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Assessment of Antifungal Properties of *Dennettia Tripetala* for Managing Fungal Rot Pathogens in Stored White Yam (*Dioscorea rotundata* L.)

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ABSTRACT

Dioscorea rotundata hold a vital position as a dietary staple across tropical Africa, with Southeast Nigeria specially relying on them as a substantial provider of calories, minerals, and vitamins for its population. Yet, the effective preservation of these tubers faces hurdles due to fungal pathogens causing rot in inadequate traditional storage methods. In this study, we assessed the impact of 35% (w/v) aqueous extracts derived from *D. Tripetala* components (leaf, seed, root bark) as well as benomyl on spore germination, in vitro fungal rot pathogen radial growth, and in vivo tuber rot development caused by the fungus. The experiment followed a Completely Randomized Design (CRD) with five treatments replicated six times. From the rotted tubers, *Rhizopus stolonifer*, *Botryodiplodia theobromae*, *Fusarium oxysporium*, and *Aspergillus flavus*, *Aspergillus niger* and *Mucor* species were isolated. Among these, the first two exhibited high virulence in inducing tuber rot during pathogenicity tests. In vitro findings demonstrated that the test organism exhibited the highest sensitivity to the leaf extract, with inhibitions of 90.27% for spore germination and 75.57% for mycelia growth. On the other hand, the root bark extract, recording 68.22% and 64.20% for the respective parameters, displayed the lowest fungitoxic effects. Similarly, in vivo, the leaf extracts exhibited a notably low mean percentage of rot severity (22.3%) on inoculated tubers, which was statistically comparable to the 20.1% recorded for benomyl-treated tubers. In contrast, tubers treated with root extract displayed the highest severity at 26.7% on an 8-point scale. These results suggest that *D. Tripetala* extract holds potential for safeguarding yam tubers against fungal rot pathogens during storage.

Keywords: *Dioscorea rotundata*, *D. Tripetala*, *Botryodiplodia theobromae*, *Rhizopus stolonifer*

INTRODUCTION

Dioscorea rotundata are important staple crops in southeast Nigeria. They are rich sources of metabolizable dietary energy, minerals and vitamins. They can be prepared boiled, roasted or fried and consumed with legumes, fresh oils or sauces. In some cases, they are prepared into pounded pastes used in eating a variety of tropical African cuisines. However, besides a few biotic challenges such as bacteria, nematodes and insect pests which target the crop for attacks in the field, the tubers suffer tremendous constraints by fungal pathogens in storage. These fungal pathogens make their shelf-life and quality to be reduced. Many fungal pathogens in their bids to compete with man for nutrients and other growth factors have been implicated with rots of yams in storage in Umudike. These mucus-pathogens include *Botryodiplodia theobromae*, *Fusarium solani*, *F. oxysporium*, *Aspergillus niger*, *A. fumigatus*, *Rhizopus stolonifer* and *Penicillium* spp. amongst others (Tijani *et al.*, 2013; Enyiukwu *et al.*, 2014). The organisms are reported to be assisted by myriads of enzymes to solubilize middle lamella and macerate the tissues of the tubers leading to loss of vital nutrients and electrolytes, watery ooze in some cases and

emission of foul odour (Nwaneri, 2017; Amadioha and Enyiukwu, 2019).

Control of postharvest losses due to fungi has been carried out using a variety of strategies or compounds cutting across physical and synthetic chemicals (Okigbo and Nmeko, 2015). Whereas physical control may be cumbersome, chemical controls using synthetic fungicides have been reported to lead to mammalian toxicities as a result of chemical residues such as polychlorinated biphenyls in treated tubers (Amadioha, 2004; Amadioha *et al.*, 2019; Nwaneri *et al.*, 2020). Recently scientists are screening tropical higher plants as sources of potential fungicides (Enyiukwu *et al.*, 2014). Antimicrobial and fungitoxic activities of *D. Tripetala* have been variously reported (Okwu and Morah, 2004; Duru *et al.*, 2015; Pallant *et al.*, 2012; Uwumongie *et al.*, 2011; 2018). However, the use of *Dennettia. Tripetala* for control of fungal rot of white yam (*Dioscorea rotundata* L.) is scarcely available.

Therefore the objectives of this paper are to isolate and identify the fungal rot pathogens associated with white yam, and to assess the antifungal potential of aqueous extract (leaf, seed and root bark) of *D. Tripetala*

against the most virulent rot pathogen(s) of white yam in storage.

MATERIALS AND METHODS

Source and preparation of plant samples

The experiment was conducted at the Plant Pathology Laboratory of the National Root Crops Research Institute, Umudike, Abia State, Nigeria. *Dennettia tripetala* (leaves, seed and root barks) used for this study were obtained from a rural community at Emii in Owerri, Imo State, and validated at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. The plant materials were washed thoroughly in tap water, rinsed in sterile distilled water and the seed and root barks cut into bits with a sharp knife before air-drying them on the Laboratory bench for 20 days. Thereafter, they were enveloped and oven-dried (40°C) for 20 minutes, and then milled separately into fine powder using a Thomas Wiley machine (Model: A500, USA) to obtain 180 g of each specimen which were stored separately in air-tight bottles until required. Each powder was weighed out separately into 60g and put into different 250 ml conical flasks, to which 200 ml of sterile distilled water was added and the flask was covered with foiled stoppers. These were allowed to stand for 8 h. Thereafter they were strained separately through 4-folds of sterile cheesecloth into different

$$\text{Percentage occurrence} = \frac{A}{B} \times 100$$

Pathogenicity test of the organisms

This was conducted based on the procedure adopted by Amadioha (2004) and Okigbo and Nmeka (2005). All the isolates were tested individually for their ability to initiate rot disease symptoms on relatively healthy (uninfected) yam tubers. The healthy yam tubers were surface sterilized with 10% sodium hypochlorite 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 yam 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 Vaseline to prevent contamination. All the inoculated yam tubers containing different isolates were incubated at room temperature (27–30 °C) for 10 days and examined daily for rot symptoms such as wetting, softening, discolouration (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

200 ml beakers to obtain the respective 30% aqueous concentrations of the extracts (Amadioha, 2003, Amadioha *et al.*, 2019).

Preparation of culture medium (PDA)

About 40 grams of potato dextrose agar (PDA) (Oxoid™ Thermo Scientific Product, England, UK) was dissolved in 1000 ml of sterile distilled water in a 1000 ml conical flask to which 4 drops of lactic acid was added, and then stirred vigorously before the flask was covered with foiled cotton wool, and autoclaved at 15 pounds per square inch (Psi) (152 mmHg, 120 °C) pressure for 15 minutes.

Isolation and identification of fungal rot organisms of yam

The fungal rot organisms associated with yam were isolated based on standard protocols as adopted by Amadioha (2004) and Amadioha *et al.*, 2019). 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 microscope to ascertain the identity of the pathogens regarding Barnett and Hunter (1998). Records of the type and number of times each kind of organism was observed on the yam tissues were also taken and used to determine the percentage incidence of the mycotic organisms using the formula adopted by Maranzu (2019):

Where: *A* = Number of observations in which a species appeared in a specimen

B = Total number of observations in the specimens

re-plated on PDA and re-isolated (Amadioha, 2004). The isolates that caused rot and bore true morphological and cultural resemblance to the original fungal cultures were regarded as pathogens whereas those isolates that did not cause any significant 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 them with 200 ml of sterile distilled water and pouring the spore suspension in another beaker through 4-folds of sterile cheesecloth to remove agar and mycelia fragments and then centrifuged the filtrate for 10 minutes (Amadioha, 2004). The spores suspension was then standardized using a haemocytometer counting slide to 10⁵ spores/ml of sterile distilled water.

Evaluation of extracts of *Dennettia tripetala* on spore germination of the test pathogen

A disc (3mm) of each of the most virulent pathogenic fungi (*R. stolonifer*) of yam was placed separately in 3ml of the 30% crude aqueous extracts from the different parts of *D. Tripetala* or 3g/L of benomyl (a placebo) placed in different test tubes and centrifuged for 10 minutes, and then filtered through 4-folds of cheesecloth. A drop (0.05 ml) of the different preparations was placed separately on 5 sterile slides and incubated for spore germination at 27 °C for 24 h in a humid chamber. The control consisted of sterile water or benomyl instead of crude plant suspensions. The whole experiment was repeated two times. At the end 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 *D. Tripetala* on the germination of the spores of the 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 or replicate were taken and then used to determine the percentage inhibition of spore germination of the pathogen compared to the control using the formula adopted by Enyiukwu *et al.* (2021):

$$\text{Inhibition of spore germination} = \frac{(m-n)}{m} \times 100$$

where m = average number of germinated spores of the test fungus with control

n = average number of germinated spores of the test fungus with treatment.

Evaluation of effects of the extracts on radial growth of the test pathogen

One millilitre of the 30% concentration of the crude aqueous extracts of the plant materials was 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 a 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):

$$\% \text{ Growth inhibition} = \frac{(b-c)}{b} \times 100$$

Where: b = average diameter of the fungal colony in the control experiment

c = 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 30 tubers weighing about 300g each was inoculated with a 5 mm diameter disc of *R. stolonifer* (the most fungitoxic pathogen isolated from the rotted yam specimen based on pathogenicity tests) and incubated on the Laboratory bench for 21 days. At the end of the study period, the tubers were cut longitudinally as in the pathogenicity tests. The fungitoxicity of the extracts of *D. Tripetala* was taken as a reduction of the rotted area of the tubers per treatment per replicate compared to the control based on a modified 8-point descriptive key adopted by Enyiukwu *et al.* (2021) as:

1 = No rot

2 = slightly rotted

4 = moderately rotted

6 = heavily rotted

8 = tuber completely rotted and

Rot initiation and development in the tubers were calculated as the weight of the percentage area of the treated tubers macerated by the rot pathogens against the final weight of the treated tubers using the formula adopted by Agu *et al.* (2016) and Enyiukwu *et al.* (2021) as:

$$\text{Rot severity (\%)} = \frac{(x-y)}{x} \times 100$$

Where: x = final weight of the inoculated tubers

y = weight of rotted regions of the treated tubers

DATA ANALYSIS

The whole experiment were repeated two times. Data collected from the study were analyzed by analysis of variance (ANOVA) using the general linear model procedure in Genstat Release (Windows/PC Vista, version 12.10) at a significant level of 5%. Means were separated and compared using Fisher's LSD at the 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 oxysporium*, *Aspergillus niger* and *Aspergillus flavus* and *Mucor* sp (Figure 1). *Rhizopus stolonifer* was the most frequently isolated organism (39.05%), followed by *Botryodiplodia theobromae* (34.90%), *Fusarium oxysporium*

(20.01%) and *Mucor* species with incidence of 0.91% was the least. Tests of pathogenicity revealed that *R. stolonifer* and *B. theobromae* were pathogenic causing rot of the test yam tubers, but the former was

significantly more virulent than the later organism, whereas the other fungal agents only induced slight rots and therefore were discarded.

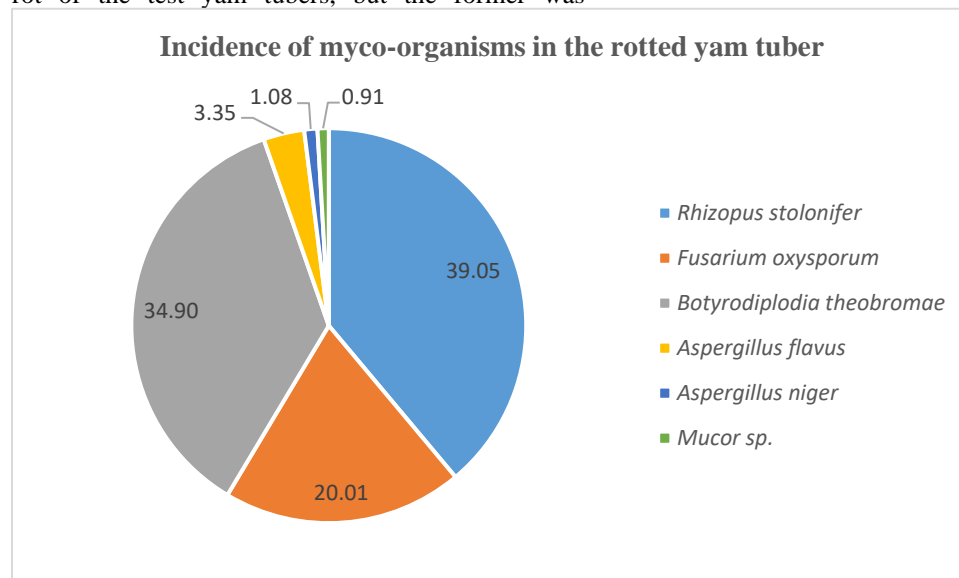


Figure 1: Frequency of occurrence of myco-organisms in the rotted yam

Results presented in Figure 2 indicated the effect of *D. Tripetala* and benomyl on the inhibition of spore germination of *R. stolonifer* on the test yam tubers. It shows that the plant-derived fungicides significantly ($P < 0.05$) retarded the radial growth of *R. stolonifer* by varying degrees. Aqueous leaf extract recording 90.20% demonstrated the highest inhibition of germination of spores of *R. stolonifer* followed by stem bark extract which had 76.73%; whereas the root bark with 68.20% was the least fungitoxic. Similarly, aqueous leaf extracts of *D. Tripetala* demonstrated the highest mean percentage inhibition of radial growth of

the organism with 75.57%. This was followed by seed extract (69.99%) and root bark having mean percentage retardation of mycelial growth of 64.20% was the least effective. In both cases, benomyl which gave 95.12% and 97.09% inhibition of spore germination and radial growth of the fungus respectively was statistically superior ($P < 0.05$) to the test botanical fungicides, notwithstanding they compare well the fungitoxicity of benomyl. On the other hand, all the botanical fungicides statistically ($P < 0.05$) out-matched the control experiment which recorded no inhibition effects (Figure 2).



Figure 2: Effect of extracts *Dennettia tripetala* on the spore germination *Rhizopus stolonifer*

Results also presented in Fig. 3 indicated that all extracts of *D. tripetala* sufficiently ($P < 0.05$) reduced the development and severity of rot due to the *R. stolonifer* in the tubers. The highest reduction of rot severity was obtained from leaf extracts of the plant material (22.30%), followed by seed extracts (24.70%) while root bark which gave a rot profile of 28.8% was the least toxic bio-fungicide. The trend of

fungitoxicity of extracts of *D. tripetala* in the *in vivo* evaluation was similar to what was obtained in the *in vitro* trials; in that benomyl which had 20.01% rot severity was significantly greater in rot reduction than all the fungicides derived from *D. tripetala* except for leaf extract of the plant with which it compared ($P < 0.05$) favourably well in reducing rot severity (Figure 3).

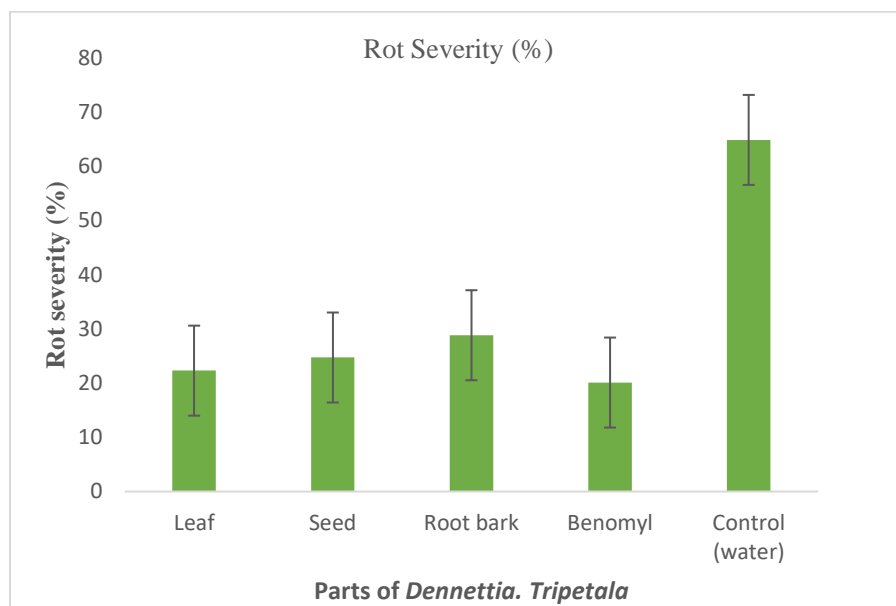


Figure 3: Percentage severity of sweet potato tubers treated with different plant extracts

DISCUSSION

The results from this study indicated that *R. stolonifer* was the most frequently isolated and virulent myco-pathogen associated with microbial deterioration of white yam tubers in storage. This finding is in agreement with the reports of Markson (2010) and Maranzu (2020) who also observed that *R. stolonifer* and *B. theobromae* were the most commonly occurring and most virulent fungal rot pathogens involved in the postharvest spoilage of white yam (*Dioscorea rotundata*) and sweet potato (*Ipomea batatas* L.) in storage. This finding is also in conformity with the reports of Tijjani *et al.* (2013) who also found *R. stolonifer* as the most frequently occurring and virulent pathogen associated with sweet potato tubers in Northern Nigeria. However, the findings in this study are not consistent with the submissions of Eze and Amah (2011) who reported that *R. stolonifer* was the least virulent pathogen in an evaluation involving the identification of causal agents of postharvest rots of cocoyam corms in Southeast Nigeria. Rot pathogens are reported to secrete a wide array of potent pectolytic and hydrolytic enzymes which they inject well in advance of their hyphae or haustoria to dissolve cementing middle lamella and cell walls of host tissues to initiate

rots (Okeke, C.O., 2004, Amadioha and Enyiukwu, 2019). Differences in inoculum potentials of pathogenic moulds (Nelson, 2009; Markson *et al.*, 2014), structure and composition of starch granules (Ballogou *et al.*, 2013) or other biochemical and nutrient composition of the tuber species (Enyiukwu *et al.*, 2021) used in the studies may account for the incongruent observations.

R. stolonifer in this study was highly sensitive to extracts of *Dennettia tripetala* incorporated in the PDA medium resulting in the inhibition of its growth and reproduction *in vitro* (Figure 2). Many workers have reported that bio-fungicides inhibit the germination of spores and mycelia growth or biomass accumulation of pathogenic fungi (Amadioha, 2003; 2004). The presence of phytochemicals such as alkaloids and certain fatty acids has been adduced as factors responsible for their antifungal activity (Okwu and Morah, 2004; Edeoga *et al.*, 2005; Uwumongiet *et al.*, 2007, 2018, Sinan *et al.*, 2020). The presence of high amounts of dodecanoic and 9-Octadecanoic, and 9, 12-Octadecadienoic acids were detected in the methanol extract of leaf specimen of *D. tripetala* (Okwu and Morah, 2004). Conversely, Uwumorongiet *et al.* (2018) reported hexadecanoic

acid as the most occurring principle in methanol stems bark extract of the plant. Differences in the type and amount of alkaloids and certain fatty acid contents in the leaf, stem bark and root bark of *D. tripetala* or their solubility in water may be the reason for the varying degrees of inhibition of the fungus observed in the study. Awurum and Nwaneri (2011) and Enyiukwu *et al.* (2021) showed that spore germination and mycelia elongation of *Colletotrichum destructivum* causal agent of anthracnose disease of tobacco, lucerne and the southern pea was significantly inhibited in culture medium poisoned with aqueous extracts of *Carica papaya* root, *Piper guineense* seeds and *D. tripetala* leaves. Therefore findings in this study where the spore germination and radial growth of *R. stolonifer* were inhibited by the aqueous extract of *D. tripetala* agree with the observation of the workers as stated above.

Aqueous extracts of leaf, seed and root bark of *D. tripetala* effectively protected treated tubers from deterioration due to attacks by *R. stolonifer* in this study. This finding conforms with the view also held by many workers where extracts obtained from leaves of *Dennetia tripetala*, *Cassia alata*, *Ocimum basilicum*, *Annona muricata*, *Alchornea cordifolia* etc. potentially protected cassava, sweet potato and cocoyam tubers against deteriorations due to attacks by *R. oryzae*, and *R. stolonifer* amongst others fungi *in vivo* (Amienyo and Ataga, 2007; Amadioha and Markson, 2007a, b; Nwachukwu and Osuji, 2008; Ugwuoke *et al.*, 2008; Banso, 2009; Anukworji *et al.*, 2012; Tijani *et al.*, 2013; Enyiukwu *et al.*, 2014).

Therefore based on the results of this study aqueous extracts obtained from the leaf, seed and root bark of *D. tripetala* in this order could be used to protect yam tubers in storage and enhance their shelf-life. In addition, we recommend that the extracts of *D. tripetala* be subjected to further characterization for active ingredients to hasten the development of bio-pesticides from it.

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