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# *In-vitro* and *In-vivo* Antimicrobial Potency of Selected Plant Extracts Against Postharvest Rot-Causing Pathogens of Stored Yam Tubers

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## Abstract

Potency of *Piper guineense* Linn., *Zingiber officinale* Rosc., *Azadirachta indica* A. Juss., *Carica papaya* Lam. and *Nicotiana tabacum* Linn. againt *in vitro* control of *Curvularia eragrostide* and *in vivo* inhibitions of rot-causing microorganisms in storage were studied. *Rotted Ogoja* and *Ghini* white yam tubers were picked from yam farmers at various locations at Lafia, Nigeria. Rot-causing organisms from *Ghini* and *Ogoja* that were isolated for a period of four months included *Botryodiplodia theobromae, Aspergillus flavus, A. niger, Fusarium moniliforme, Colletotrichum sp, F. oxysporum*, *C. eragrostide* and *Penicillium purpurogenum*. Pathogenicity test confirmed all the isolated fungi as rot causing organisms. Result showed that *Z. officinale*, *P. guineense, A. indica, C. papaya* and *N. tabacum* exhibited more antifungal properties against *C. eragrostide* at 60 g/L and 90 g/L than at 30 g/L. Results further confirmed that *Z. officinale*, *P. guineense, A. indica* and mancozeb were more efficacious *in vitro. In vivo* test using the most potent extracts; *Z. officinale*, *P. guineense* and *A. indica* and mancozeb revealed that the selected plant extracts were effective against postharvest pathogens of yam. Mean decay reduction index (DRI) of more than 0.6 indicated that the extracts and the chemical inhibited the growth of the rot causing organisms by more than 60% throughout the five months storage period. It is therefore recommended that extracts from these plants could be formulated at appropriate concentrations and used to inhibit the growth of postharvest pathogens of yam tubers because of their cheapness, ease to purchase and environmental friendliness.

**Keywords:** Plant extracts; Postharvest; 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 [1-3]. The most cultivated yam species is white yam (*Dioscorea rotundata*) followed by water yam (*D. alata*). The largest producer of yam in the World is Nigeria accounting for 38.92 million metric tonnes per annum [4,5]. In spite of large scale production, postharvest losses of yam tubers caused by pathogens continue unabated starting from the field through harvest to storage [6-8]. According to Arya [9], postharvest losses caused by pathogenic organisms are the costliest than any other loss. These pathogenic organisms consistently found to incite rot in yam tubers include *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *Fusarium oxysporum, F. solani, F. moniliforme, Penicillium chrysogenum, P. digitatum, P. oxalicum, P. purpurogenum, Rhizoctonia spp, Botryodiplodia theobromae, Rhizopus nodosus* and *Colletotrichum spp,* [8,10-15]. 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 [16]. 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 [6,17-19]. Chemical method of control is fast and, in most cases, most effective [18]. Chemical residues are not safe and also the likelihood of inflicting toxicity to human beings, pollution of the environmental as well as being non-biodegradable [20,21]. However, pesticides formulated from plant origin are biodegradable, cheap, easily available, and environmentally safe compared with synthetically made pesticides [22]. Hence, extracts from plant origin could go a long way in serving as an alternative to synthetically formulated pesticides in controlling pathogens of plant [14,23,24]. The research was therefore, carried out to test the potency of some selected plant extracts on *in vitro* and *in vivo* management of pathogens associated with storage rot of yam tubers.

## Materials and Methods

### Study area

The experiment was carried out at the department of crop and environmental protection, laboratory and in the University farm, Federal University of Agriculture, Makurdi, Nigeria.

### Collection of rotted samples

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

### Isolation of pathogenic fungi

Rotted sample of yam tubers were washed and neatly cut between healthy and diseased tissues. Cut sections were washed in running tap water and were aseptically sterilized by dipping them in 5% Sodium hypochlorite solution for about 2 min. The dipped tissues were later removed, and four successive changes of sterile distilled water were used

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to rinsed them from the chemical. The yam tissues were then dried for two minutes on sterile filter papers that were placed in the laminar Air flow cabinet before inoculation.

## Inoculation

Infected sections that were dried were later picked from the Laminar Air flow Cabinet using sterile forceps. Petri dishes containing acidified sterile potato dextrose agar (PDA) were used to aseptically plate the sections before incubation at ambient room temperature (30 ± 5°C) for 7 days for establishment of growth.

### Identification of fungi organisms

Fungal mycelial that grew were sub-cultured and incubated for 5 days before obtaining pure cultures of the pathogens. When growths of the isolates were fully established, the growths were microscopically examined; morphologically characterized, identified, and were compared with established findings [25].

### Frequency of occurrence of fungal isolates

This was done by keeping records of the organisms isolated from time to time. Isolation and identification were done at monthly interval based on the frequency of occurrence of each fungus isolated in each month for four months. This was calculated as a percentage of the sum of all the other organisms isolated per month as described by Okigbo and Ikediugwu, [26] as follows:

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

Where,

x= number of times of occurrence of a particular isolates in a month.

n= sum of occurrence of microorganisms isolated in the study area in a month.

Stock culture of *C. eragrostide* was maintained on slant of acidified potato dextrose agar (PDA) in McCartney bottles for further experiments.

### Pathogenicity test of isolated fungi

Isolated fungi organisms were pathogenetically tested using healthy yam tubers. Yam tubers were aseptically washed in running tap water with 5% Sodium hypochlorite solution for 5 min and the tubers were successively rinsed in four changes of sterile distilled water to remove the adhering chemicals on the yam tubers before inoculation. A sterile 5 mm cork borer was used to remove a 4 mm tissue from the healthy yam. A 5 mm diameter disc of pure culture of the fungi were each cut and inserted in the holes created in the healthy yam tubers separately. Same procedure was replicated as control experiment but in place of inoculum, sterile agar discs were used [24]. The remaining portions of the holes created in the yam tubers were sealed with sterile petroleum jelly to prevent microbial penetration. The treatments were replicated three times before storing them under sterile condition at ambient room temperature (30 ± 5°C). Growth and establishment of the fungi organisms in the yam tubers were observed after 14 days of incubation.

### Preparation of plant extracts

Preparation of plant extracts was done as described by Gwa and Akombo [18] and Gwa et al. [24]. Leaves of *Carica papaya (*Pawpaw*),* seeds of *Piper guineense* (Black Pepper), rhizomes of *Zingiber officinale* (Ginger), leaves of *Nicotiana tabacum* (Tobacco) and leaves of *Azadirachta indica* (Neem) were collected from different botanical gardens, identified, carefully washed with clean tap water, plant materials were air-dried before they were separately ground into fine powder using a mortar and pestle. A mixture concentration of 30 g/L, 60 g/L and 90 g/L were prepared by dissolving 30 g, 60 g, and 90 g powder of each plant extracts into 1 L of hot sterile distilled water (100°C) separately in 1000 ml Pyrex flask. The mixtures were allowed to stay for 24 h before filtration using a four folds sterile cheese cloth. The filtrates of the various plant extracts at their different concentrations were used as plant extracts for the *in vitro* management of *C. eragrostide*. Mancozeb was prepared by dissolving 4 g, 8 g, and 12 g separately in 1 L of sterile distilled water to give concentrations of 4 g/L, 8 g/L and 12 g/L respectively. The effectiveness of the extracts and mancozeb were tested *in vitro* against *C. eragrostide* and the most potent plants found *in vitro* were selected for *in vivo* management of pathogenic yam tubers in storage.

### Measurement of mycelial extension 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 [27]. It involves drawing two perpendicular lines at the bottom of the plate to create four equal sections. The intersection of the lines shows it center where the organism will be inoculated. In each of the Petri dishes, 15 ml of PDA was poured with 5 ml of each plant extract and mancozeb at their respective concentrations [28]. The mixtures were given some time to solidify before inoculation at the center of the plates where the two perpendicular lines met at the bottom of the plates with a 5 mm diameter disc of a 7-days old culture of *C. eragrostide* [29]. The treatments were replicated three times. In the control experiments, 5 ml of sterile distilled water was instead added to plates containing solidified PDA in place of plant extracts and mancozeb. Both the control and treated experiments were incubated for 120 h at ambient room temperature (30 ± 5°C). Measurements of growth were done at 24 h interval for five consecutive days [16]. The effectiveness of the extracts and mancozeb were determined as absence of growth of *C. eragrostide* in the treated plates compared with the control. Potency was calculated as percentage growth inhibition (PGI) as described by Korsten and De Jager, [30].

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

Where,

PGI = Percentage Growth Inhibition.

R = the distance of *C. eragrostide* growth from the point of inoculation to the colony margin in control plate,

$R_1$ = the distance of *C. eragrostide* growth from the point of inoculation to the colony margin in treated plate.

### To test the effectiveness of plant extracts and mancozeb in managing yam pathogens in storage

The efficacy of seeds of *P. guineense,* leaves of *A. indica* and rhizomes of *Z. officinale* and mancozeb that have been found to posses' more fungicidal properties *in vitro* were used to manage yam pathogens *in vivo*. *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 and 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, they were allowed to dry before storing them for five months. Each treatment comprises of three tubers which were replicated three times bringing the total to 9 tubers. There were 11 treatments. A total of 99 tubers of *Ghini* yam were used for the experiment. Data on the potency of the extracts and chemical fungicide in controlling pathogens of yam during storage were collected after each month for a period of 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 unrotted and rotted tubers in each treatment were recorded. The effectiveness of the different concentrations of extracts and mancozeb in managing yam pathogens in storage were evaluated. The Decay Reduction Index by Amadioha, [31] defined below, was calculated as a measure of the effectiveness of various extracts and mancozeb in managing yam tuber rot pathogens in storage after final data collection as:

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

### Statistical analysis

Data collected were analysed using Analysis of variance (ANOVA) and GenStat Discovery Edition 12 and Graph Pad Prism 6 for trend graphs. Statistical F-tests were evaluated at $P \leq 0.05$ using Fisher's least significant differences (FLSD) [32].

## Results

### Identification of *C. eragrostide*

Fungi isolates identified 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 (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).

### Occurrence of fungal isolates in Lafia

Figure 2 shows identified fungal organisms in Lafia as *B. theobromae, A. flavus, A. niger, F. moniliforme, F. oxysporum, P. purpurogenum, C. eragrostide* and *Colletrichum sp.* The 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 the rest of the isolation period. *A. niger* showed higher occurrence in *Ghini* compared with *Ogoja* throughout the period. *A. flavus* was highest in *Ogoja* and lowest in *Ghini* in the month of February, March and May but showed higher occurrence in *Ogoja* than *Ghini* in April. *B. theobromae* was highest in *Ogoja* compared with *Ghini* in February, April and May. *B. theobromae* showed the highest level of occurrence in *Ghini* than in *Ogoja* in March. The occurrence of *Colletotrichum sp.* in *Ghini* rose from March to April and declined steadily in May. The result showed 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* was not encountered in *Ghini* but occurred in *Ogoja* in all the months of isolation and was found to be highest in April. The occurrence of *F. oxysporum* was less in *Ogoja* than *Ghini* in February but more in *Ogoja* than *Ghini* in March, April, and May.

There were no significant differences ($P \leq 0.05$) in 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 between *Ghini* and *Ogoja* tubers (Table 1). Significant differences ($P \leq 0.05$) were however, observed in mean percentage frequency of occurrence between the two cultivars for *P. purpurogenum* and *C. eragrostide* (Table 1).

### Pathogenicity test

Results of pathogenicity test conducted on *Ghini* tubers using *C. eragrostide* in Figure 3 show that the pathogen incited rot symptoms in the apparently good-looking yam tubers 14 days after incubation. Rot symptoms were observed on the healthy-looking tubers. Tubers inoculated without the test fungus in the control experiments however, showed no symptoms of rot in the bored yam tissues (Figure 4).

### *In vitro* effect of plant extracts and mancozeb on growth of *C. eragrostide*

The results of *C. eragrostide* radial mycelial growth on PDA amended with plant extracts and synthetic fungicide in Table 2 show that *Z. officinale, P. guineense, A. indica, C. papaya* and *N. tabacum* exhibited more antifungal properties against *C. eragrostide* at 60 g/L and 90 g/L compared with 30 g/L. There was no significant difference

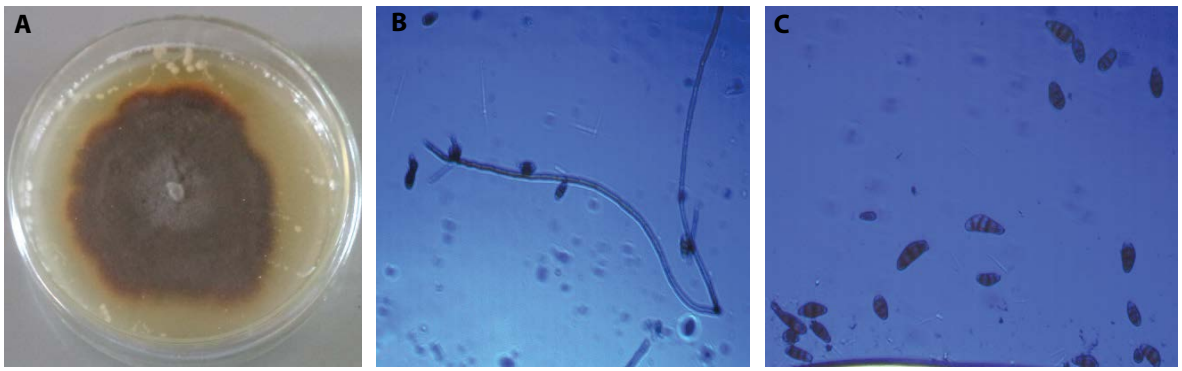


**Figure 1:** Culture of *C. eragrostide* growing on PDA (10x) **(A)**; Photomicrograph of *C. eragrostide* showing conidiophores bearing conidia (10x) **(B)** and conidia of *C. eragrostide* (10x) **(C)**.

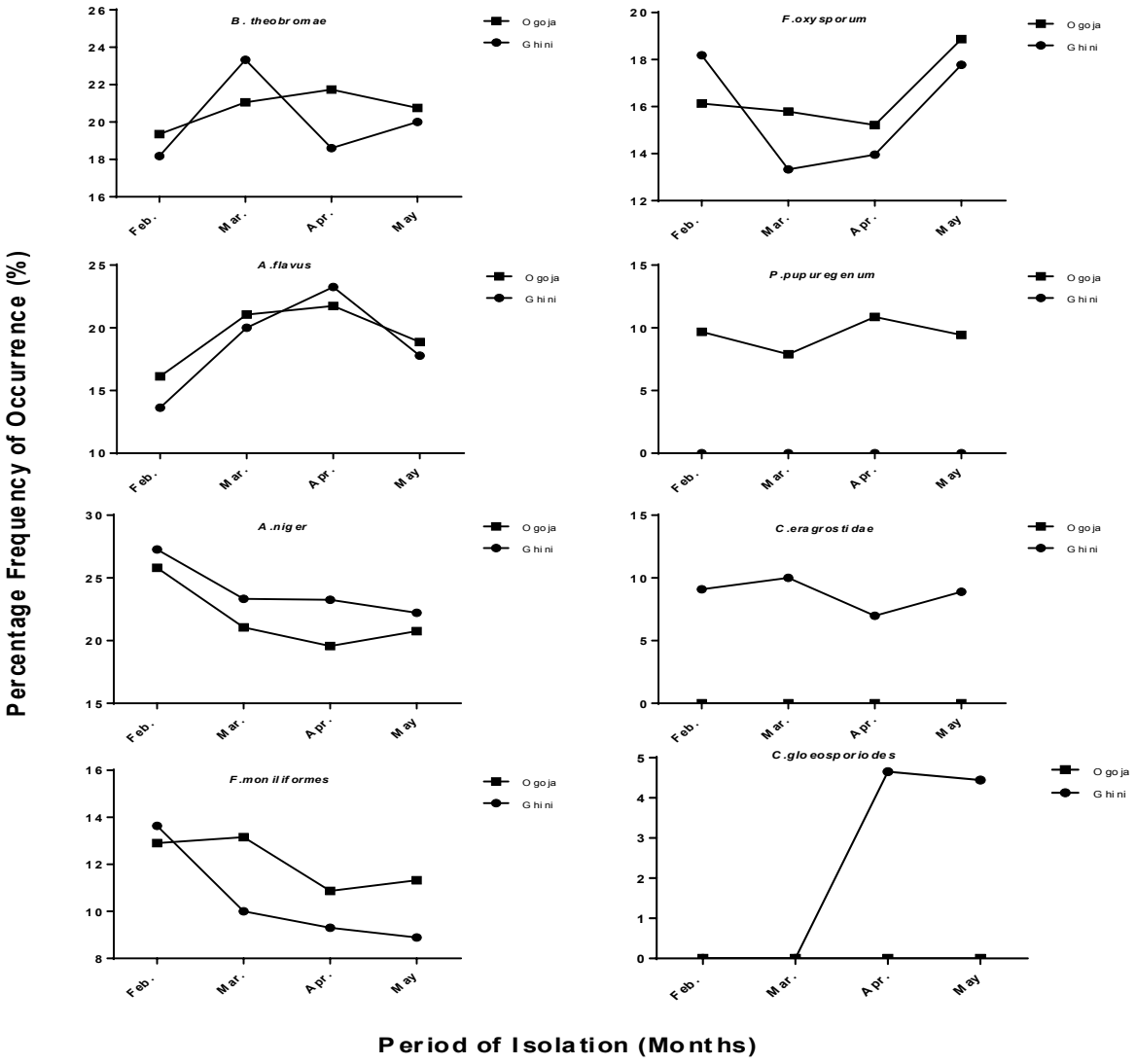


**Figure 2:** Percentage frequency of occurrence of fungal pathogens from *Ogoja* and *Ghini* white yam tubers from february to may 2015 in Lafia.

| 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) | | | | |

**Table 1:** Mean percentage frequency of occurrence of fungal isolates from *Ghini* and *Ogoja* cultivars of white yam tuber after four months of isolation in Lafia.

($P \leq 0.05$) at 30 g/L at 24 h but significantly differed ($P \leq 0.05$) for the remaining period of incubation (Tables 2 and 3). Mean percentage growth inhibition of the tested plant extracts 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 showed the highest 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

**Figure 3:** Rot caused by *C. eragrostide*.

**Figure 4:** Control (No organism inoculated).

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. Mancozeb exhibited the highest inhibition of *C. eragrostide* followed by *Z. officinale* and *P. guineense*.

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

## Effect of concentrations of plant extracts and mancozeb in controlling tuber rots of *Ghini* in storage

Figure 6 shows the effect of concentrations 30 g/L of *Piper guineense* on stored yam tubers for five months. 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 decay reduction index value of 1 was recorded in February 2016 and 0.33 was recorded in December 2015. The performance of *Z. officinale* and *A. indica* were both better at 60 g/L and 90 g/L compared with 30 g/L while mancozeb performed exceedingly better in February, March, and April 2016.

## Effect of mean concentrations of plant extracts mancozeb in controlling tuber rot of *Ghini* after five months of storage

Table 4 shows results of the performance of mean of 30 g/L, 60 g/L and 90 g/L of plant extracts and 4 g/L of mancozeb 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 the extracts and mancozeb in controlling *Ghini* tubers after five months 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. Significant differences ($P \leq 0.05$) were not observed in potency among the plant extracts for each month of storage. There was also no significant difference ($P \leq 0.05$) in mean decay reduction index among treatments.

## Discussion

The experiments were able to identify these fungi to be responsible for postharvest deterioration in storage yam. The rot organisms 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 [7,11,18,24]. The isolated fungi with the highest rate of occurrence includes: *Fusarium oxysporum Aspergillus niger, A. flavus, F. moniliforme* and *Botryodiplodia theobromae.* These results correspond with earlier findings by Okigbo et al. [33]; Ogunleye and Ayansola, [14]; Gwa and Ekefan, [24]. The low occurrence of *C. eragrostide* confirms earlier report by Amusa, [34] who reported 13% of *C. eragrostide* 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 [35-37]. When healthy yam tubers were inoculated with *C. eragrostide* rot symptoms were produced. This means that the *C. eragrostide* utilize the nutrients that were in the yam tubers for growth and development. The absent of growth in

| 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 tabacum* | 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$^{d}$ |
| | 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 2:** Percentage growth inhibition of *C. eragrostide* using plant extracts and chemical fungicide after 120 h of incubation.

| Plant extract | Period of incubation (Hours) and mean percentage growth inhibition (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | 120 | |
| **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$^{a}$ |
| Note: 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). | | | | | | |

**Table 3:** Percentage growth inhibition of *C. eragrostide* using plant extracts and mancozeb after 120 h of incubation.

the control experiment indicates that there was no infectious agent to initiate growth.

The findings demonstrated that plant extracts and mancozeb contain antimicrobial compounds potent enough to inhibit the growth of *C. eragrostide in vitro*. The most potent plants in the *in vitro* management were *P. guineense, Z. officnale* and *A. indica.* The susceptibility of *C. eragrostide* depended on the type of extract, concentration, and duration of incubation. This is in conformity with investigations carried out by Gwa and Nwankiti [14]; Gwa and Ekefan [15]; and Gwa et al., [24]. Amadioha and Obi [27] showed that anthracnose disease of cowpea caused by *Colletotrichum lindemuthianum* could be controlled by seed extracts of *A. indica* (neem) and *Xylopia aethiopica*. Similar report was obtained by Hycenth [36] who reported the antifungal potency of *A. indica* against *Rhizopus stolonifer* causal agent of yam tuber rot. The inhibition of *C. eragrostide* mycelial is as a resent of presence of antimicrobial compounds such as tannins, terpenes glycosides, alkaloids, saponins and flavenoids in *A. indica* [37]. The results revealed that rhizome extract of *Z. officinale* reduced the growth of *C. eragrostide* at all levels of concentrations. This confirms the findings of Yeni [38] 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*) found out that the extract was capable of arresting the growth of all the tested pathogens. The inhibition of

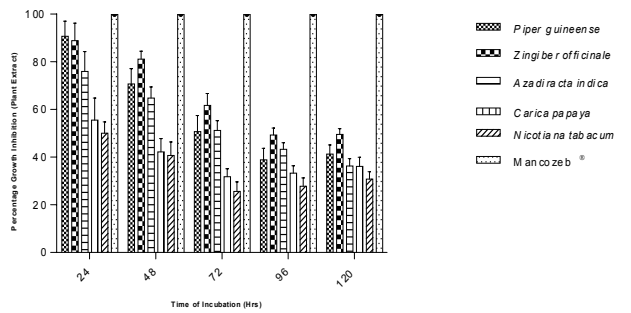


**Figure 5:** Mean percentage growth inhibition of 30 g/L, 60 g/L and 90 g/L of plant extracts and 4 g/L, 8 g/L and 12 g/L of mancozeb on inhibition of *C. eragrostide*.

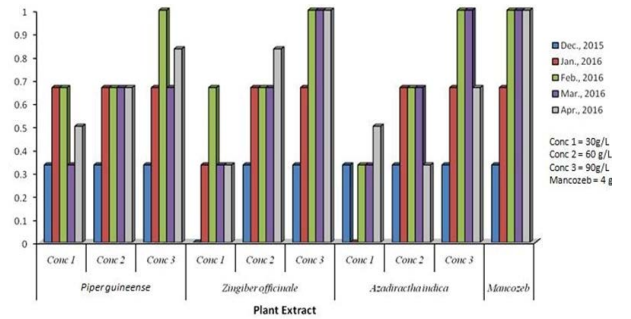


**Figure 6:** Decay reduction index of some plant extracts and mancozeb at different concentrations after five months of storage of *Ghini* tubers.

| Period of Storage | Plant extract | | | |
|---|---|---|---|---|
| | Mancozeb | *A. indica* | *P. guineense* | *Z. officinale* |
| Dec 2015 | $0.33 \pm 0.33^{ns}$ | $0.33 \pm 0.16^{ns}$ | $0.33 \pm 0.16^{ns}$ | $0.22 \pm 0.14^{ns}$ |
| Jan 2016 | $0.66 \pm 0.33^{ns}$ | $0.44 \pm 0.17^{ns}$ | $0.66 \pm 0.16^{ns}$ | $0.55 \pm 0.17^{ns}$ |
| Feb 2016 | $1.00 \pm 0.00^{ns}$ | $0.66 \pm 0.16^{ns}$ | $0.77 \pm 0.14^{ns}$ | $0.77 \pm 0.14^{ns}$ |
| Mar 2016 | $1.00 \pm 0.00^{ns}$ | $0.66 \pm 0.16^{ns}$ | $0.55 \pm 0.17^{ns}$ | $0.66 \pm 0.16^{ns}$ |
| Apr 2016 | $1.00 \pm 0.00^{ns}$ | $0.50 \pm 0.16^{ns}$ | $0.66 \pm 0.14^{ns}$ | $0.72 \pm 0.14^{ns}$ |
| Mean | $0.80 \pm 0.11^{ns}$ | $0.52 \pm 0.08^{ns}$ | $0.60 \pm 0.12^{ns}$ | $0.58 \pm 0.11^{ns}$ |
| Note: Means on the same row (comparing plant extracts) with different superscript are statistically different ($P \leq 0.05$); ns=not significant. | | | | |

**Table 4:** Mean decay reduction index of 30 g/L, 60 g/L and 90 g/L of plant extracts and 4 g/L of mancozeb in controlling tuber rot of *Ghini* after five months of storage.

*B. theobromae* and *F. oxysporum* mycelial in culture and on stored yam tubers with seed extract of *P. guineense* conforms to the work of Aidoo [39] who used rhizome of *Z. officinale* and *P. guineense* seeds and inhibited *B. theobromae* and *F. oxysporum D. rotundata* and *D. alata*. Taiga et al., [40] demonstrated that *N. tabacum* cold extract has the potency of inhibiting the mycelial growth of *F. oxysporum* yam rot pathogen. Gwa and Akombo [18] showed that *P. nigrum*, *Z. officinale, A. indica, C. papaya* and *N. tabacum* significantly ($P \leq 0.05$) inhibited the *in vitro* growth of *A. flavus* causal agent of yam tuber rot. The authors demonstrated that concentration, period of incubation as well as the type of plant extract influenced the potency of the extracts on growth of *A. flavus in vitro*.

Effect of concentrations of plant extract and chemical fungicide in controlling rot organisms of *Ghini* stored for five months showed that *P. guineense, Z. officinale* and *A. indica* extracts possess fungicidal properties at different concentration levels storage pathogens. 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 antimicrobial compounds in the extracts [41,42]. Mean decay reduction index for the various extracts on *Ghini* cultivar of white yam tubers tested showed mean values above 0.6 for each plant extract indicating 60% control with the extracts for on storage yam. The result agreed with findings of Okigbo et al., [7] who recorded high rot reduction (62.80%) with *A. sativum* and Udo et al. [43] who used garlic (*Allium sativum*) to inhibit growth and sporulation of fungal pathogens on sweet potato and yam.

## Conclusion

Plant extracts such as *P. guineense, Z. officinale, A. indica, C. papaya* and *N. tabacum* and synthetic pesticide such as mancozeb are capable of inhibiting pathogenic organisms both *in-vitro* and *in-vivo*. All the plant extracts inhibited the growth of *C. eragrostide in-vitro* and stopped the growth of yam pathogens *in-vivo* irrespective of type of extract or concentration used. High decay reduction index (DRI) indicated 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 so as to prolong the storage life of the tubers.

### Conflict of Interest Disclosure

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

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