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Control of white yam (*Dioscorea rotundata)* rot pathogen using peel extract of water yam (*Dioscorea alata*)

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ABSTRACT

Antimycotic effects of water yam peel extract using ethanol and methanol as extractants at different concentration on post harvest pathogenic fungi were investigated. Pathogenicity test revealed that Fusarium oxysporum, Rhizopus stolonifer, Botryodiplodia theobromae and Trichoderma viride were the causal organisms of deteriorations in the white yam tuber. Gentamicin, ethanol and methanol extracts were effective in controlling the establishment of the test pathogens in vitro and in vivo. Ethanol extract exhibited the highest potency in inhibition with increase in concentration, followed by ethanol extract. This was attributed to the inherent biochemical constituents of water yam peel extracts that was found to contain alkaloids, tannins, flavanoids, saponins and sterols. Gentamicin, though a known strong antibiotic had the minimum total inhibition. The possibilities that these peels extracts used can serve as source of alternatives to chemical control of yam rot is quite obvious.

Keywords: Water yam, Antifungal, Phytochemicals, Rots, Control, Pathogens.

INTRODUCTION

Yam belongs to the genus *Dioscorea* in the family Dioscoreacea and is one of the most important staple foods in the world, especially some parts of Tropics and Subtropics (Okigbo and Ogbonnaya, 2006). The edible varieties of yam are important food crops and serve as an important carbohydrate staple for millions of people in both the Tropical and Subtropical countries in West Africa, the Carribeans, the Northerrn and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania (Coursey, 1967). The FAO (1989) estimated that the world production is around 20 million ton per year. Nigeria alone produces three quarter of the world total output of yams. Okigbo (2004) noted that of the ten cultivated species, the six most important in Nigeria. Okigbo (2004) noted that of the ten cultivated species, Nigeria are *Dioscorea rotundata* Poir (White yam), *D. cayenensis* Lam (yellow yam), *D. alata* L. (water yam), *D. dumetorum* (cluster or bitter yam), *D. esculenta* loir bark (Chinese yam) and *D. Builbifera* L. (aerial yam). Besides their importance as food source, yams also play significant role in socio-cultural lives of some producing regions. The ritual 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; Ogbo and Agu, 2014a). Coursey, 1967, reported that yam tubers are 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, thiamine and vitamins B and C. Although yams are grown throughout Africa, including countries like Cameroon, Togo, Ghana, Nigeria and Ivory Coast. Nigeria is said to be the world's largest producer of yam accounting for over 70-76 percent of the world total output (FAO, 1989, Frank and Kingsley, 2014a). FAO reported that Nigeria alone in 1989 produced 18.3 million tonnes of yam from 1.5 million hectares, representing 73.8 percent of 28.8 million tonnes of yams produced in Africa. Yam can be grown in nearly all the tropical countries provided water is not the limiting factor (Pius *et al.*, 2006). They also observed that yam in Nigeria is grown within the coastal region up to latitude 12^0N and correspond to the rain forest, wood and savanna and southern savanna belt. Rot is a major factor limiting the post-harvest life of yams and losses can be very

high. Losses due to post harvest rot significantly affect farmers' and traders' income, food security and seed-yams stored for planting. The quality of yam tubers are affected by rots which makes then unappealing to consumers (Ogbo and Agu, 2014b; Agu *et al.,* 2015). Losses of yams in storage mostly to rot are considered to be heavy in Nigeria; as a result the demand for yam tubers has always exceeded its supply (FAO, 1998). Statistics have shown that an average of over 25% of the yield is lost annually to diseases and pests (FAO, 1998). Over 50% of the yam tubers produced and harvested in Nigeria are lost in storage. Most rots of yam tubers are caused by pathogenic fungi such as *Aspergillus flavus, Aspergillus niger* (Tiegh), *Fusarium oxysporum, Fusarium solani, Botryodiplodia theobromae, Penicilllium chrysogenum, Rhizoctonia spp, Penicillium oxalicum, Trichoderma viride*and *Rhizopus nodosus* (Okigbo and Ikediugwu, 2000; Okigbo, 2004; Aidoo, 2007; Frank and Kingsley, 2014b; Agu *et al*., 2014). Microbial deterioration of yam start in the soil reducing its capacity to germinate and its survival in the field and then progressed in storage which occur when infected tubers do not have any sign of external symptoms (Okigbo and Ikediugwu, 2000). The incidence of rotting varies with the species and with varieties within each species of yam (Aidoo, 2007). Nwakiti (1982) reported that rot vary due to variations in the distributions of the microorganisms and does related to the soil mineral status because the differences in the mineral status are not known to be correlated with the type of organism isolated nor total percentage of rot. Several methods have been adopted for controlling losses due to post harvest disease of yam; these include the use of chemicals, biological method of control, curing uses of natural plant extracts, as reported by Amusa *et al.,* (2003). Because of the low capital income of farmers in Nigeria and lack of expertise in the safe handling of chemical, farmers resorted to the method of crop rotation, fallowing, planting of healthy material and destruction of infected crop cultivars in controlling the diseases of yam tubers (Nwakiti, 1982). Chemical method of control has helped to reduce the rate of storage losses and also increases yield obtained, but the problem arising with the use of chemicals is that it is expensive, can cause environmental pollution and may also induce pathogen resistance. Biological control method 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- propagations and self-perpetuating (Okigbo and Ikediugwu, 2000). Some plants are known to synthesize phytochemicals with antimicrobial activities and are used successfully in the control of diseases in humans and crops like yam, cowpea, rice, etc. (Bediako *et al.,* 2007). The advantages of these natural plant products includes its local availability, has little or no toxicity to humans and simple preparation procedures (Okigbo and Nmeka, 2005). Among these plants are the peel extract of water yam (*Dioscorea alata)* Lam of the family Dioscoreaceae. It is a monocotyledonous (Coursey, 1967). They are cultivated for their edible tubers, which can grow up to about 2cm long and with weight up to 45kg. This research work is aimed at Isolation and identification of fungi associated with yam tuber rot and to establish the pathogenicity of fungal organisms associated with tuber rots of white yam varieties as well as to determine the potentiality of the methanolic and ethanolic extract of the peel of *Dioscorea alata* on the identified pathogens and the effectiveness of gentamicin on the identified pathogens.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

Infected white yam yam tubers (*Dioscorea rotundata Poir)* were collected from *Eke*-Awka and *Nkwo-Amaenyi* Markets, as well as healthy tubers of white yam used for pathogenicity testing and were packaged in sterile cellophane bags and taken to the Botany and Microbiology Laboratories of Nnamdi Azikiwe University, Awka for analyses. Water yams (*Dioscorea alata* Lam) were also purchased from the afore-mentioned markets

ISOLATION OF MICRO-ORGANISMS FROM INFECTED WHITE YAM

Infested or rotten tubers of yam (*Dioscorea rotundata* Poir) were rinsed in sterile distilled water and then surfaced disinfected using 70% ethanol. Isolation was done by cutting the decayed tubers to about 3mm-4mm pieces with a sterile knife and dropped into 70% ethanol in a beaker and later washed twice in sterile distilled water. The washed pieces were blotted with sterile filter paper to remove water droplets. Then a sterilized forceps was used in picking up two pieces of the disinfected cut pieces of the yam and inoculated onto SDA medium. All the plates at temperatures of 25-26 ^{0}C for 3-5 days and observed daily for growth of fungi.

CHARACTERIZATION AND IDENTIFICATION

Subculturing of different mycelia colonies from the inoculated plates were done to obtain pure cultures. Sterilized surgical blade was used to cut different mycelia growth and transferred into a newly prepared SDA. The plates (inoculated) were then incubated at 25-26 ^{0}C for 3-5 days. The purified isolates were kept in slants and stored for characterization and pathogenicity test carried out. Macroscopic and Microscopic examinations and referenced against Fungal Atlases *viz*. Barnett and Hunter (2000); Watanabe (2002).

PATHOGENICITY TEST

Fresh healthy yam tubers were washed with sterile distilled water. Thereafter, the tubers were disinfected with 70% ethanol and washed with sterile water. The tubers were allowed to air dry and a flamed 5mm cork borer was used to bore hole into the healthy tubers (Okigbo and Ikediugwu, 2000), Disc of five day old culture of pure isolates of fungi were used to plug the holes created in the *Dioscorea rotundata* (Poir) tubers (healthy) and the disc of the yam in the cork borer was used to cover the surface of the inoculated portion and subsequent sealin with vaseline (Okigbo and Ikediugwu, 2000). The inoculated tubers were incubated for 7 days at 80 ^{0}C. Regular observations were made for fungal infection. These infected tubers were compared with the initial decayed tubers.

EXTRACT PREPARATION

Four to five tubers of water yam (*Dioscorea alata)* were obtained from *Eke*-Awka market and *Nkwo-Amaenyi* and identified. The peel was washed with sterile water and shade dried in a dust free and clean environment. The dried peels were taken to an electric miller and pulverized into powder.

EXTRACT DILUTIONS

The desired percentage weight of solid was weighed (10g-50g) respectively and turned into empty containers, 100ml of each solvent (ethanol, methanol and gentamicin) was mixed with the solid, shaken and allowed for 24 hours to ensure that the yam peels gives forth its extracts. The solution was properly shaken, centrifuged and decanted thereafter.

ANTIFUNGAL BIOASSAY

Toxicity of *Dioscorea alata* was evaluated against the yam tuber rot. The observations were recorded in terms of radial growth of the test fungi on the medium with and without extracts and results were analyzed on the basis of percentage growth inhibitions of test fungi. The inhibition of fungal growth on SDA medium was used to quantify the toxicity of extracts. Percentage growth inhibition for 5 days was calculated.

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

Where R_1 = is the furthest radial distance of pathogens in control plates
Where R_2 = is the furthest radial distance of pathogens in extract (treated) plates.

The inhibition percentage was determined as a guide in selecting the minimum inhibition concentration (M.I.C) that will be effective in controlling the rot causing fungi.

Extracts were rated for their inhibitory effect using a scale.
< 0% inhibition (not effective)
> 0-20% inhibition (slightly effective)
> 20-50% inhibition (moderately effective)
> 50-100% inhibition (effective)
100% inhibition (highly effective)

PHYTOCHEMICAL SCREENING (QUALITATIVE ANALYSIS)

In the establishment of the presence of phytochemicals of interests, qualitative tests were conducted using different standard methods as shown below:

TEST FOR TANNINS

The Ferric chloride test described by Harbone (1973) was employed. An aqueous extract of the test sample was obtained by dispensing 2g of the paste sample in 10ml of distilled water. The mixture was shaken for 30 minutes in a mechanical shaker and then filtered through whatman filter paper no 40. The filtrate was used as the aqueous extract. 2ml of the extract was put into a test tube and 3ml of distilled water added to the test tube. After shaking gently to mix well, 2 drops of dilute Ferric chloride ($FeCl_2$) solution was added to the mixture. The test was repeated two more times and conducted against a blank control consisting of distilled water and Ferric chloride without the extract.

TEST FOR SAPONINS

The presence of saponins in the test sample was determined by the froth test as well as the emulsion test described by Harbone (1973).The test involved the use of aqueous extract of the sample 2ml of the aqueous extract was mixed with 6ml of distilled water in a test tube. The mixture was shaken well and observed for the presence of saponins (Stable froth).

For the emulsion test, 3 drops of groundnut oil was added to the test tube, the mixture was shaken well and observed for the presence of table emulsion. The formation of a stable emulsion gives a positive test for the presence of saponins. Froth tests were repeated two more times and conducted against a blank for confirmation.

TEST FOR FLAVONOIDS

The presence of flavonoids in the test sample was determined by the acid alkaline test described by Harbone (1973). An aqueous extract of the test sample was obtained by dispensing 1g of the ground sample in 5ml of distilled water. The mixture was shaken for 5 minutes and then filtered using Whatmann No. 1 filter paper. The filtrate was used as aqueous extract. Thus, 2ml of the aqueous extract was dispensed into a test tube and a few drops of bench concentrate ammonia (NH_3) was added followed by addition of concentrated hydrochloric acid (HCl). Yellow solution on addition of concentrated NH_3 which turns colourless upon addition of concentrated hydrochloric acid is a positive test for flavonoids.

TEST FOR ALKALOIDS

The presence of alkaloids in the test sample was determined using the test described by Harbone (1973). Ethanol extract of the samples was used and was obtained by dispensing 2g of the ground sample in 10ml of ethanol. The mixture was shaken properly and filtered using Whatmann No. 1 filter paper. The resulting filtrate was used for the tests. In the first experiment, 3 drops of Iodine was added to 2ml of the filtrate and shaken well to mix. The presence of dark brown green colouration in the test sample tube showed the presence of alkaloids. In the second experiment, three drops of picric acid was added to 2ml of the filtrate and the mixture was shaken well to mix. The formation of light green colouration confirmed the presence of alkaloids.

TEST FOR STEROLS

Ethanol extracts of the samples were used, 1ml of acetic anhydride was added to 2ml of ethanol in a test tube and also 3 drops of concentrated Tetraoxosulphate (IV) acid (H_2SO_4) was added. The mixture was shaken well and examined of the formation of dark brown – green sterols.

TEST STARCH

The presence of starch in the test samples was determined. Two drops of water was put in 1g of the ground sample to make it pasty. Then 2 drops of Iodine was introduced to the pasty sample. Blue-black colour or dark blue colour indicated a positive test for starch.

EXPERIMENTAL DESIGN

The one way ANOVA factorial design was employed for Statistical analyses.

RESULTS

ISOLATION OF FUNGAL PATHOGENS FROM SAMPLES

The fungi that were isolated from rotten white yam (*Dioscorea rotundata*) resulting from the sampling survey above included *Fusarium oxysporum, Rhizopus stolonifer, Trichoderma viride, Aspergillus flavus, Penicillium oxalicum* and *Botryodiplodia theobromae.* The most frequently occurring was *Fusarium oxysporum* at a frequency of 35.32 (table 1).

PATHOGENICITY TEST

The fungi tested included *Fusarium oxysporum, Rhizopus stolonifer, Trichoderma viride* and *Botryodiplodia theobromae* and were confirmed to cause the same disease and rot type noticed on the rot infested sample. The fungus *Fusarium oxysporum* was the most predominant, causing 69.0% rot on the *Dioscorea rotundata* (table 2).

SCREENING THE PEEL EXTRACT of *Dioscorea alata* for ITS EFFECT ON THE MYCELIA GROWTH OF THE FOUR TEST FUNGI

Effects of Ethanol, Methanol and Gentamicin Extracts of *Dioscorea alata* against *Fusarium oxysporum*

All the extract tested at the different concentrations had effective inhibitory effect on the mycelia growth of *Fusarium oxysporum.* The extract at 20% concentration level showed the highest inhibition on the fungus of 44 percent inhibition. At 10%, 30%, 40% and 50% concentration level, 35, 34, 24 and 34 percent inhibition were observed respectively (table 3). The ethanolic extracts were found to have the highest effect on *Fusarium oxysporum* when compared to other methanol and gentamicin extracts. At 10%, 20%, 30%, 40% and 50% concentration levels, the methanolic extracts had a moderate inhibitory effect on the mycelia growth. Which were 26, 28, 35, 31 and 34 percent inhibition respectively (table 4). The peel extract of *D. alata* with gentamicin showed a slight inhibitory effect on the fungus of 13,13.5, 14, 14.5, 15 percent inhibition at 10%,20%,30%,40% and 50% concentration levels respectively (table 5).

Effects of Ethanol, Methanol and Gentamicin Extracts of *Dioscorea alata* against *Rhizopus stolonifer*
Ethanolic extracts at 20%, 30%, 40%, and 50% concentration levels showed a moderate effect on the mycelia growth of *Rhizopus stolonifer* and had 41, 34, 28 and 35 percent inhibition respectively whereas at 10% concentration level the inhibitory effect on the mycelia growth was quite effective, showing 50 percent inhibition (table 3). At 10%, 20%, 30%, 40% and 50% concentration levels methanolic extracts had a moderate inhibitory effect on the mycelia growth. Having 32, 35, 30, 27 and 31 percent inhibition respectively (table 4). The peel extract of *Dioscorea alata* with gentamicin showed a slight inhibitory effect on the fungus of 10, 12.5, 13, 13.5 and 14.5 percent inhibition at 10%, 20%, 30%, 40% and 50% concentration levels respectively (table 5).

Effects of Ethanol, Methanol and Gentamicin Extracts of *Dioscorea alata* against *Botryodiplodia theorbromae*
A moderate effect was noticed at 20%, 30%, 40% and 50% concentration level with percentage inhibitions of 42, 35, 33, 47 respectively. At 10% concentration there was effective inhibition of the fungus growth with percentage inhibition of 52.0. The ethanolic extracts were found to be the most effective when compared to the other extracts (table.3). Methanolic extracts at all level of concentration exhibited a moderately effective inhibition on the test fungus (table 4). At all levels of concentration of gentamicin, there were slightly effective inhibition on the mycelia growth of the test fungus (table.5)

Effects of ethanol, methanol and gentamicin Extracts of *Dioscorea alaata* against *Trichoderma viride*
A moderate effect was observed at all levels of concentration with percentage inhibition of 36, 47, 34, 27 and 39 respectively (table 3). At 10%, 20%, 30%, 40%, and 50% level of concentration of methanol, the percentage inhibition was moderate with 28, 23, 25, 29 and 31 respectively (table 4). At all levels of concentration, there was a slight effect of gentamicin on the test fungus (table.5).

QUALITATIVE ANALYSIS OF TEST PLANT SAMPLES
The analysis showed that the water yam (*Dioscoreaalata*) used, tested positive to phytochemicals, saponins, flavonoids, tannins, sterols and alkaloids but negative to starch (table.6).

Analysis Of Variance (ANOVA) of Results
The effect on the growth of fungus *Rhizopus stolonifer* using the extract of *Dioscorea alata* was significant because Fcal was greater than Ftabulated (Fcal>Ftab). The extract showed a significant effect on the growth of the fungus (Fcal<<Ftab at 0.05). The difference in concentration level showed significant difference in the inhibition of mycelia growth of fungus. The effect on the growth of fungus *Fusarium oxysporum* using the extract of *Dioscorea alata* was significant because Fcal>Ftab. The extract showed a significant effect on the growth of the fungus (Fcal<<Ftab at 0.05). The difference in concentration level showed significant difference in the inhibition of mycelia growth of fungus. The effect on the growth of fungus *Botryodiplodia theobromae* using the extract of *Dioscorea alata* was significant because Fcal>Ftab. The extract showed a significant effect on the growth of the fungus (Fcal<<Ftab at 0.05). The difference in concentration level showed significant difference in the inhibition of mycelia growth of fungus. The effect on the growth of fungus *Trichodema viride* using *Dioscorea alata* extract was significant because Fcal>Ftab. The extract showed a significant effect on the growth of the fungus (Fcal<<Ftab at 0.05). The difference in concentration levels showed significant difference in the inhibition of mycelia growth of fungus.

DISCUSSION

Fungal pathogens are the major causative agents of rot in yam reducing the yield and productivity of yam per annum (IITA, 1985). The fungal organisms that were observed to cause rot in white yam were *Fusarium oxysporum, Rhizopus stolonifer, Botryodiplodia theobromae and Trichoderma viride (*Okigbo and Ogbonnaya, 2006).These organisms cause rot in yam tubers while still in the soil which progress during storage, and may not elicit any sign or external symptoms (Amusa *et al*., 2003). The pathogenicity test showed that pathogenic fungi inoculated into the yam tubers caused rot; this was due to the ability of the pathogen to utilize the nutrients of the tubers as substrates for growth and development. This result is similar to the report on fungi associated with Nigeria yam (Okigbo and Ikediugwu, 2000). Some other biological control measures like plant extracts have been used to control pathogens of yam (Akueshi *et al., 2002*; Okigbo and Ogbonnaya, 2006; Okigbo *et al.,* 2009).

In this study, ethanolic and methanolic peel extracts of water yam, produced a significant inhibition on the growth of pathogenic fungi on post harvest yam compared with that achieved by Gentamicin. Though ethanol extracts yielded more quantity than methanol, inhibition increased with decrease in concentration of extractants. Such inhibitory effect exhibited may be as result of the presence of phytochemicals which are antimicrobial agents. The phytochemicals include: alkaloids, flavonids, phenols, saponin, tannins and sterols. The difference in the inhibitory effect encountered was as a result of the solubility of the active compounds in the extractants used (Okigbo and Nmeka, 2005).

Table. 1: Percentage Occurrence Isolated Pathogenic Fungi

Organisms	Percentage rot (%)
Fusarium oxysporum	69
Rhizopus stolonifer	56
Botryodiplodia theobromae	65
Trichoderma viride	60

Table. 2: Percentage Occurrence of Fungal Isolates associated with *Dioscorea rotundata*

Organisms	Percentage Occurrence
Fusarium oxysporum	35.32
Botryodiplodia theobramae	31.41
Rhizopus stolonifer	25.20
Penicillium oxalicum	14.20
Aspergillus flavus	10.01
Trichoderma viride	29.01

Table. 3: Minimum Percentage Inhibition of Test Fungi Using Ethanolic Extracts *of Dioscorea alata* Peel

Isolates	10%	20%	30%	40%
Fusarium oxysporum	35	44	34	24
Rhizopus stolonifer	50	41	34	28
Botryodiplodia theobromae	52	42	35	33
Trichoderma viride	36	47	34	27

Table. 4: Minimum Percentage Inhibition of Test Fungi using Methanolic Extracts of *Dioscorea alata* Peel

Isolates	10%	20%	30%	40%
Fusarium oxysporum	26	28	35	31
Rhizopus stolonifer	32	33	30	27
Botryodiplodia theobromae	32	21	27	34
Trichoderma viride	28	23	25	29

Table. 5: Minimum Percentage Inhibition of Test Fungi using Gentamicin Extracts of *Dioscorea alata* Peel

Isolates	10%	20%	30%	40%
Fusarium oxysporum	13	13.5	14.5	15
Rhizopus stolonifer	10	12.5	13	13.5
Botryodiplodia theobromae	9.5	10	11	12
Trichoderma viride	13	13.5	14	15

CONCLUSION

The peel extract of water yam (*Dioscorea alata*) have the potential to control rot in post-harvest yam tubers. This can provide alternative ways in reducing rot in yams than the use of chemical fungicides. The biological antagonists are less expensive and environmentally friendly; an advantage over chemical (synthetic) fungicides.

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