Makurdi and their control in vitrowith aqueous extracts of Moringa oleifera and Eucalyptus globulus. Rhizopus stolonifer and Aspergillus niger were isolated from rotted sweet potato tubers. The pathogenicity test revealed that both pathogens were pathogenic on healthy potato tubers with Rhizopus stolonifer being the most virulent.Three extract concentrations (25, 50 and 75 %) were obtained from the leaves of M.oleifara and E.globulus by blending 25, 50 and 75g of air-dried leaves in 100 ml of sterile distilled water to produce 25, 50 and 75 % concentrations respectively. The extracts of both pathogens were more effective in inhibition of mycelial at higher concentration. The fungitoxic effects of the extracts of M.Oleifera induced mycelial inhibition in the ranges of 26.31-75.65 %, while extracts of E. globulus induced mycelial inhibition in the ranges of 21.28- 61.25 %. Extracts of E. globulus at 75 % concentration exhibited the strongest fungitoxicity against the text pathogens. This study indicated that M. oleifera and E. globulus were able to suppress rot-causing fungi of sweet potato. Therefore, they will serve as a good natural plant fungicide against sweetpoato tubers.

Keywords: *Sweet potato, Rhizopus stolonifer, Aspergillus niger, Moringa oleifera, Eucalyptus globulus.*

1. Introduction

Sweet potato (*Ipomoea Batatas* L. Lam) belongs to the family Convolvulaceae. It is a tuberous-rooted perennial native to the American tropics. It is vegetatively propagated and can be grown in relatively infertile soils with few inputs. It can withstand periods of irregular drought and rainfall (Horton *et al.*, 1984). It is one of the six important root and tuber crops grown in Nigeria. The other root crops are cassava, Yam, Irish potato, Cocoyam and Ginger. Within Sub-Sahara Africa, sweet potato is the third most important root tuber crop after cassava (*Manihot esculenta*) and Yam *Discorea* spp. (Ewell and Matura, 1991). In 2013, Nigeria produced 3.450.000 t of sweet potato, making her the third largest producer in the world, behind Tanzania (3.470.304 t) and China (70.741.161 t) (FAO, 2012)

In Nigeria, sweet potato is eaten boiled, roasted or fried in vegetable oils. The chips are also packaged and sold as snacks and may also be processed into flakes. The roots are peeled, chopped and boiled and then used as an ingredient in meat pies. The tender leaves are also used as vegetables while vines are used as livestock feed especially during the dry season. Industrial uses of sweet potato roots in Nigeria are very limited. However, studies show starch content of 15.08 to 24.68 % from the high-yielding varieties developed by the National Root Crop Research Institute, Umudike, Nigeria (Tewe *et al.,* 2003). These are therefore good sources of starch and amylose for food, alcohol, pharmaceuticals and the textile industries in Nigeria. Culture media produced from sweetpotato flour compare favourably with standard laboratory nutrient agar media (Tewe *et al.,* 2003).

The production of sweet potato in Nigeria is constrained by several factors among which storage rot is one of the most important (Echerenwa and Umechuruba 2004).Post-harvest loss of root and tuber crops has been a very serious problem to farmers as more than 40% of their harvest may be lost because of decay (Olurinola *et al*., 1992).

The principal species of microorganisms associated with the sweet-potato rot in Nigeria include: *Fusarium oxysporum, Ceretocysts fimbriata, Fusarim solani, Monilochaetes infuscans, Macrophomina phaseolina and Botryodiplodia theobromae* (Clark and Hoy, 1994).Onuegbu (2002) implicated *Penicillium sp*. *Cerocystis fimbriata*, *Diaporthe batatalis*, *Aspergillus flavus* and *Aspergillus niger.*

Oyewale (2006) repoted fungi associated with postharvest fungal rot to include *Motierella ramanniana, Rhizopus stolonifer, Muco pusiluss, Botrytis cinerea, Erysiphe polygoni and A. flavus*. These fungi create local discolouration and disruption of surrounding tissues of infected tubers (Snowdon, 1991). They cause reduction in the market value of affected tubers due to their unsightly appearance.

The use of fungicides is one of the most effective means of protecting root and tubers from storage rots. These chemicals, though valued for their effectiveness are costly and may constitute health hazards to farm households and the environment. Thus, there is a need for the development of alternative disease control materials that are both effective in plant disease control and at the same time environmentally friendly. Therefore, this study was undertaken to isolate and identify the causal organisms and determine the potency of some selected local plant extracts in the control of post-harvest storage rot of sweet-potato in Makurdi.

2. Materials and Methods

2.1. Source of Materials

Healthy and diseased sweet potato tubers were sourced from markets in Makurdi. Fresh leaves of Eucalyptus and Moringa were sourced from the University of Agriculture, Makurdi

2.2. Isolation and Identification of fungi Associated with Sweet Potato

Small tissue pieces of diseased sweet potato tubers were surface sterilized for 3 min in 10%sodium hypochlorite solution, rinsed in 5 changes of sterile distilled water and dried on sterilized paper towels before inoculation on Potato Dextrose Agar (PDA) amended with Streptomycin to prevent the growth of bacteria. The inoculated plates were incubated at 27 °C for 5–7 days during which pure cultures of microbial growth were established for identification. Confirmation of associated causal pathogens of rot was carried out, based on the morphological and cultural characteristics on PDA, and microscopic observation following the fungi identification key of Barnett and Hunter (1972).

2.3. Preparation of Crude Extracts of the Leaves

Fresh leaves of *Moringa oleifera* and *Eucalyptus globulus* were washed thoroughly under running water and surfaced sterilised with 10 % sodium hyphochlorite. It was rinsed in several changes of sterile distilled water and air dried at room temperature for 2 weeks. The air-dried leaves were milled into powder with the aid of a blender. From which 25, 50 and 75g of each extract were taken. These were then mixed with 100 ml of distilled water each separately in a bottle to produce 25, 50 and 75 % extract concentrations respectively. The mixture was shaken in a mechanical shaker for 1hour and left for 24 hours. It was sieved through three layers of sterile cheese cloth.

2.4. Pathogenicity Tests

Each of the fungi isolate obtained from the diseased sweet potato tubers was tested for its ability to cause the same disease condition in a healthy sweet potato tuber.

A healthy sweet potato tuber was first washed with a sterile water and then surface sterilized by in 70% ethanol solution. With the aid of a sterile 3 mm diameter cork borer, a cylindrical core was removed from the sweet potato tuber. A reconstituted culture of the isolate was then introduced into the open core and the core was replaced and sealed with sterile vaseline jelly. The control was left blank and kept in a clean dish in the laboratory for daily observation. The tubers were kept at room temperature for 7 days. On establishment of disease condition, inoculums were taken again from the infected sweet potato tubers and cultured. The resulting mixed cultures were sub-cultured and the resulting pure cultures were characterized and identified as the previously isolated organisms, this was taken as evidence that they incite the disease and was thus identified as pathogenic isolates.

2.5. Effects of the Extracts on fungal Growth

Food poisoning technique was used to the effect of the extracts on fungal growth (Okigbo *et al*., 2009). One millilitre of each extract concentration was dispensed into 9 ml of molten PDA to obtain agar-extract mixture in a petri dish. A 6mm diameter mycelial disc obtained from the colony edge of 7 day old culture of each text fungi was inoculated into the centre of each petri dish. The control consisted of an inoculated agar without an extracts. The experiment was replicated three times and incubated at room temperature. Radial growth was measured daily for 7 days. Colony diameter was taken as the means along two directions on two pre drawn perpendicular lines on the reverse sides of plates. Percentage inhibition was calculated according to the method described by Whipp (1987).

$$\text{Percentage inhibition} = \frac{R1 - R2}{R1} \times 100$$

Where

R1= Furthest radial distance of the pathogen in control plates

R2 = Furthest radial distance of the pathogen in extract incorporated plates

Inhibition percentage was determined as a guide in selecting the minimum inhibitory concentration that will be effective in controlling rot – causing fungi.

Extracts were rated for their inhibitory effects using the scale described by Okigbo *et al*., (2009).

≤ 0 % inhibition (Not effective)

≥ 0-20 % inhibition (Slightly effective)
>50 - < 100 % inhibition (Effective)
100 % inhibition (Highly effective)

3. Results

Two fungi species were isolated from rotted sweet potato tubers. The isolated fungi are *Rhizopus stolonifer*Link. and *Aspergillus niger* Van Tiegh. The range of occurrence were 63 % (*Rhizopus stolonifer*) and 37 % (*Aspergillus niger*).Table 1.The two isolated fungi species were pathogenic as they cause rot in healthy sweet-potato tubers 7 days after inoculation. The most virulent was *R. stolonifer* with rot incidence of 57 % (Table1). Aqueous extracts of all test plants reduced mycelial growth of *R. stolonifer* and *Moringa oleifera*At 25 % extract concentration inhibition of *R.stolonifer* ranged from 21.28- 26.31 %; *A. niger* (23.28- 29.31%). At 50 % extract concentration inhibition of *R.stolonifer* ranged from 39.22 – 46.24 %; *A. niger* (53.22- 56.24 %). At 75 extract concentration inhibition of *R.stolonifer* ranged from 61.25- 75.65 %, *A.niger* (65.65- 71.25). Extracts of both plants were effective at 50 and 75 % concentrations, while they were slightly effective at 25 % concentrations.

Fungi	Occurrence (%)	Rot incidence (%)
Rhizopus stolonifer	63	57
Aspergillus niger	*37*	*34*

Table1: Occurrence and Pathogenicity of fungi associated with sweet potato tuber rot

Treatment (Plant extracts)	Concentrations (%)			
	25	50	75	LSD (P≤ 0.05)
M. oleifera	26.31	46.24	75.65	4.81
M. oleifera	26.31	46.24	75.65	4.81
LSD (P≤ 0.05)	4.21	5.43	3.32	

Table 2: Inhibition of mycelial growth of Rhizopus stolonifer by varying concentrations of aqueous extracts of Moringa oleifera and Eucalyptus globules

Treatment (Plant extracts)	Concentrations (%)			
	25	50	75	LSD (P≤ 0.05)
M. oleifera	29.31	56.24	71.25	7.00
M. oleifera	23.28	53.22	61.25	6.76
LSD (P≤ 0.05)	5.02	2.91	4.32	

Table 3: Inhibition of mycelial growth of Aspergilus niger by varying concentrations of aqueous extracts of Moringa oleifera and Eucalyptus globulus.

4. Discussion

Micro-organisms associated with post-harvest deterioration of sweet potato in the study area are *Rhizopus stolonifer* and*Aspergillus niger*. The organisms were also confirmed to be pathogenic on healthy sweet potato tuber. Several workers including Clark and Hoy (1994), Onuegbu (2002), Oyeyipo (2012) had isolated these organisms on rotted potato tubers.

The pathogenicity test revealed that *R.stolonifer* and *A.niger* induced rot in sweet potato tubers with *R.stolonifer* being the most virulent. This is in agreement with the findings of Oyeyipo 2012 who reported *R. stolonifer* and *A. niger* as being highly virulent pathogen on sweet potato.

The isolation of more than one pathogenic organisms from a particular cormel confirms the
possibility of multiple infections whose cumulative effect may cause rapid rottening of root and tuber crops this agrees with the reports of Sangayomi, (2004) on yam.

Sites of infection are primarily wounded, necrotic storage root periderms (wounded sweet potato “skins”). These sites are usually the broken tips of sweet potatoes damaged during harvest, holes in the periderm caused by tools or insects such as weevils, or bruises or wounds created by overly rough handling of sweet potatoes after harvest and during processing (Nelson, 2009).

It was revealed that fugitoxic compound were present in *Moringa oleifera* and *Eucalyptus globolus*
since they were able to inhibit the growth of the test fungi. This is in agreement with the findings of several workers (Adandooon *et.al*., 2006,Suyoq *et.al*., 2012 Tijjani *et.al*., 2013).

However, the efficacy of the extracts differed with the plant material, concentration and with each test fungus.

The fungicidal effects of plant extracts on different pathogens of crops have been widely reported (Onuegbu, 2002, Oyeyipo, 2012). However the efficacy of the extracts differed with the material concentration and with test fungus.

M. oleifera extracts exhibited relatively stronger fungitoxity than E. globolus extracts. This study reveals the potentials of botanicals in the management of potato tuber rot.

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