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Full Length Research Paper

Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on postharvest yam (*Dioscorea* spp.) rot

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Effects of leaf extracts of *Ocimum gratissimum* and *Aframomum melegueta* on spore germination and mycelial reduction of the most occurring fungal pathogen causing soft rot of yam tuber were investigated. Fungi isolated from rotted yams were *Aspergillus niger, A. flavus, Fusarium oxsporium Rhizopus stolonifer, Botryodiplodia theobromae* and *Penicillium chrysogenum*. The leaf extracts with ethanol extraction were most effective followed by cold-water and hot water extraction. The fungicidal activity with *Ocimum gratissimum* leaf extracts was more effective. *In vitro* inoculation of fresh yam with *A. niger, A. flavus* and *F. oxsporum* at room temperature for 3 months showed typical rot symptoms characteristic of the disease. The significance of these findings is discussed in relation to plant chemical or non-chemical means of disease control on yams in Africa.

Key words: *Ocimum gratissimum*, *Aframomum melegueta*, leaf extracts, fungi, rot, storage, yam.

INTRODUCTION

Yams (*Dioscorea Spp.*) are one of the most highly regarded food products in tropical countries of West Africa and are closely integrated into social, economic, cultural and religious aspects of life. The ritual ceremony and superstition often- surrounding yam and utilization in West Africa is a strong indication of the antiquity of use of this crop (Norman et al., 1995). Yam is of a very high value, as in food, where it is a major source of carbohydrate, minerals of calcium, phosphorus, iron and vitamins such as riboflavin, thiamin and vitamins B and C (Coursey, 1967). Some species of yam have been used medically to treat disease like diabetes mellitus, to increase coronary flow and prevents high hyperchosterolemia (Undie and Akubue, 1986.)

Yams are subject to several diseases. There are different genera of fungi that have been reported in association with storage deterioration in yam tubers (Noon, 1978; Okigbo and Ikediugwu, 2000). The major microorganism causing diseases in yams are *Aspergillus flavus* Lark ex Fr., *Aspergillus niger* Van Tiegh, *Botryodiploidia theobronmae* Pat, *Fusarium* Oxysporum Schlecht ex Fr., *Fusarum solani* (Mart.) Sacc., *Pencillium chrysogenum* Thom *Rhizoctonia* sp., *Penicillum oxalicum* Currie and Thom, *Trichoderma viride* Pers. ex S. F. Gray and *Rhizopus nodosus* N' amyslowski (Adeniji 1970; Ogundana et al., 1970; Okigbo and Ikediugwu, 2000, 2001, 2002; Okigbo, 2004). Yam rots usually start in the soil and progressed in storage, which occur when infected tubers do not have any sign of external symptoms.

The use of chemicals has helped increase of yields obtained but one of the major problems with the constant use of chemicals is that resistance can be induced in target organisms. Biological method of control has been preferred in some cases because it is selective with no side effect and cheap. Resistance to biological control is rare and biological control agents are self-propagating and self-perpetuating (Okigbo and Ikediugwu, 2000; Okigbo, 2003, 2004, 2005).

Plant extracts have been used successful to control disease in plants and tuber crops (Amadioha and Obi, 1999; Onifade, 2000; Okigbo and Emoghene, 2004; Okigbo and Nmeka, 2005). *Aframomum melegeuta* Schumann of family Zingiberacea is used as spices in food and for treatment of dysentery and gastrointestinal

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trouble (Schum, 2000). In Nigeria, the plant is used for inflamed conditions of throat, fevers and exanthemata (Apata, 1979) when crushed and rubbed on the body. It is also applied for headache as well as made into paste for wounds or sores. *Ocimum gratissimum* L; family Leguminoceae is grown in gardens and used as a tea leaf for fevers. It is widely distributed in tropical and warm temperature regions (Dalziel, 1937). *O. gratissimum* is commonly used in folk medicine to treat different diseases such as upper respiratory tract infection, diarrhea, skin diseases, pneumonia and also cough and conjunctivitis (Onajobi, 1986).

It is desirable to search for new types of pesticides for control of yam rot which are not of chemical origin. Therefore, this investigation reports on the antifungal activity of *O. gratissimum* and *A. melegueta* on yam rot.

MATERIALS AND METHODS

Collection of yam

Yams with symptoms of soft rot were obtained from the traditional yam barn of National Root Crop Research Institute, Umudike, Nigeria. The yams with softness of tissues were identified as being rotted. Fresh yams were also collected from the barn.

Collection of plant materials

Aframomum melegueta and *Ocimum gratissimum* were collected in April 2001 from forest of Umudike, Nigeria. A voucher number FRIN 1148 is deposited at forestry Research Institute of Nigeria, Umuahia, herbarium.

Isolation of spoilage fungi from rotted yam

Pieces of yam tuber 3 x 3 x 2 mm in dimension cut from advancing edge of a rot were surface sterilized in 70% alcohol for 1 min, dried on sterile tissue paper and plated out on PDA amended with moisture of penicillin and streptromycin. A minimum of five replicates pieces from each of the rots were plated out. The plates were incubated at room temperature for up to 5 days and fungal growth associated with rot affected tissue identified and their frequency of occurrence determined (Okigbo and Ikediugwu, 2000).

Pathogenicity test

The method of Okigbo and Ikediugwu (2000) was adopted. Cylindrical cores, 1 cm deep, were removed from different regions of a healthy yam tuber using a 5 mm cork borer and then 4 mm discs taking from the edge of a colony of test fungus were placed downwards into each of the holes in the tubers. The core of the yam from the tuber was replaced after 2 mm pieces has been cut off to compensate for the thickness of the agar inoculum and the replaced core sealed with melted candle wax. Sterile PDA discs used in place of the culture discs served as control.

Preparation of leaf extracts

Fresh leaves of *O. gratissimum* and *A. melegueta* were washed thoroughly under running that water and sterile distilled water and air dried at 28ºC for 2 h. This was further homogenized into a paste for each leaf with a blender (mixer model 830 L, Hong Kong). A cold water extract of the ground leaves was prepared by adding 100 g of the dried leaf to 100 ml of cold sterile water in a 500 ml beaker, stirring vigorously, allowing 1 h for settling, and then filtering the extract through folds of sterile cheese cloth. Hot water extracts were obtained by infusing the ground or paste of leaf materials from each plant separately with 100 ml sterile distilled water using 250 ml conical flasks in water bath at 90^{o}C for 1 h. Thereafter, the suspension was filtered 5 times through sterile muslin cloth. An ethanol extract was prepared by mixing 20 g of ground leaf with 100 ml of 70% ethanol in a 500 ml beaker. The extract was filtered through 4 folds of sterile cheese cloth. Different concentrations of 10, 25, 50 and 100% were prepared. 5 ml from each of the concentrations was dispensed into 9 cm diameter Petri dishes after which 20 ml of melted PDA were poured into the plate, shaken together and allowed to solidity.

Activity of extract *in vitro*

The method of Amadioha (2000) was used. Three PDA disc of 1 mm diameter of the pathogen in 1 ml of each extracts were used to prepare suspension of 5-day-old cultures in test tubes. Control was prepared with similar spore suspension using distilled water or alcohol (95%). A four fold cheese cloth was used as filter. A drop of 0.5 ml each of the filtrate was placed on sterile slides in humid chambers and incubated at 28ºC for 24 h. The fungi tested were *Aspergillus niger* and *Fusarium oxysporum*. Four replicate were set up for each treatment. The germinating spores from each slide were carefully counted under low power (x10) of the microscope. Percentage inhibition was calculated from the data obtained.

Mycelia extension of fungi

Effect of extract on mycelia extension of the fungi was obtained by placing one disc (3 mm diameter) of 5 day-old culture of the pathogens in each of five Petri-dishes (11 cm diameter) with 170 ml PDA medium and 3 ml leaf extract. The control experiments were set up with 3 ml of sterile distilled water. Five replicates plates of leaf extract agar per isolate were incubated at room temperatures (28±2ºC) for seven days. Daily measurements of the mycelial extension of the cultures were determined by measuring culture along two diameters. Mycelial growth inhibition is taken as growth of the fungus on the leaf extract agar expressed as percentage of growth on the PDA.

Activity of extracts *in vivo*

A modified traditional barn method of storage of yams was adopted (Okigbo and Ikediugwu, 2002). The barn was delimited on all sides and at the top with 30 mm^2 wire netting to discourage rodents. Palm fronds were placed on the top in traditional practice of providing shade for the tubers. Wooden platforms, one meter wide, raised one meter above the floor and on all four sides of the barn served for storage. Twenty healthy tubers of one cultivar of white yam from 300 to 400 mm long and 40 to 80 mm girth were used on the same day of harvest in the storage experiment. The tubers were first washed under running tap water to dislodge soil particles and were then mechanically wounded with sterile scalpel and dipped in each of the hot water, alcoholic extracts, sterile distilled water or alcohol for 1 h or 6 h. The sterile distilled water or alcohol served as control. The treated tubers were air-dry for 1 h before spore suspension of 5 x 10^4 spores per ml of distilled water of pathogen was sprayed. All the treated tubers were incubated under polyethylene sheets in the

Table 1. Occurrences of spoilage fungi of yam rot in storage barn from November to February.

Fungi isolates	Period (%)			
	Nov	Dec	Jan	Feb
Aspergillus niger	7.6	18.5	50.1	62.5
Rhizopus stolonifer	14.5	8.6	30.1	33.3
Fusarium oxysporum	18.6	14.5	34.6	44.7
Aspergillus flavus	13.1	15.1	27.5	33.3
Penicilhum oxalicum	14.6	8.5	18.5	30.6
P. chrysogenum	10.5	10.6	32.3	41.1
B. theobromae	11.5	14.5	18.6	33.3
Fusarium solani	8.5	17.1	19.1	34.3

Table 2. Percentage inhibition of spore germination of *Fusarium oxysporum* and *Aspergillus niger* by leaf extracts of *Ocimum gratissimum* and *Afromomum melegueta*.

Plant species	Inhibition (%)					
	Fusarium oxysporum			*Aspergillus niger*		
	Hot water	Cold water	Ethanol	Hot water	Cold water	Ethanol
Ocimum gratissimum	27.0	20.2	70.1	9.4	38.6	66.6
Afromomum. melegueta	49.0	39.0	67.9	15.6	24.1	67.3

barn for five days when the sheets were removed to expose all the tubers fully for 30 days at 28±2ºC. During storage the tubers were examined on a weekly basis for the occurrence of rot. The decay reduction index (DRI) adopted from Amadioha (1996) was used to determine the percentage decay reduction.

$$DRI = \frac{\text{\% decay in control - \% decay in treated tuber}}{\text{\% decay in control}}$$

RESULT

The fungi which were consistently isolated from rot affected tissue of all the yams in the storage barn included *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Aspergillus flavus, Penicillium chrysogenum, Penicillum oxalicum Botryodiploidia theobromae* and *Fusarium solani* (Table 1). All the fungi were found to be cause of spoilage in yams. The degree of virulence of all the fungi was not significant. Rot was observed by the softness of tissues.

The two plant extracts inhibited the spores of *F. oxysporum* and *A. niger* (Table 2). Ethanolic extraction of *O. gratissimum* inhibited more than that of *A. melegueta.* There was less inhibition with the plant extracts using cold water (Table 2). However, the spores of *A. niger* were inhibited more by *A. melegueta* ethanolic extraction. There was a reduction of mycelia of *B. theobromae* by the plant extracts (Table 3), with ethanolic extraction having the highest followed by cold water and then hot water. The antifungal effects of the extracts against mycelia growth varied with plant species, extraction solvent and period of incubation (Table 4).

The effect of the extract on rot development in yams in storage showed that the yams dipped for longer time were more protected against rot pathogens (Table 4). The unwounded yam tubers were effectively protected against the fungal infection after 6 h dip in ethanol extract of *O. grathissimum* and *A. melegueta* (Table 4).

DISCUSSION

The organisms associated with the rot of white yam in the present study were *Aspergillus niger* , *Rhizopus stolonifer*, *Fusarium oxysporum*, *Aspergillus flavus*, *Penicillium chroysogenum*, *Botryodiploidia theobromae* and *Fusarium solani.* These organisms have been associated with post harvest rot (Ogundana et al., 1970; Okigbo, 2002, 2005). Roting in storage probably starts in the soil and progress in storage. This may happen when infected tubers do not show perceptible external symptoms (Ogundana et al., 1970). Each type of rot is characteristic of causal organism (Ekundayo and Naqvi, 1972).

The fungicidal activity of some plant extracts in controlling different plant pathogens have been reported by several workers (Tewari and Nayak, 1991; Amadioha, 2000; Okigbo and Emoghene, 2004; Okigbo and Nmeka, 2005) and also use of biological control of yam with *Bacillus subtilis* (Okigbo, 2002, 2005) but little or no work has been done to investigate the use of natural plant products as pesticide for control of storage rot diseases of yam (Okigbo and Nmeka, 2005).

Table 3. Percentage reduction of mycelium of *Botryodiploidia theobromae* by leaf extracts of *Ocimum gratissimum* and *Afromomum melegueta*.

Plant Species	Reduction (%)		
	Hot water	Cold water	Ethanol
Ocimum gratissimum	18.1	30.2	72.1
Afromomum melegueta	15.6	28.6	68.2

Table 4. Percentage reduction of rot by leaf extracts of *Ocimum gratissimum* and *Afromomum melegueta*.

Plant Extract	Percentage rot reduction							
	Hot water extract				Alcohol Extract			
	1 h dip		6 h dip		1 h. dip		6 h dip	
	Wounded	Unwounded	Wounded	Unwounded	Wounded	Unwounded	Wounded	Unwounded
O. gratissimum	34.6	48.9	54.3	62.1	68.1	79.3	72.3	98.6
A. melegueta	31.3	46.5	50.6	63.1	66.5	76.5	74.6	96.1

Data are average of three replicates in two experiments.

Present investigation showed that *O. gratissimum* and *A. melegueta* have proved effective against mycelia inhibition and spore germination of many rot causing microorganisms. Amadioha (2000) was able to show that *O. gratissimum* leaf extracts was able to control spore germination and mycelia growth of *Rhizopus oryzae*. The leaf extracts from *O. gratissimum* and *A. melegueta* did not show any significant difference in their potency against the rot pathogens. The active principles present in plants are influenced by many factors which include the age of plant, extracting solvent, method of extraction and time of harvesting plant materials (Qasem and Abu-Blan, 1966; Amadioha and Obi, 1999; Okigbo and Ajalie, 2005; Okigbo et al., 2005). The presence of antifungal substances in the different extracts which caused the inhibition of radial growth and spore germination *in vitro* and the reduction in rot development by the pathogen *in vivo* agree with reports of other workers (Qasem and Abu-Blan, 1996; Amadioha, 2000 and Okigbo and Nmeka, 2005). The difference observed in fungitoxic activity to the extracts is likely to be due to the solubility of the active compound(s) in water or ethanol or the presence of inhibitors to the fungitoxic principle. This also agrees with the report of Qasem and Abu-Blan (1966) and Amadioha (2000).

The extracts could be used as protectant pesticide since rot development in yam tubers caused by pathogen was effectively controlled when unwounded tubers were dipped in ethanol extracts for 6 h. This study has shown that that the use of plant extracts from *O. gratissimum* and *A. melegueta* to control rot of yam in storage has potential as a substitute for chemical pesticide. This approach to plant disease management is economically viable and poses little environmental risk and the plants are available to farmers in Nigeria that do not have ready access to other synthetic fungicides.

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