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Effects of ten plant extracts on mycelial growth and conidial production of four fungi associated with yam tuber rot

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Abstract

The effects of extracts from ten plant species on the mycelial growth and conidial production of four major fungi associated with yam tuber rot were investigated. Extracts of *Allium sativum, Ocimum gratissimum, Cassia alata, Azadiracta indica,* and *Hibiscus rosa-sinensis* were found to be effective in reducing mycelial growth as well as conidial production at varying degrees *in vitro.* Their incorporation into plant protection programs will be a good complement in integrated crop disease management.

Key words: Plant extracts, mycelial growth, fungi and yam rot

Introduction

Yam (*Dioscorea* spp.) constitute a major food crop of West and Central Africa, with West Africa as the most important yam-producing region of the world (Coursey 1967). The West African yam belt comprises Nigeria, the Republic of Bénin, Togo, Ghana, Cameroon, and Côte d'lvoire. This, sub-region produces about 90% of the world's yam, estimated at 43 million t and Nigeria alone produced 31.1 million in 2007 (FAO 2007).

There are about 600 species of *Dioscorea*, however *D. rotundata* is the most important yam species in West Africa. It is the most widely cultivated in the yam belt of Nigeria because of its economic value and uses (Degras 1993). The tuber is the only economically important part of the crop and it is consumed roasted, boiled, pounded, or as dough prepared in hot water from flour. Cultivated yams are a source of carbohydrate, protein, amino acids, and minerals (Degras 1993). In addition to the nutritional value, yam has considerable social and cultural significance, especially among the people of south-east Nigeria (Nweke and Winch 1980; Okorji and Obiechina 1985).

Pests and diseases affect the crop both in the field and in storage. A conservative estimate indicates that about 15 % of yam produced annually never reach the market, because of post harvest losses. Coursey (1967) reported that over one million t of yam tubers are lost annually in storage in West Africa. Okoh 1997 also reported an economic loss of 10.45% of the expected revenue during storage. The Food and Agriculture Organization reported that 7.9 of the 26.4 t of yams produced in Nigeria in 1999 were declared as wasted (FAO 2000). The invasion of yam tubers by microbial pathogens, especially fungi, is considered the critical factor in yam decay (Degras 1993). Early work on fungi associated with the post harvest rot of yam in Nigeria include those of Okafor (1966), Adeniji (1970), Ogundana et al (1970), Noon (1978) and Ikotun (1983). The colour, magnitude, and texture of the symptoms vary with the organisms responsible for the decay (Efiuvwevwere and Nwachukwu 1998). Three fungal genera, *Botryodiplodia, Fusarium,* and *Penicillium,* have been reported to cause extensive rotting of tubers. These genera are highly pathogenic and are extremely widespread but the level of damage varies with the region, yam variety, and season (Degras 1993). *Botryodiplodia theobromae* causes dark brown rot which may be pink at the beginning on *D. trifida* L.*,* or dirty grey on *D. rotundata* (Ricci and Arnolin 1973; Ogundana et al 1970). *Fusarium oxysporum* causes pale-pinkish dry rot which may be darker around the edges (Degras 1993; Efiuvwevwere and Nwachukwu 1998). The *Fusarium* rots are often considered to be secondary to nematode attacks

because they are superficial (Bridge 1972). *Penicillium* species cause a hard but dry brown rot which turns wet and soft when invaded by bacteria (Adeniji 1970). *Penicillium oxalicum* is one of the most widely studied of the *Penicillium* species. Lesions on infected tubers are usually covered with green sporulation (Degras 1993). Ikotun (1983) reported *Aspergillus niger* to be among the fungi responsible for severe decay of yam tubers in Nigeria. These fungal pathogens may cause infections either singly or in combination with several others. Other fungi that have been reported to cause storage rot of yam include *Rhizopus nodosus* (Ikotun, 1983), *Sclerotium rolfsii* (Ejechi and Ilondu 1998), and *Rhizoctonia solani* (Sangoyomi 1995).

Preservation of yam using chemicals (Ogundana 1972) and gamma irradiation (Adesuyi 1978) are either too sophisticated or too costly for peasant farmers who are responsible for nearly all yam produced in Nigeria. The use of plant products and extracts in disease control has been reported for some crops such as maize and cowpea, (Awuah 1989, 1994; Ekpo 1991, 1999; Owolade et al 1999) but has been sparsely used in the control of yam diseases.

Materials and Methods

Effects of 10 plant extracts on mycelial growth inhibition. The experiment was conducted in the Yam Pathology Laboratory of IITA using a completely randomized design with four replicates. Fully expanded leaves of *Acalypha wilkersiana, Azadirachta indica, Cassia alata, Chromolaena odorata, Cymbopogon citratus, Ocimum gratissimum,* flowers of *Hibiscus rosa-sinensis,* bulbs of *Allium sativum*, stems of *Enantia chlorantha* and rhizomes of *Zingiber officinale* were used in the preparation of extracts. Five water extract concentrations were prepared by blending 1 g, 5 g, 10 g, 50 g, and 100 g of the plant part in sterile distilled water that was made up to 100 ml to produce 1%, 5 %, 10 %, 50 %, and 100 % extract concentrations. The extracts were sieved through four layers of cheese-cloth and their effects were studied on the growth of four fungal pathogens (*Botryodiplodia theobromae, Sclerotium rolfsii, Fusarium oxysporum* and *Penicillium oxalicum*) using the food poisoning technique (Nene and Thapliyal 1979). Extracts were used soon after preparation. One millilitre of each extract was dispensed per Petri dish and 9 ml of molten potato dextrose agar (PDA) was added to prepare a PDA-extract mixture giving corresponding 0.1%, 0.5%, 1%, 5% and 10% extract concentrations. These concentrations were used to test their effects on mycelial growth and the production of the conidia, pycnidia, and sclerotia of appropriate fungi.

The plates were gently rotated to ensure even dispersion of the extracts. The agar-extract mixture was allowed to solidify and then inoculated at the centre with a 4 mm-diameter mycelial disc obtained from the colony edge of a 7-day old culture of each of the test fungi. Four plates per replicate and three replicates per fungus per extract were inoculated. The control set-up consisted of blank agar plates (no extracts) inoculated with the test fungi as described above. All plates were incubated at 27 ºC and radial growth was measured daily for four days. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. Percentage inhibition of mycelial growth was calculated according to the method described by Whipps 1987:

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_1} x \frac{100}{1}$$

where R_1 is the furthest radial distance of pathogen in control plates
and R_2 is the furthest radial distance of pathogen in extract-incorporated agar plates

The inhibition percentage was determined as a guide in selecting the minimum inhibitory concentrations that will be effective in controlling rot-causing fungi. Extracts were rated for their inhibitory effects using the scale:

≤ 0% inhibition (not effective);
>0-20% inhibition (slightly effective);
>20-50% inhibition (moderately effective);
>50-<100% inhibition (effective);
100% inhibition (highly effective).

Effects of 10 extracts on the production of conidia, pycnidia, and sclerotia. Plant extracts at a concentration of 1% were prepared and used as described above. Inoculated plates were incubated at 27 °C for 10 days after which the production of conidia, pycnidia, and sclerotia was investigated using completely randomized design with four replicates. For conidial production, extract-impregnated plates were inoculated with 4-day old mycelial discs of test fungi. The control set-up consisted of blank agar plates with no extracts incorporated but inoculated with the mycelial discs of the test fungi. Conidial suspensions were obtained from the 10-day old cultures of *Fusarium oxysporum* and *Penicillium oxalicum* using the method of Fajola and Nwufo 1985. Ten ml of sterile distilled water containing 0.01%

Tween 80 was added to the surface of each culture and the surface was gently rubbed using a fine brush to dislodge conidia into suspension and then filtered through four layers of cheese cloth. Each replicate plate was washed into a conical flask and the number of conidia was counted using a hemacytometer (Fisher scientific CIAT 0267110). The pycnidia produced by *Botryodiplodia theobromae* and sclerotia produced by *Sclerotium rolfsii* were directly counted on plates using a binocular stereo miscroscope. Two perpendicular lines were drawn on the reverse sides of the plates and then pycnidia and sclerotia were counted in each of the four sectors in four replicate plates of *Botryodiplodia theobromae* and *Sclerotium rolfsii*. Data obtained were subjected to the general linear model (GLM) of Statistical Analysis System (SAS 1999). Means separation was carried out using standard error of difference.

Results

Effects of ten plant extracts on mycelial growth of *Botryodiplodia theobromae.* The effects of plant extracts on the mycelial growth inhibition of *Botryodiplodia theobromae* are shown in Table 1. At 0.1% extract concentration, extracts of *A. wilkersiana* , *C. odorata*, *E. chlorantha*, *H. rosa-sinensis* and *O. gratissimum* had no inhibitory effect on mycelial growth. At 0.5% extract concentration, mycelial growth inhibitions ranged from 0.8 to 88.5% in *O. gratissimum* and *A. sativum*. At 1% extract concentration, extract of *A. sativum* caused 100% inhibition of mycelial growth. Extract of *E. chlorantha* and *H. rosa-sinensis* had no inhibitory effects on mycelial growth of *B. theobromae* while extracts of *A. indica, C. alata,* and *C. citratus* caused 51-71% inhibition of mycelial growth. The inhibitory effects of plant extracts at 5% concentration were similar to those at 1% concentration and were only slightly lower than those of the corresponding 10% concentration.

Effects of ten plant extracts on mycelial growth of *Penicillium oxalicum.* The effects of plant extracts on mycelial growth of *Penicillium oxalicum* are shown in Table 2. There was a general increase in mycelial growth inhibition of *P. oxalicum* with an increase in extract concentration. At 0.1% extract concentration, inhibition ranged from 0.7 to 31.0%. Extracts of *A. wilkersiana*, *C. citratus,* and *E. chlorantha* had no inhibitory effect on mycelial growth while others were either slightly effective (<20% inhibition) or moderately effective (21–50% inhibition). At 0.5% extract concentration, all extracts inhibited mycelial growth to varying degrees (2.4–75.1%). Extracts of *C. odorata*, *A. indica,* and *O. gratissimum* were moderately effective (21–50% inhibition) and *A. sativum* had 75.1% inhibition. At 1% extract concentration, *A. sativum* extract caused 100% inhibition of mycelial growth. In general, inhibition percentages (14.7–57.5%) at 1% extract concentration in the remaining nine extracts were only slightly lower than those of the corresponding 5 and 10% extract concentrations.

Effects of ten plant extracts on mycelial growth of *Fusarium oxysporum.* All plant extracts, except that from *E. chlorantha* at 0.1 and 0.5% concentrations, caused mycelial growth inhibition of *F. oxysporum* (Table 3). There was a general increase in percentage inhibition with an increase in extract concentration. At 0.1% extract concentration, inhibitions ranged from 1.4 to 38.8% and extracts of *A. sativum* (38.8%) and *Z. officinale* (23.7%) were moderately effective. At 1% extract concentration, five of the tested extracts (*A. indica, A. sativum, C. citratus, H. rosa-sinensis* and *Z. officinale* were effective (>50 <100% inhibition) in mycelial growth inhibition. The least inhibition was observed using the extract from *A. wilkersiana* (7.8%). At 5% extract concentration, extract of *A. sativum* caused 100% inhibition of mycelial growth and the inhibition percentages (8.6–89.9%) at 10% extract concentration in the remaining nine extracts were slightly higher than those of the corresponding 1% concentration.

Effects of ten plant extracts on mycelial growth of *Sclerotium rolfsii.* The effects of plant extracts on mycelial growth of *Sclerotium rolfsii* are shown in Table 4. At 0.1, 0.5, and 1% extract concentrations, extracts of *A. wilkersiana*, *C. odorata* and *H. rosa-sinensis* had no inhibitory effect on the mycelial growth of *Sclerotium rolfsii*. However, the extract of *E. chlorantha* had a stimulatory effect on the mycelial growth of the pathogen at 0.1% concentration. At 0.5% extract concentration, mycelial growth inhibition ranged from 1.8 to 38.4% and *A. sativum* extract caused 100% inhibition. At 1% concentration, extracts of *A. indica, C. alata,* and *C. citratus* were effective (>50<100% inhibition) in reducing the mycelial growth of *S. rolfsii* while others were slightly effective (<20% inhibition). Radial growth inhibition percentages at 5% extract concentration ranged from 0.8 to 87.0%. At 10% extract concentration, inhibition percentages were slightly higher than those of the corresponding 5% extract concentration.

Effects of ten percent concentration of plant extracts on production of pycnidia, sclerotia, and conidia. There were significant differences in the number of pycnidia produced on different PDA/ extract combinations (Table 5). Crude extracts of *A. sativum, C. citratus, and H. rosa-sinensis* completely inhibited pycnidium formation while significantly ($p \leq 0.05$) fewer pycnidia (2 or 3) were recorded on plates containing extracts of *C. alata* and *Z. officinale* compared to a count of 16 pycnidia in the control. Significantly ($p \leq 0.05$) more sclerotia (31) were recorded on medium containing *E. chlorantha* extract than on the control. The remaining extracts had no significant effects on the production of pycnidia.

Crude extracts of *A. sativum* and *Z. officinale* completely inhibited formation of sclerotia. Fewer ($p \leq 0.05$) sclerotia (7–160 per plate) were produced on PDA containing the other extracts than on the control (225 per plate). There were significant differences in the number of *P. oxalicum* conidia produced in different PDA/extract combinations. *Allium sativum* extract completely inhibited the production of conidia. There were no significant differences in the number of conidia of *S. rostrata* produced in various PDA/extract combinations. However, significantly ($p \leq 0.05$) higher conidia counts were recorded on media containing *C. odorata* extract than on the control.

Discussion

Crude extracts from ten plants were used in this study to develop cheap and simpler methods of yam rot control for use by farmers. The investigation showed that the most fungitoxic extracts obtained were from *Allium sativum, Ocimum gratissimum, Cassia alata, Azadiracta indica,* and *Hibiscus rosa-sinensis*. These extracts were able to inhibit growth of mycelia and reduce production of conidia in the four major fungi associated with yam rot during storage. This agrees with the reports of Okigbo and Nmeka (2005); and Okigbo and Ogbonnaya (2006). This study identifies the potential of these plant extracts and therefore recommends their use as natural fungicides. *Allium sativum* extracts, even when used at low concentrations, were found to be effective against the test fungi in this study. Allicin (the main biologically-active compound of garlic) has been reported to be soluble in water as well as a natural antimicrobial botanical that can disable an unusually wide variety of infectious organisms (Shashiskanth et al 1986) although it is highly unstable (Freeman and Kodera (1995). Further studies into how to stabilize the active ingredients are essential. *Ocimum gratissimum* was also effective *in vitro* in the control of rot-causing fungi in agreement with the reports of Awuah (1994), where it was reported to reduce radial growth of *Rhizopus* spp. and *Ustilaginoidea virens.* Crude steam distillate sprayed onto infection courts also inhibited the pathogen and lesion development (Awuah 1989).

The preparation of crude extracts from plants for disease control is relatively cheap and can easily be accepted by peasant farmers. From the point of view of environmental impact assessment, it is also a safer alternative to the hazardous and expensive conventional fungicides. The use of plant extracts in the control of fungal rot of yam in storage presents no potential toxicity to man. The extracts investigated in this study are from plants that are commonly used for medicinal purposes and have been widely studied.

Table1. Effects of plant extracts on the mycelial growth inhibition of *Botryodiplodia theobromae* incubated at 27 ºC for 4 days.

	Mycelial growth inhibition (%) at specified extract concentration				
Botanical	0.1%	0.5%	1%	5%	10%
Acalypha wilkersiana	0.0	0.0	9.3	9.5	9.8
Allium sativum	28.3	88.5	100.0	100.0	100.0
Azadirachta indica	8.0	26.5	70.6	76.4	80.2
Cassia alata	2.4	8.3	53.2	59.1	66.0
Chromolaena odorata	0.0	0.0	1.9	1.9	2.1
Cymbopogon citratus	10.0	19.9	60.2	65.6	65.9
Enantia chlorantha	0.0	0.0	0.0	0.0	0.0
Hibiscus rosa-sinensis	0.0	0.0	0.0	0.5	0.7
Ocimum gratissimum	0.0	0.8	2.7	2.9	3.6
Zingiber officinale	0.4	8.2	3.9	39.6	39.7
Mean	4.9	15.2	30.2	35.6	36.8
S E D	1.4	2.4	2.8	2.8	2.8

Table 2. Effects of plant extracts on the mycelial growth inhibition of *Penicillium oxalicum* incubated at 27 ºC for 4 days

	Mycelial growth inhibition (%) at specified extract concentration				
Botanical	0.1%	0.5%	1%	5%	10%
Acalypha wilkersiana	0.0	18.4	34.8	35.0	35.2
Allium sativum	20.0	75.1	100.0	100.0	100.0
Azadirachta indica	2.0	24.4	57.5	59.2	61.1
Cassia alata	4.8	10.6	49.2	49.8	52.0
Chromolaena odorata	0.0	20.3	38.0	41.0	42.0
Cymbopogon citratus	0.0	20.0	46.9	48.0	48.0
Enantia chlorantha	0.0	2.4	31.4	35.8	38.1
Hibiscus rosasinensis	2.3	12.3	14.7	15.0	16.1
Ocimum gratissimum	24.8	31.0	50.8	61.3	71.5
Zingiber officinale	0.7	6.0	40.5	42.8	46.6
Mean	5.5	22.1	46.3	48.8	51.1
S E D	1.5	2.0	21.0	2.1	2.2

Table 3. Effects of plant extracts on the mycelial growth inhibition of *Fusarium oxysporum* incubated at 27 ºC for 4 days.

	Mycelial growth inhibition (%) at specified extract concentration				
Botanical	0.1%	0.5%	1%	5%	10%
Acalypha wilkersiana	1.4	6.4	7.8	8.2	8.6
Allium sativum	38.8	50.1	63.8	100.0	100.0
Azadirachta indica	21.4	32.0	62.8	71.0	76.5
Cassia alata	8.0	20.1	43.0	60.4	64.4
Chromolaena odorata	3.9	25.0	30.5	31.2	36.4
Cymbopogon citratus	15.1	25.0	61.5	64.5	68.0
Enantia chlorantha	-2.3*	-2.2	9.4	12.0	12.9
Hibiscus rosa-sinensis	13.0	33.1	81.0	86.8	89.9
Ocimum gratissimum	4.3	14.2	43.0	46.0	49.1
Zingiber officinale	23.7	50.2	62.0	66.0	71.0
Mean	12.7	25.4	46.5	48.1	57.7
S E D	1.6	1.9	2.2	2.5	2.5

* Negative sign indicates stimulatory effects of extracts

Table 4. Effects of ten plant extracts on the mycelial growth inhibition of *Sclerotium rolfsii* incubated at 27 ºC for 4 days.

	Extract concentrations				
Botanical	0.1 %	0.5 %	1 %	5 %	10 %
Acalypha wilkersiana	0.0	0.0	0.0	5.0	7.0
Allium sativum	9.6	100.0	100.0	100.0	100.0
Azadirachta indica	32.1	38.4	71.6	78.5	82.6
Cassia alata	9.6	23.8	86.4	87.0	87.9
Chromolaena odorata	0.0	1.8	7.3	8.7	12.2
Cymbopogon citratus	9.9	23.2	59.0	61.0	63.5
Enantia chlorantha	-1.2*	0.0	0.0	0.8	1.0
Hibiscus	0.0	0.0	0.0	9.5	9.6
Ocimum gratissimum	4.7	6.3	9.0	9.6	9.9
Zingiber officinale	2.8	11.9	16.0	22.7	29.1
Mean	5.7	17.5	34.9	38.3	40.3
S E D	1.4	2.5	2.8	2.7	2.7

* Negative sign indicates stimulatory effects of extracts

Table 5. Effects of 10% concentration of plant extracts on the production of pycnidia by *Botryodiplodia sclerotia* by *Sclerotium rolfsii* and conidia by *Penicillium oxalicum* after 10 days of incubation at 27 ºC

Extracts	Number per plate		Sporulation intensity		
	B. theobromae pycnidia	*S. rolfsii* sclerotia	*P. oxalicum* ($x10^5$)	*F. oxysporum* Micro ($x10^5$)	*F. oxysporum* Macro ($x10^5$)
Acalypha wilkersiana	21.5	159.80	18.2	8.0	5.1
Allium sativum	0.00	0.00	0.0	4.0	1.1
Azadirachta indica	19.00	10.80	6.0	1.6	0.1
Cassia alata	2.50	7.30	1.7	7.0	0.5
Chromolaena odorata	11.80	122.50	15.8	3.4	0.1
Cymbopogon citratus	0.00	0.30	5.2	1.1	0.1
Enantia chlorantha	31.30	85.50	13.9	37.3	2.9
Hibiscus rosa-sinensis	0.00	149.00	5.1	28.1	2.4
Ocimum gratissimum	12.30	6.80	11.3	0.0	0.0
Zingiber officinale	2.30	0.00	0.5	1.5	0.4
Control	15.80	224.50	20.0	10.8	0.6
Mean	12.50	69.70	8.9	9.3	1.0
S.E.D.	4.37	18.38	0.8	0.2	0.3

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