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ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2009, **6(4),** 1274-1280

Post-Harvest Deterioration of Cassava and its Control Using Extracts of *Azadirachta Indica* and *Aframomum Melegueta*

R. N. OKIGBO, RAMESH PUTHETI* and C. T. ACHUSI

Department of Botany,
Nnamdi Azikiwe University Awka, Anambra State, Nigeria.
*Member, An American Association of Pharmaceutical Scientists (AAPS),
236-203, Saint David Court, Maryland, USA.
rutwikusa@yahoo.com; okigborn17@yahoo.com

Received 28 January 2009; Revised 22 April 2009; Accepted 28 April 2009

Abstract: Post-harvest deterioration is the most important cause of loss in cassava production and this is mainly as a result of microbial invasion of the tubers. This research was therefore carried out to identify and control the organisms responsible for post-harvest deterioration of cassava tubers. Ethanolic and water extractions of *Azadirachta indica* (A. Juss) leaves and *Aframomum melegueta* (Schumann) seeds were used as antifungal agents and the susceptibility of four of the isolated pathogenic fungi to them was observed in culture. The tested organisms were *Aspergillus niger* Van Tiegh, *Botryodiplodia theobromae* Pat, *Fusarium solani* (Mart). Sacc and *Penicillium oxalicum* Currie and Thom. All tested organisms showed different degrees of inhibitions by the extracts with *A. niger* being the most inhibited by ethanolic extraction of *A. melegueta.* The overall result showed that *A. indica* was more active on the organisms though it's effects and that of *A. melegueta* were not significantly different (P>0.05).

Keywords: Cassava, Plant extracts, Fungi, Post harvest deterioration.

Introduction

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphobiaceae. It is a perennial woody shrub that is commonly grown as an annual throughout the lowland tropics. Since its introduction to Africa in the late sixteenth century, cassava had served as a staple food source for the teeming population of people in this region, and this is a result of its place as the major source of low-cost carbohydrate. It is the major carbohydrate food for an estimated 500 million people and in tropical Africa, it is the single most important source of calories in the diet[1].

The rate of post–harvest deterioration in cassava tubers is high and this is as a result of invasion by microorganism. The following microorganisms had been successfully isolated from cassava tubers rots: *Aspergillus flavus* Lark ex Fr., *Betryodioplodia theobromae* Pat., *Fusarium solani* (Mart). Sacc., *Mucor sp., Rhizopus sp, Trichodema harizianum* Rifai, *Cylindrium clandestrium* Corda and *Diplodia manihortis* Sacc[2-4].

Several studies by the National Resources Institute (NRI) and International Centre for Tropical Agriculture have led to the development of some storage systems based on root curing in polyethylene bags and treatment with a thiabendazole–based chemical to prevent the onset of deterioration[5]. The curing process involves exposure of the fleshly harvested tubers to temperatures of 25-35 ^{0}C and relative humidity of 80-90 percent for 7-14 days[6].

However, farmers in developing countries like Nigeria either cannot afford the cost of curing materials or they lack the expertise or equipments required to maintain the required temperature and relative humidity for the needed number of days. As a result of these restrictions and many more, a lot of natural plant extracts had been tried in the control of post harvest rot in many root and tuber crops[7-9]. The advantages of these natural plant products include local availability, little or no toxicity to humans and simple preparation procedures[10].

Azadirachta Indica A. Juss which belongs to the family Maliacea is an evergreen tree that is abundantly found in tropical and sub-tropical climates. In Nigeria, it has been used as a cure for fever, intestinal disorders, skin problems, cough and ulcers. They are used as oral medicine, cosmetics, tooth powders and even as tooth brushes.

Aframomum melegueta Schumann, a member of the family Zingiberaceae, bears tiny hot-tasting seeds that are of traditional importance to the people of Eastern Nigeria. It is used for inflamed conditions of the throat, fevers and exanthemata[11]. Traditionally, it is made into powdered form which is subsequently dissolved in hot water for treatment of common cold and toothaches.

The necessity of anti-microbial agents which are cheap, easier to prepare and that would be available for the control of post-harvest rot of cassava tubers makes this research a necessity. The study therefore tries to investigate the effects of extracts from *Azadirachta indica* and *Aframomum melegueta* on post-harvest tuber rot of cassava.

Objectives of research

(i) To isolate and identify the organisms responsible for the post-harvest deterioration in cassava.
(ii) To determine the frequency of occurrence for each of the isolated organisms.
(iii) To determine the extent of fungal inhibition by extracts from *A. indica* and *A. melegueta.*

Experimental

Sources of materials

The cassava tubers with signs of rots and healthy cassava tubers were obtained from a local farm at Awka, Anambra State, Nigeria. The *Aframomum melegueta* (Schumann) seeds and *Azadirachta indica* (A. Juss) leaves were also obtained from bushes around Awka. The plants were identified by Prof. C.U. Okeke of the Department of Botany, Nnamdi Azikiwe University, Awka, and samples were kept in the Department.

Isolation of fungal pathogens

Diseased cassava samples were surface sterilized by cleaning with 70% ethanol solution. Then with a sterile kitchen knife, it was cut open to reveal the boundary portion between the rotted and healthy parts. Carefully, portions (about 3 mm in diameter) were taken from the boundary and inoculated on the solidified SDA medium. The plates were then incubated on the laboratory bench at room temperature (27±2 ^{0}C) for 2-5 days. They were examined daily for presence of fungal growth. The fungi isolated were put into pure culture and stored in slants in the refrigerator at 4 ^{0}C.

Determination of percentage of fungal occurrence

This was done to determine the frequency of occurrence of the different fungal isolates. Isolations were made from eight different rotted cassava tubers and were cultured differently. The number of occurrence for each of the isolates in the eight different samples were recorded and calculated as a ratio of the total number of occurrence and was then expressed as a percentage (Table 1). It was given by the formula below;

$$\text{Percentage occurrence} = \frac{\chi}{N} \times \frac{100}{1}$$

χ = Total number of each organism in all the samples.
N = Total number of all the organism in all the samples screened.

Table 1. Percentage frequency of occurrence of fungi on rot cassava**.**

Isolate	Frequency of occurrence, %
Fusarium solani	15.10
F. oxysporum	2.14
Candida spp	1.73
Aspergillus niger	16.25
A. tamarii	2.42
Mucor spp	6.90
A. flavus	8.92
Penicillium oxalicum	6.80
P. digitatum	1.71
Trichoderma viride	8.62
Neurospora spp	3.45
P. chrysogenum	3.56
Botryodiplodia theobromae	5.17
Geotrichum candidum	6.90
Rhizopus stolonifera	10.34

Identification of isolates

The pure culture isolates obtained from the diseased cassava tubers were used for the purpose of identification.

Each isolate was subjected to colony and microscopic examinations during which their structural features were observed. The structures of the growing fungi were first studied under the hand lens. This was followed by a slide mount of each Isolate under the lactophenal cotton blue stain. The characteristics observed were matched against those available in manuals of Barnett and Hunters[12]. They were then identified accordingly.

Pathogenicity tests

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

A healthy cassava tuber was first washed with a sterile water and then surface sterilized by dipping cotton wool into 70% ethanol solution and then used to clean the surface. With the aid of a sterile 30 mm diameter cork borer, a cylindrical core was removed from the cassava tuber. A reconstituted culture of the isolate was then introduced into the open core and the core was replaced and sealed with sterile petroleum jelly. The tubers were kept at room temperature for ten days.

On establishment of disease condition, inoculums were taken again from the infected cassava tubers and cultured. The resulting mixed cultures were subcultured 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.

Preparation of extracts

The plant samples, *Aframomum melegueta* (Alligator pepper) and *Azadirachta indica* (Neem), were sorted to select the desirable ones. Those selected were spread in a laboratory tray and dried in a moisture extraction oven at 65 ^{0}C for three hours. They were separately ground in an Arthur Thomas laboratory mill, USA. They were afterwards sieved through a 1mm test sieve to obtain powdered processed plant materials.

A measured weight of each processed plant material was soaked separately in the appropriate medium (*i.e* water and ethanol). The mixture was shaken and allowed to stand for three hours. It was then filtered through Whatman No 42 filter paper. The filtrate was evaporated to dryness over a water bath at low temperature (45-55 ^{0}C). The dried extract was used for the test after reconstituting it with sterile distilled water. This was done by embedding sterile paper discs into it and the discs were then used.

Determination of yield of extract

A measured weight (20 g) of each plant materials (*A. indica* and *A. melegueta*), was soaked in excess of the extracting solvent and allowed to stand for three hours. It was shaken and filtered. The filtrate was put in a weighed evaporation dish and evaporated to dryness. The dish and extract was weighed after cooling in desiccators. By difference, the weight of extract was obtained and expressed as a percentage of weight of sample analyzed. It was calculated as shown below;

$$\text{Percentage yield} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

W = Weight of processed sample analyzed
W_1 = Weight of empty evaporation dish
W_2 = Weight of dish and dried extract.

Sensitivity test on pathogens using the plant extracts

To determine how sensitive each pathogen isolate is to the two plant extracts, pure culture of the isolates were directly inoculated on a sterile SDA plate. The extract was reconstituted in minimal (1 mL) of sterile distilled water and embedded on sterile paper discs. The sterile paper discs were placed on the surface of the inoculated plates and were incubated at room temperature for 7 days.

The cultures were examined for the presence of clear zones. The extent of inhibition was determined by the diameter of the clear zones around the paper discs (Table 4).

Determination of minimum inhibition concentration

The minimum inhibition concentration of each extract was determined as the least concentration of the extract which caused inhibition of the test organism.

The method involved reconstituting the extracts in dilutions of 0%, 25%, 50% and 100% of water and ethanol respectively.

A sterile paper disc was placed in each concentration and aseptically transferred to the SDA plates inoculated with the isolates, incubated for 7 days and presence of clear zones if any was measured and recorded.

Results and Discussion

Many different fungi was successfully isolated from different cassava tuber rots, they include; *Fusarium oxysporum*, *Fusarium solani*, *Botryodiplodia theobromae*, *Aspergillus niger* and *Penicillium oxalicum* all of which were implicated as pathogens when tested on healthy tubers (Table 1). Of all the isolated fungi, *A. niger* and *F. oxysporum* had the highest percentage frequency of occurrence (Table 1), with *A. niger* being the most virulent, exhibiting a dry rot contrary to the wetness that characterized the areas infected with *F. oxysporum.*

Azadirachta indica and *Aframomum melegueta* showed some inhibitory activities on the isolated fungi from the diseased cassava tubers. The extract of *A. indica* and *A. melegueta* which showed no significant difference ($p > 0.5$) in yield was found to be fungitoxic on *F. oxysporum, P. oxalicum, A. niger* and *B. theobromae* when used to control their growth *in vitro* (Tables 2-6). The most inhibited was *A. niger* when *A. melegueta* was extracted with ethanol (Table 5). In general however, *A. indica* proved to be more fungitoxic than *A. melegueta* both with water and ethanolic extractions (Tables 2-6).

Table 2. Inhibition of some pathogens causing rot in cassava tubers using *Azadirachta indica* and *Aframomum melegueta* extracts.

Pathogen	*A. indica*		*A. melegueta*	
	Ethanol	Water	Ethanol	Water
F. oxysporum	11.2	9.1	10.4	7.5
P. oxalicum	12.6	11.8	15.3	10.0
A. niger	14.1	10.8	16.0	14.2
B. theobromae	13.4	12.2	8.6	8.1

Note: All dimensions are in mm and include the diameter of the disc.

Table 3. Minimum inhibition concentration for *Azadirachta indica* using ethanolic extractions

Isolate	Concentration, mg/mL			
	0%	25%	50%	100%
A. niger	14.6	10.4	8.20	6.5
B. theobromae	15.6	9.20	-	-
F. oxysporum	12.4	-	-	-
P. oxalicum	13.8	10.6	-	-

Note: All dimensions are in mm and includes the diameter of the disc.

Table 4. Minimum inhibition concentration for *Azadirachta indica* using water extractions.

Isolate	Concentration, mg/mL			
	0%	25%	50%	100%
A. niger	13.2	9.80	-	-
B. theobromae	13.80	-	-	-
F. oxysporum	9.80	-	-	-
P. oxalicum	12.4	-	-	-

Note: All dimensions are in mm and include the diameter of the disc.

Table 5. Minimum inhibition concentration for *Aframomum melegueta* using ethanolic extractions**.**

Isolate	Concentration, mg/mL			
	0%	25%	50%	100%
A. niger	17.00	13.2	10.8	-
B. theobromae	10.60	9.40	-	-
F. oxysporum	10.90	8.50	-	-
P. oxalicum	16.20	14.10	9.30	-

Note:All dimensions are in mm and includes the diameter of the disc.

Table 6. Minimum inhibition for *Aframomum melegueta* using water extractions**.**

Isolate	Concentration, mg/mL			
	0%	25%	50%	100%
A. niger	14.80	12.50	9.80	-
B. theobromae	9.40	-	-	-
F. oxysporum	9.20	-	-	-
P. oxalicum	10.40	9.30	-	-

Note:All dimensions are in mm and include the diameter of the disc.

Water and ethanolic extractions showed no significant difference ($p>0.5$) in their rates of fungitoxicity on *A. niger, P. oxalicum,* and *F. oxysporum* but exhibited a difference only on *B. theobromae* inhibitions.

The different concentrations of both plant extracts used for MIC tests showed a difference ($P<0.5$) among each other. The four tested fungi also showed a significant difference in their different rates of susceptibility to ethanolic extractions of *A. indica* and *A. melegueta*. The different organisms showed no significant difference ($P>0.5$) in their sensitivity to water extractions of *A. indica* but significantly differs ($P<0.5$) in their sensitivity to water extraction of *A. melegueta.*

The isolated fungi with the highest rate of occurrence include; *Aspergillus niger, Fusarium oxysporum, Penicillium oxalicum, Botryodiplodia theobromae, Aspergillus flavus, Rhizopus stolonifera and Trichoderma viride.* These correspond with previously isolated microorganisms[2-4,13]. These organisms probably gained access into the tubers through the area where the tuber is separated from the stem at harvest, or from the root tip which often got broken during harvest, or through natural cracks and openings on the surface of the tubers.

The pathogenicity test showed *Aspergillus niger* as the most virulent organism, and in culture, it is the most abundant. This suggests that *A. niger* could be the leading cause of post-harvest deterioration in cassava especially here in south–east Nigeria from where all the samples were obtained. This is a reconfirmation of the result obtained by Okigbo and Ikediugwu[14] when they reported *A. niger* as the most frequently occurring fungi in yam rot isolates.

Several works had been done on the use of plant materials as antimicrobial agents[7-9,15, 16]. *Azadirachta indica* (Neem) was specifically shown to be fungitoxic by Amadioha and Obi[8]

when they used it's seed extract to control the growth of *collectotrichum lindemuthianum,* a cowpea fungus. Subsequently, Onifade[9] also recorded the use of Neem leaf, bark, fruit and seed in controlling *C. lindemuthianum. Aframomum melegueta* (Alligator pepper) extracts was used by Okigbo and Ogbonnaya[16] in the control of *F. oxysporum* and *A. niger.* Iwu[17] also showed that *A. melegueta* has antimicrobial and antifungal effects and also effective against schistosomes.

A. niger was the most inhibited when *A. melegueta* was extracted with ethanol. This showed that *A. niger* is more sensitive to the active principle present in *A. melegueta* than that present in *A. indica.* Since ethanolic extractions of both *A. indica* and *A. melegueta* are generally more fungitoxic than water extractions, the active principles are probably more soluble in ethanol than in water. This agrees with some workers who observed that factors like the type extracting solvent and age of plant could influence the active principles present in plants[18,19]. *B. theobromae* probably reacts differently to the two plants (*A. indica* and *A melegueta*) tested, this is because the two plants showed a significant difference in their fungitoxicity only on *B. theobromae,* but showed no difference on *A. niger, F. oxysporum,* and *P. oxalicum* inhibitions.

Conclusion

Since the results of this study have shown the potentials of extracts from *A. indica* leaves and *A. melegueta* seeds to control the post–harvest rot of cassava, it would be necessary to adopt them in prolonging the shelf–life of fresh cassava tubers since it is available, easily prepared and with little or no adverse environmental effects. This will provide the much needed economic benefits from cassava cultivation and production. Subsequent research in this area is expected to identify the active principles that are present in this plant (*A. indica and A. melegueta)* and find ways of specifically using them in the control of these organisms.

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