



Response of Fungal Rot Pathogens of Sweet Potato (*Ipomoea Batatas* (L) Lam. in Storage to Aqueous Extracts of Some Tropical Plant Materials and Benomyl

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ARTICLE INFO

Article No.: 112119209

Type: Research

Accepted: 22/11/2019

Published: 31/01/2020

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Keywords: Postharvest rot; sweet potato; storage diseases; bio-pesticides; *R. stolonifer*; *B. theobromae*

ABSTRACT

Sweet potato is ranked amongst the seven most important tropical tubers in the world. Its production in sub-Saharan Africa is however deeply constrained by poor shelf life due in part to microbial deterioration during storage. This study evaluated aqueous extracts of some plants (*Ocimum gratissimum*, *Curcuma longa*, *Azadirachta indica*, *Ficus exasperata* and *Achornea cordifolia*) and benomyl as possible low-input bio-pesticides for the control of storage rot of the crop. The experiment was laid out in a Completely Randomized Design (CRD) made up of 7 treatments, replicated 4 times. The results revealed that *Rhizopus stolonifer* (70.01%) and *Botryodiplodia theobromae* (67.9%) were the most frequently occurring fungal isolates from the rotted samples; that virulently initiated rot development in the healthy tubers. Exposure of these pathogens to test extracts-impregnated medium showed that aqueous extracts of *Cucuma longa*, *Azadirachta indica* and *Ficus exasperata* significantly ($P \leq 0.05$) reduced the mean spore germination and radial growth of the pathogens by 77.22 and 89.04 %; 76.51 and 85.29 %; and 61.93 and 75.69 % respectively over the control experiment: and these results thus compared well with 91.37 and 90.37 % recorded for benomyl treated samples. Therefore aqueous extracts of the test plants (*Cucuma longa*, *Azadirachta indica* and *Ficus exasperata*) in this order could be used for the control of rot development in sweet potato due to these fungal pathogens. However determination and characterization of the active principles of the plant materials is strongly recommended.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L) Lam) is a member of the plant family Convolvulaceae. The crop is a dicot with a large starchy, sweet tasting root tuber. The leaves of the crop are edible and are used as leafy vegetable. Both the tuber and the leaves are eaten in several cuisines of some parts of tropical Asia and Africa (Nielsen *et al.*, 1997). Sweet potato is suspected to have originated from America, from where it spread throughout the entire tropical world. Tubers of the crop is highly rich in calories (energy), low glycemic index carbohydrates and sugars, fibre, vitamins A (usually in the form of β -carotene) and C and minerals such as calcium. It is also reported to contain large amounts of polyphenolics and anthocyanins related to slowing down effects of aging, dementia and diabetes in humans (Maranzu, 2019).

Sweet potato in 2016 was ranked 16th most valuable crop in Hawaii, 3rd most important tuber crop in Nigeria and classified amongst the 7th most important tuber crop in the world (Nelson, 2009; Enyiukwu *et al.*, 2014). 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, and 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). Losses due to postharvest fungal attacks on stored tubers ranging 20-100 % have been reported because of inadequate farm and village-level storage (Arinze, 2005; Anyim, 2010) and this is especially true in sweet potatoes known to have thin rind where postharvest loss of about 85 % has been documented (Maranzu, 2019).

Toxic chemical residues have been reported to trail treatment of stored agro-products with synthetic pesticides. In most instances consumption of such toxic chemical residues-contaminated products leads to harmful effects such as teratogenicity, allergies and even death on mammals (Enyiukwu *et al.*, 2014). Against this backdrop, many workers have adopted plant-derived compounds as viable and safer alternatives (Markson, 2010). Extracts of *Azadirachta indica* and *Moringa oleifera* significantly frustrated the initiation and development rot caused by *R. stolonifer* in sweet potato in Northern Nigeria. In another evaluation conducted in Southern Nigeria by Amienyo and Ataga (2007), these workers demonstrated that extracts of *Garcinia kola*, *Zingiber officinale* and *Alchornea cordifolia* inhibited rot development due to *B. theobromae* in artificially wounded tubers of the crop. Similarly, Banso (2009) found extracts of *Annona muricata* and *Monodora myristica* to sufficiently impede the growth and reproduction of *R. stolonifer* associated with rot of the crop.

The objectives of this study were therefore to evaluate the response of fungal rot pathogens associated with sweet potato tubers to aqueous extracts of some medicinal plants (*Ocimum gratissimum*, *Curcuma longa*, *Azadirachta indica*, *Ficus exasperata* and *Achornea cordifolia*) commonly found around the University as possible sources of storage bio-fungicides useable by low-input farmers for preservation of tubers of the crop in Umudike, Southeast, Nigeria.

MATERIALS AND METHODS

Source and preparation of plant samples

The experiment was conducted at the Plant Health Laboratory of the National Root Crops Research Institute, Umudike, Abia State, Nigeria (Latitude 5° 29' N, longitude 7° 33' E, and altitude 122 meters above sea level). The plant materials *Ocimum gratissimum* (leaves), *Curcuma longa* (rhizomes), *Azadirachta indica* (leaves), *Zingiber officinale* (rhizomes) and *Xylopia aethiopica* (pods) collected locally around the University community and authenticated by the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike (MOUUAU) were used for the study. The plant materials were washed thoroughly in tap water, rinsed in sterile distilled water and air-dried on the laboratory bench for 20 days. Thereafter, they were milled separately into fine powder using a Thomas Wiley machine (Model: A500, USA) to obtain 300 g of each specimen which were stored separately in air-tight bottles. Each powder was weighed out separately into 30 g and put into different 250 ml conical flasks, to which 100 ml of sterile distilled water was added and the flask stoppered with foiled stoppers. These were allowed to stand for 2 h. Thereafter they were strained separately through 4-folds of sterile cheese cloth into different 200 ml beakers to obtain the respective aqueous filtrates of 30 % strength of the extracts (Amadioha, 2003).

Preparation of culture medium (PDA)

About thirty nine point five (39.5) grams of potato dextrose agar (PDA) (Oxoid™ ThermoScientific Product, England, UK) was dissolved in 1000 ml of sterile distilled water in 1000 ml conical flask to which 4 drops of lactic acid was added, and then stirred vigorously before the flask was stoppered with a foiled cotton wool and autoclaved at 15 pounds per square inch (Psi) (152 cmHg, 120 °C) for 15 minutes.

Isolation and identification of the fungal rot organisms of sweet potato

The fungal rot organisms associated with sweet potato were isolated based on standard protocols as adopted by Amadioha (2004) and Amadioha and Markson (2007a, b). The colour and colony characteristics of the isolates were observed under the microscope and recorded. Slides of the organisms were prepared, fixed,

mounted and examined under a low-high power of the microscope to ascertain the identity of the pathogens with reference to Barnett and Hunter (1998). Records of the type and number of times each type of organism was observed on the incubated sweet potato tissues was also taken and used to determine the percentage occurrence of the mycoflora using the formula adopted by Maranzu (2019) as:

$$\text{Percentage occurrence} = \frac{\text{No. of observations in which a species appeared in a specimen}}{\text{Total no. of observations in the specimens}} \times \frac{100}{1}$$

Pathogenicity test of the organisms

This was conducted based on the procedure adopted by Amadioha (2004) and Okigbo and Nmeko (2005). All the isolates were tested individually for their ability to initiate rot disease symptom on healthy (uninfected) sweet potato tubers. The healthy sweet potato tubers were surface sterilized with 70% ethanol and bored on the surface with a 3 mm cork borer. Disc (3 mm) of the pure culture isolate was separately inserted into the hole and plugged with the sweet potato tuber tissue previously removed from the tuber after 1 mm had been cut to compensate for the thickness of the disc. The inoculated points were sealed with the sterile gel to prevent contamination. All the inoculated sweet potato tubers containing different isolates were incubated at room temperature (27–30 °C) over a period of 8 days and examined daily for rot symptoms such as wetting, softening, discoloration (darkening) and emission of offensive odour. Those that showed rot symptoms at the end of the incubation period were each cut longitudinally through the uninfected portion to expose the rotted regions. The rotted regions were re-plated on PDA and re-isolated (Amadioha, 2004). The isolate which compared with the original culture introduced. The isolates that caused rot and bore true morphological and cultural resemblance of the original culture were regarded as pathogens whereas those isolates that do not cause any rot were regarded as saprophytes and discarded.

In vitro Experiment

Preparation of spore suspension

The spores of the pathogenic organisms (*R. stolonifer* and *B. theobromae*) were collected from 10-day old culture-agar stock in Petri dishes by lifting 60 cm² pieces into a beaker containing 200 ml of sterile distilled water. This was sieved through 4-folds of sterile cheese cloth to remove agar and mycelia fragments and the filtrate centrifuged for 10 minutes (Amadioha, 2004). The spores suspension was then standardized using a haemocytometer counting slide to 10⁵ spores/ml of sterile distilled water.

Effect of plant extracts on spore germination of the test pathogens

A disc (3mm) of each of the pathogenic fungi of sweet potato was placed separately in 3 ml of the 30 % crude aqueous extracts from the different plant materials or 3 g/L of benomyl as standard check placed in different test tubes and centrifuged for 10 minutes, and then filtered through 4-folds of cheese cloth. A drop (0.05 ml) of the different preparations was placed separately on 3 sterile slides and incubated for spore germination at 27 °C for 24 h in a humid chamber. The controls consisted of sterile water or benomyl instead of the crude plant suspensions. At the lapse of the evaluation time, further spore germination was stopped by adding one drop of lactophenol in cotton blue to each preparation on the slides. The effect of the tissue extracts from the plant materials on the germination of the spores of the test fungus was determined by randomly examining 100 spores of the pathogen under a high-power (x400) microscope field. Records of the number of germinated spores for each treatment/replicate were taken and then used to determine the percentage inhibition of spore germination of the pathogen compared to the controls using the formula by Amadioha (2003) as:

$$\text{Inhibition of spore germination} = \frac{gc - gt}{gc} \times \frac{100}{1}$$

where gc = average number of germinated spores of the test fungus with control
gt = average number of germinated spores of the test fungus with treatment.

Effects of the extracts on radial growth of the test pathogens

One (1) ml of the 30 % concentration of the crude aqueous extracts of the plant materials were smeared separately on the surface of solidified PDA contained in Petri dishes by gentle swirling motion (Amadioha, 2003; 2004). A disc (3mm) of the 10-day old cultures of the pathogenic fungi was transferred to the centre of the solidified PDA-extract medium in the Petri dishes which had been marked underneath with two perpendicular lines intersecting at the center. The dishes were covered and incubated at 27 °C for 7 days. The controls

consisted of PDA medium impregnated with sterile distilled water or benomyl in the dishes. The radial growth of the pathogen was measured along the perpendicular lines with a meter rule 7 days after incubation. This experiment was laid out in a Completely Randomized Design (CRD) consisting of 7 treatments and 4 replications. The antifungal effects of the extract was determined as a percentage of mycelial growth inhibition and calculated by the formula as adopted by Amadioha (2003):

$$\% \text{ Growth inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

where dc = average diameter of fungal colony in the control experiment
dt = average diameter of fungal colony with treatment.

In vivo experiment

This was conducted based on the protocols adopted by Amadioha (2004) and Agu *et al.* (2016). A total of 24 tubers each were impregnated with 5 mm diameter disc of *R. stolonifer* while another set of 24 of tubers were inoculated with *B. theobromae* and incubated on laboratory bench for 21 days. Rot initiation and development in the tubers were assessed as percentage area of the treated tubers macerated by the rot pathogens using the formula by Agu *et al.* (2016) as:

$$\text{Rot severity (\%)} = \frac{W-w}{W} \times \frac{100}{1}$$

Where W = final weight of the inoculated tubers
w = weight of rotted regions of the treated tubers

DATA ANALYSIS

The whole experiments was repeated twice. Data collected from the study were subjected to analysis of variance (ANOVA) using the general linear model procedure in Genstat Release (Windows/PC Vista, version 12.10) at significant level of 5 %. Means were separated and compared using Fishers LSD at probability of 0.05.

RESULTS

A total of six fungi were isolated from the rotted tuber of sweet potato in storage in Umudike. The six fungi isolated were *Rhizopus stolonifer*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Penicillium expansum*, *Aspergillus niger* and *Aspergillus flavus* (Table 1). *Rhizopus stolonifer* was the most frequently isolated organisms (70.01%), followed by *Botryodiplodia theobromae* (67.9%). Tests of pathogenicity revealed that these two organisms were virulently pathogenic causing soft and dry rot respectively on the test tubers, whereas the other fungal agents only induced slight rot and therefore were discarded.

Table 1: Frequency of occurrence of fungi associated with storage rot of Sweet potato

Fungal isolate	Frequency (%)	Type of rot
<i>Aspergillus niger</i>	40.04	Dry rot
<i>Aspergillus flavus</i>	25.92	Dry rot
<i>Botryodiplodia theobromae</i> ,	67.90	Dry rot
<i>Rhizopus stolonifer</i>	70.01	Soft rot
<i>Fusarium oxysporum</i>	20.01	Dry rot
<i>Penicillium expansum</i>	31.61	Dry rot

Table 2 indicated the effect of four plant materials and benomyl (a synthetic fungicide) on the inhibition of spore germination of the fungal rot pathogens (*R. stolonifer* and *B. theobromae*) of the test sweet potato tubers. Amongst the botanical treatments, *A. indica* recording 83.08 % demonstrated the highest inhibition of germination of spores of *R. stolonifer* followed by *C. longa* with 80.02 %; whereas *C. longa* (74.41 %) and *A. indica* (69.93 %) showed ultimately and pen ultimately the highest inhibition of spores germination of *B. theobromae*. Extracts of *C. longa* recorded the best mean percentage inhibition of spore germination of the pathogens (77.22%). This was followed by extract of *A. indica* (76.51%), *F. exasperata* (61.93%); and the least effective in inhibition effects was the extract of *A. cordifolia* which recorded 40.89% reduction in mean spore germination of the pathogens. Benomyl recording mean inhibition of (91.37 %) was significantly ($P \leq 0.05$) superior to the inhibition effects from the plant extracts on the pathogens. However, effects obtained from *C. longa* and *A. indica* were favourably ($P \leq 0.05$) comparable to it (Table 2).

Table 2: Effect of the plant extracts on the spore germination of the Sweet potato tuber rot pathogens

Treatments	<i>B. theobromae</i>	<i>R. stolonifer</i>	Mean
<i>Alchornea cordifolia</i>	38.03	43.74	40.89
<i>Curcuma longa</i>	74.41	80.02	77.22
<i>Azadirachta indica</i>	69.93	83.08	76.51
<i>Ficus exasperate</i>	60.06	63.79	61.93
Benomyl	91.03	91.71	91.37
Sterile water	0.00	0.00	0.00
LSD (0.05)	0.42	0.45	0.34

Table 3 also shows that the plant extracts significantly ($P \leq 0.05$) retarded the radial growth of *B. theobromae* and *R. stolonifer* to varying degrees. Extract of *C. longa* exerted significant ($P \leq 0.05$) retardation of the growth of the pathogen recording a mean percentage radial growth reduction of 89.04 %. This was closely followed by the extract of *A. indica* (85.29%) and then *F. exasperata* (75.67%) while the extract of *A. cordifolia* which

recorded 49.20% inhibition of the radial growth was the least. However, highest mean radial growth inhibition was obtained from benomyl where a mean radial growth inhibition value of 90.37% was obtained. Generally, the results indicated that the test organisms were most sensitive to benomyl followed by *C. longa*, then *A. indica*, *F. exasperata* but least to *A. cordifolia*.

Table 3: Effect of the plant extract on the radial growth of pathogens causing Sweet potato tuber rot

Treatments	<i>B. theobromae</i>	<i>R. Stolonifer</i>	Mean
<i>Alchornea cordifolia</i>	40.38	58.01	49.19
<i>Curcuma longa</i>	85.03	93.04	89.04
<i>Azadirachta indica</i>	80.53	90.61	85.29
<i>Ficus exasperata</i>	74.54	76.79	75.67
Benomyl	90.02	90.72	90.37
Sterile water	0.00	0.00	0.00
LSD (0.05)	0.47	0.51	0.39

Results presented in Fig. 1 indicated that all the plant extracts sufficiently ($P \leq 0.05$) reduced the development and severity of rot due to the test pathogens in the tubers. The best reduction of rot was recorded with *C. longa* (12.08 %), followed by *A. indica* (15.22 %) while *A.*

cordifolia with 27.33 % was the least performing bio-fungicide. As in the *in vitro* trials benomyl (11.09) outperformed all the botanical treatments but results obtained from *C. longa*, *A. indica* and *F. exasperata* were favourably comparable with it (Fig. 1).

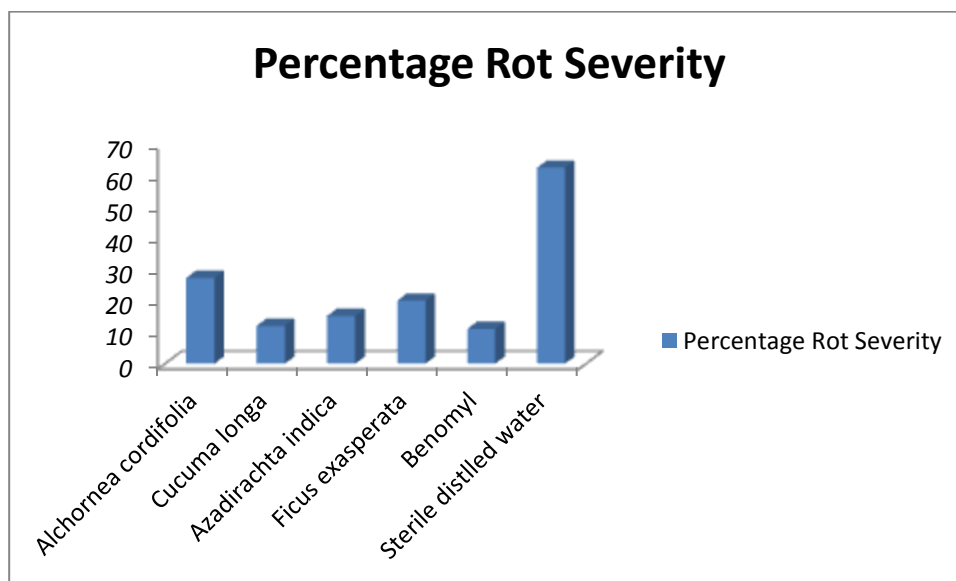


Figure 1: Percentage severity of sweet potato tubers treated with different plant extracts

DISCUSSION

The results from this study indicated that *R. stolonifer* and *B. theobromae* doubled as the most frequently isolated and most virulent myco-pathogens associated with the postharvest microbial spoilage of sweet potato. This observation is in tandem with the reports of Markson (2010) and Maranzu (2019) who also observed that *B. theobromae* and *R. stolonifer* were the most

commonly occurring and most virulent fungal rot pathogens involved in the postharvest deterioration of white yam (*Dioscorea rotundata*) and sweet potato (*Ipomea batatas* L.) in storage in Southeast and Northwest Nigeria respectively. This finding is also in conformity with the findings of Tijjani *et al.* (2013) who also found *R. stolonifer* as the most frequently occurring and virulent pathogen on mechanically wounded sweet potato tubers in Northern Nigeria. However, findings in

this study are not in agreement with the submissions of Eze and Amah (2011) who reported that *R. stolonifer* was the least virulent pathogen in an evaluation of postharvest rots of cocoyam corms in Southeast Nigeria. Differences in the inherent genetic potentials of the fungal biotypes (Nwaneri, 2017) or differences in structure and composition of the starch granules (Ballogou *et al.*, 2013) or other biochemical dissimilarities in the different varieties of tubers used in the studies may be the reason for the divergent observations.

The test fungi (*R. stolonifer* and *B. theobromae*) of sweet potato in this study were strongly sensitive to the plant extracts incorporated in the study medium resulting in the inhibition of their growth and reproduction (Tables 2 and 3).. Many workers have reported that plant-derived pesticides act by impeding the germination of spores and retarding mycelia growth or biomass accumulation of pathogenic fungi (Amadioha, 2003; 2004). Ethanol extracts of *Aframomum melegueta* and *Zingiber officinale* as well as aqueous extracts of *Garcinia kola* massively inhibited spore germination and strongly retarded radial growth of *B. theobromae* in culture. Studies conducted by Enyiukwu and Awurum (2011; 2012) showed that the spore germination and mycelia elongation of *Colletotrichum destructivum* responsible for anthracnose disease of southern pea were significantly inhibited in culture medium impregnated with aqueous extracts of *Piper guineense* seeds. Therefore findings in this study where the spore germination and radial growth of *R. stolonifer* and *B. theobromae* were frustrated by the different plant extracts agree with the observation of the workers as stated above.

Several extracts of plant origin has been reported to effectively inhibit mycelial elongation and biomass accumulation of pathogenic rot fungi as such foster protection of treated tubers for many months post-harvesting (Enyiukwu *et al.*, 2014). Seed tissue extracts obtained from *Piper nigrum*, *A. melegueta*, *Garcinia kola*, and leaves of *Dennetia tripetala*, *Cassia alata* and *Ocimum basilicum* potentially protected cassava and cocoyam tubers against deteriorations due to attacks by *B. acerina*, *B. theobromae*, *R. oryzae*, and *Sclerotium rolfsii* *in vivo* (Amadioha and Markson, 2007a, b; Nwachukwu and Osuji, 2008; Ugwuoke *et al.*, 2008; Anukworji *et al.*, 2012). In like manner yam and sweet potato tubers have been reported to be protected *in vivo* from postharvest spoilage due to *B. theobromae* and *R. stolonifer* by extracts of *Annona muricata*, *Monodora myristica*, *M. oleifera*, and *A. cordifolia* (Banso, 2009; Amienyo and Ataga, 2007; and Tijani *et al.*, 2013). Findings in this study in which the test plant extracts demonstrated significant inhibition of the attacks of the test pathogens on the tubers leading to their protection are in agreement with the reports of the earlier workers as mentioned above.

Therefore based on the results of this study aqueous extracts obtained from *C. longa*, *A. indica*, and *F. exasperata* in this order) could be used to protect sweet potato tubers in storage and enhance their shelf-life. In addition, we recommend that these plant materials especially *C. longa* be subjected to phytochemical profiling to isolate and characterized their active ingredients so as to hasten development of bio-pesticides from them.

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Cite this Article: Nwaneri, JA; Enyiukwu, DN; Cyprain, UEC; Nicholas, JC (2020). Response of Fungal Rot Pathogens of Sweet Potato (*Ipomoea Batatas* (L) Lam. in Storage to Aqueous Extracts of Some Tropical Plant Materials and Benomyl. *Greener Trends in Plant Pathology and Entomology* 3(1): 18-24.