Journal of
Plant Pathology & Microbiology
ISSN: 2157-7471

Ekefan et al., J Plant Pathol Microbiol 2018, 9:7
DOI: 10.4172/2157-7471.1000444

Research Article Open Access

Comparative Assessment of Antimicrobial Potency of Some Selected Plant Extracts Against Seed Borne Pathogens of Germinating Yam Setts

Ekefan EJ[1], Nwankiti AO[1] and Gwa VI[1,2]*

[1]*Department of Crop and Environmental Protection, Federal University of Agriculture, Makurdi, Nigeria*
[2]*Faculty of Agriculture and Agricultural Technology, Department of Crop Production and Protection, Federal University, Dutsin-Ma, Katsina State, Nigeria*

Abstract

Comparatives studies of the potency of seeds of *Piper guineense* Schumach. (Black peper), rhizomes of *Zingiber officinale* Rosc. (Ginger), leaves of *Azadirachta indica* A. Juss. (neem), leaves of *Carica papaya* Lam. (pawpaw) and leaves of *Nicotiana tabacum* Linn. (Tobacco) were tested against growth of *F. solani in vitro*. Pathogenicity tests were carried out on *Botryodiplodia theobromae, Aspergillus flavus, A. niger, A. ochraceus, Fusarium moniliforme, F. oxysporum, F. solani, Curvularia eragrostide* and *Colletotrichum sp* which confirmed that all the fungi organisms elicited rot on healthy Hembankwase cultivar. *In vitro* tests of the different plant extracts on *F. solani* at 30 g/L, 60 g/L and 90 g/L revealed that all the extracts were fungitoxic. However, *P. guineense* and *Z. officinale* were more potent compared with *A. indica, C. papaya* and *N. tabacum*. The synthetic fungicide mancozeb consistently gave higher inhibition of 100% throughout the period of incubation in spite of the concentration used. Application of these extracts in the control of seed borne pathogens of yam during germination of yam setts also proved effective in both years with decay reduction index (DRI) ranging from 0.22 in Hembankwase to 0.88 in Pepa using *Z. officinale* in 2015 compared with 0.66 in Hembankwase and 0.77 in Pepa using *P. guineense* in 2016. Mean decay reduction index showed that all the extracts were more potent on Pepa cultivar than *Hembankwase*. It is therefore, concluded that plant extracts could be used as alternative to chemicals in controlling fungal pathogens of yam both *in vitro* and *in vivo*.

Keywords: Antimicrobial; Comparative; Decay reduction index; Pathogenicity test; Plant extracts; Seed borne

Introduction

Yam (*Dioscorea rotundata* Poir) is an important food crop in West Africa, the Caribean's, South America, and South-East Asia [1] Production and consumption of yam is mostly in West Africa with Nigeria been the highest producer with about 38.92 million metric tonnes annually [2]. The production and storage of yam is constrained by several factors such as pathogens mostly from fungi which are considered heavy in Nigeria [3-5]. Rot of yam tubers in storage caused by pathogenic organisms is of particular importance because the pathogens reduce viability of the tubers and subsequently yield and quality [6,7]. Fungi implicated as rot causing organisms of yam tubers in storage include *Fusarium oxysporum, F. moniliforme, F. solani, Penicillium chrysogenum, P. oxalicum, P. purpurogenum, P. digitatum, Rhizopus nodosus, Rhizoctonia spp, Aspergillus flavus*, *A. ochraceus*, *A. niger*, *Colletotrichum spp, Botryodiplodia theobromae,* [5-13]. Fungi pathogens have also been reported to be major cause of storage rots of cassava and sweet potatoes [14-16]. Synthetic chemicals have been used in reducing rot of yam tubers both *in vitro* and *in vivo* [3,17-20]. These chemical pesticides are found to be costly, may even cause environmental pollution and induce pathogen resistance [21,22]. Pesticides of plant origin are generally considered to be better in controlling pathogens of plants both in the field and in storage barns because they are cheap, biodegradable, safe, easily available and environmentally free from pollution compared with the chemically formulated fungicides [23,24]. Ability of plants to synthesize aromatic secondary metabolites such as coumarins, flavones, flavonoids, flavonols phenols, phenolic acids, quinines, tannins, terpenoids, alkaloids [25,26] have made them effective against rot-causing pathogens [26-28]. Thousands of these phyto-chemicals metabolites have been demonstrated to have inhibitory effects on all types of pathogens *in vitro* and are therefore subjected to *in vivo* testing in order to evaluate the efficacy of the plant extracts in controlling diseases in crops [19,27,29,30]. It is against this backdrop that different plants were selected and formulated to test their potencies in inhibiting pathogens of yam in storage hence could be considered as alternatives in the control of pathogens of yam both in culture and in the field.

Materials and Methods

Experimental site

The experiment was carried out at the Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria.

Collection of diseased yam tubers

Diseased white yam (*Dioscorea rotundata*) varieties (Ghini, Hembankwase) showing different types of symptoms were collected from farmers' barns in Kadarko, Keana local government area of Nasarawa State, Nigeria. The settlement is located between longitudes 8°30' and 8°35'E, and latitudes 8°10′ and 8°14′N. Tubers were collected and sealed in sterile polyethylene bags to prevent wounding and possible infection by other pathogenic organisms. The tubers were taken to the laboratory for subsequent isolation and identification of rot causing fungi two days after collection of samples. Potato Dextrose Agar (PDA) medium was prepared according to manufacturer's recommendation and used for isolation of the rot causing fungi pathogens. Pathogenicity tests were done using the healthy yam tubers collected from same location.

Isolation and identification of rot causing fungi

Diseased yam tubers showing different symptoms of rots were washed in clean water before cutting into small pieces of about 2 × 2 mm from the advancing edge of lesion using sterile scalpel. A 5% Sodium

***Corresponding author:** Gwa VI, Department of Crop and Environmental Protection, Federal University of Agriculture, PM B 2373 Makurdi, Nigeria, Tel: +2348039357109; E-mail: igwa@fudutsinma.edu.ng

Received June 11, 2018; **Accepted** July 26, 2018; **Published** July 31, 2018

Citation: Ekefan EJ, Nwankiti AO, Gwa VI (2018) Comparative Assessment of Antimicrobial Potency of Some Selected Plant Extracts Against Seed Borne Pathogens of Germinating Yam Setts. J Plant Pathol Microbiol 9: 444. doi: 10.4172/2157-7471.1000444

hypochlorite solution was used to surface sterilized the cut tissues for 2 minutes in order to clean them off from contaminants. The sterilized cut pieces were then rinsed in four successive changes of sterile distilled water in order to remove the chemical before drying on sterile filter paper for 5 minutes [31]. Plates containing sterile solidified potato dextrose agar (PDA) were inoculated with four pieces of the sterilized diseased yam. Inoculated plates were neatly covered with mastic tapes to prevent contamination by air borne fungi and incubated at ambient room temperature (30 ± 5°C) for 7 days. The growths of the fungi were observed daily. Fungi colonies were sub-cultured and identified after 7 days of incubation when pure cultures were fully grown [31]. Different fungal colonies were seen from the rotted yam samples, from which *B. theobromae, A. flavus, A. niger, A. ochraceus, F. moniliforme, F. oxysporum, F. solani, C. eragrostide* and *Colletotrichum sp* were identified, purified and multiplied on PDA. *F. solani* wass choosen for further studies since it has not been previously isolated in this location. Identifications of grown pure cultures the fungi were done using morphological characteristics as well as microscopic and identification guide [32,33].

Pathogenicity tests

Pathogenicity tests of *B. theobromae, A. flavus, A. niger, A. ochraceus, F. moniliforme, F. oxysporum, F. solani, C. eragrostide* and *Colletotrichum sp* isolated from the two varieties of rotted yam lesions were confirmed by inoculating these organisms into healthy Hembankwase yam tubers. Running tap water was used to wash the healthy-looking yam tubers before sterilization with a 5% Sodium hypochlorite solution for 2 minutes. Tubers were then rinsed in four successive changes of sterile distilled water. Cylindrical discs of 5 mm were removed from the healthy yam tubers using a sterile cork borer. Mycelial discs of each fungus measuring 4 mm in diameter were taken from 5 day-old cultures of each of the fungi and each fungal disc was put into the hole created in the tubers. The control experiments had sterile agar discs inserted in the holes created instead of the inoculum which was obtained from the fungi [19]. Sterile petroleum jelly was used to completely seal the remaining parts of the holes to prevent pathogenic invasion. The inoculated yam tubers were replicated three times for each of the tested pathogens and control experiments and were arranged in completely randomized design. The treatments were incubated at ambient room temperature (30 ± 5°C) under sterile condition for 14 days for growth of the fungi to establish. When growths were fully established, the tubers were cut transversely at point of inoculation to determine the extent of infection and disease development. Disease symptoms that developed on the artificially inoculated yam tubers with the pathogens after the incubation period were compared with those naturally infected tubers initially collected from farmers' barns. Re-isolation of the fungi pathogens from the inoculated diseased yam tubers were done and cultured on PDA plates. The characteristic culture of each of the pathogens obtained was compared with the culture initially collected from the naturally infected tubers.

Preparation of plant extracts

The preparations of plant extracts were carried out using the methods of Gwa and Akombo, [11] and Gwa and Nwankiti [12]. Extracts prepared from healthy plant materials were from leaves of *Azadirachta indica* (Neem), rhizomes of *Zingiber officinale* (Ginger), seeds of *Piper guineense* (Black Pepper), leaves of *Nicotiana tabacum* (Tobacco) and leaves of *Carica papaya*. These materials were washed neatly with cold running tap water; air-dried and separately ground into fine powder using pestle and mortar. About 30 g, 60 g and 90 g of the ground plant materials were dissolved in 1000 ml of sterile distilled hot water (100°C) separately in 1500 ml Pyrex flask. The mixtures were stirred and left for 24 hours and subsequently filtered using four-fold of sterile cheese cloth. These gave concentrations of 30 g/L, 60 g/L and 90 g/L, respectively. The filtrates obtained were used as the plant extracts in the experiment. Mancozeb, a chemical fungicide was prepared by dissolving 4 g, 8 g and 12 g in one litre of sterile distilled water separately to give concentrations of 4 g/L, 8 g/L and 12 g/L, respectively. The potencies of the extracts and the chemical fungicide were compared for their *in vitro* fungicidal activity in inhibiting mycelial growth of *F. solani*.

Effect of plant extracts on *F. solani*

The effects of the prepared extracts were evaluated on radial mycelial growth of *F. solani* using the method of Amadioha and Obi [34]. Four equal sections were created at the bottom of the plates by drawing two perpendicular lines. The intersections of the two lines were considered as the centre of the plates before dispensing PDA into each of the plates. About 15 ml of the prepared medium was poured into sterilized Petri dishes containing 5 ml of each plant extract and chemical fungicide at their respective concentrations [35], mixed well and allowed to solidify. The solidified medium was inoculated centrally at the point of intersection of the two perpendicular lines drawn at the bottom of the plate with discs 5 mm diameter of one-week-old cultures [36] of *F. solani*. The treatments were replicated three times for each of the concentrations. In the control experiments, 5 ml of sterile distilled water was added to PDA in place of plant extracts respectively; the treatments and control were completely randomized [37] and incubated for 120 hours at ambient room temperature (30 ± 5°C).

Measurement of mycelial radial growth of *F. solani*

Growth of *F. solani* was measured after 24 hours for five consecutive times using a transparent ruler. The absence of growth in any of the plates was an indication of the efficacy of the extracts and the chemical fungicide against *F. solani*. Fungitoxicity was calculated as percent growth inhibition (PGI) of *F. solani* over control based on the formula stated by Korsten and De Jager [38].

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

Where,

PGI=Percentage Growth Inhibition,

R=The distance (measured in mm) 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.

The potencies of the aqueous plant extracts and the chemical fungicide that were found to be effective against *F. solani in vitro* were used to control other seed pathogens of yam in the field.

Potency of plant extracts and chemical fungicide in controlling rot causing pathogens of white yam setts planted in the field

The potencies of the five plant extracts in controlling yam tuber rot *in vitro* were tested on yam setts planted *in vivo*. The extracts were applied on four cultivars of white yam namely: Ogoja, Pepa, Hembankwase and Ghini to test their efficacies in controlling rots pathogens on germination of white yam setts in the field using the different aqueous extract concentrations. The four cultivars were each sprayed with the five botanical extracts at concentration of 30 g/L, 60 g/L and 90 g/L, respectively while mancozeb was applied at

concentrations of 4 g/L, 8 g/L and 12 g/L, respectively using a hand sprayer. After spraying, the tubers were allowed to dry before planting. The treatments were completely randomized, and control treatments were sprayed with sterile distilled water (no plant extract or chemical applied). The experiments were monitored regularly for the first eight weeks after planting to allow enough time for germination of all the tubers. The tubers that did not germinate were removed thereafter and examined for signs of rots. The numbers of unrotten (germinated) and rotten (ungerminated) tubers in each treatment were recorded for Ogoja, Pepa, Hembankwase and Ghini cultivars, respectively. The effectiveness of each plant extract and chemical fungicide in controlling yam tuber rot and increasing germination of yam setts at different concentrations was calculated using the Decay Reduction Index (DRI) as proposed by Amadioha [39].

$$Decay\ \mathrm{Re}ductionIndex(\mathrm{DRI}) = \frac{\%\text{decayin control} - \%\text{decayin treated tubers}}{\%\,\text{decay in control}}$$

Statistical analysis

Analysis of variance (ANOVA) using GenStat Discovery Edition 12 for ANOVA and means separation 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) [40].

Results

Isolation of fungal pathogens from rotted yam tubers

B. theobromae, *A. flavus*, *A. niger*, *A. ochraceus*, *F. moniliforme*, F. oxysporum, *F. solani, C. eragrostide* and *Colletotrichum sp.,* were isolated from Hembankwase and Ghini cultivars of white yam tuber samples collected from farmers' barns in Kadarko a major yam producing settlements in Nasarawa State, Nigeria. Figure 1 shows the characteristics pure culture of *F. solani* which was chosen for this study because it has not been previously studied in this location. The fungus was grown on Potato dextrous agar (PDA) as shown in Figure 2.

Pathogenicity tests

Pathogenicity tests conducted on *B. theobromae, A. flavus, A. niger, A. ochraceus, F. moniliforme, F. oxysporum, F. solani, C. eragrostide* and *Colletotrichum sp* using Hembankwase cultivar of yam tubers show that all the organisms were pathogenic. Results presented in Figure 3 show that *F. solani* induced rot in healthy looking Hembankwase cultivar of yam 14 days after inoculation. Re-isolation of the test organism, *F. solani* from the artificially inoculated yam tubers shows symptoms similar with the naturally infected tubers. Tubers that were not inoculated with *F. solani* mycelial (control) did not show symptoms of rot in the hole created in the yam tubers (Figure 4).

Figure 1: Culture of *F. solani* on Potato Dextrose Agar (10x).

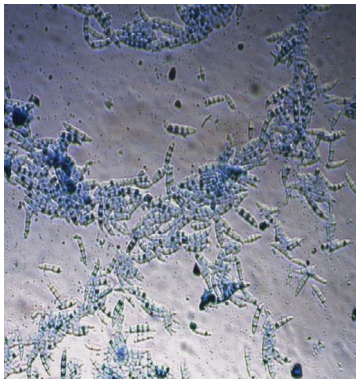

Figure 2: Photomicrograph of *F. solani* showing macroconidia (10x).

Figure 3: Rot caused by *F. solani.*

Effect of some plant extracts on growth of *F. solani in vitro*

Results presented in Table 1 show that *P. guineense, Z. officinale, A. indica, C. papaya* and *N. tabacum* extracts possess fungicidal properties at different concentration levels against mycelial growth of *F. solani in vitro*. However, only mancozeb gave 100% inhibition of mycelial growth of *F. solani*. All the plant extracts were able to reduce the radial growth of the test fungus throughout the period of incubation irrespective of the concentration level used. Though the potency of the extracts decreased with increase in the period of incubation; the test fungus was effectively controlled throughout the test period. *P. guineense, Z. officinale* and *A. indica* were considered better extracts compared with *C. papaya* at all the levels of concentrations. At concentration I (plant extract 30 g/L and mancozeb 4 g/L), the percentage growth inhibition of *F. solani* decreased at 24 hours from 79.44%, 79.44%, 73.89%, 75.60%, 53.33% and 100% to 48.20%, 48.27%, 35.54%, 29.75%, 23.92% and 100% at 120 hours with *P. guineense, Z. officinale, A. indica, C. papaya, N. tabacum* and mancozeb, respectively. At concentration II (plant extract 60 g/L and mancozeb 8 g/L), the percentage growth inhibition of *F. solani* decreased at 24 hours from 100%, 87.89%, 79.44%, 93.33%, 60.00% and 100% to 55.22%, 55.18%, 47.04%, 39.11%, 34.39% and 100% after 120 hours with *P. guineense Z. officinale, A. indica, C. papaya, N. tabacum* and mancozeb, respectively. At concentration III (plant extract 90 g/L and mancozeb 12 g/L), the percentage growth inhibition of *F. solani* decreased at 24 hours from 100%, 93.33%, 79.44%, 94.44%, 80.60% and 100% to 61.96%, 63.11%, 56.32%, 56.09%, 43.51% and 100%

Period of Incubation (Hours) and Percentage Growth Inhibition (%)								
Plant Extract	**Concentration (g/L)**	**24**	**48**	**72**	**96**	**120**	**LSD**	**Mean**
Piper guineense	30	79.44 ± 2.42a	59.62 ± 3.76b	55.11 ± 1.32bc	47.33 ± 1.76c	48.20 ± 2.61c	7.93	57.94 ± 3.26
	60	100.00 ± 0.00a	72.86 ± 3 .15b	60.04 ± 3.38c	58.11 ± 1.16c	55.22 ± 1.10c	6.88	69.25 ± 4.49
	90	100.00 ± 0.00a	78.42 ± 2.52b	69.10 ± 1.64c	64.94 ± 3.16c	61.96 ± 2.75c	7.27	74.89 ± 3.77
Zingiber officinale	30	79.44 ± 2.42a	59.62 ± 3.76b	49.82 ± 2.69c	51.28 ± 2.96bc	48.27 ± 1.79c	8.79	57.69 ± 3.27
	60	87.89 ± 6.19a	70.09 ± 5.90ab	69.40 ± 15.30ab	58.06 ± 1.94b	55.18 ± 1.73b	25	68.10 ± 4.29
	90	93.33 ± 6.67a	78.42 ± 2.52b	67.25 ± 0.58bc	63.50 ± 2.36c	63.11 ± 3.00c	11.42	73.12 ± 3.37
Azadiracta indica	30	73.89 ± 3.89a	51.50 ± 3.59b	55.28 ± 1.60b	47.33 ± 1.76b	35.54 ± 2.33c	8.82	52.71 ± 3.50
	60	79.44 ± 2.42a	56.84 ± 1.50b	55.01 ± 2.52b	54.06 ± 3.46bc	47.04 ± 2.15c	7.85	58.48 ± 3.09
	90	79.44 ± 2.42a	67.52 ± 4.89b	58.35 ± 4.19bc	58.06 ± 1.94bc	56.32 ± 0.59c	10.11	63.94 ± 2.62
Carica papaya	30	75.60 ± 12.40a	27.14 ± 3.15b	38.01 ± 4.68b	41.94 ± 5.01b	29.75 ± 4.17b	21.25	42.48 ± 5.30
	60	93.33 ± 6.67a	51.28 ± 4.98b	47.97 ± 4.54b	48.61 ± 1.81b	39.11 ± 1.36b	13.73	56.06 ± 5.36
	90	94.44 ± 5.56a	57.05 ± 5.56b	55.37 ± 5.96b	55.50 ± 4.19b	56.09 ± 5.86b	15.85	63.69±4.53
Nicotiana tabacum	30	53.33 ± 3.33a	18.80 ± 5.26b	22.19 ± 3.69b	23.00 ± 1.53b	23.92 ± 5.50b	12.99	28.25 ± 3.73
	60	60.00 ± 10.00a	27.14 ± 3.15b	36.35 ± 1.62b	35.11 ± 2.47b	34.39 ± 2.93b	15.9	38.60 ± 3.54
	90	80.60 ± 10.00a	35.26 ± 3.29b	41.55 ± 1.85b	43.28 ± 3.69b	43.51 ± 4.18b	17.01	48.83 ± 4.77
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
	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
	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
Means on the same row (for each Plant Extract) with the different superscript (a, b and c) are statistically significant ($p \leq 0.05$) by period of incubation								

Table 1: Percentage growth inhibition of *F. solani* at concentrations of plant extracts and chemical fungicide after 120 hours of incubation in culture.

Period of Incubation (Hours) and Percentage Growth Inhibition (%)						
Plant Extract	**24**	**48**	**72**	**96**	**120**	**Mean**
I						
Azadiracta indica	73.89 ± 3.89b	1.50 ± 3.59b	5 55.28 ± 1.60b	47.33 ± 1.76bc	35.54 ± 2.33c	52.71 ± 3.50b
Carica papaya	75.60 ± 12.40b	27.14 ± 3.15c	38.01 ± 4.68c	41.94 ± 5.01c	29.75 ± 4.17cd	42.48 ± 5.30c
Nicotiana tabacum	53.33 ± 3.33c	18.80 ± 5.26c	22.19 ± 3.69d	00 ± 1.53d	23. 23.92 ± 5.50d	28.25 ± 3.73d
Piper guineense	79.44 ± 2.42b	59.62 ± 3.76b	55.11 ± 1.32b	47.33 ± 1.76bc	48.20 ± 2.61b	57.94 ± 3.26b
Zingiber officinale	79.44 ± 2.42b	59.62 ± 3.76b	49.82 ± 2.69b	51.28 ± 2.96b	48.27 ± 1.79b	57.69 ± 3.27b
Mancozeb	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
LSD	17.39	11.16	8.63	8.19	7.97	--
II						
Azadiracta indica	79.44 ± 2.42b	56.84 ± 1.50c	55.01 ± 2.52bcd	54.06 ± 3.46bc	47.04 ± 2.15c	58.48 ± 3.09bc
Carica papaya	93.33 ± 6.67ab	51.28 ± 4.98c	47.97 ± 4.54cd	48.61 ± 1.81c	39.11 ± 1.36d	56.06 ± 5.36c
Nicotiana tabacum	60.00 ± 10.00c	27.14 ± 3.15d	36.35 ± 1.62d	35.11 ± 2.47d	34.39 ± 2.93d	38.60 ± 3.54d
Piper guineense	100.00 ± 0.00a	72.86 ± 3.15b	60.04 ± 3.38bc	58.11 ± 1.16b	55.22 ± 1.10b	69.25 ± 4.49b
Zingiber officinale	87.89 ± 6.19ab	70.09 ± 5.90b	69.40 ± 15.30b	58.06 ± 1.94b	55.18 ± 1.73b	68.10 ± 4.29b
Mancozeb	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
LSD	17.27	11.37	20.89	6.47	5.52	--
III						
Azadiracta indica	79.44 ± 2.42b	67.52 ± 4.89bc	58.35 ± 4.19c	58.06 ± 1.94bc	56.32 ± 0.59b	63.94 ± 2.62c
Carica papaya	94.44 ± 5.56ab	57.05 ± 5.56c	55.37 ± 5.96c	55.50 ± 4.19c	56.09 ± 5.86b	63.69 ± 4.53c
Nicotiana tabacum	80.60 ± 10.00b	35.26 ± 3.29d	41.55 ± 1.85d	43.28 ± 3.69d	43.51 ± 4.18c	48.83 ± 4.77d
Piper guineense	100.00 ± 0.00a	78.42 ± 2.52b	69.10 ± 1.64b	64.94 ± 3.16b	61.96 ± 2.75b	74.89 ± 3.77b
Zingiber officinale	93.33 ± 6.67ab	78.42 ± 2.52b	67.25 ± 0.58b	63.50 ± 2.36bc	63.11 ± 3.00b	73.12 ± 3.37bc
Mancozeb	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
LSD	16.95	11.45	7.14	8.95	10.43	--
Means on the same column (for each concentration) with different superscript (a, b and c) are statistically significant ($p \leq 0.05$). (I=30 g/L of Plant extract, 4 g/L of Mancozeb; II=60 g/L of Plant extract, 8 g/L of Mancozeb; III=90 g/L of Plant extract, 12 g/L of Mancozeb)						

Table 2: Comparative assessment of plant extracts and chemical fungicide at different concentrations after 120 hours of incubation.

at 120 hours in *P. guineense, Z. officinale, A. indica, C. papaya, N. tabacum* and mancozeb respectively (Table 1). There were significant differences *($p \leq 0.05$)* in the activity of plant extracts at each level of concentration throughout the period of incubation (Table 2). Mean percentage growth inhibition of *F. solani* after 120 hours of incubation revealed increase in the performance of the extracts from the lowest concentration to the highest concentration. The most potent extracts at concentrations I (30 g/L) were *P. guineense* (57.94%), followed by *Z. officinale* (57.69%), *A. indica* (52.71%), *C. papaya* (42.48%) and *N. tabacum* (28.25%) respectively while mancozeb consistently gave 100%

Yam Varieties and Mean decay Reduction Index					
Plant Extracts	**Ghini**	**Hembankwase**	**Ogoja**	**Pepa**	**LSD**
Year 2015					
P. guineense	0.55 ± 0.17	0.33 ± 0.16	0.66 ± 0.16	0.77 ± 0.14	0.47ns
Z. officinale	0.66 ± 0.16	0.22 ± 0.14	0.55 ± 0.17	0.88 ± 0.11	0.43
A. indica	0.44 ± 0.17	0.33 ± 0.16	0.33 ± 0.16	0.88 ± 0.11	0.45
C. papaya	0.55 ± 0.17	0.33 ± 0.16	0.66 ± 0.16	0.55 ± 0.17	0.49ns
N. tabacum	0.44 ± 0.17	0.33 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.48ns
Mancozeb	0.55 ± 0.17	0.33 ± 0.16	0.66 ± 0.16	0.77 ± 0.14	0.47ns
LSD	0.49ns	0.46ns	0.47ns	0.41ns	--
Year 2016					
P. guineense	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.77 ± 0.14	0.46ns
Z. officinale	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.47ns
A. indica	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.47ns
C. papaya	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.47ns
N. tabacum	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.47ns
Mancozeb	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.66 ± 0.16	0.47ns
LSD	0.47ns	0.47ns	0.47ns	0.46ns	--
Mean (2 years)					
P. guineense	0.61 ± 0.11	0.49 ± 0.12	0.66 ± 0.11	0.77 ± 0.10	0.46ns
Z. officinale	0.66 ± 0.11	0.44 ± 0.12	0.60 ± 0.11	0.77 ± 0.10	0.47ns
A. indica	0.55 ± 0.12	0.49 ± 0.12	0.49 ± 0.12	0.77 ± 0.10	0.47ns
C. papaya	0.61 ± 0.11	0.50 ± 0.12	0.66 ± 0.11	0.60 ± 0.11	0.47ns
N. tabacum	0.55 ± 0.12	0.50 ± 0.12	0.66 ± 0.11	0.66 ± 0.11	0.47ns
Mancozeb	0.61 ± 0.11	0.49 ± 0.12	0.66 ± 0.11	0.72 ± 0.02	0.47ns
LSD	0.24ns	0.16ns	0.25ns	0.29ns	--
Means on the same row (comparing yam varieties) and column (comparing plant extracts) with the different superscript a, b and c are statistically significant ($p \leq 0.05$); ns=not significant					

Table 3: Mean decay reduction index of some plant extracts and chemical fungicide against rot-causing pathogens of white yam setts in the field.

Figure 4: Control (without *F. solani* inoculum).

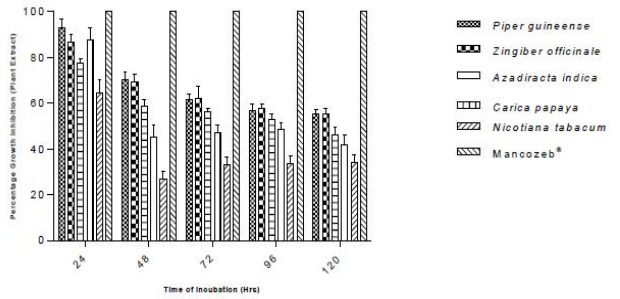

Figure 5: Mean of three concentrations of each plant extract (30 g/L, 60 g/L and 90 g/L) and mancozeb (4 g/L, 8 g/L and 12 g/L) on growth of *F. solani.*

inhibition in *F. solani* throughout the period of incubation and at all level of concentrations respectively (Table 2). Mean percentage growth inhibition of three concentrations (I, II and III) of each plant extract and chemical fungicide showed that *P. guineense* and *Z. officinale* were the most effective extracts in reducing the mycelial growth of *F. solani* in culture throughout the period of incubation (Figure 5).

Effect of plant extracts and chemical fungicide in controlling rot causing pathogens of white yam setts planted in the field

Table 3 compares the potency of some plant extracts and chemical fungicide in controlling rots causing pathogens of white yam setts planted in the field using decay reduction index method. Result showed that all the plant extracts were able to inhibit growth of rot causing pathogens on all the cultivars of white yam setts planted for 2015 and 2016 seasons. In 2015, *P. guineense* was more potent on Pepa (0.77) cultivar followed by Ogoja (0.66) while the least inhibition was recorded on Hembankwase (0.33) cultivar. *Z. officinale* was more effective on Pepa (0.88) but least on Hembankwase (0.22). *A. indica* (0.88) *N. tabacum* (0.66) and Mancozeb (0.77) respectively gave a higher decay reduction index value in *Pepa* compared with Hembankwase which recorded values of 0.33 for each of the extracts respectively. *C. papaya* was however, more potent on Ogoja (0.66) compared with Hembankwase (0.33). Only *Z. officinale* and *A. indica* gave significant difference ($p \leq 0.05$) among the four cultivars of white yam setts tested in the field. There was also no significant difference ($p \leq 0.05$) among extracts for each cultivar of white yam tested. The result of 2016 revealed that only *P. guineense* proved more effective on Pepa (0.77)

cultivar but less effective (0.66) on other cultivars respectively. There was no significant difference ($p \leq 0.05$) among cultivars for each plant extract tested and no significant difference among extracts for each cultivar used. Mean decay reduction index for two years for each of the cultivars used and each of the extracts tested showed no significant differences for each extract and for each cultivar respectively (Table 3).

Discussion

Chemical fungicides have been found to be most effective in the control of fungal pathogens of crops both in the field and in store. In order to find out fungicides which are effective, plant fungicides were tested at different concentrations both *in vitro* and *in vivo*. The result revealed that *P. guineense, Z. officinale, A. indica, C. papaya* and *N. tabacum* and the synthetic chemical; mancozeb all possess' antimicrobial properties potent enough to inhibit mycelial growth of *F. solani* in culture. The inhibitory activity of *F. solani* by the different plant extracts depend on the magnitude of concentration as well as the duration of incubation and the type of plant extract used [11,17,41]. Percentage growth inhibition of *F. solani* increased with increase in concentrations of plant extracts indicating that the effects of the active compounds of the extracts used were persistent and increased with the incubation period [11,12,42,43]. This result is similar to the finding of [19] that *P. guineense, Z. officinale, A. indica, C. papaya* and *N. tabacum* significantly inhibited the growth of *B. theobromae* in culture at higher concentrations compared to lower concentrations. The inhibition of *F. solani* by *P. guineense* extracts may be attributed to presence of phyto-chemical compounds such as *piperine*. Antimicrobial activity of piperine increased as the concentration increases against *F. solani*. Similar study was carried out by Aidoo, [44] who used the seed extract of *P. guineense* and rhizome of *Z. officinale* to reduce the growth of *B. theobromae* and *F. oxysporum* on two varieties of white yam (*D. rotundata* and *D. alata*).

The study also revealed that the rhizome extract of *Z. officinale* completely inhibited the growth of *F. solani* at different concentrations. Result of Okigbo and Nneka [45] and Gwa and Akombo [11] confirmed that *Z. officinale* inhibited the growth of rot fungi in culture and reduces rot development in yam tubers. Results obtained by Anukwuorji et al., [46] revealed the abundance of saponins in *Z. officinale* was responsible for inhibition of pathogens.

In other studies, conducted by Suresh et al. [47] Wang et al. [48] and Gwa et al. [19] the higher the concentration (nicotine) of *N. tabacum* the more the inhibition of mycelial growth of pathogens. This is in agreement with the result obtained here as the growth of *F. solani* was reduced more at higher concentrations compared with application of *N. tabacum* at lower concentration. Amadioha and Obi [34] showed that *A. indica* (neem) and *Xylopia aethiopica* seed extracts have fungitoxic activity against the anthraconose fungus (*Colletotrichum lindemuthianum*) of cowpea. The inhibitory activity of *C. papaya* is attributed to its active compound papain [26,49] *C. papaya*, *C. odorata* and *Acalypha ciliata* has proved very effective against pawpaw fruit rot fungi. In similar studies, Ogwulumba et al. [50] used *C. papaya* leaf extracts to reduce incidence of foliar mycopathogens of groundnut *(Arachis hypogea)* while result obtained by Suleiman [51] showed the inhibition of mycelial growth of *Alternaria solani*, causal agent of yam rot using leaf extracts of *C papaya*. The inhibitory activity of *C. papaya* may be due to the presence of phyto-chemical compounds such as tannins, glycosides, alkaloids, and flavonoids [52].

Study has shown that *A. indica* as a medicinal plant possesses potent antifungal properties in the leaves which inhibit the growth of pathogens both *in-vitro* and *in-vivo*. According to Amadioha [53], *A. indica* contains phytochemical compounds such as azadirachtin, betasiterol and 3- desciacetyl alamine which show antifungal properties. Hycenth [54] demonstrated the antimicrobial effect of *A. indica* against yam rot pathogens (*Rhizopus stolonifer*). Mallesh and Narendrappa [55] showed that leaf extract of *A. indica* caused maximum inhibition of mycelial growth of *F. solani* and *R. solani*. Similarly, Yelmame et al., [56] showed inhibitory effect against *F. solani* using neem cake. Mohammad et al., [57] investigated antifungal activity of ten plant extracts under laboratory conditions and they found out that only higher doses of *A. indica* and *Calotropis procera* extracts caused maximum inhibition followed by *Citrus hystrix* and *Capsicum annuum* extracts. The five extracts (*P. guineense, Z. officinale, A. indica, C. papaya* and *N. tabacum*) and chemical fungicides (Mancozeb) which were applied on yam setts before planting were able to protect the yam setts against rot pathogens by 22% in Hembankwase and 77% in Pepa varieties. It was observed that the second year of planting gave a similar but better result with all the plant extracts and the chemical fungicide reducing rot by at least 66% in each cultivar of the white yam setts. The reason for the occurrence of more disease in the first year could probably be due to the interaction between the pathogens, host and environmental conditions which favoured the production of inoculum in the first year more than in the second year hence a better control in the second year. This result agreed with the work of Ekundayo [58] and Agrios [59] who found that favourable environmental condition favoured the production of inoculum. The work is similar to the result obtained by Aidoo [44] who recorded the decay reduction index value of Mancozeb in controlling the rot of 'dente' white yam tuber at 0.46 and that of 'pona' white yam tuber at 0.60 which corresponded to reduction in rot by 46% for 'dente' and 60% for 'pona' varieties of white yam, respectively. It was observed that *Z. officinale* and *A. indica* extracts significantly differed across varieties in the first year and that all the extracts were more potent on Pepa cultivar but less effective on Hembankwase cultivar in both years. The different levels of reductions of rot by the plant fungicides may probably be due to the varying extent of interference of the different phytochemical compounds with the metabolism of the rot organisms involved.

Conclusion

Yam tubers are susceptible to different fungal pathogens both in storage and in the field. Crude extracts of plant from *P. guineense, Z. officinale, A. indica, C. papaya* and *N. tabacum* and the synthethetic chemical mancozeb could be used in controlling pathogens of yam both in storage and in field. Since chemical fungicides are costly, non-biodegradable, toxic to man and environment, it is therefore, recommended that application of plant extracts as natural fungicides for the control of different plant pathogens of yam setts before planting be adopted for small-scale farmers to reduce rot of yam setts and increase germination as well as increase shelf life of harvested produce.

References

1. Food and Agricultural Organization (2013) Food and Agricultural Organisation of the United Nations.
2. Kleih U, Phillips D, Ogbonna M, Siwoku B (2012) Nigeria-Scoping yam value chain analysis. Yam Improvement for income and food security in West Africa. ABK Publishers pp: 1-53.
3. Amusa NA, Adegbite AA, Muhammed S, Baiyewu RA (2003) Yam diseases and its management in Nigeria. African Journal of Biotechnology 2: 497-502.
4. Okigbo RN, Putheti R, Achusi CT (2009a) Post-harvest deterioration of cassava and its control using extracts of *Azadirachta indica* and *Afromonium meleguata*. E-J Chem 6: 1274-1280.
5. Gwa IV, Bem AA, Okoro JK (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.

6. Arinze AE (2005) Plant pathology and post –harvest food loss. An Inaugural Lecture Series 43: 29-72.

7. Okigbo RN, Anuagasi CL, Amadi JE (2009) Advances in selected medicinal and aromatic plants indigenous to Africa. Journal of Medicinal Plant Research 3: 86-95.

8. Markson AA, Amadioha AC, Omosun G, Madunagu BE, Udo SE, et al. (2012) Control of *Botryodiplodia theobromae* causing tissue rot of White Yam (*Dioscorea rotundata Poir*). Scholarly Journal of Agricultural Science 2: 1-7.

9. Ogunleye AO, Ayansola OT (2014) Studies of some isolated rot-causing Mycoflora of yams (*Dioscorea Spp*.). Amer J Microb and Biot 1: 9-20.

10. Okigbo NR, Enweremadu CE, Agu CK, Irondi RC, Okeke BC, et al. (2015) Control of white yam (*Dioscorea rotundata*) rot pathogen using peel extract of water yam (*Dioscorea alata*). Advances in Applied Science Research 6: 7-13.

11. Gwa VI, Akombo RA (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: 1-8.

12. Gwa VI, Nwankiti AO (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: 8-16.

13. Gwa VI, Ekefan EJ (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: 555787.

14. Amienyo CA, Ataga AE (2007) Use of Indigenous plant extracts for the protection of mechanically injured sweet potato (Ipomea batatas (L.) Lam) tubers. Scientific Research and Essay 2: 167-170.

15. Yusuf C, Okusanya BAO (2008) Fungi associated with the storage rot yam (*Dioscorea rotundata Poir*) in Yola, Adamawa state. Jour of Sustainable Dev in Agric and Environ 3: 99-103.

16. Okigbo RN, Agbata CA, Echezona CE (2010) Effect of leaf extract of *Azadirachta* India and *Chromolaena odorata* on post-harvest spoilage fungi of yams in storage. Current Res J of Soil Sci 2: 9-12.

17. Okigbo RN, Emeka AN (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: 18-23.

18. Gwa VI, Nwankiti AO (2017b). *In Vitro* antagonistic potential of *Trichoderma harzianum* for biological control of *Fusarium moniliforme* isolated from *Dioscorea rotundata* tubers. Virol-mycol 6: 1-8.

19. Gwa VI, Abdulkadir KH (2017) Biological control using *Trichoderma harzianum* against *Penicillium purpurogenum*, causal agent of white yam tuber (*Dioscorea rotundata Poir*) rot. J Biores Commun 1: 1-6.

20. Nwankiti AO, Gwa VI (2018) Evaluation of antagonistic effect of *Trichoderma Harzianum* against *Fusarium oxysporum* causal agent of white yam (*Dioscorearotundata poir*) tuber rot. Trends Tech Sci Res 1: 555554.

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

22. Lakshmeesha TR, Sateesh MK, Vedashree S, Sofi MS, Umesha S (2013) Efficacy of botanicals on soybean seed-borne Fusarium equiseti VCFL Sciences 3: 10-16.

23. Okigbo RN (2005) Biological control of postharvest fungal rot of yam (*Dioscorea spp.*) with *Bacillus subtilis*. Mycopathologia 159: 307-314.

24. Riaz T, Khan SN, Javaid A (2010) Management of corm-rot disease of gladiolus by plant extracts. Nat Prod Res. 24: 1131-1138.

25. Cowan MM (1999) Plant products as antimicrobial agents. Clinical Microbiolology Reviews 12: 564–582.

26. Dahanukar SA, Kulkarni RA, Rege NN (2000) Pharmacology of medicinal plants and natural products. Ind J Pharmacol 2: S81-S118.

27. Das K, Tiwari RKS, Shrivastava DK (2010) Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research 4: 104-111.

28. Aidah N, Abdullah N, Oskoueian E, Sieo CC, Saad WZ (2014) Membrane active antibacterial compounds in methanolic extracts of *Jatropha curcas* and their mode of action against *Staphylococcus aureus* S1434 and *Escherichia coli* E216. Int J Agric Biol 16: 723-730.

29. Jamil AMS, Khan MM, Ashraf M (2007) Screening of some medicinal plants for isolation of antifungal proteins and peptides. Pak J Bot 39: 211-21.

30. Singh ND, Sharma AK, Dwivedi P, Kumar M, Patil RD (2011) Immunosuppressive effect of combined citrinin and endosulfan toxicity in pregnant wistar rats. Veterinarski Arhiv 81: 751-763.

31. Gwa VI, Ekefan EJ, Nwankiti AO (2017) Antifungal potency of some plant extracts in the control of white yam (*Dioscorea rotundata poir*) tuber rot. Adv Biotech & Micro 7: 555703.

32. Ahmed KM, 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.).

33. Navi SS, Bandyopadhyay R, Hall AJ, Bramel-Cox PJ (1999) A pictorial guide for the identification of mold fungi on *Sorghum* grain. Information Bulletin no. 59 (In En. Summaries in En, Fr). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics pp:118.

34. Amadioha AC, Obi VI (1999) Control of anthracnose disease of cowpea *Cymbopogon citratus* and *Ocimum gratissimum*. Acta phytopathol Entomol Hungerica 34: 85-89.

35. Nene ZH, Thapilyal (2002) Management of mushroom pathogens through botanicals. Ind Phytopathol 58: 189-193.

36. Vedashree S, Sateesh MK, Lakshmeesha TR, Sofi MS, Vedamurthy AB (2013) Screening and assay of extracellular enzymes in *Phomopsis azadirachtae* causing die-back disease of neem. J Agricultural Technol 9: 915-927.

37. Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research 2nd Edition John Wiley and sons pp: 680.

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

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

40. Cochran GW, Cox GM (1992) Experimental designs. 2nd Edn John willey and Sons Inc pp: 611.

41. Bobbarala VP, Katikala PK Naidu KC, Penumajji S (2009) Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* F2723. Indian Journal of Science and Technology 2: 87-90.

42. Amadioha AC (2003) Evaluation of some plants leaf extracts against *Colletotrichum lindemuthianum* in Cowpea. Acta Phytopathologia et Entomologica Hungarica 38: 259-265.

43. Chiejina NV, Ukeh JA (2012) Antimicrobial properties and phytochemical analysis of methanolic extracts of *Aframomum melegueta* and *Zingiber officinale* on fungal diseases of tomato fruit. J Nat Sci Res 2: 10-15.

44. Aidoo KA (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.

45. Okigbo RN, Nneka IA (2005) Control of yam tuber with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. African Journal Biotechnology 4: 804-807.

46. Anukwuorji CA, Anuagasi CL, Okigbo RN (2013) Occurrence and control of fungal pathogens of potato (*Ipomoea batatas* L. Lam) with plant extracts. Ph Tech Med 2: 278-289.

47. Suresh K, Saravana Babu S, Harisaranraj R (2008) Studies on *in vitro* antimicrobial activity of ethanol extract of *Rauvolfia tetraphylla*. Ethnobotany Leaflets 12: 586-590.

48. Wang H, Zhao M, Yang B, Jiang Y, Rao G (2008) Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. Food Chemistry 107: 1399-1406.

49. Ebele MI (2011) Evaluation of some acqueous plant extracts used in the control of pawpaw (*Carica papaya L.*) fruits rot fungi. Journal of Applied Biosciences 37: 2419-2424.

50. Ogwulumba SI, Ugwuoke KIb, Iloba C (2008) Prophylactic effect of pawpaw leaf and bitter leaf extracts on the incidence of foliar mycopathogens of groundnut (*Arachis hypogaea L.*) in Ishiagu, Nigeria. African Journal of Biotechnology 7: 2878-2880.

51. Suleiman MN (2010) Fungitoxic activity of neem and pawpaw leaves extracts on *Alternaria Solani*, causal organism of yam rots. Advances in Environmental Biology 4: 159-161.

52. Chima N (2012) Antifungal potencies of leaf extracts of *Carica papaya* on fungi implicated in soft rot of yam. Annals of Food Science and Technology 3: 11-16.

53. Amadioha AC (2002) Fungicidal activity of leaf extracts of four plants against pathogenic fungi of rice in Nigeria. Archives of Phytopathology and Plant Protection 51: 50-61.

54. Hycenth N (2008) Effect of different plant extracts in the control of yam (Dioscorea sp) in Yola, Adamawa State, Nigeria. Nigerian Agricultural Journal 3: 382-387.

55. Mallesh SB, Narendrappa T (2009) Management of root rot of sage, *Salvia officinalis* caused by *Fusarium solani* and *Rhizoctonia solani*. Ind J of Plt Prot 37: 119-122.

56. Yelmame MG, Mehta BP, Deshmukh AJ, Patil VA (2010) Evaluation of some organic extracts in *in-vitro* to control *Fusarium solani* causing chilli wilt. Int J Pharma & Bio Scie 1: 1-4.

57. Muhammad AK, Muhammad T, Sultan AM, Jamal H, Aziz AU, et al. (2016) Comparative efficacy of chemical fungicides, plant extracts and bio-control agents against *Fusarium solani* under laboratory conditions. Pak J Phytopathol 28: 133-139.

58. Ekundayo CA (1986) Biochemical changes caused by mycoflora of yam slices during storage. African Journal of Biotechnology 3: 207-214.

59. Agrios G (2005) Plant pathology 5th ed. Elsevier, London.