In-Vitro Study of Biofungicidal Effects of *Cassia alata* Leaf Extracts on Some Pathogenic Organisms of White Yam (*Dioscorea rotundata*) Tuber in Storage

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Abstract

A preliminary experiment was conducted to evaluate the efficacy of Cassia alata leaf extract as a biofungicide and also to determine the minimum inhibition concentration (MIC) of the extract on organisms that cause soft rot of yam tuber in storage. The study was done at the plant pathology laboratory of University of Nigeria, Nsukka. The fungal organisms were isolated from the decaying yam tuber obtained from the yam storage barn of the Department of Crop Science, University of Nigeria, Nsukka. The Cassia alata leaf was obtained from the Botanical garden of the Department of Botany, University of Nigeria, Nsukka and ethanolic extraction was got using cold extraction method. The extract was further diluted to four levels of concentration thus: 250, 175, 87.5 and 48.75 mg/ml. The sensitivity pattern of the isolated fungal organisms was carried out using Agar cup diffusion technique. Organisms found sensitive to the extract were Scopulariopsis brevicaulis and Rhodorula mucilaginesa but Penicillium restrictum and Penicillium digitalum were not. Result of the sensitivity test showed that the extract was able to inhibit the growth of R. mucilaginesa at 56.23 mg/ml while the MIC of the extract for S. brevicaulis was 50.10 mg/ml. A major finding in this study is that the ethanol leaf extract of C. alata is selective in its bioactivity on the rot-causing organisms of yam.

Keywords: *In-Vitro,* Biofungicidal effects, *Cassia alata* extracts, Pathogenic organisms, *Dioscorea rotundata*

Introduction

Yam (*Dioscorea spp*) is an important food crop grown in humid and sub-humid tropics. World yam production amounts to 30 million metric tonnes annually and 90% are grown in the yam belt of West Africa (FAO, 2002). It has been reported (IITA, 1995) that yam rot is a major factor in spoilage of tuber during storage. About 30 different fungi have been reported to be associated with yam storage of rots (Ikotun, 1989). Microbial infection of yam tubers is rapid and severe especially in areas where high temperature and high humidity favours rapid microbial growth. Rotting is a major factor limiting the post harvest life of yams (Osagie, 1992). Losses in yam tuber in storage due to rot are considered heavy in Nigeria. Microbial rotting of yam tubers accounted for a substantial proportion of the annual losses in yam production in Nigeria. Okigbo and Ikediugwu (1999) associated different forms of tuber rotting in the storage barn to microbial attacks that probably took place in the field and increase in storage. Fungi which have been associated with storage losses are *Botryodiplodia theobromae, Fusarium moniliforme, Penicillim sclerotigenum, Rosellina bunnode, Aspergillus niger, Hendersonula forulvidae, Macrophomina phasseoli* and *Rhizopus nodosus*. The use of chemicals has been found to be effective in reducing fungal rots of yams (Ogundana and Denis, 1981).

There is a growing awareness on the dangers associated with the use of some chemical for storage and preservation of farm produce. Some of these chemicals have high mammalian toxicity and are considered not safe and even on the environment.

Consequently, emphasis is now being placed on the use of non-toxic and environmentally friendly chemicals particularly those of plant origins. Plant extracts have found greater acceptance in modern storage methods of agricultural produce. There are several local plant species whose extracts have proved effective in protecting yam produce before and after harvest. Other plants extracts include ashes from oil palm (*Elaesis guineensis*) inflorescence, kola (*Cola natida*) and mango (*Mangifera indica*) (Ogbeni, 1995). The medicinal usefulness of *Cassia alata* plant has been the object of many chemical and pharmacological studies. It is an erect ornamental shrub, annual herb with leathery compounded leaves and yellow flowers which is widely available in the tropics, in the grasslands and around towns and villages throughout West Africa. Earlier reports in the scientific literature indicated that some leaves and roots of *Cassia alata* can be used as remedy for boils and wound (Benjamin and Lamikama, 1981). The leave extracts have also been reported as purgative, as an expectorant, as an astringent and as mouthwash (Quisumbring, 1978). These medicinal properties of the plant have stimulated interest in investigating the bioactivity of the plant extract against pathogens of crops especially yam. The objective of this study was therefore:

1. To test the biopesticide effects of *Cassia alata* leaf extract on wet or soft rot-causing organisms of white yam (*D. rotundata*)
2. To determine the minimum inhibitory concentration (MIC) of the extracts on the organisms.

Materials and Methods

The experiment was conducted at the plant pathology laboratory of the Department of Crop Science, University of Nigeria, Nsukka. The area is located by latitude 60 52, N and longitude 7^0 23, E, altitude 400m above sea level and has a humid tropical climate. The mean annual rainfall ranges from 1600 to 2000mm. The temperature is uniformly high throughout the year but the annual mean maximum temperature does not exceed 35^0C. A decaying yam tuber with evidence of soft rot was collected from the Department of Crop Science, UNN yam storage barn. *Acacia alata* leaves were collected from the Department of Botany (botanical garden) of the University of Nigeria, Nsukka. The leaves were cured under a shade for 48 hours to reduce the moisture content and also to make them less brittle during crushing.

Preparation of Extract: Sixty grams of the pulverized leaves of *C. alata* were weighed out using mettler sensitive balance and poured into 500 ml flat bottom flask and these were soaked in 250 ml of absolute ethanol. This was strred with magnetic stirrer for 24 hours before it was filtered with a clean muslin cloth and then concentrated in the oven at 60^0C. One gram of the dark solid material obtained after evaporation was dissolved in 4 ml of Diomethylsulphoxide (DMSO). The substance was then subjected to serial dilutions to obtain four levels of extract concentrations thus: 250, 175, 87.5, and 48.75 mg/ml.

Isolation of rot-causing organisms: With the aid of flame sterilized wire loop, the soft/wet part of the tuber was scooped into a prepared growth medium, sabouraud dextrose agar (SDA) which was allowed to stay for 48 hours to enable the rot-causing organisms grow. The organisms were isolated, identified and characterized as described by Pitt and Hocking (1997). The morphological structures of the organisms in lactolphenol blue stain were captured and filmed with Motic MCC 1.1 camera for ease of identification. The organisms were identified as *Scopulariopsis brevicaulis, Rhodotorula mucilaginesa, Penicillium restrictum and Penicillium digitalum.* These are the micro-organisms that are associated with soft/wet rot in yam tubers.

Sensitivity test: The preliminary tests of the *C. alata* ethanol extract were evaluated by the bore plate and agar diffusion method as described by Agboke et al (2005).

Determination of the inhibition zone diameter (IZD) of the extract on Penicillium spp: The determination of the inhibition zone diameter (IZD) of *C. alata* leaf extract was as follows: Sterile Petri dishes were aseptically seeded with 0.1ml of freshly prepared suspension of *Penicillin restrictum* using a sterile pipette. A 20 ml aliquot of a sterile molten sabouraud dextrose agar at 45^0C in MacCartney bottle was poured into each plate and swirled clockwise and anti-clockwise for even distribution of the organism.

After solidifying, the agar plates were marked into four sections representing the four two-fold dilution of the extract and labeled 1-4 with an indelible marker made in each of the four divisions. The two fold dilutions of the *C. alata* extracts were aseptically added to the cups using standard sterile dropper starting with the highest concentration of the extract.

The plates were incubated at 32^0C for 24hours and then, the zones of inhibition were measured with meter rule. This was repeated and the average values of the zones of inhibition were calculated. The same procedure was used in determining the 1ZD for the other three organisms.

Determination of the MIC of the extracts: A scatter analysis was performed using the 1ZD squared against the logarithm of the concentrations of the extract dilutions. The point at which the straight line that passed through the scatter plots cut the log conc was estimated and the anti log of the value was calculated to be the MIC of the extract for each organism.

Results

Microphotographs of the organisms isolated from rotting yam tuber which were associated with soft rot in yam are shown in Fig. 1. Out of the four organisms tested, only two, *Rhodotorula mucilaginesa* and *Scopulariopsis brevicaulis* were moderately sensitive to *Cassia alata* ethanol leaf extract while *Penicillium restrictum* and *Penicillium digitalum* were not sensitive (Table 1).

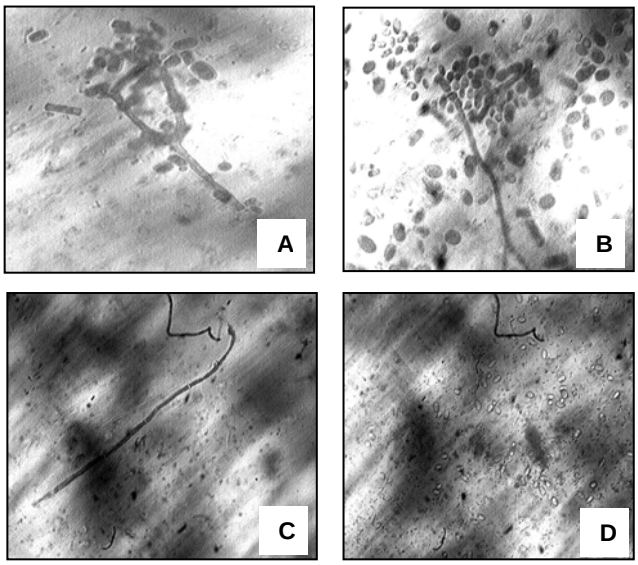

Fig.1: Microphotographs of the organisms associated with soft rot in yam tuber (*Dioscorea rotundata*) (a) *Penicilium restrictum,* (b) *Penicilium digitalum,* (c) *Rhodotorula mucilaginesa* and (d) *Scopulariopsis brevicaulis*

The concentration of 48.75 and 87.5 mg/ml of the extract resulted in very low inhibition diameter of the extract on *R. mucilaginesa* but as the concentration increased up to 250mg/ml, the inhibition diameter became reasonably high (Table 2).

Table 1: Culture and sensitivity test

Antimicrobial agent (*C. alata*)	Activities
Extract on *P. restrictum*	-
Extract on *P. digitalum*	-
Extract on *R. mucilaginesa*	+ +
Extract on *S. brevicaulis*	+ +

Table 2: Effects of varying concentration of the extract on the mycelia growth of *R. mucilaginesa*

S/N	Conc. mg/ml	Zones of inhibition (cm)	Zones of inhibition (mm)	Log of conc. (mg/ml)	IZD^2
1	250	0.55	5.5	2.398	30.25
2	175	0.35	3.5	2.243	12.25
3	87.5	0.15	1.5	1.942	2.25
4	48.75	0.1	1.0	1.688	1.04

Table 3: Effects of varying concentration of the extract on the mycelia growth of *S. brevicaulis*

S/N	Conc. mg/ml	Zones of inhibition (cm)	Zones of inhibition (mm)	Log of conc. (mg/ml)	IZD^2
1	250	0.75	7.5	1.75	56.0
2	175	0.55	5.5	1.45	30.25
3	87.5	0.40	4.0	1.20	16.0
4	48.75	0.15	1.5	0.35	2.25

Similarly, the inhibition zone diameter of the extract increased as the concentration was increased thereby reducing the mycelial growth (Table 3).

Discussion

Yam storage studies over the last decade have shown that despite the high production of yam tubers, rotting accounted for a substantial proportion of the losses during storage. This preliminary investigation of ethanol leaf extract of *Cassia alata* for its effectiveness as anti-fungal agent of soft rot in yam gave encouraging and interesting result. The four micro-organisms that were found to be associated with soft rot in yam tuber were *Penicilium restrictum, Penicilium digitalum, Rhodotorula mucilaginesa* and *Scopulariopsis brevicaulis.* The last two organisms were moderately sensitive to ethanol extract of *C. alata* at the in vitro environment while the other two, the Penicilium sp were not sensitive at all.

The sensitivity of the organisms to *C. alata* extract may be related to the nature and structure of the organisms. The *Penicilium* group has thick spores and dense morphological features which probably made it difficult or impossible for the extract to penetrate and interfere with the mycelial growth of the organisms. On the other hand, *R. mucilaginesa* and *S. brevicaulis* which were sensitive to the extract have less dense structure and light spores which probably could not resist the antimicrobial activities of the extract.

It is possible that the resistant *P. restrictum* and *P. digitalum* were capable of degrading the extract as it has been reported (Falodum *et al*, 2006) that extract of higher plants have served as food for bacterial and fungal pathogens. It is also possible that the method of extraction affected the bioactivity of *C. alata* leaf extract in this study. This is because El-mahmood and Amey (2007) reported in their study that extract activities varied between and among solvents with the ethanol extract demonstrating the highest activity against all the organisms tested in order of ethanol extracts being more potent than chloroform and chloroform more potent than aqueous extracts. Differences in minimum inhibition concentrations (MICs) of the extracts on different organisms and non sensitivity of some organisms to the extracts as observed in this study are indicators that the extract is selective in action against pathogenic micro-organisms.

Conclusion: Evidences in this study indicate that ethanol leaf extract of *Cassia alata* possesses some level of bioactivity against some fungal pathogens of yam. A major finding in this study is that the ethanol leaf extract of *C. alata* is selective in its bioactivity. It is therefore, recommended that further research is needed to test its compatibility with other plant extracts so that a broad spectrum biofungicide could be achieved from *C. alata* based formulations.

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