EVALUATION OF SOME PLANT EXTRACTS ON THE CONTROL OF FUNGI INFESTATION ON YAM IN STORAGE

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ABSTRACT

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The fungicidal potentials of leaf extract of Azadirachta indica L. (neem) Piper nigrum Schum and Thornn (bush pepper), Vernonia amygdalina L. (bitter leaf) and Ocimum basilicum L (basil plant) plants on the control of fungi infestation on yam in storage were assessed in the Laboratory of Department of Plant Science and Biotechnology, College of Natural Science, Michael Okpara University of Agriculture, Umudike. Nigeria. Specifically, the study assessed the effect of leaf extracts on sprouting potentials of yam tubers in storage, proximate analysis of treated yam tubers, fungi establishment on yam tubers in storage and Phyto-chemical composition of the extracts. The experimental design used was of 5 levels of treatments and dilution rate Completely Randomized Design (CRD) with each treatment replicated four times and data generated subjected to analysis of variance The results on the effect of leaf extracts of A. indica, P. nigrum, V. amygdalina and O. basilicum on the sprouting of yam tubers, proximate composition and pathogenicity test indicated that the extract of P. nigrum, V. amygdalina and the control were significant ($p<0.05$) on the sprouting of the yam tuber for a period of four weeks. The extract of Vernonia amygdalina show stronger sprouting potentials than the other plant treatments and the various plant extracts had an increase in the amount of protein, fibre, and moisture of the stored yam. The phytochemical composition of the test plants are found potent for the growth inhibition of the fungi on the yam tubers.

Keywords: *Leaf extracts, fungi, yam tubers*

INTRODUCTION

One of the most pressing problems facing developing and underdeveloped countries of the world is food scarcity. It has been reported that nearly one billion people are challenged by severe hunger of which approximately 10% die from hunger-related complications annually (Kana *et al.,* 2012). A substantial part of this hunger stems from inadequate agricultural storage facilities and produce preservation from microbes induced spoilage (Kana *et al.,* 2012). However, post-harvest deterioration caused by microbial invasion of tuber crops is a major cause of losses in production which contributes hugely to the unsuccessful long-term storage of root and tuber crops (Amadioha and Markson, 2007). These pathogenic organisms gain entry into tubers through the area where the tubers are separated from the stems at harvest, or the root tips which break during harvesting or even through cracks on the tuber surfaces sustained during harvesting, and in (Okigbo *et al.,* 2009). The effect of storage bio-deterioration in yam cannot be over emphasized because postharvest losses attributed to fungal diseases and some other microorganisms associated with bio--deterioration in yams includes *Aspergillus flavus, A. niger, Botryodiplodia theobronmae, Fusarum oxysporum, Fusarum solani, Penicullum chrysogenum, Penicullum oxaleum, Trichoderma viride and Rhizopus nodosus* (Okigbo, 2004).

It has been reported that fungi and related rot organisms produce extra-cellular enzyme such as amylases, celluloses, zylanases, polygalactunases and pectin-methyl esterases which degrade cell wall component of susceptible produce leading to emission of foul odor and water (Oladoye *et al.,* 2013). Hence fungi attack reduces the market value of the affected yam tubers and hampers the value addition. Fungal pathogens produce various mycotoxins on produce like oil seeds, maize and cereals. They are dangerous in minute quantities and present extreme toxicity due to their ability to withstand heat (Shukla *et al.,* 2012). Bankole *et al.* (2003) reported that mycotoxins of most agricultural importance are aflatoxins, fumonisins, ochratoxin zearalenone and deoxynivanelol and that fungal toxin contamination of food products causes acute or chronic intoxications, which results into reduced shelf life, exacerbate disease conditions in humans and loss of economic productivity. And that fungi attack on yam has led to hunger and food scarcity in Nigeria where yam is a staple food. Against this backdrop, the study seeks to assess the fungicidal potentials of leaf extract of *Azadirachta indica L. (*neem), *Piper nigrum* Schum and Thornn (bush pepper), *Vernonia amygdalina* L. (bitter leaf) *and Ocimum basilicum* L (basil plant) plants on the control of fungi infestation on yam in storage. Specifically, the study assessed the effect of leaf extracts on sprouting potentials of yam tubers in storage, effect of leaf extract on proximate analysis of treated yam tubers, the effect of leaf extract on fungi establishment on yam tubers in storage and also ascertained Phyto-chemical composition of test plant extracts.

MATERIALS AND METHODS

The experiment was conducted in the laboratory in the Department of Plant Science and Biotechnology, College of Natural Science, Michael Okpara University of Agriculture, Umudike. Nigeria. The trial leaf extract of

*Azadirachta indica L. (*neem), *Piper nigrum* Schum and Thornn (bush pepper), *Vernonia amygdalina* L. (bitter leaf) *and Ocimum basilicum* L (basil plant) plants *were* collected within Umudike area. The fresh leaves (*O. basilicum, P. nigrum, A. indica and V. amygdalina)* were air-dried for three weeks and ground separately into powder with a grinding machine (Attrition milling machine). Acetone extraction of the leaf powder was prepared by adding 5.0 g of each leaf powdered into 50 ml of acetone in glass specimen bottle and allowed for one week (Salawu, 1992). The organic solvents were allowed to evaporate in open-space for three days, after which, 40 ml of water was added to each plant extract in the specimen bottle and mixed thoroughly for the active substance to dissolve. The plant extracts were diluted with tap water at the ratio of 1: 5 (1 ml of plant extracts to 5 ml of water) in buckets and the infested yam tubers soaked for 24 hours. The yam tubers used were visually rated as 40% nematode infested while four samples of healthy yam tubers were also collected and used for pathogenicity test.

The isolation and identification of fungi was done using rotten yam tubers rinsed in sterilized water with 70% ethanol for 1 minute. The yam was carefully cut open using sterilized knife to show both infected and healthy parts. A portion was cut out aseptically from the boundary area between the healthy and the rotted part. This was quickly crushed into small pieces and teased out in 10 ml of distilled water in a universal bottle. The bottle was corked and allowed to stay for 15 minutes and shaken to dislodge the pathogens from the yam. A loopful of the inocula was collected from the suspension of each test sample and separately cultured on the already prepared slant agar (PDA). The inoculated plates were incubated at room temperature (28 °C) for 2-4 days at the same temperature. Observation of the plates were made daily for emergence of colonies. On establishment of growth, each culture plate were examined for distinct colonies, from which inocular were collected and sub-cultured onto fresh sterile agar plates and incubated in order to obtain a pure culture and were observed daily. On establishment of growth in sub-culture plates, they were re-examined for uniformity and consistency as a mark of purity. The fungi isolated were identified through their respective colony and structural characteristics compared with those of known-existing taxa in standard manuals (Bergey's Manual). The slide mounts of each isolate was made and stained with lactophenol/cotton-blue dye. The slide mount was viewed under a light microscope and the structures were noted and later compared with those in the fungi manual (CMI). Each fungi isolate was tested on its ability to initiate disease on a healthy yam tuber. Holes were bored on the surface of the tubers and a core sample of the isolate from the pure culture was inserted into the hole and it was closed back with the yam flesh. The inoculated point was sealed up with sterile gel and marked. Similar hole was made on an adjacent position in the yam tuber and closed back again with the flesh but without any pathogen which serves as control. All the inoculated samples were kept at room temperature (28-30 °C) and watched for a period of 7-14 days after which the tubers were cut open transversely along the point of each inoculation to ascertain the extent of rot infestation.

Data was collected on tuber germination, fungi establishment and on yam composition. The experiment was laid out in completely randomized design replicated four times. Data generated were subjected to analysis of variance (ANOVA) using SAS (1999). Significantly different treatment means were separated using least significant difference (LSD) at 5% probability level.

Proximate test of yam tubers

Moisture determination

2g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100 °C to dry to a constant weight for 24 hours overnight. At the end of the 24 hours period, the crucible plus sample was removed from the oven and transferred to desiccator, cooled for ten minutes and weighted. If the weight of empty crucible is W_o, weight of crucible plus sample is W1 weight of crucible plus oven dried sample W_3.

% Moisture = W_1-W_3x100

= W_1-W_o

or % Moisture = 100-%DM

Determination of crude protein

The crude protein was determined by the Kjeldahl method with slight modification. 0.5 g of the powdery form of each *Dioscorea* spp was digested with 5 ml of concentrated sulphuric acid in the presence of Kjeldahl catalyst. The nitrogen from the protein in the sample was converted to ammonium sulphate that reacted with 2.5 ml of 2.5% brucine reagent, 5 ml of 98% sulphuric acid to give a coloured derivative and the absorbance read at 470 mm. the percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein (AOAC, 1990).

Estimation of crude lipid

This estimation was performed using the soxhlet extraction method. Ten grammes of the powdery form of *Dioscorea* spp. were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was concentrated to a condenser. 200ml of n-hexane was used to extract the lipid (AOAC, 1990). The flask which now contains the fat or oil is detached, its exterior cleaned and dried to a constant weight in the oven. If the initial weight of dry soxhlet flask is W_o and the final weight of oven dried flask+oil fat is W_1, percentage fat/oil is obtained by the formula: $\frac{W1-Wo \times 100}{\text{wt of sample}}$

Determination of crude fibre

The estimation was done using the method of AOAC (1990). Five grammes of the powdery form of each *Dioscorea spp* and 200 ml of 1.25% H_2SO_4 were heated for 30 minutes and filtered with a Buchner funnel. The residue was washed with distilled water until it was acid free. 200ml of 1.25% NaOH was used to boil the residue 30min, it was filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCl and twice with ethanol. Finally it was rinsed with petroleum ether three times. The residue was put in a pre-weighed crucible and dried at 105 °C in an oven overnight. After cooling in a desiccators, it was ignited in a muffle furnace at 550 °C for 90 minutes to obtain the weight of the ash. Percentage fibre content was estimated by the formular: $\frac{\text{W1}-\text{Wo x 100}}{\text{wt of sample}}$

Where: Wo is weight of crucible and dried sample

W1 is weight of crucible and ash

Determination of ash content

This was done using the method of AOAC (1990). The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited. It was then cooled in a desiccator and weighted at room temperature to get the weight of the ash. The percentage ash was calculated from the formula below:

Ash content = $\frac{\text{wt.of ash}}{\text{original wt. of sample}} \times \frac{100}{1}$

Carbohydrate determination

The carbohydrate content was determined by subtracting the summed up percentage composition of moisture, protein, lipid, fibre, and ash contents from 100

RESULTS and DISCUSSION

Effect of leaf extracts on sprouting potentials of yam tubers in storage

The results on the effect of leaf extracts of *A. indica, P. nigrum, V. amygdalina* and *O. basilicum* are shown on Table 1 and this includes the pathogenicity test, sprouting of yam tubers and proximate composition of yam tubers after 4 weeks in storage. The various means of the extracts on sprouting potentials for 4 weeks did not show any significant difference from each other, however, the extract of *P. nigrum, V. amygdalina* and the control had significant effect (<0.05) on the sprouting of the yam tuber for a period of four weeks. The extract of *Vernonia amygdalina* showed stronger sprouting potentials than the other plant treatments and this was in line with Aghale *et al.* (2016) who also reported on the application of *Vernonia amygdalina* at the rate of 5.0 kg ha^{-1} to induce early seed yam sprouting. This could be due to possible positive allolopatic chemical presence which may have aided in the sprouting process of the tuber (Osuagwu, 2011).

Table 1: Effect of leaf extracts on sprouting potentials of yam tubers in storage

Name of Plants	Week 1	Week 2	Week 3	Week 4	$LSD_{0.05}$
Azadirachta indica	1.0	1.0	0.75	1.00	0.385
Piper nigrum	0.50	0.75	0.75	1.00	0.802
Vernonia amygdalina	0.50	0.50	0.50	0.75	0.861
Ocimum basilicum	1.00	1.00	1.00	1.00	0.000
Water	0.50	0.75	0.75	0.75	0.802

Effect of leaf extract on proximate analysis of treated yam tubers

Table 2 Shows that yam tubers treated with the various plant extracts had an increase in the amount of protein, fibre, and moisture. *Vernonia amygdalina* had 5.45 mg per 100g while *P. nigrum* extract had 4.75 mg per 100g lower than the control in protein content. Fiber, ash and carbohydrate contents were lowest (0.70, 0.70, 59. 3) mg per 100g respectively. *Vernonia amygdalina* had the highest level of moisture content (32.4 mg per 100g) followed by the control (27.3 mg per 100g). The control had the higher fat content (0.60 mg per 100g) than all the tubers treated with the various plant extracts. The high moisture content of the untreated control suggest possible inhibition action of the fungi organisms by the plant extracts and Nweke (2015) reported that tuber with high fungal presence had higher moisture contents. The high level of fat observed on the control could be attributed to the distribution of phytoalexins on the treated yam tubers which was also responsible for the different rate in fungi control. Arinze, (1989) observed that the distribution of pytoalexin within the tuber play a role in the susceptibility to rot. The increase in carbohydrate within the treated yam tubers may be due to possible inhibition of some organisms which ordinarily will absorb the nutrient in such stored tuber for cellular growth and reproduction. Amadioha (2011) reported that attack by pathogenic fungi on plant products is aimed at absorbing stored nutrients in the plant. Plant extracts had no deleterious effect on the yam tuber composition.

The fungi associated with bio-deterioration of yam tubers as shown in Table 3 were identified as *Rhizopus* spp., *Penicillum, Aspergillus,* and *Fusarium* spp. and *B. theobrone.* The reduced presence of *Fusarium* spp. may

suggest that the different treatments inhibited their presence on the treated yam tubers. The readily presence of all other reported organism in yam deterioration is also in line with the reports of Opara and Nwokocha (2015).

Table 2: Proximate analysis of treated yam tubers mg per 100g

Treatments	Protein	Fat	Fibre	Ash	Moisture	CHO
Control (water)	4.95	0.60	0.86	2.10	27.3	64.1
Azadirachta indica	5.17	0.50	0.90	2.40	21.7	69.2
Piper nigrum	4.75	0.53	1.05	2.10	20.7	70.8
Vernonia amygdalina	5.45	0.40	0.70	1.73	32.4	59. 3
Ocimum basilicum	5.333	0.46	0.83	2.57	23.7	67.1
$LSD_{0.05}$	0.10	0.07	0.05	0.16	0.15	0.28

Table 3: Occurrence of fungi isolate in rotten yam

Sample	*Rhizopus*	*Penicillum*	*Aspergillus*	*Fusarium*	*B.thcobrone*
A.indica	+ve	+ve	+ve	+ve	+ve
P.nigrum	+ve	+ve	+ve	+ve	+ve
V.amygdalina	+ve	+ve	+ve	-ve	+ve
O. basilicum	+ve	+ve	+ve	+ve	+ve
Total	4	4	4	4	4
No. of +ve	4	4	4	3	4
% occurrence	100%	100%	100%	75%	100%

Effect of leaf extract on fungi establishment on yam tubers in storage

Tables 4 and 5 presents the effect of leaf extract on fungi establishment of yam tubers in storage. There was significant different ($p<0.05$) between all the plant extracts throughout the four weeks. The means of *A. indica* ranges from 0.00±0.00, *P. nigrum* 0.50±0.58–1.00±0.00, and other treatments where almost at the same range.

Table 4: Effect of leaf extract on fungi establishment on yam tubers in storage

Name of Plants	Week 1	Week 2	Week 3	Week 4	$LSD_{0.05}$
Azadirachta indica	0.00	0.25	0.50	1.00	0.59
Piper nigrum	0.50	0.75	0.50	1.00	0.74
Vernonia amygdalina	1.00	1.00	1.00	1.00	0.00
Ocimum basilicum	0.75	0.25	0.25	1.50	0.59
Water	0.50	0.75	0.75	0.75	0.80

Table 5: Occurrence of fungi isolate in rotten yam

Sample	*Rhizopus*	*Penicillum*	*Aspergillus*	*Fusarium*	*B.thcobrone*
A. indica	+ve	+ve	+ve	+ve	+ve
P. nigrum	+ve	+ve	+ve	+ve	+ve
V. amygdalina	+ve	+ve	+ve	-ve	+ve
O. basilicum	+ve	+ve	+ve	+ve	+ve
Total	4	4	4	4	4
No. of +ve	4	4	4	3	4
% occurrence	100%	100%	100%	75%	100%

Phyto-chemical composition of test plant extracts in mg per 100g

Table 6 summaries the phytochemical composition of the plant extracts for the trial. The extract of *V. amygdalina* had the highest level (2.36 mg per 100g) of alkaloids, followed by *P. nigrum* (1.24 mg per 100g) and *A. indica* (1.22 mg per 100g). *Vernonia. amygdalina* also had the highest (2.16 mg per 100g) level of saponine followed by *A. indica* (0.54 mg per 100g), *O. basilicum* and *P. nigrum.* The flavonoids of the test plants showed that *P. nigrum* had highest content (0.46 mg per 100g) followed by *A. indica* (0.38 mg per 100g). Phenol content levels were 0.22 mg/100g in *P. nigrum* and 0.18 mg per 100g in *O. basilicum.* Alkaloid in plant has been reported to contain dihydroioscorine, a compound that is convulsant and causes central nervous system of paralysis in animals (Oliver-Bever, 1989). Okwu *et al.* (2006) reported that both alkaloids and saponin are considered important due to their toxicity which occurs in varying concentration in each plant. This explained why some plants are used in the preparation of pesticides. Flavonoids act as anti- oxidant in various biological systems and also protect against allergies, inflammation, free radicals, microbes, ulcers viruses and tumours (Okwu, 2005). Flavonoids inhibit estrogen synthesease, an enzyme that binds estrogen receptors in several organs and behaves as a powerful protective agent against inflammatory disorder, thus reducing edema formation and platelet aggregation. Phenolic

compound helps to prevent death of crops due to their anti-microbial activities and hormone modulators (Ofokansi *et al*., 2005) while Okwu (2004) had it that tannine in plants act as repellant against rots in crop system. The phytochemical composition of the test plants may be responsible for the growth inhibition of the fungi on the yam tubers.

Table 6: Phyto-chemical composition of test plant extracts in mg per100g

Plants	Alkaloids	Flavonoids	Saponin	Tannin	Phenol
A. Indica	1.22	0.38	0.54	0.22	0.18
P.nigrum	1.24	0.46	0.30	0.26	0.22
V. amygdalina	2.36	0.34	2.16	0.26	0.12
O.basilicum.	0.56	0.30	0.42	0.36	0.18

CONCLUSION

The different plant extracts inhibited fungi presence on the treated yam tubers. The extract of *Vernonia amygdalina* showed stronger sprouting potentials than the other plant treatments and could be used as seed dressing material in the sprouting process of the tuber. The higher moisture content of the untreated yam tubers (control) as against the plant extracts suggest possible inhibition action of the fungi organisms by the plant extracts and the high level of fat observed on the control could also be attributed to the distribution of phyto alexins on the treated yam tubers which was also responsible for the different rate of fungi control. The increase in carbohydrate within the treated yam tubers may be due to possible inhibition of fungi which ordinarily will absorb the nutrient in such stored tuber for cellular growth and reproduction. Plant extracts had no deleterious effect on the yam tuber composition rather it improves the nutritive value of the tubers and could be adopted in Integrated Pest Management (IPM) system. The botanicals can commercially be used as alternative to synthetic fungicides due to its availability, accessibility and affordability as safe treatment in a sustainable organic farming system.

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