



FUNGITOXIC EFFECT OF AZADIRACHTA INDICA AND VERNONIA AMYGDALINA EXTRACTS ON ASPERGILLUS NIGER AND FUSARIUM OXYSPORIUM THE CAUSAL ORGANISMS OF YAM (*DIOSCOREA ROTUNDATA*) ROT.

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ABSTRACT

This study investigated the antifungal effects of *Azadirachta indica* and *Vernonia amygdalina* (leaf extract) on rot causing organisms of yam: *Aspergillus niger* and *Fusarium oxysporium*. Pathogenicity test showed that all the four tested organisms induced rot in healthy yam tubers, with *Aspergillus niger* (74%) being the most virulent while *Rhizopus stolonifer* (10%) was the least virulent. Although, all the extracts showed varying degrees of antimycotic efficacy, there was no significant difference across the organisms tested. The potency of the extracts varied with plant material, extraction medium and concentration of the extract. Inhibition of fungal growth increased with a corresponding increase in the concentration of extract. *Azadirachta indica* proved to be more effective than *Vernonia amygdalina*. Methanolic extract of *Azadirachta indica* (80.88 ± 2.84) was the most fungitoxic and significantly better than other interactions. The significance of these findings was discussed in relation to biological control method of yam rot using leaf extracts (*Azadirachta indica* and *Vernonia amygdalina*).

Key word: Yam, Antifungal effect, Rot, Plant extracts

INTRODUCTION:

Yam (*Dioscorea spp*) of the family *Dioscoreaceae* and of the genus *Dioscorea* is a herbaceous annual climbing plant with edible underground tubers, they are largely twiners and are easily propagated by means of bulbils or portions of the tuber (Orkwor *et al*, 1998). Members of the genus *Dioscorea* are monocot and have been reported to comprise about 600 species (IITA, 1985). The most cultivated species in Nigeria are the *Dioscorea rotundata* (white yam), *Dioscorea alata* (water yam) and the yellow yam (*Dioscorea cayensis*). Yam constitutes a valuable source of carbohydrates to the people of the tropical and sub-tropical

Africa, Central and South America, part of Asia, the Caribbean and the Pacific Islands. (Adelusi and Lawanson, 1987). It is also a staple food in the tropics (Hahn *et al*, 1987) hence West Africa is the most important yam producing region of the World (Coursey, 1967, Okigbo, 2005). The West African yam belt comprises of Nigeria, Cameroun, Republic of Benin, Togo, Ghana and Coted'Ivoire, where over 90% of 69 world's production takes place (Orkwor *et al*, 1998). According to FAO (2003) Nigeria is the largest producer of yam in the world accounting for about 72% of the total World's output estimated to annual production of about 27million tones

followed by Ghana with more than 3million tones and Coted'Ivoire with 2.9million tones.

However, yam is susceptible to several diseases, both in the field and during storage. The invasion of yam tubers by microbial pathogens especially fungi is considered the most critical factor in yam rot (Degoas, 1993). Several authors have reported the isolation of different fungi genera associated with post-harvest rot either singly or in combination with several others (Ejechi and Iiondu, 1988 and Sangoyomi *et al*, 2002). These organisms includes *Fusarium solani*, *Rhizopus stolonifer*, *Botryodiplodia theobromae*, *Geotricum candida*, *Penicillium oxalicum*, *Aspergillus tamari* and *Aspergillus niger* (Arene *et al*, 1985; Okigbo and Ikediugwu 2000; Okafor, 1966; Coursey, 1967; Adeniji,1970; Sangayomi 2004; Okigbo 2005).

There have been attempts to control tuber rots of yams using synthetic pesticides inform of chemicals dip and fumigation (Ogundana and Dennis, 1981). These chemicals are fungistatic and act by penetrating through the wounded surface on the yam tuber to stop the mycelia growth of fungi until it is surrounded by suberised cortical tissues (Ricci *et al*, 1978). However, post-harvest use of chemicals on crop is often regulated by law due to their carcinogenicity, tetratogenicity and non-biodegradability or pollutive nature (Bajaj and Gosh, 1975). But biological control is generally favoured as a method of plant disease control (Okigbo and Ikediugwu, 2000; Okigbo, 2002; 2005), hence many plant extracts have been used to control yam diseases (Okigbo and Ogbonnaya, 2006) and some of the plants with fungicidal properties include *Azadirachta indica* and *Vernonia amygdalina*.

Azadirachta indica belongs to the family Meliaceae. It's common name is Neem which is borrowed from Hindi, in English it is know as Indian lilac. Although native to Pakistan, India and Bangladesh, it has spread throughout the tropic, Nigeria inclusive. In medicine, neem products are believed to be anthelmintic, antifungal, antidiabetic, antibacterial, anitviral, contraceptive and sedative (Biswas *et al* 2002).

Vernonia amygdalina (L) commonly know as bitter leaf is a shrub that grows up to 3 meters high in the African tropics and other parts of Africa, particularly Nigeria, Cameroon and Zimbabwe. Organic fraction extract of the plant was show to posses cytotoxic (Kupchan *et al*, 1969). It is effective against amoebic dysentery (Mounidipa *et al*, 2000) gastro intestinal disorder (Akeh and Ekekwe,1995) and has anti microbial and antiparasitic activities. The biological active compounds of *Vernonia amygdalina* are saponin and alkaloids (Muraina *et al*, 2010) terpenes, steroids, flavnoids, phenolic acids and antraquinone (Cimangn *et al*, 2004) edotides (Izeubigie, 2003) and sesquiterpenes (Kupchan *et al*, 1969).

On this note, it is important to search for a method of controlling post-harvest yam rot that will be affordable, durable and environment friendly; hence this research work studied the relative effectiveness of aqueous (Cold water), ethanolic and methanolic extracts of *Vernonia amygdalina* and *Azadirachta indica* on *Fusarium oxysporium* and *Aspergillus niger* casual organisms of post-harvest yam rot.

MATERIALS AND METHODS

Collection of Plant Materials

Healthy yam tubers alongside samples of yam tubers showing rot diseases were randomly selected from yam barn of

National Root Corps Research Institute, Umudike, Abia State, Nigeria. The leaves of the two local plants used (*Vernonia amygdalina* (L) and *Azadirachta indica* (L)) were sourced from the neighborhood of Umuariaga and along the road side of Michael Okpara University Umudike respectively. The Horticulture unit of National Root Crops Research Institute (NRCRI) verified and authenticated the plants.

Isolation of fungal pathogens associated with rotten yam tubers

Rotten yams were cut into pieces about 3 – 4cm. The yam piece were washed in 70% ethanol for 1minutes (Fawole and Oso, 1988) and later washed in sterile distilled water twice. A bale of sterile filter paper was used in blotting out the sterile water on the pieces of yam. The pieces of yam (5 in number were equidistantly placed with the aid of a sterile forceps on a newly prepared and solidified Potato Dextrose Agar in Petri dishes). The inoculated plates were transferred into an incubator at 26⁰C for 5 days. The plates were examined daily for any mycelia or colony growth.

Subculturing and purification

After incubation at 26⁰C for 5 day, the emerging different mycelia (colour) were aseptically transferred with a flamed surgical knife onto a newly prepared Potato Dextrose Agar plate. The plates were incubated at 26⁰C for 5 days. Further sub-culturing was done for the purification of all the isolated organisms. The Petri dishes of pure cultures of the test fungi were then sealed with paraffin to prevent contamination. The resulting pure cultures were used for subsequent identification of fungi isolates with the aid of a compound microscope and identification guide (Sulton, 1980).

Characterization of the Purified Micro Organisms

Wet mount method (Fawole and Oso, 1988) was used in viewing the purified micro organisms and it was later matched with a mycology atlas (Hunter and Bornette, 1987) for the identification of the micro organism.

Pathogenicity of the Isolated Fungi

Fresh healthy yam tubers were surface sterilized with 70% ethanol and washed in five changes of distilled water. Cylindrical discs were removed from the tuber with a sterile 5mm cork borer. A disc of a five days old pure cultures of the test fungi was transferred into the holes created in the tubers, then covered with a portion of the tuber removed earlier, which was cut-off a little to compensated for the disc size. The edge was sealed completely. The same procedure was used for the control except that discs of uninoculated PDA were placed in the holes created in the tubers with the cork borer. All the inoculated tubers were incubated for 14days in a humidity chambers, thereafter the tubers were examined for infection and disease development.

Plant Extracts Preparation

Vernonia amygdalina and *Azadirachta indica* (leaves) were collected and washed with sterile distilled water, shade dried for four days. The dried leaves were milled, sieved into powdery form and were kept in air – tight container. Powdered samples of 25g, 50g, 75g and 100g were weighed out and mixed separately with 100ml of each of the solvents (ethanol, methanol and water) used for the extraction, this gave rise to corresponding 2.5%, 5.0%, 7.5% and 100% extract concentration. The sample was filtered with a Whatman filter paper and the filtrate used as the extract.

Evaluation of Extract Purity

All the plant extracts used in this study were screened to ascertain their purity; this was done by streaking the plant extracts separately onto sterile plates of the test media. The plates were incubated at a temperature of 27°C for 24 hours (Cheesbrough, 2000) and was examined for possible growth of contaminants. The absence of growth confirms the purity of the test extracts.

Inoculation of Target Micro Organisms into Extract Media Plates

One ml from each concentration of the extracts 2.5%, 5.0%, 7.5%, 100% was transferred into a sterile Petri dish with the aid of a sterile pipette and 9ml of molten Potato Dextrose Agar (PDA) was aseptically poured into the plates. The plates were rotated gently for easy mixing of the media and the inoculums. This gave rise to PDA extract mixture with corresponding 2.5%, 5.0%, 7.5%, and 10% extract concentration. The plates were allowed to solidify. A 5mm cork borer was used in cutting an established culture of *Aspergillus niger* and *Fusarium oxysporium* and dropped with an aid of sterile forcep into the centre of the solidified plates (Okigbo and Ikediugwu, 2000). Each treatment consists of three replicates. The control set up consists of blank agar plates (no extracts) inoculated with the test fungi as described above. The inoculated Petri dishes were incubated at 27°C for 5 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. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Whips (1987).

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

Where R_1 is the farthest radial distance of pathogen in control plate while R_2 is the farther radial distance of pathogen in extract incorporated agar plates. The mycelia growth on each plate were measured daily and recorded with a transparent ruler.

Experimental Design

The experimental design used was Complete Randomized Design (CRD) with three replicates. All the data obtained were subjected to statistical analysis of variance and treatment means were separated using least significant difference at $P < 0.05$ level of probability.

RESULTS

Isolation of Fungi Pathogens from Rotten Yam Tubers and Pathogenicity Test

The fungi that were consistently isolated from the rot infested tissues of the yam tubers included *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium*, *Botryodiplodia theobromae* and *Trichoderma viride*. (Table 1).

The pathogenicity test revealed that all the organisms tested (*Aspergillus niger*, *Fusarium oxysporium*, *Botryodiplodia theobromae* and *Rhizopus stolonifer*) induced rot on healthy yam tubers 14 days after inoculated, hence pathogenic. *Aspergillus niger* with rot incidence of 74% was more virulent than *Fusarium oxysporium* with rot incidence of 48%, whereas other two organisms: *Botryodiplodia theobromae* and *Rhizopus stolonifer* depicted a lower pathogenic effect with rot incidence of 25% and 10% respectively (Table 2).

Effects of Extracts, Extract Concentrations and Extraction Medium on the Growth of *Aspergillus niger* and *Fusarium oxysporium*.

The statistical analysis showed that the two plants extract used depicted a significant ($P > 0.05$) different values on the two organisms tested. *A. indica* gave the highest mean value of 57.069 ± 4.837 on *Aspergillus niger* and 56.231 ± 4.584 on *Fusarium oxysporium*, while *V. amygdalina* showed lower mean microbial inhibition of 39.584 ± 4.206 and 37.533 ± 4.184 on *Aspergillus niger* and *Fusarium oxysporium* respectively (Table 3).

The inhibitory effectiveness of *V. amygdalina* extraction medium concentration and interaction across the two test organisms (*A. niger* and *F. oxysporium*) revealed that methanol extract with mean inhibitory effect of 62.83 ± 2.38 was the highest, this was followed by mean value of 45.66 ± 3.33 recorded by ethanol extract whereas the least was aqueous extract with mean inhibition of 11.07 ± 1.96 (Table 4).

For extract concentration 10% showed the highest mean value of 52.89 ± 5.32 but this was not significantly ($P > 0.05$) higher than 46.67 ± 6.11 value recorded by 7.5% extract concentration. Extract concentration of 2.5% and 5.0% gave a lower mean value of 25.91 ± 4.67 and 33.93 ± 5.70 respectively; this was significantly different from each other (Table 4). For their interaction methanol at 7.5% and 10% extract concentration gave the highest inhibitory effect ranging from 62.83 ± 72.58 whereas aqueous extract at 2.5% and 5.0% extract concentration gave the least inhibitory effect of 1.82 ± 1.54 and 5.52 ± 1.17 respectively (Table 4).

The inhibitory effectiveness *A. indica* extraction medium, concentration and interaction across *Aspergillus niger* and *Fusarium oxysporium* depicted a mean value (inhibitory effect) of 80.88 ± 2.84 by methanol this was significantly ($P > 0.05$)

higher than the values recorded by the other extraction medium, ethanol gave a mean value of 63.88 ± 3.12 which was significantly ($P > 0.05$) better than the mean inhibitory effect of 25.19 ± 3.58 recorded by aqueous extract (Table 5).

For extract concentration, 10% had the highest inhibitory effect of 75.66 ± 5.23 which was significantly greater than 61.23 ± 5.99 and 51.07 ± 6.16 inhibitory value recorded by 7.5% and 5.0% extract concentration. The least value and significantly ($P > 0.05$) lower inhibitory effect was observed in 2.5% extract concentration with a value of 38.65 ± 6.20 . For their interaction, methanol at 7.5%, 10% extract concentration gave the highest inhibitory effect of 85.42 ± 5.27 and 96.83 ± 1.20 respectively, this was significantly ($P < 0.05$) greater than the values recorded by ethanol at 10% extract concentration with a value of 82.25 ± 2.06 . Aqueous extracts at 2.5% and 5.0% extract concentration gave the least inhibitory effect of 6.05 ± 0.87 and 16.6 ± 2.16 respectively (Table 5).

The effect of plant extract, their concentration and plant extract – by – concentration interaction in the inhibition of *Aspergillus niger* revealed that *A. indica* with mean inhibitory effect of 57.067 was the highest, hence significantly ($P < 0.05$) greater than 42.172 value obtained with *V. amygdalina*. The control did not show any inhibitory effect; hence mean value of 0.00 (Table 6).

For extract concentration, there was no significant difference across all the mean concentrations; their mean inhibitory value ranges from 22.387 to 42.193. For interaction, *A. indica* at 7.5% and 10% extract concentration exhibited the highest inhibitory of 67.978 and 71.567 respectively. The least inhibitory effect was observed in *V. amygdalina* at 2.5% extract

concentration, which gave a percentage inhibition of 26.911. Other interactions were not significantly different ($P < 0.05$) from each other. The control showed uninhibited growth (Table 6).

The effect of plant extract, their concentration and plant extract – by – concentration interaction in the inhibition of *Fusarium oxysporium* depicted that *A. indica* which gave a mean inhibition of 56.231 was significantly ($P < 0.05$) higher than 37.533 value recorded for *V. amygdalina*. There was no significant ($P < 0.05$) difference among the mean values observed in 7.5% and 10% extract concentration which were 33.052 and 43.507 respectively, although this 43.507 value obtained in 10% extract concentration was significantly greater than the mean values obtained in 2.5% and 5.0% extract concentration which are 20.474 and 27.985 respectively. For their interaction *A. indica* at 10% gave an inhibition of 79.744 which was statistically better than other interactions, next to it were *A. indica* at 7.5% and 5.0% and *V. amygdalina* at 10% and 7.5%, they gave an inhibition percentage ranging between 44.678 to 54.478%, other interactions showed a lower inhibition rate, they gave an inhibitory value ranging between 24.911 and 36.511 whereas the control did not show any inhibition (Table 7).

Table 1: Frequency of Occurrence (%) of Isolated Fungi on Rotten Yam Tubers.

Fungal Isolates	% Occurrence
<i>Aspergillus niger</i> (Eurotiomycetes)	35
<i>Rhizopus stolonifer</i> (Zygomycetes)	10
<i>Aspergillus flavus</i> (Eurotiomycetes)	8.6
<i>Fusarium oxysporium</i> (Sordariomycetes)	30.4
<i>Botryodiplodia theobromae</i> (Dothideomycetes)	9.4
<i>Trichoderma virida</i> (Hypocreaceae)	6.6

Table 2: Pathogenicity Test/fungi inoculated percentage rot

Inoculated Fungi	Percentage Rot
<i>Aspergillus niger</i>	74%
<i>Fusarium oxysporium</i>	48%
<i>Botryodiplodia theobromae</i>	25%
<i>Rhizopus stolonifer</i>	10%

Table 3: Percentage mean of microbial inhibition of plant extract

Plant extract	<i>Aspergillus niger</i> % mean \pm SE inhibition	<i>Fusarium oxysporium</i> % mean \pm SE Inhibition
<i>A.indica</i>	57.069 \pm 4.837	56.231 \pm 4.584
<i>V.amygdalina</i>	39.574 \pm 4.296	37.533 \pm 4.184
Control	0.00	0.00
FLSD 0.05	15.648	15.648

Table 4: Inhibitory Effectiveness of *Vernonia* extraction medium, concentration and their interaction across *A. niger* and *F. oxysporium*

Extraction Medium	Concentration (%)				Mean for Extraction Medium
	2.5	5.0	7.5	10.0	
Aqueous	1.82 ± 1.54	5.52 ± 1.17	13.68 ± 3.30	23.27 ± 1.66	11.07 ± 1.96
Ethanol	28.42 ± 2.33	37.40 ± 6.01	54.00 ± 2.80	62.83 ± 2.94	45.66 ± 3.33
Methanol	47.50 ± 2.22	58.88 ± 2.33	72.33 ± 1.61	72.58 ± 2.05	62.83 ± 2.38
Mean for concentration	25.91 ± 4.67	33.93 ± 5.70	46.67 ± 6.11	52.89 ± 5.32	

FLSD_{0.05} for comparing extraction medium = 3.91

FLSD_{0.05} for comparing concentration = 4.51

FLSD_{0.05} for comparing extraction medium and concentration interaction = 11.52

Table 5: Inhibitory Effectiveness of *Azadiratcha indica* extraction medium, Concentration and their interaction across *A. niger* and *F. oxysporium*

Extraction Medium	Concentration (%)				% Mean for Extraction Medium
	2.5	5.0	7.5	10.0	
Aqueous	6.05 ± 0.87	16.67 ± 2.16	30.00 ± 3.76	47.88 ± 4.51	25.19 ± 3.58
Ethanol	42.67 ± 1.82	62.48 ± 2.16	68.10 ± 2.15	82.25 ± 2.06	63.88 ± 3.12
Methanol	67.23 ± 2.91	74.05 ± 2.99	85.42 ± 5.27	96.83 ± 1.20	80.88 ± 2.84
Mean Conc.	38.65 ± 6.20	51.07 ± 6.16	61.23 ± 5.99	75.66 ± 5.23	

FLSD_{0.05} for comparing extraction medium = 4.16

FLSD_{0.05} for comparing concentration = 4.80

FLSD_{0.05} for comparing extraction medium and concentration interaction = 11.76

**Table 6: Effectiveness of plant extract, their concentration and plant extract -
by- concentration interaction in the inhibition of *A. niger***

Plant extract	Concentration (%)				Mean extract
	2.5%	5.0%	7.5%	10.0%	
<i>A.indica</i>	40.789	47.944	67.978	71.567	57.069
<i>V.amygdalina</i>	26.911	38.100	48.667	55.011	42.172
Control	0.000	0.000	0.000	0.000	0.000
Mean Conc.	22.387	28.681	38.881	42.193	

FLSD_{0.05} for comparing plant extract = 11.637

FLSD_{0.05} for comparing concentration = 15.023

FLSD_{0.05} for comparing plant extract and concentration interaction = 26.020

Table 7: Effectiveness of plant extract, their concentration and plant extract-by-concentration interaction in the inhibition of *F. oxysporium*

Plant extract	Concentration (%)				Mean extract
	2.5%	5.0%	7.5%	10.0%	
<i>A.indica</i>	36.511	54.189	54.478	79.744	56.231
<i>V.amygdalina</i>	24.911	29.767	44.678	50.778	37.533
Control	0.000	0.000	0.000	0.000	0.000
Mean Conc.	20.474	27.474	33.052	43.507	

FLSD_{0.05} for comparing plant extracts = 12.106

FLSD_{0.05} for comparing concentration = 13.979

FLSD_{0.05} for comparing plant extract and concentration interaction = 24.212

DISCUSSION

Fusarium oxysporium and *Aspergillus niger* were consistently associated with post harvest rot of yam tuber in this study and was found to be pathogenic on yam during storage. These pathogens have been previously linked with post – harvest yam rot (Amusa *et al*, 2003; Ogundana *et al*, 1970, Okigbo, 2002; 2005 Okigbo and Odurukwe 2009; Onuoha, 2005). The pathogenicity test which showed that the pathogenic fungi inoculated in the yam tubers caused rot was due to the ability of the pathogen to utilize the nutrients of yam as a substrate for growth and development; this is in consonance with the reports on fungi associated with Nigerian yams (Adeniyi, 1970; Okigbo and Emeka, 2010; Okigbo and Ikediugwu ,2000). However, these pathogenic organisms infect the tubers during pre harvest stage while in the soil or through natural opening and wounds created during harvesting, transportation, handling and marketing, hence damages incurred by the tubers during post harvest operations and then manifest fully during storage, this is in agreement with the reports of many workers (Amusa *et al*, 2003; Okigbo and Nmeke, 2005). It was observed that the three extraction solvents gave satisfactory results in controlling the two test organisms that causes tuber rot, although the water extracts were less effective than the corresponding ethanol and methanol extracts. Methanol extract proved to be the best extraction medium than ethanol and water, this agrees with the reports of Amadioha and Obi, (1998) who stated that extraction of the active compounds in the leaves of the test plants are affected by solvent used in the extraction, this is also in line with the observations of Amadioha (2000) and Ekweuige and Elegalam (2005) who reported that water used in the extraction

process was probably not able to dissolve all the fungicidal principle present in the plants, which are contained in the ethanol extract. The difference in the fungitoxic between the extraction medium can be as a result of the different susceptibility of each of the test isolates to different concentrations of the extracts, this is in line with the findings of some researchers (Amadioha, 2000; Anukwuorji *et al*, 2012; Okigbo and Nmeke, 2005; Okigbo and Odurukwe 2009).

Observations made on the effect of the various plant extracts used depicted that *A. indica* proved to be more fungitoxic and exhibit the highest percentage growth inhibition on the rot pathogen; this suggests that the plant contain more active compounds/phytochemical that affect the growth of the rot pathogen. This agrees with the report of Anukwuorji *et al* (2012) who stated that *A. indica* was highly effective against mycelia growth of *Botryodiplodia theobromae*, *Aspergillus niger*, *Fusarium solani*, and *Sclerotia rolfsii* with inhibition values ranging from 40.57% to 79.63%, hence *A. indica* may have acted by the production of more antibiotics substances that inhibited the growth of *A. niger* and *F. oxysporium*; this has been reported by Okigbo and Emeka (2010). There was a similar trend in the fungitoxic effect of all the plant extracts with respect to concentration, extract concentration of 7.5% and 10% were more effective in the control of all the organisms, whereas 2.5% and 5.0% extract concentration gave a lower inhibitory effect this is in consonance with the results of Suleiman (2010) who stated a significant difference between mycelia growth value recorded on the various plant extract concentration. The observations of Anukwuorji *et al* (2012) and that of Okigbo *et al* (2009b) all reported a significant (P

<0.05) difference in the inhibitory effect of all the plant extract concentration, this suggested that there is difference in the solvent soluble antifungal element in the respective leaf extracts as reported by Iwu (1993) and Sofowora (1997).

The result of this work has shown that *A. indica* and *V. amygdalina* have potential to control post harvest yam rot this can provide an alternative ways of reducing and controlling rot by farmers because fungicides of plant origin are environmentally safe, nonphytotoxic and the extract of these plant materials can be easily prepared by farmers.

CONCLUSION AND RECOMMENDATION:

The result of this study proved that *A. indica* and *V. amygdalina* have inhibitory potential on rot causing fungi of yam in storage, the antimycotic potential of these two plants suggest their ability to prolong the shelf life of tubers in storage. Prolonging the shelf life

of tuber would go a long way in reducing the scarcity of the tubers. It can also be deducted from this study that methanolic extract of *A. indica* and *V. amygdalina* could be an alternative to synthetic chemicals in controlling yam rot. Aqueous extracts of these plants, which inhibited the growth of these pathogens to a recommendable level, can also be used as a second option to methanolic extract since it is environment friendly, readily available and affordable.

However, further research should be done to step up the development of the plant extracts in controlling pathogenic fungi. To achieve this, further pharmacological evaluation, toxicological studies and possible isolation of the therapeutic antifungal should be conducted. Moreso, further work can be carried out by testing these plant extracts on other fungi pathogenic to other tuber corps. Finally, further investigation can combine the plant extracts for possible synergistic effect.

REFERENCES

- Adeniji, M.O. (1970). Fungi associated with storage decay of yam in Nigeria. *Phytopathology*. 60:590 – 592.
- Amadioha, A.C. (2000). Fungitoxic effects of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. *Arch phytopathol pflanzl*. 34:1 – 9.
- Amadioha, A.C. and Obi, V.I. (1998). Fungitoxic activity of extracts of *Azadirachta indica* and *Xylopiya aethiopia* on the *Collectotrichum lindmurtianum* in cowpea. *J.Herb spiece and medicinal plants (in press)*.
- Amusa, N.A., Adegbite, A.A., Mohammed, S. and Baiyewu, R.A. (2003) Yam diseases and management in Nigeria. *Journal of Biotechnology*. 2(12):497 – 502.
- Anukwuorji, C.A., Ramesh, R. Putthi and Okigbo, R.N. (2012). Isolation of fungi causing rot of cocoyam (*Colocasis esculata* (L.) schott) and control with plant extracts: (*Allium sativum* (L.), *Garcinia kola*, heckle; *Azadirachta indica* L. and *Carica papaya* L. *Globa Advanced Research Journal of Agricultural sciences* 1(2): 033 – 048.
- Arene, O.B., Nwankiti, A.O. and Okafor, N. (1985). The chemical basis of the pathology of yam tuber. In Osuji G. (Ed.) *Advances in yam Research. The biochemistry and Technology of the yam tuber*. Biochemical Society of Nigeria. Anambra State University of Technology Nigeria. Pp. 251 – 258.
- Bajay, S.S. and Ghosh, A.K. (1975). Antifungal antibiotics in Perspectives: Pages 297 – 309: in *Advanced in mycology and plant pathology*, edited by Rxychavohiri, S.P., Verma, A., Bhargara, K.S. and Mehrotia, B.S. Sagar Printers, New Delhi, India.
- Degras, L. (1993). *The yam (A tropical Root crop)*. Macmillian press, London. 408pp.
- Dorosh, L. (1988). The economic of Root and Tubers crops in Africa RCMP Research Monograph No. 1. 46pp.
- Efiuvwevwere, B.J.O. and Nwachukwu E. (1998). Incidence of yam (*Dioscorea rotundata* Poir) rots, inoculation-induced quality charges and control by chemical fungicides and modified atmosphere. *Post – harvest Biology and Technology* 14: 235 – 243.
- Egedigwe C.A. (2010). Effects of dietary incorporation of *Vernonia amygdalina* and *Vernonia* on blood lipid profile and relative organ weighs in albino rats. (Thesis) department off biochemistry, MOUAU, Nigeria.
- Ejечи B.O. and Souzey J.A. (1999). Inhibition of low – deterioration of yam tuber (*Dioscorea rotundata* Poir) in storage with phenolic extract of *Acalyphahispida Borm F. leave a Journal of stored Product Research* 35:127 – 134.
- Ejечи, B.O. and Ilondu, M.E. (1998). Control of yam tuber (*Dioscorea rotundata*) rot agent *Sclerotium rolfsii* with camwood (*Baphia nitida* Lpdd) saw dust extract. *African Journal of Root and Tuber crops* 3:13 – 15.
- Ekwealor, K.U., Nnabude, P.C., and Anukwuorji, C.A. (2012). Effects of different irrigation levels and mulch rates on the performance of *Amaranthus* hybrids (H). *African Journal of Sciences*.
- Ekwenye, U.N., and Elegalam, N.N. (2005). Antibacterial activity of ginger (*Zingiber officinale* Roscoe) and garlic (*Allium sativum* L.) extracts of *E.coli* and *S.typhi* *Journal of Molecular medicine and Advance Sciences* 1(14):411 – 416.
- Enyi, B.A.C. (1992). Yams in Africa. *Proceedings of the 2nd international symposium on Tropical Root and Tuber crops* Hawall, 93pp.

- FAO (2000). Food and Agricultural Organization Food Balance Sheet. <http://apps.fao.org/lim500/wrap.pl?> Food Balance Sheet & Domain Food Balance Sheet. Accessed 19 July, 2002.
- FAO (2003). *Food and Agriculture Organization production year book*. FAO Rome.
- Fawole, M.O. and Oso, B.A. (1988) Laboratory manual of microbiology. Spectrum Books Ltd. Sunshine house Ibadan, Nigeria
- Ferrguson T.V., Hayneo P.H. and Spence T.A. (1984). The effect of sett size, sett type and spacing on some aspect of growth, development and yield white libson yams (*Dioscoreaalata*) *Proceedingsofthe 6th symposiumofISTR*C. Perv.
- Gonzalez, M.A. and Collazo de River, A. (1972). Storage of fresh yam (*Dioscoreaalata*L.) under controlled conditions. *J. Agric. Viniu R.R.* 56: 46 – 56.
- Green, K.R. (1994). *Studies on the epidermology and control of yam anthracnose*. Ph.D Thesis, Viniv of reading.
- Hahn, S.C, Osiru D.S.O, Akoroda, M.O. and Otoo, J.A. (1987). Yam production and its future prospects. *Outlook on Agriculture*, 16:105 – 109.
- Hahn, S.K, Osiru, D.S.O., Akoroda, M.O. and Otoo, J.A. (1987). Yam production and its future prospects. *Outlook on Agriculture*. 16:105 – 109.
- Ijeh I.I., Ejike C.E. (2011). Current perspectives on the medicinal potentialof *Vernonia amygdalina* Del J. medicinal plant Resources 5(7): 1051 – 1061.
- International Institute of tropical Agriculture (IITA) (1985). Yam diseases. *In common African pests and diseases of cassava, yam, sweet potato and cocoyam*. IITA Ibadan, Nigeria pp.40 – 49.
- Iwu, M.T. (1993). Handbook of African Medicinal plants CRC Press. Boca Raton, Ann Aobor London 435pp.
- Kokwaro, J. (2009). Medicinal plants of East Africa 3rd edition. Nairobi, Kenya. University of Nairobi Press ISBN 9966 – 846 – 84 – 0.
- Ogundana, S.K. and Dennis, C. (1981). Assessment of fungicides for prevention of storage rot of yam tubers. *Pesticides Science* 12:491 – 494.
- Ogundana, S.K. Naqvi, S.H. and Ekundayo, J.A. (1970). Fungi associated with soft rot of yam (*Dioscorea spp*) in Nigeria *Trans Br. Mycol Soc.* 54:445 – 451.
- Okafor, N. (1966). Microbial rotting of stored yam (*Dioscorea spp*) in Nigeria. *Expl. Agric* 2:179 – 182.
- Okigbo, R.N. (2005). Biological control of post-harvest fungal not of yams (*Dioscorea spp*) with *Basillus subtilis*. *Mycopathologia* 159(2):307 – 314.
- Okigbo, R.N. and Ikediugwu, F.E.O (2000) Studies on Biological control of post-harvest rot of yams (*Dioscorea spp.*) with *Trichoderma viride* *J . phytropathol* 148:351.
- Okigbo, R.N. and Nmeka, I.A. (2005). Control of yam tuber rot with leaf extracts of *Xylophia aethiopic*s and *Zingiber officinalis*. *Afri J. Biotechnol* 4(8): 804 – 807.
- Okigbo, R.N. and Odurukwe, C.N. (2009). Occurrence and control of fungal rot pathogens of yam (*Dioscorea rotundata* Poir) with leaf extracts of *Chromolinae odorata*, *Carica papaya* and *Aspilia africana* *Nigerian Journal of Mycology*. 2(1): 154 – 165.
- Okigbo, R.N. and Ogbonnaya, U.O. (2006). Antifungal effects of 2 tropical plant leaf extract of *Ocimum gratissimum* and *Aframomium melegueta* on post-harvest yam (*Dioscorea spp*) rot. *African .J. Biotechnology* 5(9):727 – 731.

- Okigbo, R.N., and Emeka, A.N. (2010). Biological control of rot-inducing fungi of water yam (*Dioscorea rotundata*) with *Trichoderma harizianum*, *Pseudomonas syringae* and *Pseudomas chlororaphis*. *Journal of stored product and post-harvest Research*. 1(12): 18 – 23.
- Okigbo, R.N., Anukworji, C.A. and Eguae, C.T. (2012). Control of microorganism causing the deterioration of yam chips with *Vernonia amygdalina* (L) and *Zingiber officinale* (L). *Nigerian Journal of mycology* 4:45 – 46.
- Onuoha, A.I. (2005). Antifungal potentials of *Azadirachta indica* and *Ocimum grastissimum* leaf extracts in the control of yam rot. *International Journal of Applied Science* 1:20 – 25.
- Orkwor, G.C., Asiedu, R. and Ekanayakel, J. (1998) *Food yam, Advances in Research*. IITA and NRCRI. Nigeria.
- Osagie, A.U. (1992). *The yam tuber in storage*. Post harvest research unit, Department of Biochemistry. University of Benin, Nigeria, 247pp.
- Ricci, P. and Amolin, R. (1973). Fungicidal treatment of yam tubers useful storage decay. *Proc. 3rd inter. Symp. Trop Root crops*. IITA, Ibadan, Nig.
- Sangoyomi, T.E. (2004). Post-harvest fungal deterioration of yam (*Dioscorea rotundata* poir) and its control. Ph.D thesis IITA, Ibadan Nigeria, 179pp.
- Sangoyomi, T.E., Ekpo, E.J.A and Asiedu, R. (2002). First Report of *Naltrassia Mangifera* as a post-harvest fungal pathogen of white yam (*Dioscorea rotundata*.) in Nigeria *plant diseases* 86(8):919.
- Schmotterer H. (1995). *The Neem Trec: Source of Unique Natural products for intergrated pest management, Medicine, Industry and other purpose*, VCH Weinheim, Germany pp. 1 – 696.
- Singh, R.P., Chari, M.S., Raheja, A.K., and Kraus, W. (1996). *Neem and Environment*, Oxford and IBH Publishing, New Delhi, Vols 1 and II, pp 1 – 1198.
- Sofowora, A. (1997). *Medicinal plants used in traditional medicine in Africa*. 1st edition. John Wiley and Sons, New York. pp 128 – 129.
- Suleiman, M.N. (2010). Fungitoxic activity of neem and pawpaw leaves extracts of *Alternaina solani* causal organism of yam rots. *Advanced Environmental Biology* 4(2): 159 – 161.