Evaluation Of Antifungal Effects Of Extracts Of *Allium Sativum* And *Nicotiana Tobacum* Against Soft Rot Of Yam (*Dioscorea Alata*).

Ijato James Yeni

DEPARTMENT OF PLANT SCIENCE, FACULTY OF SCIENCE, UNIVERSITY OF ADO-EKITI P.M.B 5363, EKITI STATE, NIGERIA.

E-mail: jamesyeni@yahoo.com; GSM: 08067335124

Abstract: The antifungal effects of *Allium sativum* (rhizome) and *Nicotiana tobacum* (leaf) extracts on rot causing organisms on yam: *Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer, Botryodiplodia theobromae, Aspergillus flavus* and *Fusarium solani*. Treatment with aqueous and ethanolic extracts of the test plants significantly reduced the radical growth of the pathogens *in vitro*. Value 76.66% inhibition of *Botryodiplodia theobromae* was obtained using 80% aqueous extract of *Allium sativum*, 60% inhibition of *Fusarium oxysporum* was obtained using 80% aqueous of *Nicotiana tobacum*, 86.66% inhibition of *Botryodiplodia theobromae* was obtained using 30% ethanol on *Allium sativum* and 60.5% inhibition of *Aspergillus niger* was obtained using 25% ethanol to extract 70% inhibition of *Fusarium solani* was obtained using 30% ethanol to extract on *N. tabacum* both the aqueous and the ethanolic extract of the test plants were found to be more active as bio-killer on yam rot organisms.

[Ijato James Yeni**. Evaluation Of Antifungal Effects Of Extracts Of *Allium Sativum* And *Nicotiana Tobacum* Against Soft Rot Of Yam (*Dioscorea Alata*).** Researcher. 2011;3(2):1-5]. (ISSN: 1553-9865). http://www.sciencepub.net.

Key-word: *Allium sativum*, yam, *Nicotiana tobacum*, plant extract, rot organisms.

Introduction

Cultivated yams belong to the family *Dioscoreacea* and the genus Dioscorea (Coursey, 1967) .The most cultivated species in Nigeria are the *D. rotundata* (white yam) D. *cayenesis* (yellow or guinea yam) and D. *alata* (water yam). There are all also species of wild yam found growing in Nigeria whose tubers are collected for eating during food scarcity yams are good sources of carbohydrate to the people of South and Central America, Caribbean, Pacific Islands Asia, tropical and subtropical Africa (Coursey, 1967; Adelusi and Lawanson, 1987). *Dioscorea* sp are staple foods in the tropics (Han *et al*, 1987). Yam tubers, after peeling can be cooked in various ways by boiling and then pound; roasting and frying are also employed in preparing yam. Yam tubers in Nigeria are also processed into intermediate and end product forms. (Okaka and Anajekwu, 1990), which are consumable by animals, yam could also be processed into snacks or made into flour useful for man (Coursey, 1983; Okaka and Okechukwu, 1987).

From the Global production of yam annually, Nigeria alone produced 22 million metric ton FAO (1998). Through this figure is high, demand for yam has always being more than its supply. It is estimated that an average of over 25% are lost to diseases and pest (Arene, 1987; FAO, 1998; FAO 2008; Onayemi, 1983) over 50% of the yam tuber in Nigeria are lost in storage. Chemicals have proved useful over the ages to control yam rot but recently these pathogens are now developing resistance, therefore, the need of alternative method such as the use of plant extracts to control the menace is vital, as plant extracts are eco–friendly, accessible to rural peasant yam producers, less costly and little or no literacy is needed in handling the extract and these extracts are not phytotoxic on plant and animals.

Therefore, it is needful to carry out phytochemical elucidation of these test plants, to precisely determine their toxicity effect on mammal. Plant extracts have been used successfully to control disease in plant and tuber crops (Amadioha and Obi 1999; Okigbo and Emoghene 2004; Okigbo and Nmeka, 2005). This investigation is on the fungicidal efficacy of two tropical plants: *Allium sativum* and *Nicotiana tabacum* on yam rot organisms.

Methodology

Collection of yam

Yam tubers that showed symptoms of softness were collected from a market at Ado–Ekiti, Nigeria. Yam tubers with softness of tissues were identified as being rotten, fresh/healthy yam tubers were also collected, packed into a polyethene bag already lined and taken to be laboratory for further studies.

Collection of plant materials

Allium sativum was purchased from the market of Ado–Ekiti, and *Nicotiana tabacum* 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 piece of yam tuber 3 x 3 x 2 mm in division was cut from advancing edge of a rot they were surface sterilized in 70% alcohol for 1 minute, dried on sterile tissue paper and plated out on (Potato dextrose agar) PDA already incorporated with moisture streptomycin. A minimum of three replicates pieces from each of the rots were plated out. The plates were incubated at room temperature for up to five days and fungi associated with rot affected tissue were identified and their frequency of occurrence determined using methodology of Okigbo and Ikediugwu (2001).

Pathogenicity test.

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

Preparation of extracts

Allium sativum (bulb) and *Nicotiana tabacum* (leaf) where 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 24hr. The sample was filtered with a Whatman filter paper (no1) and the filtrate used as the extract sample process was followed using 30 and 20% ethanol extract.

Effect of the extract on fungal mycelia growth

The method of Amadioha and Obi (1999) was used to determine the effect of extract on fungal growth. This involves 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 dish (4mm diameter) of the pure culture of each isolate was placed on the extract just at the point of intersection of the two lines drawn at the bottom of the Petri-dish. Control experiments were set up without the addition of any plant material. Fungitoxicity was recorded in terms of percentage colony inhibition and calculated according to this formula:

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

Where DC – Average Diameter of control and

DT – Average Diameter of fungal colony with treatment.

Results

The fungi which were isolated from rot affected tissue included *Aspergillus niger*, *Fusarium oxysporum*, *Aspergillus flavus*, *Botryodiplodia theobromae*, *Rhizopus stolonifer* and *Fusarium solani*. All the fungi were found to be responsible for spoilage of yam tubers. Rot was observed by the softness of tissues. Extract of both *A. sativum* and *N. tabacum* (cold aqueous and ethanol extract) were found to have inhibitory effect on the mycelia of all the rot fungi isolated (Table l and 2). Aqueous extract of *A. sativum* (80%) was found to have more inhibitory effect on the mycelia of rot fungi more than 60% aqueous extract. The highest inhibition of 80% was recorded on *B. theobromae* 76.66% while 66.70% inhibition was observed using 60% aqueous extract (Table 1).80% aqueous extract of *N. tabacum* had the highest inhibitory effect on *F. oxysporum* (60.00%) also 60% aqueous extract of *N. tabacum* was not as effective as 80% aqueous extract (Table 1). Ethanol extract of *A. sativum* (30%) was found effective than 20%, the highest inhibition on *B.* theobromae 86.66. 30% ethanol extract of *N. tabacum* was also more efficacious than 20% ethanol extract (Table 2). There was reduction of mycelia of *B. theobromae* most by the plant extracts (Tables 1 & 2) with ethanol extraction having the highest inhibition cumulatively than cold water.

Table 1. Percentage inhibition of mycelia growth of fungi grown in potato dextrose agar incorporated with plant extracts of 60% and 80% cold aqueous concentrations.

Rot fungi	Plant extracts (% inhibition of mycelia growth)				
	Allium sativum		*Nicotiana tabacum*		Control
	60%	80%	60%	80%	
A. niger	53.33bc	60.00d	46.66ab	53.33ab	30.00
F. oxysporum	46.66cd	66.70bc	46.66ab	60.00a	30.00
R. stolonifer	41.17c	64.70cd	44.12c	52.94bc	30.00
B.*theobromae*	66.70a	76.66a	53.33a	53.30ab	30.00
A. flavus	45.16dc	64.50cd	51.62c	51.62c	30.00
F. Solani	53.33bc	53.33c	50.00c	50.00c	30.00

Means with the same letters in the same column are not significantly different at ($P=_{0.05}$) levels according to Duncan Multiple Range Test.

"Table 2. Percentage inhibition of mycelia growth of fungi in potato dextrose agar incorporated with plant extracts of 20% and 30% ethanol concentrations."

Rot fungi	Plant extracts (% inhibition of mycelia growth)				
	Allium sativum		*Nicotiana tobacum*		Control
	60%	80%	60%	80%	
A. niger	60.00a	66.66e	53.33b	60.00b	30.00
F.oxysporum	53.33bc	73.33cd	56.00bc	66.66a	30.00
R. stolonifer	44.11d	70.00cd	47.00c	60.66a	30.00
B.theobromae	53.33bc	86.00a	66.66a	66.66a	30.00
A. flavus	54.85ab	73.33cd	51.62b	66.66a	30.00
F. Solani	51.62c	80.00b	54.85b	70.00a	30.00

Means with the same letters in the same column are not significantly different at ($P=_{0.05}$) levels according to Duncan Multiple Range Test.

Discussion.

The organisms found associated with the rot of white yam in this study were: *Aspergillus niger*, *Fusarium oxyporum*, *Rhizopus stolonifer*, *Botryodiplodia theobromae*, *Aspergillus flavus* and *Fusarium solani*. These organisms were found associated with post harvest rot of yam (Okigbo, 2002, 2005; Ogundana *et al*, 1970). Rot in the store is believed to commence from the soil / field and progresses while in storage. This tends to occur when infected tubers do not reveal visible external symptoms (Ogundana, *et al*, 1970, Ekundayo and Naqvi 1972). This showed characteristic of causal organism.

Antifungal efficacy of some tropical botanical extracts in controlling various phytopathogens has been reported by many workers (Okigbo and Emoghene,

2004; Tewari and Nayaki 1991; Amadioha, 2000; Okigbo, 2009; Okigbo and Nmeka 2005; Amadioha and Obi, 1999; Okigbo and Ikediugwu 2000) but there is dearth of investigations on the use of botanicals as bio-protectors and for control of storage rot of yam (Okigbo and Nmeka 2005).

This study revealed that *A. sativum* and *N. tabacum* have proved effective against mycelia growth of several rot causing microbes using cold water and ethanol extract. Amienyo and Ataga (2007) were able to show that *A. sativum* cold extract was able to control mycelia growth of all the yam rot fungi isolated in this study. Taiga *et al* (2008) showed that *N. tabacum* cold extract inhibited the Mycelia of *F. oxysporum* yam rot organism. The active principle 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 (Quasem and Abu – blan 1996: Okigbo *et al* 2005: Amadioha and Obi 1999; Okigbo and Ajalie 2005). The presence of fungicidal compounds in *A. sativum* and *N. tabacum* which caused the inhibition of mycelia growth in vitro agreed with the reports of other investigators (Okigbo and Nmeka 2005, Quasem and Abu – blan, 1996 and Amadioha 2000). The variation noted in the antimycotic effects of the extracts may be as a result of solubility of the active substances in water or ethanol or the presence of inhibitor against fungicidal principles. This is in tandem with the investigations of Quasem and Abu-blan (1996) and Amadioha (2000).

In conclusion, cold and ethanol extracts of *A. sativum*, and *N. tobacum* could serve as bio – protective agents against rot fungi of yam. These have potentials as alternatives to synthetic fungicide. This method of phyto disease control is eco–friendly, economically viable and not phytotoxic to plants and animals. This method is also at the disposal of peasant yam growers and marketers who may not be able to afford the cost of chemical fungicides.

Correspondent Author:

Ijato james yeni

DEPARTMENT OF PLANT SCIENCE, FACULTY OF SCIENCE, UNIVERSITY OF ADO-EKITI P.M.B 5363, EKITI STATE, NIGERIA.

jamesyeni@yahoo.com

GSM: 08067335124

References.

Okigbo, R.N. Variation in phytochemical properties of selected fungicidal aqueous extract of some plant leaves in Kogi State, Nigeria. A*merican-Eurasian J. of Sust. Agric*. 2009; 3 (3): 407-409.

FAO. Food and Agricultural Organization corporate statistical database. 2008.

Tewari, S.N, Nayak, N. Activity of four plant extracts against three fungal pathogens of rice. *Trop. Agric.* (Trinidad). 1991; 68. 373 - 375

Okaka, J.C and Anajekwu, B. Preliminary studies on food and quality evaluation of dry yam snack. *Trop, Sci .J*. 1990; 30: 67-72.

Onayemi, O. Observations on the dehydration of different varieties of yam and cocoyam. *Abstract, The Int. Soc. For. Trops.* Peru. 1983

Okigbo, R.N Ikediugwu, F.E.O. Studies on biological control of post harvest rot of yams (*Dioscorea spp*) with *Trichoderma viride . J. Phytopathol.* 2000; 148; 351 – 355

Amienyo, C.A and Ataga, E.A. Use of indigenous plant extract for the protection of mechanically injured sweet potato. (*Ipomea batatas* {L} lam) tuber *Science Research and Essay.* 2006; Vol. 2 (5) pp 167 – 170

Taiga, A, Suleiman, M.N, Sule, W, Olufolaji, D.B. Comparative in vitro inhibitory effects of cold extracts of some fungicidal plants on *Fusarium oxysporum* mycelium. *Afr. J. of Biotechnol*. 2008; Vol. 79(18), pp 3306 – 3308.

Quasem, J.R, Abu- blan, H.A. Fungicidal activity of some common weed extracts against different plant pathogenic fungi. *J. phytopath*ol.1996; 44:157 – 161

Okigbo, R.N, Ajallie, A.N. Inhibition of some human pathogens with tropical plants extracts *Chromolaena odorata* and *Citrus aurantifolia* and some antibiotics. *Int. J. Mol .Med. Adv. Sci.* (Pakistan) .2005; 1 (1): 34–40

Ogundana, S.K, Naqvi, S.H.Z and Ekundayo, J.A. Fungi associated with soft rot of yams in storage in storage in Nigeria. *Trans. Br. Mycol . Soc.* 1970; 54:445 – 451

Okigbo, R.N, Mbajuka, C and Njoku, C.O. Antimicrobial potential of (UDA) *Xylopia aethiopica* and *Ocimum gratissimum* L on some pathogens of man. *Int . J. Mol. Med. Adv. Sci.* (Pakistan). 2005; 1 (4): 392 – 397

Amadioha, A. C. Fungitoxic effects of some leaf extract against *Rhizopus Oryzae . Int. J. pest. Manage.* 2000; 52: 311 – 314

Okigbo, R.N. Mycoflora of tuber surface of white yam (*Dioscorea rotundata*) and post harvest control of pathogens with *Baccilius subtilis Mycopathol.* 2002; 156:81-85.

Amadioha, A.C and Obi, V.I. Control of anthracnose disease of cowpea by *Cymbopogon citratus* and *Ocimum gratissimum*. *Acta Phytopathol Entomol. Hungerica.* 1999; 34(2): 85 – 89.
Ekundayo, J.A and Naqvi S.H.Z. Pre-harvest rot of yam (*Dioscorea* spp) in Nigeria. *Trans Mycol. Soc.* 1972; 58(1):15-18
Coursey, D.G. Yams. Longmans London. 1967; p. 230
Okigbo, R.N and Emoghene, A.O. Antifungal activity of leaf extract of some plants species on *Mycosphaerella fijiensis* Morelet, the causal organism of black sigatoka disease of banana (*Musa acuminata*) *KMITL SCI. J.* 2004; 4. 20 –31
Okigbo, R.N and Nmeka, A. Control of yam tuber with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. *Afr. J. Biotechnol.* 2005; 4(8). 804 – 807
F.A.O. Food and Agriculture Organization production, FAO, Rome. 1998.
Adelusi, A.A and Lawanson, A.O. Disease induced carotenoid content of edible yam (Dioscorea spp) by *Botryodiplodia theobromae* and *Aspergilius niger*. *Mycopathologia.*1987; 98: 49 – 58.
Okaka, J.C and Okechukwu, P.E. Yam processing: prospect in quality evaluation of sundried yam. 1987; 30:267-275.
Han, S.K, Osiru, D.S.O, Akoroda and M.O, Otoo, J.A. Yam production and its future prospect outlook on Agriculture.1987; 16: 109-118.
Coursey, D.G. Potential utilization of major root crops emphasis on human, animal and industrial uses part of second Triennial Symposium of the ISTRC Cameroon. 1983.
Arene, O.B. Advances Integrated Control of economic diseases of cassava in Nigeria. In: Haln S K, Cavenes FE, eds Integrated Pest Management for Tropical Root and Tuber Crops. 1987; pp167-175
Okigbo,R.N. Biological control of post harvest fungal rot of yam (*Dioscorea* spp) with *Bacillus subtilis*. Mycopathologia. 2005; 159 (2): 307-314.

9/3/2010