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Effects of Leaf Extracts of *Azadirachta indica* and *Chromolaena Odorata* on Post Harvest Spoilage Fungi of Yams in Storage

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Abstract: Investigation was carried out to test the potency of some plant extracts for control of yam tuber rot caused mostly by *Fusarium oxysporum, Asperigillus niger* and *Botryodiploidia theobromae* which were isolated from rooten tissues of yam tubers obtained from National Root Crop Research Institute (N.R.C.R.I) Umudike, Nigeria. These organisms were isolated from stored yam tubers. They caused soft rot leading to tissue discoloration and production of foul smell from the rotted portion of the tuber. Extract used were obtained from two plant materials – leaves of *Chromolaena odorata* and *Azadirachta indica* and the extracting solvent were ethanol and water. The extract of the plant material were found to be fungitoxc against the yam rot spoilage fungi that were tested. *Azadirachta indica* was found to inhibit organisms more than *Chromoleana odorata.* This study indicated that *Azadiracta indica* and *Chromoleana odorata* were able to suppress rot-causing fungi of yam. Therefore, they will serve as a good natural plant fungicide (Protectranct) against yam tubers in storage.

Key words: *Azadirachta indica*, *Chromoleana odorata,* extract, rot, spoilage fungi and yam

INTRODUCTION

Cultivated yams belong to the family *Dioscoreceae* and to the genus *Dioscorea* (Coursey, 1967) the most cultivated species in Nigeria are the *Dioscorea rotundata* (white yam) *Dioscorea alata* (water yam). There are also species of wild yam growing in Nigeria whose tubers are collected for eating in times of food shortage. Yams are a valuable source of carbohydrate to the people of the Tropical and Subtropical Africa, Central and South America, parts of Asia, the Caribbean and Pacific Islands (Coursey, 1967; Adelusi and Lawanson, 1987). *Dioscorea* plants are large twiners and are easily propagated by means of the bulbils or portions of the tubers.

Yams after peeling the tuber can be cooked in various ways by boiling and mashing, but roasting and frying are also widely used, yam in Nigeria is also processed into various staple intermediate and end product forms (Okaka and Anajekwu, 1990), which are used for direct consumption by animals, used as the basic ingredient for snacks or made into flour used for making instant puree (Coursey, 1967; Okaka and Anajekwu, 1990). Out of the world production of over 30 million tonne per annum, Nigeria alone products 22 million tones (FAO, 1998). Despite this, the demand for yam tubers in Nigeria has always exceeded it's supply. However, it has been estimated that an average of over 25% of the yield is lost annually to diseases and pests (Arene, 1987; FAO, 1998). Okafor (1966) and Okigbo (2005) also reported that over 50% of the yam tubers produced and harvested in Nigeria are lost in storage. The disease causing agents not only reduce the quantity of yam produced, but also reduce the quality by making them unappealing to the consumers.

There are many microorganisms associated with yam in Nigeria, which may include *Fusarium solani*, *Rhizopus, stolonifera, Botrodiploidia theobrome, Geotriclum candidum* (Okafor, 1966; Coursey, 1967; Adeniji, 1970) some such as *Trichoderma viride* and *Bacillus subtilis* are also effective in the control or reducing storage rot in yam (Okigbo, 2005). The use of synthetic chemicals such as sodium orthiophenylphenate and borax has been found to reduce storage rot in yam (Booth, 1974). But biological control is generally favoured as a method of plant disease management (Okigbo and Ikediugwu, 2000; Okigbo, 2002; 2005).

Plant extracts have been used to control yam diseases (Okigbo and Ogbonnaya, 2006). It is important to search for a method of control of yam rot that will be affordable, durable and free of chemicals that can pollute environment. Therefore, in this report the antifungal properties of *Azadiracta indica* A. Juss and *Chromolaena odorata* (L.) King and Robinson against some spoilage fungi responsible for yam tuber rot in storage were reported.

MATERIALS AND METHODS

Source of materials: Yam samples (rotted and healthy tubers) were obtained from the traditional yam storage barn of National Root crop Research Institute, Umudike, Abia State, Nigeria. The experiment was conducted from

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November 2006 to June 2007. this trespasses through two seasons (Rainy and Dry season). Test plant samples *Azadiracta indica* A. Juss and *Chromolaena odorata* (L.) King and Robinson were also obtained from the Institute and their respective botanical identifications were authenticated by the Institute Field Officer.

Processing of test plant sample: The plant samples were processed prior to extraction. First each test plant sample was washed with sterile water and closely examined for the presence of dried, shriveled or deteriorated leaves which were removed alongside extraneous materials (dirts and insect) the good ones were spread on a laboratory tray and dried in a carbolite moisture extraction oven at 60ºC until they were brittle, the dried leaves were then grounded in an Arthur Thomas laboratory mill in which they were also sieved through a 1 mm test sieve to obtain a powdered processed sample used for the extraction.

Isolation of Fungi from rotted Yam tubers: Yam tubers (*Dioscorea rotundata)* stored in the National Root Crop Research Institute (NRCRI), Umudike traditional Yam barn and which has started rotting were obtained and used for the work. The tubers were surface sterilized by spraying with cotton wool moisturized with 70% ethanol solution. They were then cut open to reveal the boundary area of rotted and unrotted portions. Inocula were taken from such boundary areas and used for he analysis as described below.

Direct Plating: Bits of the rotting yam were cut out with sterile scapula and aseptically transferred onto solidified sterile SDA medium in petridish up to 4 pieces were placed at regular distance from one another on the medium surface and incubated at room temperature for 2-5 days. The plates were examined daily for growth.

Identification of fungi isolates: Fungi Isolates were identified following sub-culture growth. The culture plates obtained were examined and inocula were collected from distinct colonies. They were inoculated onto sterile SDA plates, incubated and when growth established, they were examined for uniformity as a mark of purity. The pure cultures so obtained were identified using Barnett and Hunter (1985).

Test for pathogenicity: Each of he fungi Isolates was tested for it's ability to elucidate disease (rot) condition in healthy tubers. A healthy yam tuber was surface sterilized by cleaning with 70% ethanol solution with the aid of sterile cork borer; a hole was made on the tuber. The flesh of the tuber was removed aseptically carefully with the sterile cork borer; a disc was cut from an established plate culture of the test fungus. The disc (inoculum) was placed inside the hole made on the test tuber sample. A portion of the tuber flesh removed earlier was cut off to compensate for the disc size. The hole was cornered with sterile petroleum jelly (Vaseline) and allowed to incubate at room temperature. Each preparation was observed daily for signs of rot or establishment of rot, the rotted sample was used as source for reisolation of the suspect pathogen.

Extraction Determination of % yield: To obtain extracts (from the plants) for use in tests, solvents, water and ethanol were used. The cold extraction method (Amadioha and Obi, 1998) was employed. Twenty grams of each test sample (processed) was weighed into conical flask. The extracting solvent (water or ethanol) was added to form a ratio of 1:10 v/w. It was corked with a rubber bring shaken well to mix and allowed to sand overnight at room temperature.

The next day, the mixture was shaken for 30 min in a reciprocal mechanical shaker filtered through Whatman No 42 filter paper to obtain the extract solution (filtrate) each extract was evaporated to dryness in a weighed disc over a steam bath, dried further in the oven at 60ºC for 1 h and cocked in a desiccators before weighing. By difference, the extract obtained from each sample used, it was calculated using the formulae below.

% Extract yield

$$= \frac{W_2 - W_1}{Weight\ of\ sample} \times \frac{100}{1}$$

W_1 = Weight of empty dish
W_2 = Weight of dish text tract (dried)

Preparation of Antimicrobial Test Dics: The test discs were prepared using the method of Cheesbrogh (2000) with the aid of an office paper perforator, a circular discs were cut out of an absorbent paper (filter paper) the discs were boiled for 1hr in distilled water (to remove any possible preservative) they were then dried in the oven at 80 ºC for 30 min, before being rapped in aluminum foil and sterilized in the autoclave at 121ºC and pressure of 15 psi for 15 min, they were stored in sterile screw capped bottle (universal bottle) until needed for use.

Direct medium treatment: This method involved direct treatment of the medium with the extract before inoculation. A measured weight of extract from each plant was placed inside a sterile petridish before the medium, 15 meals was poured into the petridish, mixed well and allowed to cool and gel. The test fungi isolate was inoculated onto the surface of the medium and incubated. The plates were examined for growths, the absence of growth in any of the plates was indicative of the potency of the extract against the test fungi Isolate (Pathogen).

Anti fungal Sensitivity test: Test of the established fungi pathogens for sensitivity to each of the plan extracts was done by the disc diffusion technique (Cheesbrogh, 2000) as well as direct medium treatment technique.

Disc diffusion test: The prepared discs were impregnated with the extracts respectively. An inoculum's from 2-day broth culture of the fungi was collected and spread over the surface of SDA plate.

The discs bearing the extract were separately placed at equal distances from one another over the surface of the plates. The plates were incubated and observed for the presence of inhibition zones as a mark of sensitivity to the test extract.

Determination of protective potency of test extract plants: The ability of the plant (processed) to protect harvested yam tubers from fungi attack was investigated; several healthy test yam tubers were used. A portion of each test tuber was cut open. The control was off untreated while the open portion of the test samples were covered very well with the test sample (dust) they were separately inoculated with the different fungi pathogens (as Isolated) they were all incubated and observed daily for signs of rot. This included discolouration, softening and foul smell. The potency of the test plants was measured by their inhibition of he fungi growth and hence preventing over the test period.

RESULTS

The most frequently occurring fungi from the yam rot samples has shown to be *Botryodiplodia theobromae, Fusarium oxysporum, Aspergillus niger, Rhizopus stolonifera, Geotrichum candidum* and *Penicilum digitatum* (Table 1) in their order of increasing frequency. The pathogenicity test revealed that *A niger, F. oxysporum B. theobromae* and *R. stolonifera* were all pathogens in yam tuber rot (Table 2). The weight of the extract yield from *Chromolinae odorata* was 0.064 g or 0.32% for water extracts, 0.066 g or 0.33% for ethanol extract while the yield in *Azadirachta indica* was 0.075 g or 0.38% for the water extract and 0.84 g or 0.42% for ethanol. Water and ethanol extracts of *Azadiracta indica* and *Chromolaena odorata* proved to be *Fungitoxic* on *A. niger, B. theobromae, F. oxysporum P. digitatum* and *R. stotlonifera* when used to inhibit their growth in culture (Table 3 and 4).

Table 1: Percentage of occurrence of fungi in yam rot samples in storage barn from November to February.

Isolate	Period %			
	Nov.	Dec.	Jan.	Feb
Fusarium oxysporum	18.5	28.1	34.6	36.4
Botryodiplodia theobromae	31.5	37.6	39.8	40.5
Aspergillus niger	17.3	20.9	19.6	20.1
Penicilium digatum	8.2	10.5	8.5	9.6
Rhizopus stoloniferat	15.3	30.1	28.8	29.1
Collectricum spp	2.6	5.6	5.9	6.2
Trichioderma viride	5.2	7.3	3.5	3.6
Geotrichum candidum	12.6	0	0	0
Erwinia carotovora	7.8	8.1	16.1	17.1
Aspergillus tamani	8.6	7.4	6.5	7.1
Penicilluim chysogenum	7.8	9.5	7.8	8.1
Rhizopus spp	*4.7*	*5.6*	*6.8*	*7.5*

Table 2: Pathogencity test result of fungi in yam tuber rot

Isolate	*A*	*B*	*C*
Fusarium oxysporum	+	+	+
Botryodiplodia theobromae	+	+	+
Aspergillus niger	+	+	+
Pencillium digitatum	+	+	+
Rhizopus Stolonifera t	+	+	+
yeast	-	-	-

Key, + = Pathogenic, - = Non pathogenic, A, B, C Indicate triplicate preparation of sample

Table 3: Anti fungal sensitivity test of disc diffusion technique ofAzadirachta indica

	Diameter of inhibition (%)	
Isolate	Waterextract	Ethanol extract
Fusarium oxysporum	0	21.2
Botryodipodiatheobromae	0	18.5
Aspergillus niger	20.5	26.2
Penicilum digitatum	18.5	24.6
Rhizopus stolonifera	28.1	26.1

Table 4: Anti fungal sensitivity test by disc diffusion techniques of *Chromolinae odorata*

	Diameter of inhibition (%)	
Isolate	Waterextract	Ethanol extract
Fusarium oxysporum	Nil	13.0
Botryodiplodia theobromae	10.5	12.5
Aspergillus niger	11.0	14.0
Penicilum digitatum	Nil	14.2
Rhizopus stolonifera	13.0	14.7

DISCUSSION

This research was able to associate the following fungi with post-harvest rot of yam *Aspergillus niger, Botryodiplodia theobroamae, Fusarium oxysporum, Penicillium digitatum* and *Rlizopus stolonifer.* These had been previously linked with post harvest yam rot (Okigbo and Ogbonnaya, 2006; Okigbo and Nmeka, 2005; Okigbo, 2004; Ogundana, 1971). Most of these microorganisms infect the tubers in the field and subsequently manifest in storage barn.

Okigbo and Ikediugwu (2000) employed some biological control measures in controlling the yam rot fungi. They were able to show that *Trichoderma viride* displayed the naturally occurring mycoflora on the surface of the yam tuber effectively. The presence of anti fungal active principles in the leaves of *Azadirachta indica* and *Chromolinae odorata* was demonstrated in this work. This was shown by their ability to inhibit the growth of the tested organisms in culture. This coincided with previously done works on the use of plant extracts in the control of fungal rots (Okigbo and Ogbonnaya, 2006; Okigbo and Nmeka, 2005; Onifade, 2002; Amadioha and Obi, 1998). However, *Azadirachta indica* proved to be generally more fungitoxic than *Chromolinae odorata* This could be as a result of higher concentration of the active principles in the leaves of *Azadirachta indica* than that of *Chromolinae odorata.* The availability of active principles in the extracting solvent is determined by the factors like age of plant and method of extraction (Okigbo and Ajalie, 2005; Okigbo and Omodamiro, 2005).

CONCLUSION

Having shown that the extracts of *Azadirachta indica* and *Chromolinae odorata* could be used to inhibit post harvest rot fungi of yam, the use of them should be encouraged in the form of protestant pesticide. Their leaf litter could also be used as manure so as to control the rot organisms present in the soil.

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