Global Science Research Journals

ISSN: 2437-1874 Vol. 6 (1), pp. 276-287, May, 2018

http://www.globalscienceresearchjournals.org/

Global Journal of Pests, Diseases and Crop Protection

Full Length Research Paper

In vitro and *In vivo* antimicrobial potency of selected plant extracts in the control of postharvest rot-causing pathogens of yam tubers in storage

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Received 23 January, 2018, Accepted 20 February, 2018

Potency of *Piper guineense, Zingiber officinale, Azadirachta indica, Carica papaya and Nicotiana tabacum* on *in vitro* control of *Curvularia eragrostide* and *in vivo* control of postharvest rot-causing pathogens of yam tubers in storage was carried out. *Rotted Ogoja and Ghini* white yam tubers were collected from farmers' barns in different locations in Lafia, Nigeria. Fungi organisms isolated from *Ghini* and *Ogoja* white yam tubers after four months *included Botryodiplodia theobromae, Aspergillus flavus, A. niger, Fusarium moniliforme, F. oxysporum*, *Penicillium purpurogenum C. eragrostide* and *Colletotrichum sp.* Only *P. purpurogenum* and *C. eragrostide* showed significant differences (*P≤ 0.05)* in mean percentage frequency of occurrence between the two cultivars. Pathogenicity test showed that all the isolated fungi were able to incite rot in the healthy yam tubers. The result revealed that *Z. officinale*, *P. guineense, A. indica, C. papaya* and *N. tabacum* exhibited more antifungal properties against *C. eragrostide* at higher concentrations (60 g/L and 90 g/L) than at lower concentration (30 g/L) throughout the period of incubation. Results also indicated that *Z. officinale*, *P. guineense, A. indica* and the synthetic chemical mancozeb were more efficacious *in vitro. In vivo* test using the most potent extracts; *Z. officinale*, *P. guineense* and *A. indica* and *mancozeb* showed that the extracts were effective against postharvest pathogens of yam at all the level of concentrations. Mean decay reduction index (DRI) of more than 0.6 indicated that the extracts as well as the chemical were able to inhibit the growth of pathogens on the yam tubers by more than 60% throughout the five months of storage. It is therefore recommended that extracts from these plants could be used to control postharvest pathogens of yam since they are eco-friendly, cheap and easily available.

Keywords: Potency; Plant extracts; Postharvest; *In vivo;* Decay Reduction Index; Pathogenicity test; *C. eragrostide*

INTRODUCTION

Yams belong to the family *Dioscoreaceae.* Cultivation of yam is carried out mostly in west and central Africa, Asia and South American countries (Okigbo and Ogbonnaya, 2006; FAO, 2007; FAO, 2013). White yam (*Dioscorea rotundata*) is the most cultivated species followed by water yam (*D. alata*). Nigeria is the largest producer of yam and accounts for over 38.92 million metric tonnes annually (FAO, 2008; Kleih *et al*., 2012). In spite of large scale production, postharvest losses of yam tubers caused by pathogens continue unabated starting from the field through harvest to storage (Amusa *et al*., 2003; Okigbo *et al*., 2009a; Gwa *et al.,* 2015). According to Arya (2010), postharvest losses caused by pathogenic organisms are the most costly than any other loss. These pathogenic organisms consistently found to incite rot in yam tubers include *Aspergillus flavus*, *A. niger*, *A. ochraceus Botryodiplodia theobromae, Colletotrichum spp, Fusarium oxysporum, F. solani, F. moniliforme, Penicillium chrysogenum, P. digitatum, P. oxalicum,*

P. purpurogenum, Rhizoctonia spp and *Rhizopus nodosus* (Okoro and Nwankiti, 2004; Markson *et al.,* 2012; Ogunleye and Ayansola, 2014; Gwa *et al.,* 2015; Okigbo *et al.*, 2015; Shiriki *et al.,* 2015, Gwa and Akombo 2016, Gwa and Nwankiti 2017a; Gwa and Ekefan 2017a). Postharvest losses of yams caused by pathogens in storage are considered to be significantly high in Nigeria; this has always put demand for yam tubers exceedingly higher than supply (FAO, 1998). The control of these postharvest rot causing pathogenic organisms has been linked to several methods such as biological control method, chemical control method and use of natural plant extracts (Amusa *et al.,* 2003, Okigbo and Emeka 2010; Gwa and Akombo 2016; Gwa and Abdulkadir 2017). Chemical method of control is fast and in most cases most effective (Gwa and Akombo, 2016). On the other hand, there is fear of the safety of the chemical residues and also the likelihood of human toxicity, pollution of the environmental as well as being non-biodegradable (Richie *et al.,* 2005 Lakshmeesha *et al.,* 2013). However, pesticides formulated from plant origin are biodegradable, cheap, easily available and environmentally safe compared with synthetically made pesticides (Okigbo, 2005). Hence, the use of plant extracts could go a long way in serving as an alternative to synthetically formulated pesticides in the control of pathogens of plant origin (Singh *et al.,* 2011; Gwa and Nwankiti, 2017a; Gwa *et al.,* 2017). The research was therefore, carried out to test the potency of some selected plant extracts on *in vitro* and *in vivo* control of postharvest rot causing pathogens of yam tubers in storage.

MATERIALS AND METHODS

Study area

The study was carried out at the Advanced Plant Pathology Laboratory and in the University farm, Federal University of Agriculture, Makurdi, Nigeria.

Collection of rotted yam tubers

Rotted yam tubers (*D. rotundata*) with various degrees of rots in Lafia settlement of Nasarawa State, Nigeria were collected from different yam farmers from various storage barns. Rotted yam tubers were packaged in sterile polyethylene bags to prevent them from further deterioration. In the laboratory, the samples were protected from rodent using wire. Potato Dextrose Agar (PDA) prepared according to manufacturer's recommendation was the medium used for growing fungal organisms. Test fungus in the *in vitro* study was *Curvularia eragrostide* which has not being studied in this area.

Isolation of pathogenic fungi

Rotted yam tubers were cut at interphase between diseased and healthy tissues. The cut tissues were washed in running tap water and surface sterilized by dipping in 5% sodium hypochlorite for 2 minutes. The dipped tissues were then removed and rinsed in four successive changes of sterile distilled water. The yam tissues were then placed on sterile filter papers in the laminar Air flow cabinet to dry for 2 minutes before inoculation.

Inoculation

The dried infected tissues were later picked from the sterile filter paper in the Laminar Air flow Cabinet using sterile forceps. The infected tissues were aseptically plated on Petri dishes containing acidified sterile potato dextrose agar (PDA) and the plates were incubated at ambient room temperature (30±5°C) for 7 days to allow for fungal growth.

Characterization and identification

The fungal colonies that grew on the incubated plates were sub-cultured into fresh separate sterile acidified PDA plates and incubated for 5 days to obtain pure cultures of pathogens. When growths were fully established, the growths were microscopically examined; morphological characteristics and identification of the isolated pathogens were made and compared with existing authorities (Ahmed and Ravinder, 1993; Agrios, 2005) for identification.

Determination of frequency of occurrence of isolates

The frequency of occurrence of the isolates was determined by keeping records of the organisms isolated from time to time. Isolation and characterization were carried out at monthly interval based on the number of times each fungi pathogen was isolated in a month. This was expressed as a percentage of the total of all the different organisms isolated over the period (Okigbo and Ikediugwu, 2000) and thus calculated as follows:

$$\%\ frequency\ of\ occurence = \frac{x}{n} \times \frac{100}{1}$$

Where,

x = number of times of occurrence of the individual isolates over the period

n = total number of micro organisms isolated in the study over the period

Stock culture of *C. eragrostide* which was used as test pathogen was maintained on slant of acidified potato dextrose agar (PDA) in McCartney bottles for subsequent studies.

Pathogenicity test of isolated fungi

The isolated fungi organisms from the rotted yam tubers were pathogenitically tested using healthy yam tubers. The tubers were washed in running tap water, sterilized with 5% sodium hypochlorite for 30 seconds, rinsed in four successive changes of sterile distilled water. A sterile 5 mm cork borer was punched into the healthy looking yam tubers to a depth of 4 mm and the bored tissues were removed. A five mm diameter disc

from the pure culture of the fungi were each cut and replaced in the holes created separately. Same procedure was used for the control experiment except that sterile agar discs were used instead of the inoculum obtained from the fungi in the holes created in the tubers (Gwa *et al.,* 2017). Petroleum jelly was used to completely seal the remaining holes to prevent contamination by other pathogenic organisms. The inoculated yam tubers were replicated three times and stored at ambient room temperature (30±5^{o}C) under sterile condition. Tubers were incubated for 14 days to allow for growth and establishment of the fungi organisms after which the tubers were examined for infection and disease development.

Preparation of Plant Extracts

The method of (Gwa and akombo 2016) and (Gwa *et al.,* 2017) were used. Seeds of *Piper guineense* (Black Pepper), Rhizomes of *Zingiber officinale* (Ginger), leaves of *Azadirachta indica* (Neem), leaves of *Carica papaya (*Pawpaw*)* and leaves of *Nicotiana tabacum* (Tobacco) were washed thoroughly with cold running tab water, air-dried and separately ground into fine powder using a mortar. Hot water (100^{o}C) extraction was obtained by adding 30g, 60g and 90g of the powder of each plant extracts to 1litre of sterile distilled water separately in 1000 Ml Pyrex flask. The mixtures were left for 24 hours and subsequently filtered through four fold of sterile cheese cloth. The filtrates obtained were used as plant extracts in the experiment. Mancozeb was prepared in sterile distilled water at 4 g/L, 8 g/L and 12 g/L concentrations respectively. The potencies of the aqueous plant extracts and the synthetic fungicide were tested *in vitro* against *C. eragrostide* and the most effective extracts were tested *in vivo* on yam tubers to determine their fungicidal activity against pathogenic rot-causing organisms of white yam (*D. rotundata*) in storage.

Measurement of mycelial growth of *C. eragrostide in vitro*

Measurement of mycelial growth of *C. eragrostide in vitro* was done based on the method developed by Amadioha and Obi (1999) to determine the fungitoxic effect of plant extracts and chemical fungicide on mycelial growth of *C. eragrostide.* The method involves creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection of the lines indicates the centre of the plates. These were done before dispensing PDA into each of the plates. In each of the sterilized Petri dishes 15 Ml of the prepared medium was poured into it and 5 Ml of each of the plant extracts and chemical fungicide at their different level of concentrations were poured into Petri dishes containing the media separately (Nene and Thapilyal 2002). They were mixed well and allowed to solidify; after which the solidified medium was inoculated centrally at the point of intersection of the two perpendicular lines drawn at the bottom of the plate with *C. eragrostide* mycelial. A 5 mm diameter mycelial disc retrieved from one-week-old fresh cultures of *C. eragrostide* grown on PDA plates served as inoculum (Vadashree *et al.,* 2013). The different levels of concentrations were replicated three times. In the control experiments, only 5 Ml of sterile distilled water was added to PDA in place of the different levels of plant extracts and chemical fungicide respectively. The treatments and control experiments were incubated for 120 hours at ambient room temperature (30 ±5^{o}C) and measurement of growth as radius of a growing fungal colony were undertaken at intervals of twenty four hours for five times using a transparent ruler (Gwa and Akombo 2016). The absence of growth in any of the plates was indicative of the potency of the extract and the chemical fungicide against the test fungus. Fungitoxicity was determined in form of percentage growth inhibition (PGI) according to the method described by Korsten and De Jager, (1995).

$$PGI\ (\%) = \frac{R - R_1}{R} \times 100$$

Where,
PGI = Percentage Growth Inhibition
R = the distance of fungal growth from the point of inoculation to the colony margin in control plate,
R_1 = the distance of fungal growth from the point of inoculation to the colony margin in treated plate.

To test the effectiveness of plant extracts and chemical fungicide in controlling yam tuber rot pathogens in storage.

The efficacy of three plant extracts (seeds of *P. guineense,* rhizomes of *Z. officinale* and leaves of *A. indica)* and chemical fungicide (mancozeb) that have been found to posses' more fungicidal properties *in vitro* were tested for efficacy in controlling yam tuber rot pathogens *in vivo* at different levels of concentrations. *Ghini* cultivar of white yam which was found to be pathogenic on many fungi was earlier planted and harvested from University of Agriculture; Makurdi research farm was collected and treated with the three plant extracts. The white yam tubers were each sprayed with three plant extracts at concentrations of 30 g/L, 60g/L and 90 g/L, respectively. The synthetic chemical, mancozeb was applied at a concentration of 4 g/L respectively on the *Ghini* tubers using a hand sprayer. After spraying, the tubers were allowed to dry after which tubers were stored for five months. Three tubers formed a treatment; this was replicated three times bringing the total to 9 tubers per treatment. There were 11 treatments. A total of 99 tubers of *Ghini* yam tubers were used for the experiment. Data on the potency of the extracts and chemical fungicide in controlling rot causing pathogens during storage were collected at monthly interval for five months. The treatments were completely randomized and control was set up for each cultivar in which sterile distilled water was sprayed on the yam tubers and allowed to dry (no plant extract or chemical applied). The numbers of unrotten and rotten tubers in each treatment were

recorded. The effectiveness of the concentrations of plant extracts and chemical fungicide in controlling yam tuber rot pathogens in storage were evaluated. The Decay Reduction Index (Amadioha, 1996) defined below, was calculated as a measure of the effectiveness of each plant extract and chemical fungicide in controlling yam tuber rot pathogens in storage at different concentrations after final data collection as:

$$\text{Decay Reduction Index (DRI)} = \frac{\%\ \text{decay in control} - \%\ \text{decay in treated tubers}}{\%\ \text{decay in control}}$$

Data analysis

Data collected were subjected to Analysis of variance (ANOVA) using GenStat Discovery Edition 12 for ANOVA and means separation, Minitab Release 17 for descriptive statistics and Graph Pad Prism 6 for trend graphs. Statistical F-tests were evaluated at $P \leq 0.05$. Differences among treatment means for each measured parameter were separated using Fisher's least significant difference (FLSD) (Cochran and Cox, 1992).

RESULTS

Sample collection, isolation and identification of *C. eragrostide*

Fungi organisms identified in this location included: *B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum, P. purpurogenum, C. eragrostide* and *Colletrichum sp.* Growth characteristics of *C. eragrostide* was slow taking more than 7 days to fill the entire plate (Figure 1A). Microscopic examination showed that the hyphae were branched, septate, colourless or brown, or with rough swellings. Conidia were borne at the apex or sides of the conidiophores (Figure 1B). Conidia were straight or curved, usually broad in the middle and narrow towards the ends, an oval, an inverted egg shape, club-shaped or pear-shaped, occasionally rounded at the base, or with a distinct point of attachment, 3 or more septate, smooth, or rough, and often with one or more middle cells larger and darker than the others (Figure 1C).

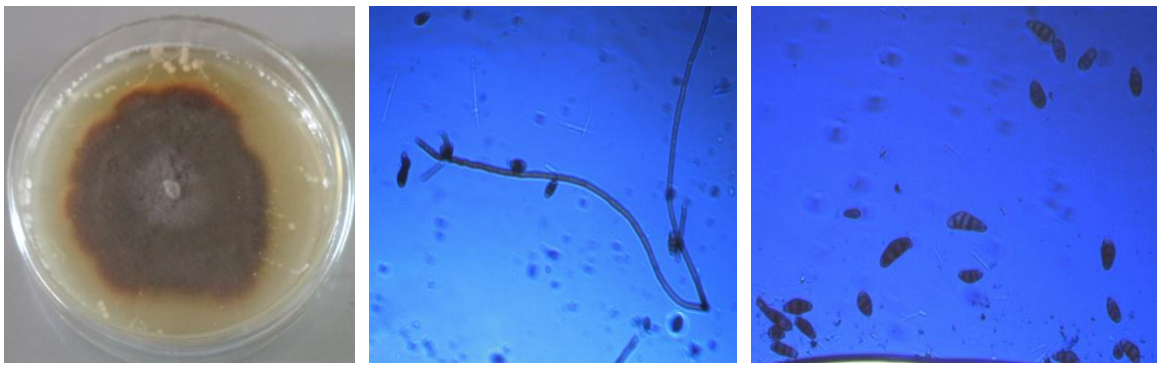

Figure 1: Culture of *C. eragrostide* on Potato Dextrose Agar (× 10) (A); Photomicrograph of *C. eragrostide* showing Conidiophores bearing Conidia (× 10) (B) and Conidia of *C. eragrostide* (× 10) (C)

Percentage frequency of occurrence of fungal pathogens in Lafia

Figure 2 shows the identified fungal organisms in Lafia as *B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum, P. purpurogenum, C. eragrostide* and *Colletrichum sp.* The percentage frequency of occurrence showed that *F. moniliforme* and *F. oxysporum* were higher in *Ghini* compared with *Ogoja* in February but the occurrences of these organisms were lower in *Ghini* compared with *Ogoja* in March, April and May. *A. niger* showed higher occurrence in *Ghini* compared with *Ogoja* in February, March, April and May. *A. flavus* showed the highest occurrence in *Ogoja* than in *Ghini* in February, March and May but was more in *Ogoja* than *Ghini* only in April. *B. theobromae* showed the highest level of occurrence in *Ogoja* than *Ghini* in February, April and May. It was only in March that the isolate showed the highest level of occurrence in *Ghini* than in *Ogoja*. The percentage frequency of occurrence of *Colletotrichum sp.* in *Ghini* rose from March to April and declined steadily in May. The result revealed that *Colletotrichum sp.* was not encountered in *Ogoja* cultivar in this location. *C. eragrostide* occurrence increased in *Ghini* from February to May except in April and the same organism was not encountered in *Ogoja. P. purpurogenum* occurred in *Ogoja* throughout the period of isolation and was higher in April but was not encountered in *Ghini.* The occurrence of *F. oxysporum* was less in *Ogoja* than *Ghini* in February but more in *Ogoja* than *Ghini* in March, April and May.

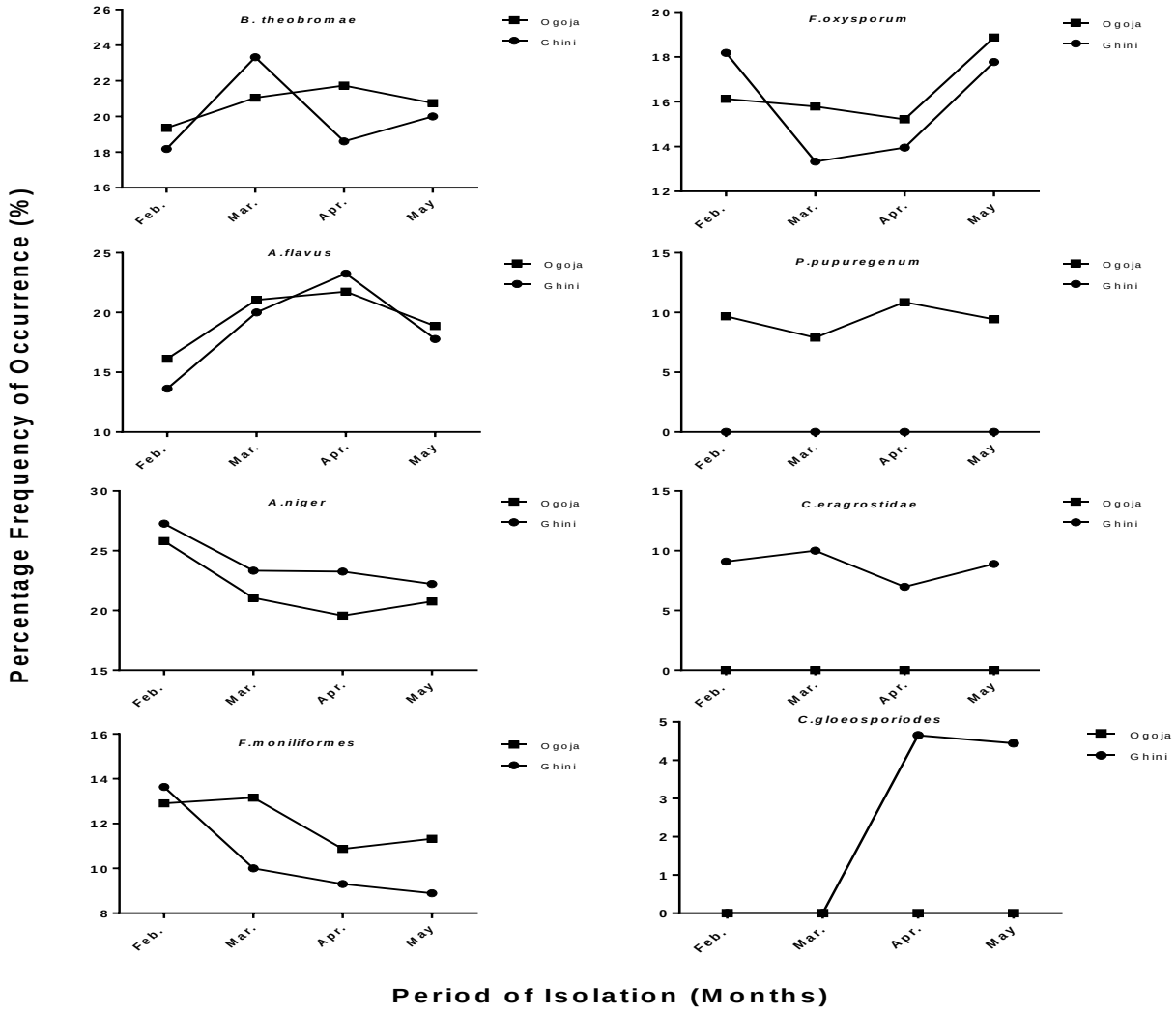

Figure 2: Percentage Frequency of Occurrence of Fungal Pathogens from *Ghini* and *Ogoja* White Yam Tubers from February to May 2015 in Lafia

Mean percentage frequency of occurrence of *B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum*, *C. eragrostide* and *Colletotrichum sp.* in Lafia after four months of isolation showed no significant difference (*P≤ 0.05)* between *Ghini* and *Ogoja* white yam tubers (Table 1). However, there were significant differences (*P≤ 0.05)* in mean percentage frequency of occurrence between the two cultivars in *P. purpurogenum* and *C. eragrostide* after four months of isolation (Table 1).

Table 1: Variation of Mean Percentage Frequency of Occurrence of Fungal Isolates from *Ghini* and *Ogoja* Cultivars of White Yam Tuber after Four Months of Isolation in Lafia

Pathogens	White Yam cultivar		T-Value	P-Value
	Ghini	***Ogoja***		
B. theobromae	20.03±20.72	20.72±0.50	-0.55	0.61
A .flavus	18.67±2.02	19.45±1.26	-0.33	0.75
A. niger	24.02±1.11	21.79±1.38	1.26	0.26
F. moniliforme	10.46±1.08	12.06±0.56	-1.31	0.26
F. oxysporum	15.81±1.26	16.50±0.81	-0.46	0.66
P. purpurogenum	0.00±0.00	9.46±0.61	-1.23	0.03*
C. eragrostidae	8.73±0.63	0.00±0.00	2.11	0.04*
Colletotrichum sp.	2.27±1.31	0.00±0.00	-0.89	0.56

*indicates statistical significance (*P≤ 0.05)*

Pathogenicity test

Pathogenicity test was carried out on *Ghini* yam tubers using *C. eragrostide.* Results presented in Figure 3 show that the pathogen incited rot in the healthy looking yam tubers14 days after inoculation. Rot symptoms were seen on the inoculated healthy yam tubers. Tubers inoculated without the test fungus in the control experiments however, showed no symptoms of rot in the bored yam tissues (Figure 4).

Figure 3: Rot caused by *C. Eragrostide*

Figure 4: Plate 31: Control (No Organism Inoculated)

In vitro* effect of plant extracts and fungicide on the mycelial growth of *C. eragrostide

The results of *C. eragrostide* radial growth on PDA amended with plant extracts and synthetic fungicide presented in Table 2 show that *Z. officinale*, *P. guineense, A. indica, C. papaya* and *N. tabacum* exhibited more antifungal properties against *C. eragrostide* at high concentrations (60 g/L and 90 g/L) than at low concentration (30 g/L) throughout the period of incubation. The tested plant extracts showed no significant difference ($P \leq 0.05$) at 30 g/L at 24 hours but the antifungal properties varied significantly for each plant extract concentration throughout the remaining period of incubation (Tables 2 and 3). Mean percentage growth inhibition of the plant extracts tested showed that extracts from *Z. officinale* and *A. indica* at low concentration (30 g/L) gave the highest growth inhibition of 58.08 % and 48.29 % respectively of *C. eragrostide* compared with the lowest of 29.02 % and 32.34 % radial growth inhibition recorded with *N. tabacum* and *C. papaya* at the same concentration respectively (Table 3).

Extracts of *Z. officinale* and *P. guineense* at concentration of 60 g/L gave the highest radial growth inhibition of *C. eragrostide* at 67.41 % and 60.38 % respectively while the lowest growth inhibition of 32.78 % and 40.63 % came from *N. tabacum* and *C. papaya* extracts respectively. Extracts from *Z. officinale, P. guineense* and *A. indica* at concentration of 90g/L gave the highest radial growth inhibition of *C. eragrostide* at 72.83 %, 72.73 % and 64.83 % respectively while the lowest percentage growth inhibition of 43.17 % and 46.35 % were recorded with extracts of *N. tabacum* and *C. papaya* respectively. It was generally observed that mancozeb had fungicidal properties at all tested concentrations which were higher than the plant extracts tested whereas *Z. officinale* and *P. guineense* showed the highest inhibition of *C. eragrostide* among the plant extracts.

Table 2: Percentage Growth Inhibition of *C. eragrostide* by different Concentrations of Plant Extracts and Chemical Fungicide after 120 Hours of Incubation

Plant Extract	Concentration (g/L)	Period of Incubation (Hours) and Percentage Growth Inhibition (%)					
		24	***48***	***72***	***96***	***120***	***Mean***
Piper guineense	30	72.20±14.70^{a}	50.00±5.77^{ab}	32.08±9.12^{b}	26.31±9.15^{b}	30.91±3.34^{b}	42.30±5.73^{cd}
	60	100.00±0.00^{a}	74.44±7.29^{b}	49.01±8.96^{c}	38.79±5.90^{c}	39.68±5.20^{c}	60.38±6.74^{bc}
	90	100.00±0.00^{a}	87.78±6.19^{b}	71.03±2.41^{c}	51.52±1.52^{d}	53.33±3.33^{d}	72.73±5.23^{b}
Zingiber officinale	30	83.30±16.70^{a}	74.44±7.29^{ab}	48.90±10.30^{bc}	41.82±6.05^{c}	41.90±4.23^{c}	58.08±5.98^{b}
	60	83.30±16.70^{a}	81.11±1.11^{a}	69.97±6.47^{ab}	51.52±1.52^{b}	51.11±1.15^{b}	67.41±4.80^{b}
	90	100.00±0.00^{a}	87.78±6.19^{a}	66.27±5.16^{b}	54.55±4.55^{b}	55.58±1.15^{b}	72.83±5.09^{b}
Azadiracta indica	30	61.10±20.00	63.33±8.82	46.23±8.59	39.60±3.50	31.19±4.52	48.29±5.26^{bc}
	60	77.80±11.10^{a}	55.56±8.01^{b}	45.30±5.35^{bc}	39.34±1.57^{bc}	31.05±1.38^{c}	49.81±4.98^{cd}
	90	88.90±11.10^{a}	75.56±4.4^{ab}	62.10±2.76^{bc}	50.96±5.59^{c}	46.67±3.33^{c}	64.83±4.80^{b}
Carica papaya	30	55.60±29.40	32.20±13.90	24.67±6.81	27.12±4.84	22.14±1.49	32.34±6.53^{d}
	60	50.00±9.62	44.44±8.01	33.13±2.58	33.54±3.68	42.04±3.27	40.63±2.89^{de}
	90	61.11±5.56^{a}	50.00±5.77^{ab}	37.43±6.23^{b}	39.09±6.58^{b}	44.13±4.32^{ab}	46.35±3.16^{c}
Nicotiana	30	38.89±5.56^{a}	37.78±2.22^{a}	20.50±3.21^{b}	23.84±4.78^{b}	24.07±5.07^{b}	29.02±2.64^{d}
tabacum	60	50.00±9.62	35.60±15.60	23.70±10.40	23.84±4.78	30.75±4.82	32.78±4.54^{e}
	90	61.11±5.56^{a}	48.89±8.89^{ab}	32.67±5.98^{b}	35.76±7.16^{b}	37.44±7.16^{b}	43.17±3.76^{c}
Mancozeb®	4	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00^{a}
	8	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00^{a}
	12	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00^{a}

Means on the same row (for each Plant Extract) with different superscript are statistically significant ($p<0.05$) by period of incubation, ns=not significant

Table 3: Percentage Growth Inhibition of *C. eragrostide* of Plant Extracts and Chemical Fungicide and Effect of different Concentrations after 120 Hours of Incubation

Plant Extract	Period of Incubation (Hours) and Mean percentage Growth Inhibition (%)					
	24	***48***	***72***	***96***	***120***	Mean
Concentration I						
Azadiracta indica	61.10±20.00^{ab}	63.33±8.82^{bc}	46.23±8.59^{bc}	39.60±3.50^{bc}	31.19±4.52^{bc}	48.29±5.26^{bc}
Carica papaya	55.60±29.40^{ab}	32.20±13.90^{d}	24.67±6.81^{cd}	27.12±4.84^{bc}	22.14±1.49^{c}	32.34±6.53^{d}
Nicotiana tabacum	38.89±5.56^{b}	37.78±2.22^{d}	20.50±3.21^{d}	23.84±4.78^{c}	24.07±5.07^{c}	29.02±2.64^{d}
Piper guineense	72.20±14.70^{ab}	50.00±5.77^{cd}	32.08±9.12^{bcd}	26.31±9.15^{bc}	30.91±3.34^{bc}	42.30±5.73^{cd}
Zingiber officinale	83.30±16.70^{ab}	74.44±7.29^{b}	48.90±10.30^{b}	41.82±6.05^{b}	41.90±4.23^{b}	58.08±5.98^{b}
Mancozeb	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Concentration II						
Azadiracta indica	77.80±11.10^{ab}	55.56±8.01^{bc}	45.30±5.35^{c}	39.34±1.57^{c}	31.05±1.38^{d}	49.81±4.98^{cd}
Carica papaya	50.00±9.62^{b}	44.44±8.01^{c}	33.13±2.58^{cd}	33.54±3.68^{cd}	42.04±3.27^{bc}	40.63±2.89^{de}
Nicotiana tabacum	50.00±9.62^{b}	35.60±15.60^{c}	23.70±10.40^{d}	23.84±4.78^{d}	30.75±4.82^{d}	32.78±4.54^{e}
Piper guineense	100.00±0.00^{a}	74.44±7.29^{ab}	49.01±8.96^{c}	38.79±5.90^{c}	39.68±5.20^{cd}	60.38±6.74^{bc}
Zingiber officinale	83.30±16.70^{a}	81.11±1.11^{ab}	69.97±6.47^{b}	51.52±1.52^{b}	51.11±1.15^{b}	67.41±4.80^{b}
Mancozeb	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Concentration III						
Azadiracta indica	88.90±11.10^{a}	75.56±4.4^{b}	62.10±2.76^{b}	50.96±5.59^{bcd}	46.67±3.33^{bcd}	64.83±4.80^{b}
Carica papaya	61.11±5.56^{b}	50.00±5.77^{c}	37.43±6.23^{c}	39.09±6.58^{cd}	44.13±4.32^{cd}	46.35±3.16^{c}
Nicotiana tabacum	61.11±5.56^{b}	48.89±8.89^{c}	32.67±5.98^{c}	35.76±7.16^{d}	37.44±7.16^{d}	43.17±3.76^{c}
Piper guineense	100.00±0.00^{a}	87.78±6.19^{ab}	71.03±2.41^{b}	51.52±1.52^{bc}	53.33±3.33^{b}	72.73±5.23^{b}
Zingiber officinale	100.00±0.00^{a}	87.78±6.19^{ab}	66.27±5.16^{b}	54.55±4.55^{b}	55.58±1.15^{bc}	72.83±5.09^{b}
Mancozeb	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Means on the same column (for each concentration) with different superscript are statistically significant (P≤0.05). (**Conc I** = 30 g/L of Plant extract, 4 g/L of Mancozeb; **Conc II** = 60 g/L of Plant extract, 8 g/L of Mancozeb; **Conc III** = 90 g/L of Plant extract, 12 g/L of Mancozeb)

Mean percentage growth inhibition of three concentrations of plant extracts (30 g/L, 60 g/L and 90 g/L) and mancozeb (4 g/L, 8 g/L and 12 g/L) on the mycelial growth of *C. eragrostide* varied with the period of incubation (Figure 5). The highest growth reduction of the pathogen by the plant materials was recorded 24 hours and 48 hours of culture and the efficacy of the plant products generally decreased thereafter; as incubation period increased indicating that the efficacy of the active compounds of the plant materials tested were not persistent in the culture medium or they depreciated in toxicity after two days of culture (Figure 5).

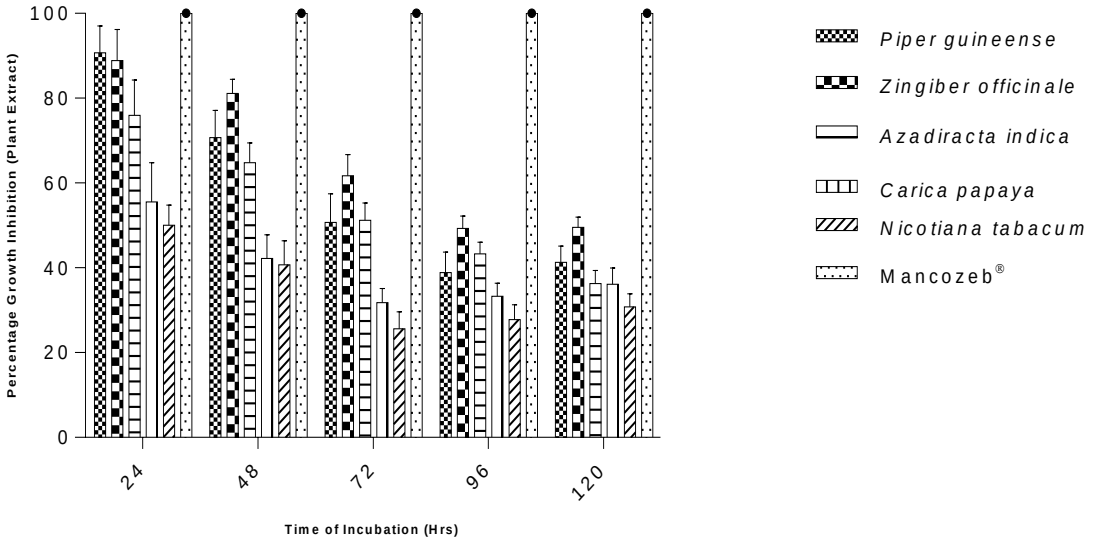

Figure 5: Mean percentage growth inhibition of three concentrations of plant extracts (30 g/L, 60 g/L and 90 g/L) and of mancozeb (4 g/L, 8 g/L and 12 g/L) on mycelial growth of *C. eragrostide*

Effect of concentrations of plant extracts and chemical fungicide in controlling tuber rots of *Ghini* in storage

Figure 6 shows the effect of concentrations I (30 g/L) of *Piper guineense.* Results revealed that the decay reduction index was lowest in December, 2015 and March, 2016 with the value of 0.33 each and highest in January, 2016 and February, 2016 with the value of 0.66 each. At 60 g/L, the performance of the extract was lowest in December with the value of 0.33 and highest throughout the remaining period of storage with the value of 0.66 for each month respectively. At 90 g/L the highest value of 1 was in February, 2016 and the lowest of 0.33 was in December, 2015. The performance of *Z. officinale* and *A. indica* were both better at concentration II (60 g/L) and III (90 g/L) compared with concentration I (30 g/L) while mancozeb performed exceedingly better in February, March and April, 2016.

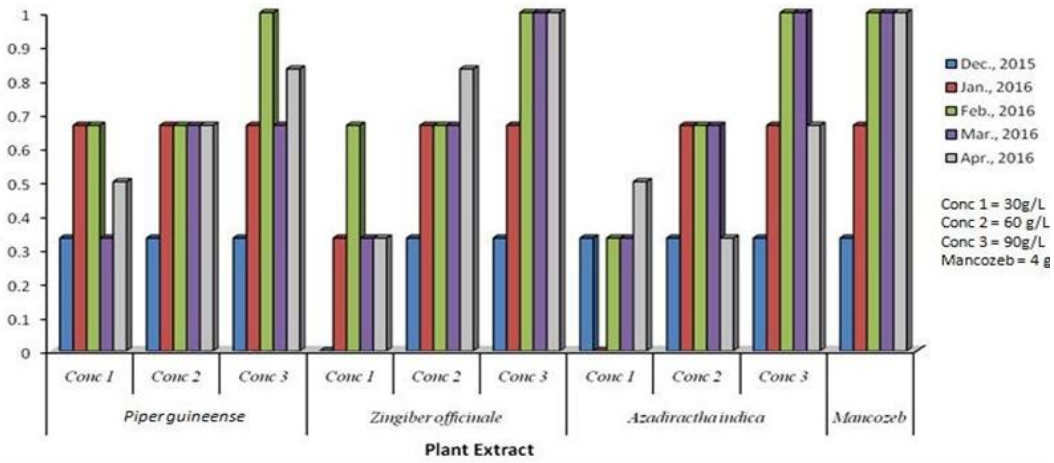

Figure 6: Decay Reduction Index of Plant Extracts and Chemical Fungicide at different Concentrations after Five months of Storage of *Ghini* Tubers

Effect of mean concentrations of plant extract and chemical fungicide in controlling tuber rot of *Ghini* after five months of storage

Table 4 shows results of the performance of mean concentrations of plant extracts (30 g/L, 60 g/L and 90 g/L) chemical fungicide (4 g/L) in controlling rot-causing fungi of *Ghini* tuber. Results indicated that mean decay reduction index in December, 2015, was 0.33 each for Mancozeb, *A. indica* and *P. guineense* while *Z. officinale* recorded the mean value of 0.22. Mean decay reduction index increased in January with mancozeb and *P. guineense* having the values of 0.66 each as against 0.44 and 0.55 for *A. indica* and *Z. officinale* respectively. The efficacy of mancozeb increased in February, March and April, 2016 to 1.00 while *A. indica* increased in February and March, 2016 to 0.66 but decreased thereafter to 0.50 in April, 2016. *P. guineense* extract attended the highest level of efficacy in February, 2016 (0.77) but declined in March, 2016 (0.55) only to rise again in April, 2016 (0.66). Extract of *Z. officinale* recorded 0.77, 0.66 and 0.72 in February, March and April, 2016 respectively. Mean decay reduction index of plant extracts and chemical fungicide in controlling *Ghini* tubers after five months of storage showed that the highest decay reduction index was recorded by *P. guineense* followed by *Z. officinale* and *A. indica* with the mean values of 0.60, 0.58 and 0.52 respectively. There was however, no significant difference (P≤0.05) in potency among the plant extracts for each month of storage. Mean decay reduction index also showed no significant difference (P≤0.05)) among treatments.

Table 4: Mean Decay Reduction Index of Plant Extracts (30 g/L, 60 g/L and 90 g/L) and Chemical Fungicide (4 g/L) in Controlling Tuber Rot of *Ghini* after Five Months of Storage

Period of Storage	Plant Extract			
	Mancozeb	***A. indica***	***P. guineense***	***Z. officinale***
Dec., 2015	0.33 ± 0.33^{ns}	0.33 ± 0.16^{ns}	0.33 ± 0.16^{ns}	0.22 ± 0.14^{ns}
Jan., 2016	0.66 ± 0.33^{ns}	0.44 ± 0.17^{ns}	0.66 ± 0.16^{ns}	0.55 ± 0.17^{ns}
Feb., 2016	1.00 ± 0.00^{ns}	0.66 ± 0.16^{ns}	0.77 ± 0.14^{ns}	0.77 ± 0.14^{ns}
Mar., 2016	1.00 ± 0.00^{ns}	0.66 ± 0.16^{ns}	0.55 ± 0.17^{ns}	0.66 ± 0.16^{ns}
Apr., 2016	1.00 ± 0.00^{ns}	0.50 ± 0.16^{ns}	0.66 ± 0.14^{ns}	0.72 ± 0.14^{ns}
Mean	$\mathbf{0.80\pm0.11^{ns}}$	$\mathbf{0.52\pm0.08^{ns}}$	$\mathbf{0.60\pm0.12^{ns}}$	$\mathbf{0.58\pm0.11^{ns}}$

Means on the same row (comparing plant extracts) with different superscript are statistically different (P≤0.05); ns = not significant

DISCUSSION

The results of the experiments were able to identify these fungi to be responsible for postharvest rot of yam tubers in storage. These pathogenic fungi include *B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum, P. purpurogenum, C. eragrostide* and *Colletotrichum sp.* Recent studies had implicated these pathogens with postharvest rot of yam tubers (Okigbo *et al.,* 2009; Ogunleye and Ayansola, 2014; Gwa and Akombo, 2016; Gwa and Ekefan, 2017). The isolated fungi with the highest rate of occurrence includes: *Aspergillus niger, A. flavus, Botryodiplodia theobromae, Fusarium oxysporum, F. moniliforme.* These results correspond with earlier findings by Okigbo *et al.,* (2015); Ogunleye and Ayansola, (2014); Gwa and Ekefan, (2017). The low frequency of occurrence of *C. eragrostide* confirms earlier report by Amusa, (1997) who reported 13 % in *C. eragrostide* of occurrence on white yam leaves in South-western Nigeria. These pathogenic organisms probably gained access into the tubers through the area where the tuber is separated from the stem at harvest, or from the root tip which often got broken during harvest, or through natural cracks and openings on the surface of the tubers or the soil adhering to the tubers (Osagie, 1992; Okigbo *et al.,* 2009). Inoculation of *C. eragrostide* into the healthy yam tubers produced rot symptoms. This was probably due to the ability of the pathogen to utilize the nutrients of the tubers as substrates for growth and development. The control tubers were however not infected suggesting the absence of inoculum in the bored yam tissues.

The results obtained demonstrated that all the plant extracts and the synthetic chemical; all possess fungitoxic substances potent enough to protect yam tubers against *Curvularia eragrostide in vitro.* The most potent extracts in the *in vitro* control were *P. guineense, Z. officnale* and *A. indica.* The susceptibility of *C. eragrostide* to the tested plant extracts varied with the duration of incubation, the type of plant extract used as well as the concentrations of the extracts. This supports the earlier investigations by Banso and Adeyemo (2007); Gwa and Nwankiti (2017); Gwa and Ekefan (2017); and Gwa *et al.,* (2017). Period of incubation also significantly influenced the efficacy of the extracts indicating that the effects of the active compounds of the extracts used were persistent and increased with the incubation period (Amadioha, 2003; Gwa and Akombo, 2016). Amadioha and Obi (1999) showed that seed extracts of *Azadirachta indica* (neem) and *Xylopia aethiopica* have fungitoxic activity against the anthracnose fungus (*Colletotrichum lindemuthianum*) of cowpea. Similar report was obtained by Hycenth (2008) who reported the antifungal potency of *A. indica* against yam rot pathogen (*Rhizopus stolonifer*). The inhibition of *C. eragrostide* mycelial is due to the presence of anti-nutrients such as tannins, terpenes glycosides, alkaloids, saponins and flavenoids in the aqueous extracts of the leaves of *Azadirachta indica* (Biu *et al.,* 2009). The results showed that the rhizome extract of *Z. officinale* inhibited the growth of *C. eragrostide* at all

levels of concentrations. This confirms the findings of Yeni (2011) who studied the antifungal properties of *Z. officinale* on *A. flavus*, *A. niger*, *F. solani* and *F. oxysporum* on post-harvest rot of yam (*D. alata*) and found out that the extract inhibited the growth of all the pathogens tested. The inhibition of *B. theobromae* and *F. oxysporum* mycelial in culture and on stored yam tubers with seed extract of *P. guineense* agreed with the work of Aidoo (2007) who used the seed extract of *P. guineense* and rhizome of *Z*. *officinale* to inhibit the growth of *B. theobromae* and *F. oxysporum* on two varieties of yam (*D. rotundata* and *D. alata*) respectively. Taiga *et al.* (2008) demonstrated that *N*. *tabacum* cold extract has the potency of inhibiting the mycelial growth of *F*. *oxysporum* yam rot pathogen. Gwa and Akombo (2016) observed that *P. nigrum*, *Z. officinale, A. indica, C. papaya* and *N. tabacum* have significant effect (P≤0.05) on the *in vitro* growth of *A. flavus* isolated from white yam tubers. The authors showed that period of incubation and concentration influenced the efficacy of the extracts on mycelial growth of *A. flavus in vitro*.

Effect of concentrations of plant extract and chemical fungicide in controlling rot organisms of *Ghini* white yam tubers in storage for five months showed that *P. guineense, Z. officinale* and *A. indica* extracts possess fungicidal properties at different concentration levels against rot pathogens of yam in storage. Generally, concentrations II (60 g/L) and III (90 g/L) were more efficacious than concentration I (30 g/L). The variations in efficacy of the extracts may be due to the presence of inhibitors to the fungitoxic principles (Qasen and Abu Blan, 1996; and Amadioha, 2003). Mean decay reduction index for each of the plant extract on *Ghini* cultivar of white yam tubers tested showed mean values above 0.6 for each plant extract indicating that at least 60 % control was achieved through the use of extracts for the control of tuber rots of white yam. This result is in tandem with the findings of Okigbo *et al.* (2009a) who recorded high rot reduction (62.80 %) with *A. sativum* and Udo *et al.* (2001) who reduced the growth and sporulation of fungal pathogens on sweet potato and yam with garlic (*Allium sativum*).

CONCLUSION

Plant extracts such as *P. guineense, Z. officinale, A. indica, C. papya* and *N. tabacum* and chemical fungicide such as mancozeb were able to inhibit pathogenic organisms both *in-vitro* and *in-vivo*. All the plant extracts inhibited the mycelial growth of pathogens *in-vitro* and stopped the growth of the pathogens *in-vivo* irrespective of the concentrations used. High decay reduction index (DRI) at all the level of concentrations showed that the extracts were very effective in controlling postharvest yam tuber rot pathogens in storage. It is therefore, recommended that extracts of plant origin be used in the treatment of yam tubers before storage in order to increase the shelf life of the tubers.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest regarding the publication of this paper.

FUNDING ACKNOWLEDGEMENT

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

REFERENCES

Agrios, G. (2004). *Plant pathology 5 ed.* Elsevier, London.

Ahmed, K. M. and Ravinder Reddy**,** Ch. (1993). A pictorial guide to the identification of seed borne fungi of sorghum, pear millet, finger millet, chickpea, pigeonpea and groundnut.

Information Bulletin no. 34: (In En summaries in Fr, Es and Ar.) Patancheru, A. P. 502324, India: Internation Crops Research Institute for the Semi Arid Tropics. 200pp. ISBN 92- 9066-254-4. Order code. IBE 034

Aidoo, K.A. (2007). Identification of yam tuber rots fungi from storage systems at the Kumasi Central market. A dissertation submitted to Faculty of Agriculture, K.N.U.S.T

Amadioha, A. C and Obi, V. I. (1999). Control of anthracnose disease of cowpea *Cymbopogon citratus* and *Ocimum gratissimum*. *Acta phytopathol. Entomol. Hungeric*a. 34(92): 85-89

Amadioha, A. C. (1996). Control of storage rot of potato caused by *Rhizopus oryzae*. *International journal of Pest Management, 52: 311-324*

Amadioha, A. C. (2003). Evaluation of some Plants Leaf Extracts against *Colletotrichum lindemuthianum* in Cowpea. Acta Phytopathologia et Entomologica Hungarica. 38(3-4)259-265

Amusa, N. A. (1997). Fungi Associated with Anthracnose Symptoms of Yam (*Dioscorea spp*.) in South-West Nigeria and their Roles in Crop. *Research, 13:177-183*

Amusa, N. A., Adegbite, A. A, Muhammed S. and Baiyewu R. A. (2003). Yam diseases and its management in Nigeria. *African Journal of Biotechnology*, 2 (12): 497-502

Arya, A. (2010). Recent Advances in the Management of Plant Pathogens: Botanicals in the Fungal Pest Management. In: Management of Fungal Plant Pathogens. Arya A. and Perello, A. E. (Eds). UK, CAB International, pp. 1-25.

Banso, A. and Adeyemo, S. O. (2007) Evalution of antibacterial properties of tannins isolated from *Dichrostachys cinerea. Afr. J. Biotechnol*, 6(15): 1785-1787

Biu, A. A., Yusuf, S. D. and Rabo, J. S. (2009). Phytochemical Screening of *Azadirachta indica* (Neem). *Bioscience Research Communications, 21:* 281-283.

Cochran, G.W. and Cox, G.M. (1992). Experimental Designs. 2nd Edn John willey and Sons Inc., pp: 611

Food and Agricultural Organization (1998). FAO Annual Report. Food and Agriculture Organisation Production Yearbook. FAO Rome P. 36

Food and Agriculture Organization**.** (2007). Production Year Book, FAO, Rome.

Food and Agriculture Organization**.** (2008). Production Year Book, FAO, Rome.

Food and Agricultural Organization (2013). Food and Agricultural Organisation of the United Nations. www.fao.org/statistics/en/ FAO, Rome

Gwa, I. V., Bem, A. A. and Okoro, J. K. (2015). Yams (*Dioscorea rotundata* Poir *and D. alata* Lam.*)* Fungi Etiology in Katsina-Ala Local Government Area of Benue State, *Nigeria. Journal of Phytopathology and Plant Health 3: 38-43*

Gwa, V. I. and Akombo, R. A. (2016). Studies on the Antimicrobial Potency of Five Crude Plant Extracts and Chemical Fungicide in in vitro Control of *Aspergillus flavus,* Causal Agent of White Yam *(Dioscorea rotundata*) Tuber Rot. *Journal of Plant Sciences and Agricultural Research,* 1(1): 1-8

Gwa, V. I. and Abdulkadir, K. H. (2017). Biological Control Using *Trichoderma harzianum* against *Penicillium purpurogenum*, Causal Agent of White Yam Tuber (*Dioscorea rotundata* Poir) Rot *J. Biores Commun 1:2 1-6.*

Gwa, V.I. and Ekefan, E. J. (2017). Fungal Organisms Isolated from Rotted White Yam (*Dioscorea rotundata*) Tubers and Antagonistic Potential of *Trichoderma harzianum* against *Colletotrichum Species*. Agri Res & Tech: Open Access J. 10(3): 555787. DOI: 10.19080/ARTOAJ.2017.10.555787

Gwa, V. I. and Nwankiti, A. O. (2017). Efficacy of some plant extracts in *in-vitro* control of *Colletotrichum* species, causal agent of yam (*Dioscorea rotundata* Poir) tuber rot. *Asian Journal of Plant Science and Research, 7(2):8-16*

Gwa, V. I., Ekefan, E. J**.** and Nwankiti, A. O. **(**2017). Antifungal Potency of Some Plant Extracts in the Control of White Yam (*Dioscorea rotundata poir*) Tuber Rot. Adv Biotech & Micro 7(1): 555703. DOI: 10.19080/AIBM.2017.07.555703

Hycenth, N. (2008). Effect of Different Plant Extracts in the Control of Yam (*Dioscorea* sp) in Yola, Adamawa State, Nigeria. *Nigerian Agricultural Journal,* 3 (5*): 382-387*.

Kleih, U., Phillips, D., Ogbonna, M. and Siwoku, B. (2012). Nigeria-Scoping Yam Value Chain Analysis. Yam Improvement for Income and Food Security in West Africa. ABK Publishers. pp 1-53.

Korsten, L. and De Jager, E. S. (1995). Mode of action of Bacillus subtilis for control of avocado post harvest pathogens*. S. Afr. Avocado Growers Assoc. Yearb*, 18: 124-130

Lakshmeesha, T.R., Sateesh, M.K., Vedashree, S., Sofi, M.S. and Umesha, S. (2013). Efficacy of botanicals on soybean seed-borne *Fusarium equiseti. VCFL Sciences*, 3, 10-16.

Markson, A. A., Amadioha, A. C., Omosun, G., Madunagu, B. E., Udo, S. E. and Umana, E. J**.** (2012). Control of *Botryodiplodia theobromae* causing Tissue Rot of White Yam (*Dioscorea rotundata* Poir) *Scholarly Journal of Agricultural Science* Vol. 2(1), pp. 1-7

Nene, Z.H and Thapilyal, (2002). Management of mushroom pathogens through botanicals. *Ind. Phytopathol*. 58:189-193.

Ogunleye, A.O., Ayansola, O. T. (2014). Studies of Some Isolated Rot-Causing Mycoflora of Yams (*Dioscorea* Spp.). *Amer. J. Microb. and Biot.* Vol. 1, No. 1, pp. 9-20.

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

Okigbo, R.N. (2005) Biological Control of Postharvest Fungal Rot of Yam (*Dioscorea* spp.) with *Bacillus subtilis*. *Mycopathologia*, **159**, 307-314.

Okigbo, R.N. and Ogbonnaya, U.O. (2006). Antifungal effects of two tropical plant leaves extract (*Ocimum gratissimum* and *Aframomum melegueta*) on post harvest yam (*Dioscorea* spp.) rot. *Afr. J. Biotech*., 5(9): 727- 731.

Okigbo, R.N., Putheti, R. and Achusi, C.T. (2009a). Post-harvest deterioration of cassava and its control using extracts of *Azadirachta indica* and *Afromonium meleguata.* E-J Chem, 6(4):1274-1280

Okigbo, R. N and Emeka, A. N. (2010). Biological Control of Rot-inducing Fungi of Water Yam (Dioscorea alata) with *Trichoderma harzianum, Pseudomonas syringe* and *Pseudomonas chlororaphis. Journal of stored product Research, 1(2): 18-23*

Okigbo, N. R., Enweremadu, C. E., Agu, C. K., Irondi, R. C., Okeke, B. C., Awah, S. N., Anaukwu, C. G., Okafor, I. O., Ezenwa, C. U. and Iloanusi, A. C. (2015). Control of white yam (*Dioscorea rotundata)* rot pathogen using peel extract of water yam (*Dioscorea alata*) Advances in Applied Science *Research, 6(10)*:7-13

Okoro, O. and Nwankiti, A.O. (2004):Post-harvest Microbial Rot of Yam in Nigeria. *Pathologia:* 35-40.

Osagie, A. U. (1992). The Yam Tuber in Storage. Postharvest Research Unit, Department of Biochemistry, University of Benin, Benin-City, Nigeria. Pp 146 – 149.

Qasem, J. R. and Abu Blan, H. A. (1996). Fungicidial Activity of some common Weed Extracts against Difference Pathogenic Fungi. *Journal of Phytopathology, 144: 157-161*

Rachie, P., Vanderlinde, R., Dutra, S and Marcon A. (2005). Control of pest and diseases on cowpea seed, text book of Agricultural science, pp 13

Shiriki, D., Ubwa, S.T. and Shambe, T. (2015). Isolation of Nine Microorganisms from Rotten *Dioscorea rotundata* (White Yam) and Antimicrobial Sensitivity Test with Five Plant Extracts. *Food and Nutrition Sciences*, 6, 825-835.

Singh, N.D., Sharma, A. K., Dwivedi, P., Kumar, M. and Patil, R. D. (2011) Immunosuppressive effect of combined citrinin and endosulfan toxicity in pregnant wistar rats. *Veterinarski Arhiv,* 81, 751-763.

Taiga, A., Suleiman, M. N., Sule, W. and Olufolaji, D. B. **(**2008) Comparative *in vitro* inhibitory effects of cold extracts of some fungicidal plants on *Fusarium oxysporium* Mycelium. ***7 (18):*** 3306-3308

Udo, S. E., Madunagu, B. E. and Isemin, C. D. (2001). Inhibition of growth and sporulation of fungal pathogens on sweet potato and yam by garlic extracts. *Nigeria Journal of Botany, 14*: 35-39.

Vedashree, S., Sateesh, M. K., Lakshmeesha, T. R., Sofi, M. S. and Vedamurthy, A. B. (2013). Screening and assay of extracellular enzymes in *Phomopsis azadirachtae* causing die-back disease of neem. *J Agricultural Technol*, 9, 915-927.

Yeni, I. J. (2011). Evaluation of Antifungal Effects of Extracts of *Allium sativum* and *Nicotiana tabacum* against Soft Rot of Yam (*Dioscorea alata*). *Journal of Agricultural Research*, *3:1-5.*