Inhibitory effects of two indigenous plant extracts (*Zingiber officinale* and *Ocimum gratissimum*) on post harvest yam (*Dioscorea rotundata Poir*) rot, *in vitro*.

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Abstract: Cold water and ethanol extracts of two fungicidal plants (*Zingiber officinale* and *Ocimum gratissimum*) were screened for their *in vitro* effects on rot fungi of yam using 60 and 80% aqueous extract and 20 and 30% ethanol extract of each concentration. The two concentrations of aqueous and ethanol extracts were found to have inhibitory effects on all the rot fungi isolated from yam, 80% *a*queous extract of *Zingiber officinale* inhibited *Fusarium oxysporum* to 66.70%, 80% aqueous extract of *Ocimum. gratissimum* inhibited *Botrydioploidia theobromae* to 60.00% also73.33% inhibition of *Aspergillus flavus* was recorded using 30% ethanol extract of *Zingiber officinale*, the same concentration of *Ocimum gratissimum* inhibited *Aspergillus niger* to 70.00%. Both aqueous and ethanol extract of *Zingiber officinale* and *Ocimum gratissimum* had potential inhibitory effect on all the rot fungi.

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Key word – *In vitro*, *Zingiber officinale*, *Ocimum gratissimum*, rot fungi, yam.

Introduction

Several fungi have been identified by different workers as causal organisms of many plant diseases. Adebanjo and Onesirosan (1986) isolated *Colletotrichum gloesporoides* as a fungal pathogen infecting minisetts through infected yam tubers. Ikotun (1989) also isolated many fungi associated with rot of yam tubers.

Synthetic fungicides control approach has proved effective in the control of phyto diseases. Yam has been protected against rot using borax (5% aqueous solution), copper 8 – hydroxyquinolinoate (4% aqueous solution) and lime ash (Coursey, 1961). Ogundana and Denis (1981); Plumbey (1985) enumerated many other synthetic fungicides active against some rot causing phyto pathogens. Nwakiti *et al.* (1990) listed some defensive synthetic fungicides which have been proved efficacious in controlling some phyto pathogens; they averred that those fungicides were costly and therefore are not economically viable. The continuous and unguided use of chemicals in agricultural processes potent an acute and chronic toxicity to man and livestock (Kamel and Manga, 1987).

The use of these chemicals can result to death by accumulating in man, poison and concentrate in the food chain as they are usually not eco – friendly. They can induce resistance and resurgence of phyto pathogens, pest population and as well as cause death of flower pollinators, predators and parasites. In this respect, it is necessary to search for dependable and sustainable antidotes that regard the requirement of man and its environment.

The use of pesticides of botanical origin has been pin – pointed by many researchers as an option to synthetic fungicides, as an alternative to the havoc and contamination difficulty identified with the indiscriminate application of fungicides (Amadioha, 1998, 2000; Amadioha and Obi, 1998, 1999). Therefore, the effectiveness of botanical extracts requires investigations. Ejechi and Ilondu (1999) used the sawdust extract from cam wood to control yam rot caused by *Sclerotium rolfsii*. The significance of natural bio pesticides as possible sources of pathotoxicity as they are systematic and easily biodegradable has been stressed in the separate work of (Al Abed *et al* 1993, Amadioha and Obi 1998, 1999, Amadioha 2000 and Olufolaji, 1999; Okigbo, 2009).

This investigation is therefore targeted at the inhibitory effects of two tropical, indigenous plant extracts of *Zinginber officinale* and *Ocimum gratissimum* on yam rot fungi at *in vitro* study.

Methodology

Collection of yam tubers that showed symptoms of softness were collected from the market at Ado-Ekiti, Nigeria. Yam tubers with softness of tissues were identified as being rotted, fresh and healthy yam tubers were also collected, packed into a polythene bag already lined with tissue paper and taken to the laboratory for further studies.

Collection of plant materials

Ocimum gratissimum was purchased from the market at Ado- Ekiti while *Nicotiana tobacum* was collected in the vegetation reserve of Ado-Ekiti, Nigeria. These plants were taken to the herbarium unit of University of Ado-Ekiti for proper identification.

Isolation of spoilage fungi from rotten yam tubers.

Pieces of yam tuber 3 x 3 x 2mm in dimension was cut from advancing edge of a rot, they were surface sterilized in 70% alcohol for 1minute and dried on sterile tissue paper and plated out on potato dextrose agar (PDA) already incorporated with streptomycin. A minimum of three replicated pieces from each of the rotted portion were plated out. The plates were incubated at room temperature for five days and fungi associated with rot affected tissue were identified and their frequency of occurrence determined using method of Okigbo and Ikediugwu (2000).

Pathogenicity tests

The method of Okigbo and Ikediugwu (2000) was used, cylindrical cores, 1cm deep, were removed from various spots of a healthy yam tuber with sterile 5mm cork borer and then 4mm discs taken from the edge of a colony of test fungus were place downward into each of the holes in the tuber. The cores of the yam tuber were replaced after 2 mm pieces has been cut off to compensate for the thickness of the agar inoculums and the replaced core sealed with melted candle wax. Sterilized PDA was used in place of the culture disc served as control.

Preparation of plant extracts.

Zinginber officinale and *Ocimum gratissimum* 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 last to stand for 24 hrs. The sample was filtered with a Whatman filter paper (No.1) and the filtrate used as the extract. Same process was followed using 30 and 20% ethanol extract concentrations.

Effect of plant extracts on fungal mycelia growth

The method of Amadioha and Obi (1999) was used to determine the effect of the extract on fungal growth. This involved creating a four equal section on each Petri-dish by drawing two perpendicular lines at the bottom of the plate, the point of intersection indicating the centre of the plate. This was done before dispensing PDA into each of the plates. About 2ml of the extract of various plant materials were separately introduced into the Petri-dish containing the media (PDA). A disc (4mm diameter) of the pure culture of each isolate was placed on the extract just at the point of intersection of the two lines draw at the bottom of the Petri dish. Control experiments were set up without addition of any plant material. Fungitoxicity was recorded in terms of percentage colony inhibition and calculated according the formula:

$$\text{Growth inhibition (\%)} = \frac{DC - DT}{DC} \times \frac{100}{1}$$

Where: DC -Average Diameter of control and

DT -Average diameter of fungal colony with treatment.

Result

The isolated fungi from rot affected yam included *Fusarium oxysporum, Fusarium solani, Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae* and *Rhizopus stolonifer*. These fungi were found to cause yam rot from the pathogenicity test. Rot was determined by the softness of tissues. Extract of both *Zingiber officinale* and *Ocimum gratissimum* (cold aqueous and ethanol extract) were found to possess fungitoxic effect on the radial growth of the rot fungal mycelia (Tables1 and 2). There was no remarkable difference in the concentrations of aqueous extract of *Z. officinale* as well as *Ocimum gratisssium*. The highest inhibition of 60.00% was recorded on 60% aqueous extract of *Z. officinale* against *B. theobromae*, whereas 80% aqueous extract of the same test plant inhibited *F. oxysporum* to 66.70%. The highest inhibition using 80% aqueous of *Ocimum gratissimum* was on *B. theobromae* of 60.00% (Table 1). 70% inhibition was observed with 30% ethanol extract of *Z. officinale* on *F.*

oxysporum, 66.66% inhibition was recorded on *B. theobromae* (Table 2) 70% inhibition was obtained using 30% ethanol extract of O. gratissium on *A. niger* being the highest followed in order by *B. theobramae* and *A. flavus* of 66.66% inhibition (Table 2). *B. theobromae* appeared to be the most inhibited by both aqueous and ethanol extracts of the test plants. The highest inhibitory of ethanolic extract was found using 20% ethanol of *O. gratissimum* on *A. flavus* of as much as 73.33% (Table 2).

Table1**.** Percentage inhibition of mycelia radial growth of rot fungi in Potato dextrose agar with aqueous plant extracts of 60% and 80% concentrations. Plant extracts (% inhibition of mycelia growth).

Rot fungi	*Zinginber Officinale*		*Ocimum gratissimum*		Control (mm)
	60%	80%	60%	80%	
A.niger	46.66cd	56.66cd	40.00bc	53.33bc	30.00
F.oxysporum	46.66cd	66.70a	46.66a	46.66d	30.00
R. stolonifer	41.17e	52.94bc	35.30c	46.66d	34.00
B.theobramae	60.00a	46.66d	40.00bc	60.00a	30.00
A. flavus	45.16de	51.62cd	45.16ab	51.62cd	31.00
F. solani	50.00bc	53.33b	50.00a	53.33bc	30.00

Each of the data is a mean of three replacements. Each data followed by the same alphabet along the columns not significantly different at P= 0.05 using (DMRT) Duncan Multiple Test to separate the means.

Table 2**.** Percentage inhibition of mycelia radial growth of rot fungi in Potato dextrose agar with plant ethanol extracts of 20 and 30% concentrations. Percentage inhibition of mycelia growth (means)

Rot Fungi	*Zingiber officinale*		*Ocimum gratissimum*		Control (mm)
	20% ethanol	30% ethanol	20% ethanol	30% ethanol	
A. niger	46.66a	66.66a	46.66a	70.00a	30.00
F.oxysporum	50.00a	70.00a	46.66a	60.00a	30.00
R. stolonifer	47.00a	52.00a	44.11a	52.00a	34.00
B.theobromae	66.66a	66.66a	50.00a	66.66a	30.00
A. flavus	51.62a	73.33a	50.00a	66.66a	30.00
F. solani	50.00a	60.00a	51.62a	53.33a	30.00

Each of the data is a mean of three replacements. Each data followed by the same alphabet along the columns not significantly different at P= 0.05. Using (DMRT) Duncan Multiple Range Test to separate the means.

Discussion.

The radial growth of all the rot fungi: *Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Fusarium solani* and *Botryodiplodia theobromae* were significantly inhibited by the two test plant: *Zingiber officinale* and *Ocimum gratissimum.* This indicates that they have fungitoxic potential, though none of these had 100% inhibition of the radial growth of the mycelia in the Petri dish within the period of observation (Tables and 2).

The results of aqueous and ethanol plant extracts on PDA medium revealed that *Z. officinale* and *O. gratissimum* extracts were mycotoxic to the fungal pathogens but 100% inhibition was not recorded. 30% ethanol extract of *Z. officinale* was found to be more effective than 20% ethanol extract of the same test plant; this was also observed with 30% ethanol extract of *O. gratissium.* Okigbo and Ogbonaya (2006) used leaf extracts of *O. gratissimum* to control post harvest yam rot through cold and hot water, and ethanol extracts. *O. gratissimum* L. is commonly used in folk medicine to treat infection, diarrhea, skin diseases, pneumonia, cough and also conjunctivitis (Onajobi, 1986). It is of family *Leguminoceae*, is grown in garden and used as a tea leaf for fever. It is widely distributed in tropical and warm temperature region (Dalziel, 1937).

Okigbo and Nmeka (2005) used extract of *Z. officinale* to control yam tuber rot. *Z. officinale* Roscoe

family *Zingiberaceae*, is a herbaceous perennial plant which has an upright stems and narrow medium, green leaves arranged in two ranks on each stem. *Z. officinale* or ginger has been used in Asia for relief from arthritis, rheumatism, coughs, fever and infectious diseases (Anonymous 2004 b). Biological control is generally favoured as a method of plant diseases management because it does not have demerit of chemicals (Amadioha and Obi, 1999). Kuhn and Hargreaves (1997) observed that substances found fungicidal in vitro in almost cases kill the fungi *in vivo*. Plants with such fungicidal properties include *Z. officinale* Roscoe (Maurice, 1993).

This work showed that fungitoxic compounds were present in *Z. officinale* and *O. gratissimum* since they were able to control the growth of microbes tested. This agreed with earlier work of some workers on effects of these plants on phyto pathogens of other crops (Amadioha and Obi 1998, 1999). Amienyo and Ataga (2007) used Z. *officinale* extracts to protect mechanically injured sweet potato tubers. Fokunang *et al* (2000) used crude extracts of *Ocimum gratissimum* to control cassava anthracnose diseases. Okigbo *et al* (2005) used *O. gratissimum* against some pathogens of man. Amadioha and obi (1999) also used *O. gratissimum* to control anthracnose diseases of cowpea. The result of this investigation showed that both *Z. officinale* and *O. gratissimum* have potential to control post harvest rot of yam. This can serve as an alternative means of reducing and controlling rot by yam growers and consumers. The pesticides of botanical origin are environmentally non – hazardous by being non phototoxic. Furthermore, the extracts of these botanicals can be easily formulated and applied with little or no literacy of the rural dwellers.

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