Antimycotic effects of aqueous and ethanol plant extracts on yam rot pathogens in Ado-Ekiti, Nigeria.

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Abstract: The metabolism of secondary plants compounds proves to be important to the plant that synthesizes them. One of the functions is to provide protection against the pathogenic attack. This work aims at studying the effect of different plant extracts on the post harvest phytopathogenic fungi. *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium solani*, *Rhizopus stolonifer*, *Botryodiplodia theobromae* and *Aspergillus flavus* that causes deterioration of tubers of yam in Ado-Ekiti, Nigeria. One gram of ethanol and water extract of different plants. *Allium sativum*, *Zingiber officinale*, *Ocimum gratissimum* and *Nicotiana tobacum* of 5 milliliters of each of 25 and 15% ethanol as well as 85 and 65% aqueous extract. After adding the extract, the PDA (Potato dextrose agar) was flowed in Petri dishes. After that, disc of 3mm diameter of each of the isolate was placed in the centre of the same one. The radial growth of the fungi was evaluated after 72 hrs. Each treatment had four replications. The highest inhibitions of 64.31 and 52.22% were recorded with *Allium sativum* against the isolates using 85 and 65% aqueous extract respectively and as well as in 25% ethanol extract of 50.93% inhibition. *Allium sativum* was the best in its fungitoxic effect against the isolates. Though all the extracts; aqueous and ethanol had inhibitory effect on the rot causing organisms of yam tubers. The possibilities that these plants can serve as sources of alternatives to chemical control of yam rot is quite obvious.

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Key words – Plant extracts, yam rot, stored yam, rot pathogens.

Introduction

Yams (*Dioscorea* spp.) are significant staple food in the sub-region of West Africa and Nigeria produces 21,814 million tons of yam tubers per year thereby placing Nigeria as the World's highest producer of yam (FAO, 1998). Yams are closely integrated into social, economic, religious and cultural aspects of life.

The ritual festival and superstition usually encompass yam and consumption in West Africa is an indispensable indication of the antiquity of the importance of yam.

(Normal *et al.*1995). Of the ten cultivated species, the most important six species of yam cultivated in Nigeria are Dioscorea *rotundata* Poir (white yam), *Dioscorea cayenensis* Lam (yellow yam), *Dioscorea alata* L (water yam), *Dioscorea dumentorum* (clusters as bitter yam), D*ioscorea esculenta* (Loir), Bark (Chinese yam) and *Dioscorea bulbifera* L (aerial yam). (Adeniji, 1970, Okigbo, 2004).

Yam tubers are susceptible to many fungal diseases. Many genera of fungi have been reported in association with storage decay in yam tubers (Okigbo and Ikediugwu, 2006). The major microbes causing diseases in yams are: *Aspergillus flavus* Lark ex Fr, *Aspergillus* niger Van Tiegh, *Botryodiplodia theobromae* Pat, *Fusarium oxysporum* Schlecht ex Fr., *Fusarium solani* (Mart) Sacc., *Penicillum chrysogenum* Thom, *Rhizoctonia spp*., *Penicillum oxalicum* Curie and Thom, *Trichoderma* viride Pers. Ex S.F. Gray and *Rhizopus nodosus* N. Amyslowski (Adeniyi,1970; Okigbo and Ikediugwu, 2000;Okigbo, 2004).

Chemicals have proved helpful in the control of yam diseases but one of the key problems is that frequent use of chemicals predisposes target organisms to resistance also chemical control leaves behind residual effect which are not eco-friendly. Erute and Oyibo (2008) used *Ocimum gratissimum* to control post harvest pathogen of *Persea americana* (avocado). Okigbo and Nmeka (2005) control yam rot with leaf of *Zingiber officinale*. Taiga *et al* (2008) inhibited the growth of *Fusarium oxysporum* mycelium with cold

extract of *Nicotiana tabacum* and Udo *et al* (2001) reduced the growth and sporulation of fungal pathogens on sweet potato and yam with garlic (*Allium sativum*). Therefore, there is need for alternative approach using plant extracts in controlling phyto pathogens.

Materials and Methods

Two sets of experiments that included the isolation and identification of the rot pathogens as well as their inoculations on healthy yam tubers followed by the application of plant extracts of aqueous and ethanol were carried out at the Department of Plant Science Laboratory, University of Ado-Ekiti, Nigeria.

Collection of infected and healthy yam isolates.

Infected yam tubers with symptoms of softness were randomly procured locally from Oja-Oba market in Ado-Ekiti. Five samples were collected from each selling point, these were taken and placed in sterile polyethylene bags and conveyed to the laboratory for isolation and subsequent identification. The identified isolates were used to infect healthy yam tubers to establish their pathogenicity.

Isolation of fungi

Diseased portion of the yam tubers were cut under aseptic condition into small bits into a sterile dish with the aid of scissors which was flamed over a Bunsen burner flame and dipped inside methylated spirit (Fawole and Oso, 1988). The cut diseased and sterilized bits with 70% ethanol were then placed on Petri dishes containing solidified PDA. The solidified plates were then incubated at room temperature (28 ± 2^0 C) in the dark for 72 hours. The fungal colonies grown on the incubated plates were sub-cultured into fresh medium until pure culture was obtained.

Microscopic examination was used after examining the colony characteristics. A sterile needle was used in taking little portion of the hyphae containing spores on the sterile glass slide stained with lactophenol-cotton-blue and examined under the microscope for fungal structures. The morphology and cultural characteristics observed were compared with structures in (Snowdon, 1990).

Pathogenecity Test

Healthy yam tubers were surface sterilized with 0.1M of Mercuric chloride ($HgCl_2$) for 1 minute and washed in five changes of distilled water. A 5ml cork borer was punched to a depth of 4mm into the healthy yam tubers and the bored tissues were removed. A five (5) mm diameter disc from the pure culture was cut and placed back. The wound was sealed with prepared candle wax according to the method of Fawole and Oso (1988). The control was set up in the same manner except that sterile agar disc was used instead of the inoculums. The inoculated yam tubers were placed in four (4) replications at room temperature (28 ± 2^0C) under sterile condition. The pathogens were re-inoculated and identified using the same procedures described earlier.

Preparation of plant extracts.

The following local plants; *Ocimum gratissimum* (leaf), *Zingiber Officinale* (rhizome), *Allium sativum* (bulb), *Nicotiana tabacum* (leaf) were air dried and grounded separately. Thirty grams of each sample was added to 15ml of distilled water in separate flasks. This was vigorously stirred and left to stand for 24 hrs. The sample was filtered with Whatman filter paper (No 1) and the filtrate used as extract.

Effect of plant extract on fungal growth.

Flat bottom flasks were used for the assay. Different percentages of extract solution were poured into separate flask containing sterilized potato dextrose broth with a sterile cork borer, different fungi were inoculated into separate flasks and incubated at room temperature (28 ± 2^0 C) for seven (7) days.

After the incubation period, mycelia from different broths were taken onto pre-weighed filter paper, oven dried at 85% and reweighed until a constant weight was obtained. The changes in weight were noted. For the control, no plant extract was added to the potato dextrose broth.

Mycelia extension of fungi *in vitro*

The method of Amadioha and Obi (1999) was used to determine the effect of extract on mycelia extension of the fungi. This was obtained by placing one disc (3mm diameter) of 5-days-old culture of the pathogens in each of five Petri dishes (1cm diameter) with 170ml PDA medium and 3ml leaf extract. The control experiments were set up with 3ml of sterile distilled water. Five replication plates of leaf extract agar per isolate were incubated at room temperature ($28\pm2\ ^0$C) for 7 days. Daily measurements of the mycelia extension of the cultures were determined by measuring culture along two diameters. Mycelia growth inhibition was taken as growth of the fungus on the leaf extract agar expressed as percentage of growth on the PDA. Fungitoxicity was determined in form of percentage growth of colony inhibition and calculated according to this formula:

Growth inhibition (%) = $\frac{dc-dt}{dc} \times \frac{100}{1}$

Where dc= Average diameter of colony with control

dt= Average diameter of colony with treatment

Results and Discussion

"Table1. Information on plants used"

Common Names	Scientific names	Families	Plant parts used
Galiki	*Allium sativum*	*Lilliaceae*	Bulb
Taba/tobacco	*Nicotiana tabacum*	*Solanaceae*	Leaf
Jinja/Ginger	*Zingiber officinale*	*Zingiberaceae*	Rhizome
Efinrin/Scent leaf	*Ocimum gratissimum*	*Legumineaceae*	Leaf

"Table 2. Inhibition % of mycelia growth of fungi grown in potato dextrose agar incorporated with 65% and 85% cold aqueous plant extract concentrations."

Test plants	% inhibition of radial growth (means)	
	65%	85%
Allium sativum	52.22c	64.31b
Nicotiana tabacum	47.01bc	50.20b
Zingiber officinale	48.27bc	54.65b
Ocimum gratissimum	42.85b	51.93b
Control	30.83	30.83

Means with the same letters in the same column are not significantly different at 0.05 levels according to Duncan multiple range test.

"Table 3. Inhibition of mycelia growth of fungi growth in potato dextrose agar incorporated in 25% and 15% ethanol extract concentrations."

Treatments/Test plants	% inhibition of radial growth (Means)	
	25%	15%
Allium sativum	50.93b	52.86b
Nicotiana tabacum	54.65b	53.91b
Zingiber officinale	51.91b	51.99b
Ocimum gratissimum	30.33a	49.91b
control	30.83	30.83

Means with the same letters in the same column are not significantly different at 0.05 levels according to Duncan multiple interest range test.

Many rot causing fungi were isolated from rotten tubers of yam such as *Botryodiplodia theobromae*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxyporum*, *Fusarium solani* and *Rhizopus stolonifer*. The pathogenicity test showed that these six spoilage fungi: *Botryodiplodia theobromae*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Fusarium solani* and *Rhizopus stolonifer* cause rot of yam. The test plants used were: *Allium sativum* (bulb), *Ocimum gratissimum* (leaf), *Zingiber officinale* (rhizome) and *Nicotiana tabacum* (leaf). From (Tables 1 & 2), *Allium sativum* was found to have the highest inhibitory effect of aqueous extract of 65 and 85% as well as 25% ethanol extract. The control experiment revealed uninhibited growth of the pathogens.

Several works have been done on the areas of tuber rot of yam caused by several micro-organisms in stored yam tubers and on the field (Ogundana *et al* 1970; Adeniyi, 1970; Okigbo and Ikediugwu, 2000; Okigbo, 2001). Micro organisms that have been reported to cause tuber rot also in storage have also been identified (Ogundana *et al.* 1970; Okigbo and Ikediugwu, 2000, Okigbo, 2002). These fungi included those that were identified in this investigation. Ogundana *et al* (1970) reported that in most cases, pathogens gain entry into yams through natural opening and wounds that occur mechanically in harvesting and transit form field to storage barn or market.

However, yam tubers during harvest might already be infected by phytopathogens derived from disease foliage, roots or parent tubers. Yam tubers which are already attacked by rot phytopathogens when harvested get spoiled to a greater extent in storage.

Okigbo and Ikediugwu (2000) used biological control measure to control yam rot, *Trichoderma viride* displaced the naturally occurring mycoflora on the surface of the yam tuber.

Okigbo (2002) also used *Bacillus subtilis* to control phytopathogens that affected white yam. Qasem and Abu-Blan (1966); Amadioha and Obi (1999); Okigbo and Ajalie (2005); Okigbo *et a l* (2005)

and Okigbo, (2009) found out that the active principles present in plants are influenced by many factors such as age of plants, extracting solvent, methods of extraction and time of harvesting plant materials.

The presence of antifungal substance in the different extracts which cause inhibition of radial growth and spore germination *in vitro* agreed with the report of other workers (Qasem and Abu-Blan 1996; Amadioha 2000; Okigbo and Nmeka, 2005). The difference observed in fungitoxic activity of the extracts might be due to the solubility of the active compounds in water and ethanol or the presence of inhibitor to the fungitoxic principles. This also agreed with the report of Qasem and Abu-Blan (1966); Amadioha (2000).

The present investigation showed that *Ocimum gratissimum, Zingiber officinale, Allium sativum* and *Nicotiana tabacum* have proved effective against mycelia inhibition and spore germination of many rot causing pathogens of yam tubers.

The extracts could be used as protective pesticide, since mycelia inhibition of the pathogens was effective. The result of this work showed that the test plants have potentials to control post harvest yam rot. This can provide an alternative way of reducing and controlling rot by farmers. Fungicides of plant origin are environmentally safe and non phytotoxic. The extracts of the local plants can easily be prepared by the local peasant farmers.

Erute and Oyibo (2008) used O*cimum gratissimum* to control post harvest pathogen of *Persea americana* (avocado)

Okigbo and Nmeka (2005) control yam rot with leaf of *Zingiber officinale*. Taiga *et al* (2008) inhibited the growth of *Fusarim oxysporum* mycelium with cold extract of *Nicotiana tabacum* and Udo *et al* (2001) reduced the growth and sporulaton of fungal pathogens on sweet potato and yam with garlic (*Allium sativum*).

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