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Efficacy of Some Plant Extracts in *In Vitro* Control of *Colletotrichum* Species, Causal Agent of Yam (*Dioscorea rotundata Poir*) Tuber Rot

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ABSTRACT

Efficacy of some plant extracts (Piper nigrum, Zingiber officinale, Azadirachta indica, Carica papya and Nicotiana tabacum) and a chemical fungicide (mancozeb) using three concentrations of hot aqueous plant extracts (30, 60 and 90 g/l) and mancozeb (4, 8 and 12 g/l) in in vitro inhibition of Colletotrichum sp. mycelia was carried out at Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria. Rotted yam tubers were collected from farmers' barns and taken to the laboratory for isolation and identification of fungal organisms. Colletotrichum sp. was subsequently isolated and identified based on microscopic examination and its morphological characteristics from the pure culture of the fungus. 5 ml of each extract and the chemical fungicide were separately amended in 15 ml of potato dextrose agar and the pathogen was inoculated in the plates and incubated for 120 h and measurement of mycelia radial growths were recorded at 24 h interval throughout the period of incubation. The results obtained showed that all the plant extracts at all concentrations significantly ($p<0.05$) inhibited the mycelia growth of Colletotrichum sp. with the highest mean percentage growth inhibition recorded at concentration of 90 g/l followed by 60 g/l and 30 g/l. At 30 g/l, Z. officinale (60.69%) was more fungitoxic followed by P. nigrum (53.83%) compared with the least effective extract of C. papaya (30.39%). At 60 g/l, Z. officinale and A. indica both inhibited the mycelia of Colletotrichum sp. by 68.50% compared with the least inhibition of C. papaya at 43. 70%. At 90 g/l, P. nigrum was the most efficacious (82.19%) followed by Z. officinale (76.98%) while the least potent extract was C. papaya with an inhibition of 51.97%. The results showed that increase in period of incubation resulted in increase in percent growth inhibition with Z. officinale and P. nigrum been more effective at all concentrations compare with extracts of A. indica, N. tabacum and C. papaya. The chemical fungicide consistently gave 100% inhibition irrespective of concentration or duration of incubation. It is therefore concluded that all the plant extracts posses' antifungal compounds and should be used to control fungal rots of yam since extracts of plants are eco-friendly, cheap and easily available.

Keywords: Efficacy, *In vitro*, Plant extracts, *Colletotrichum* sp., Inhibition, Yam Rot

INTRODUCTION

Yams (*Dioscorea* spp.) are among the oldest recorded food crops and constitute an economically important staple for millions of people in the tropics and sub tropics. West Africa is the largest producer of this crop accounting for about 95% of total world production and 93% of the total yam production area [1]. Nigeria is the largest producer of the crop, producing about 38.92 million metric tonnes annually [2,3]. In spite of the high volume of production, the demand for yam tubers has always exceeded its supply due to the presence of rot causing organisms. Rot is a major factor limiting the Post-harvest life of yams [4,5] and losses can be very high resulting to seven million metric tonnes

of yams annually [4]. Losses due to post-harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting. It is estimated that an average of 50% of the yam tubers produced and harvested in Nigeria are lost to diseases in storage [6,7]. Some yam rot causing fungi organisms include *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae, Colletotrichum* spp*., Fusarium oxysporum, Penicillium chrysogenum, Pennicillium digitatum* [8-11]. Different methods have been adopted for controlling rots caused by post-harvest pathogens of yam; some of the methods include the use of chemicals such as captan, basic copper chloride, captafol and mancozeb [12], biological control method such as the use of *Trichoderma harzianum*, *T. viride* and *Bacillus subtilis* is also adopted [13,14] as well as the use of natural plant extracts [15,16]. The intensive use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and the environment, and also in the build-up of resistance of the pathogens.

Epidemic that starts prior to or during tuber formation can have great effect on tuber formation and yield. It is against this backdrop that the investigation focuses on the use of some plant extracts such as the seeds of *Piper nigrum* (Black Pepper), Rhizomes of *Zingiber officinale* (Ginger), leaves of *Azadirachta indica* (Neem), leaves of *Carica papaya (*Pawpaw*)* and leaves of *Nicotiana tabacum* (Tobacco) in the *in vitro* control of *Colletotrichum* sp. one of the pathogens isolated from rotted yam tubers in order to increase overall production to meet the high local and overseas demand for yam tubers [17].

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Advanced Plant Pathology Laboratory, Department of Crop and Environmental Protection, Federal University of Agriculture, Makurdi, Nigeria between February and May, 2015.

Collection of infected and healthy yam tubers

Yam tubers of white yam varieties (*Dioscorea rotundata*) showing various degree of disease symptoms of dry rots were obtained from yam farmers from various storage barns in Kadarko, Keana local government area of Nasarawa State, Nigeria located between longitudes 8°30' and 8°35'E and on latitudes 8°10′ and 8°14′N. The rotten yam tubers were packaged in sterile polyethylene bags and were taken to the laboratory for isolation and identification of pathogens [18]. The healthy yam tubers were used for pathogenicity test. Test isolate in this study was *Colletotrichum* sp.

Preparation of potato dextrose agar (PDA)

Potato dextrose Agar (PDA) was prepared according to manufacturer's recommendations by dissolving 39 g of dehydrated PDA in 1 l of distilled water and autoclaved at 121°C for 15 min [19]. The medium was allowed to cool to 45-50°C. About 0.16 g/l streptomycin sulphate powder was added to suppress bacterial contaminations [20]. 15 ml of the molten PDA was poured into sterile 9 cm glass Petri dishes and were allowed to cool at room temperature before inoculation.

Isolation of *Colletotrichum* sp.

Rotted yam pieces of approximately 2 × 2 mm were cut out with sterile scalpel at the inter-phase between the healthy and rotten portions of the tubers. The pieces were first surface sterilized by dipping completely in a concentration of 5% sodium hypochlorite solution for 2 min; and rinsed in four successive changes of sterile distilled water (SDW) [21].

Inoculation

Infected yam tissues were later picked onto sterile filter paper using a sterile forceps and then blotted with filter paper for 2-3 min in the laminar Air flow cabinet. The dried 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.

Characterization and identification of *Colletotrichum* sp.

Colletotrichum sp. was identified following sub-culture of growing fungi after 7 days of incubation. The culture plates obtained were examined and spores were collected from distinct growths. This was inoculated onto sterile PDA plates and incubated. When pure cultures were obtained, the growth pattern was examined for uniformity. Microscopic examination and morphological characteristics were noted and compared with existing authorities [20,22].

Preparation of plant extracts

Plant parts were prepared according to the methods described [4,23]. Seeds of *Piper nigrum* (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 tap water, air-dried and separately ground into fine powder using a mortar. Hot water (100°C) extraction was obtained by adding 30 g, 60 g and 90 g of the powder of each plant extracts to 1litre of sterile distilled water separately in 1000 ml Pyrex flask. These were left for 24 h and subsequently filtered through four fold of sterile cheese cloth. The filtrates were used as the plant extracts in the experiment. Mancozeb was prepared at 4 g/l, 8 g/l and 12 g/l concentrations respectively. The efficacies of the botanical extracts and chemical fungicide were tested for their fungicidal activity in controlling yam tuber rot fungi *in vitro* using these concentrations.

Effect of plant extracts on mycelia growth of *Colletotrichum* sp.

Colletotrichum sp. obtained from rotten yam tissues was used in this experiment. The method involves direct treatment of Potato Dextrose Agar (PDA) medium with the plant extracts before inoculation of the fungus. To evaluate the fungitoxic effect of the plant extracts and the chemical fungicide on fungal mycelia growth, four equal sections on each plate were created by drawing two perpendicular lines at the bottom of the plate [24]. The point of intersection indicates the centre of the plates. These were done before dispensing PDA into each of the plates. The prepared medium was poured into sterilized Petri dishes and 5 ml of each plant extracts and chemical fungicide at the different levels of concentrations were poured into Petri dishes containing 15 ml of the media separately [25] 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) which was obtained from 1 week old cultures of the test fungus grown on PDA plates served as inoculum [26]. Three plates were treated with extract of each plant at each concentration. The control experiments had 5 ml of distilled water added to PDA in place of plant extracts respectively; the treatments and control were completely randomized [27] and incubated for 120 hours at ambient room temperature (30 ± 5°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. 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 as percent growth inhibition (PGI) according to the method described by [28].

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

Where,

PGI=Percent 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.

DATA ANALYSIS

Test of variance was calculated using Analysis of variance (ANOVA) and statistical F-tests were evaluated at $P \leq 0.05$. Mean separation was done using fishers least significance difference (F-LSD) [29].

RESULTS

Sample collection, isolation and identification of *Colletotrichum* sp.

Colletotrichum sp. was isolated from the samples of rotted white yam tubers. Culture of *Colletotrichum* sp. on PDA was slow taking more than 7 days to fill the plates (Plate 1A). The colour of *Colletotrichum* sp. varied from white to grey, to dark orange while the reverse side of the growth pattern was either circular with the mycelia showing a uniform growth pattern and radial in a ring. Acervuli were rounded or elongate, separate or confluent, superficial, erumpent, with conspicuous multicellular, darkly pigmented setae. The acervuli consist of a gelatinous or mucoid, salmon orange coloured conidial mass (Plate 1B). Setae were brown with a dark swollen base and a pale rounded tip. The conidia were cylindrical with one end rounded and the other end pointed (Plate 1C).

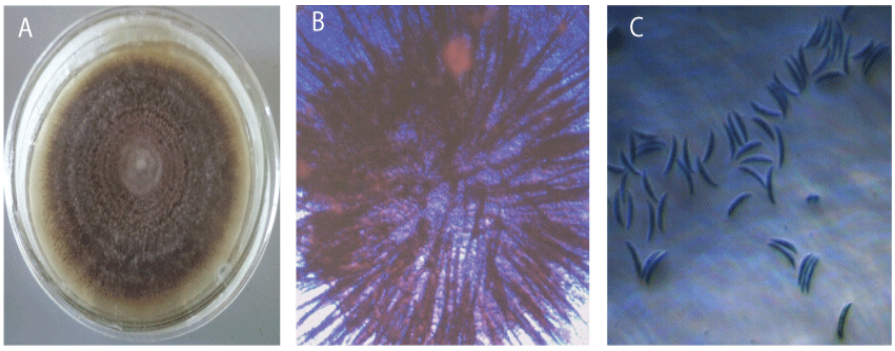

Plate 1: Colony characteristics growth of *Colletotrichum* sp. on PDA (A), Acervulus with setae of *Colletotrichum* sp. (×10) (B) and conidia (×10) (C)

Table 1: *In vitro* effect of different filtrate concentrations of some plant extracts and chemical fungicide at different concentrations on percentage growth inhibition of *Colletotrichum* sp. after 120 h of incubation (Means on the same row (for each plant extract) with the different superscript are statistically significant ($p<0.05$) by period of incubation, ns=not significant

Plant Extract	Concentration (g/L)	Period of Incubation (Hours)					
		24	48	72	96	120	LSD
Piper nigrum	Conc I (30)	50.00 ± 28.90	47.78 ± 7.78	55.19 ± 2.89	58.19 ± 1.61	58.01 ± 2.13	42.49^{ns}
	Conc II (60)	66.70 ± 33.30	56.10 ± 12.20	61.85 ± 4.27	63.55 ± 4.03	63.35 ± 3.17	50.89^{ns}
	Conc III (90)	100.00 ± 0.00^{a}	80.60 ± 10.00^{b}	82.59 ± 3.76^{b}	76.32 ± 2.07^{b}	71.47 ± 2.98^{b}	15.92
Zingiber officinale	Conc I (30)	83.30 ± 16.70	62.80 ± 14.80	54.81 ± 5.19	52.63 ± 2.63	49.89 ± 2.31	36.62^{ns}
	Conc II (60)	100.00 ± 0.00^{a}	73.89 ± 3.89^{b}	55.19 ± 2.89^{c}	58.19 ± 1.61^{c}	55.24 ± 3.37^{c}	8.63
	Conc III (90)	100.00 ± 0.00^{a}	88.90 ± 11.10^{a}	68.52 ± 7.10^{b}	65.40 ± 8.04^{b}	62.07 ± 2.05^{b}	20.1
Azadiracta indica	Conc I (30)	66.70 ± 16.70	36.70 ± 18.60	37.41 ± 8.12	49.03 ± 2.41	45.83 ± 2.41	37.82^{ns}
	Conc II (60)	100.00 ± 0.00^{a}	73.89 ± 3.89^{b}	55.19 ± 2.89^{c}	58.19 ± 1.61^{c}	55.24 ± 3.37^{c}	8.63
	Conc III (90)	83.30 ± 16.70^{a}	56.10 ± 12.20^{a}	44.44 ± 5.56^{b}	52.73 ± 1.60^{b}	49.89 ± 2.31^{b}	30.39
Carica papaya	Conc I (30)	50.00 ± 28.90	41.11 ± 4.84	7.04 ± 3.53	29.04 ± 3.45	32.16 ± 5.22	43.05^{ns}
	Conc II (60)	66.70 ± 16.70^{a}	47.78 ± 7.78^{a}	23.70 ± 6.30^{b}	38.01 ± 5.67^{a}	41.67 ± 4.81^{a}	29.33
	Conc III (90)	83.30 ± 16.70^{a}	54.40 ± 13.70^{ab}	24.07 ± 3.03^{b}	45.32 ± 3.99^{b}	52.67 ± 3.66^{ab}	31.6
Nicotiana tabacum	Conc I (30)	66.70 ± 16.70^{a}	36.70 ± 18.60^{a}	13.33 ± 6.67^{b}	41.72 ± 2.83^{a}	37.82 ± 0.32^{a}	36.61
	Conc II (60)	83.30 ± 16.70^{a}	62.80 ± 14.80^{ab}	30.74 ± 5.15^{b}	49.03 ± 2.41^{b}	45.73 ± 4.72^{b}	33.08
	Conc III (90)	83.30 ± 16.70^{a}	72.20 ± 14.70^{a}	47.78 ± 7.78^{b}	54.48 ± 2.34^{a}	51.28 ± 2.73^{a}	33.56
Mancozeb	Conc I (4)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-
	Conc II (8)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-
	Conc III (12)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-

***In vitro* effect of plant extracts on the mycelia growth of *Colletotrichum sp*. isolated from rotted white yam tubers**

Table 1 shows results of plant extracts on mycelia growth of *Colletotrichum* sp. at different concentrations. The results of *Colletotrichum* sp. growth on PDA amended with plant extracts shows that *P. nigrum, Z. officinale, A. indica, C. papaya* and *N. tabacum* had antifungal properties against the isolate at higher concentrations (60 g/l and 90 g/l) compared with lower concentration (30 g/l) in *in vitro* test. Duration of incubation showed significant difference on the plant extracts while there was no significant difference ($P \leq 0.05$) in either of concentrations in mancozeb in inhibiting the growth of the pathogen in culture. There were however, significant differences in the activity of plant extracts between extracts within concentrations except in mancozeb which showed no significant difference between concentrations (Table 2). Variation of the tested plant extracts within concentrations showed no significant difference ($P \leq 0.05$) at 24 h of incubation for all the levels of concentrations but varied significantly for each concentration among the plant extracts throughout the remaining period of incubation (Table 2). On the other hand, *N. tabacum* shows no growth inhibition difference of mycelia of *Colletotrichum* sp. at 24 h of incubation at 60 and 90 g/l. Mean percentage growth inhibition of *Colletotrichum* sp. after 120 h of incubation shows an increase in the performance of the extracts from the lowest concentration to the highest concentration. It was observed in the work that inhibition of *Colletotrichum* sp. was more by extract of *Z. officinale, P. nigrum* and *A. indica* compared with extracts of *C. papaya* and *N. tabacum*. The result shows that susceptibility of the pathogen to the tested plant extracts varied with the concentrations of the extracts and duration of incubation (Table 3). Though *Colletotrichum* sp was more susceptible to the extracts at higher concentration, the extract of *A indica* however, proved more effective at 60 g/l than at 30 g/l and 90 g/l indicating that the extract only stimulated the growth of the pathogen at higher concentration. Mean percentage growth inhibition of three concentrations (I, II and III) of *Colletotrichum* sp. throughout the period of incubation revealed that the plant extracts were able to inhibit the mycelia growth of the pathogen more at initial period of incubation than at the later periods (Figure 1).

Table 3: Mean percentage growth inhibition of *Colletotrichum* sp. at different concentrations of plant extracts and chemical fungicide after 120 h of incubation. Means on the same column with the 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=90 g/L of plant extract, 12 g/l of Mancozeb)

	Concentrations		
Plant Extract	**Conc I**	**Conc II**	**Conc III**
Azadiracta indica	47.12 ± 5.33bc	68.50 ± 4.71b	57.30 ± 5.14c
Carica papaya	30.39 ± 6.88d	43.70 ± 5.14d	51.97 ± 6.34c
Mancozeb®	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Nicotiana tabacum	39.24 ± 6.31cd	54.32 ± 6.18cd	61.82 ± 5.43c
Piper nigrum	53.83 ± 5.22bc	62.31 ± 6.17bc	82.19 ± 3.22b
Zingiber officinale	60.69 ± 5.08b	68.50 ± 4.71b	76.98 ± 4.64b
LSD	14.91	13.93	12.99

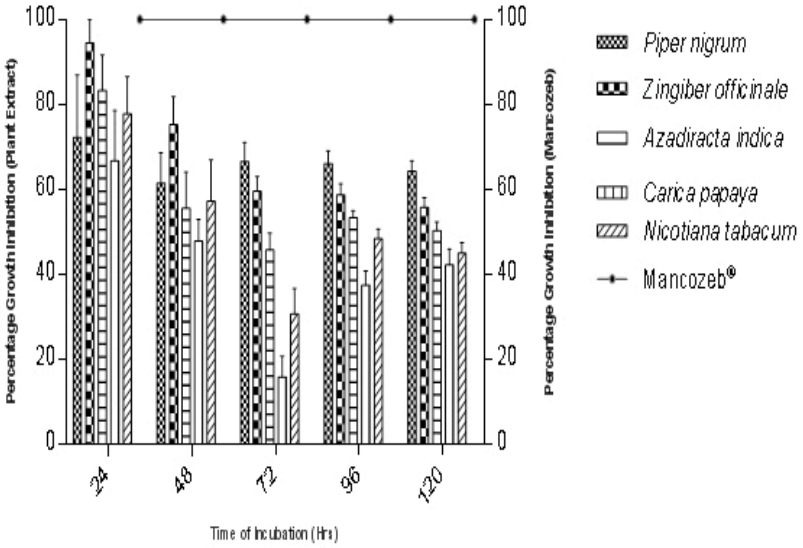

Figure 1: Mean percentage growth inhibition of three concentrations (30 g/l, 60 g/l and 90 g/l) of plant extracts and (4 g/l, 8 g/l and 12 g/l) of mancozeb on the mycelia growth of *Colletotrichumm* sp. on period of incubation

Table 2: *In vitro* percentage growth inhibition of *Colletotrichum* sp. at different concentration of plant extracts and chemical fungicide after 120 h of incubation. (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)

	Period of Incubation (h)				
Plant Extract	**24**	**48**	**72**	**96**	**120**
Concentration I					
Azadiracta indica	66.70 ± 16.70	36.70 ± 18.60^{b}	37.41 ± 8.12^{c}	49.03 ± 2.41^{cd}	45.83 ± 2.41^{cd}
Carica papaya	50.00 ± 28.90	41.11 ± 4.84^{b}	7.04 ± 3.53^{d}	29.04 ± 3.45^{e}	32.16 ± 5.22^{e}
Nicotiana tabacum	66.70 ± 16.70	36.70 ± 18.60^{b}	13.33 ± 6.67^{d}	41.72 ± 2.83^{d}	37.82 ± 0.32^{de}
Piper nigrum	50.00 ± 28.90	47.78 ± 7.78^{b}	55.19 ± 2.89^{b}	58.19 ± 1.61^{b}	58.01 ± 2.13^{b}
Zingiber officinale	83.30 ± 16.70	62.80 ± 14.80^{ab}	54.81 ± 5.19^{b}	52.63 ± 2.63^{bc}	49.89 ± 2.31^{bc}
Mancozeb	100.00 ± 0.00	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
LSD	62.90	39.61	17.01	7.46	8.25
Concentration II					
Azadiracta indica	100.00 ± 0.00	73.89 ± 3.89^{ab}	55.19 ± 2.89^{b}	58.19 ± 1.61^{bc}	55.24 ± 3.37^{bc}
Carica papaya	66.70 ± 16.70	47.78 ± 7.78^{b}	23.70 ± 6.30^{c}	38.01 ± 5.67^{d}	41.67 ± 4.81^{d}
Nicotiana tabacum	83.30 ± 16.70	62.80 ± 14.80^{b}	30.74 ± 5.15^{c}	49.03 ± 2.41^{c}	45.73 ± 4.72^{cd}
Piper nigrum	66.70 ± 33.30	56.10 ± 12.20^{b}	61.85 ± 4.27^{b}	63.55 ± 4.03^{b}	63.35 ± 3.17^{b}
Zingiber officinale	100.00 ± 0.00	73.89 ± 3.89^{ab}	55.19 ± 2.89^{b}	58.19 ± 1.61^{bc}	55.24 ± 3.37^{bc}
Mancozeb	100.00 ± 0.00	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
LSD	51.36	26.92	12.65	9.70	11.13
Concentration III					
Azadiracta indica	83.30 ± 16.70	56.10 ± 12.20^{b}	44.44 ± 5.56^{c}	52.73 ± 1.60^{d}	49.89 ± 2.31^{d}
Carica papaya	83.30 ± 16.70	54.40 ± 13.70^{b}	24.07 ± 3.03^{d}	45.32 ± 3.99^{d}	52.67 ± 3.66^{d}
Nicotiana tabacum	83.30 ± 16.70	72.20 ± 14.70^{ab}	47.78 ± 7.78^{c}	54.48 ± 2.34^{d}	51.28 ± 2.73^{d}
Piper nigrum	100.00 ± 0.00	80.60 ± 10.00^{ab}	82.59 ± 3.76^{b}	76.32 ± 2.07^{b}	71.47 ± 2.98^{b}
Zingiber officinale	100.00 ± 0.00	88.90 ± 11.10^{ab}	68.52 ± 7.10^{b}	65.40 ± 8.04^{c}	62.07 ± 2.05^{c}
Mancozeb	100.00 ± 0.00	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
LSD	36.31	35.01	16.16	9.22	7.88

DISCUSSION

This study has revealed that *Colletotrichum* sp is one of the fungi organisms responsible for storage rot of different white yam cultivars in Kadarko, Nasarawa State of Nigeria. The organism had been previously found to be associated with leaves, stems and post-harvest yam tubers in different locations in Nigeria [1,10,15]. The occurrence of *Colletotrichum* sp. in yam tubers may probably be due to the fact that *Colletorichum* sp. overwinters in leaves, stems and seeds and in infected soil as reported by [10,15]. It has been found that most of these microorganisms infect the tubers in the field reducing its capacity to germinate and its survival in the field and then subsequently manifest in storage barns which occur when infected tubers do not have any sign of external symptoms [15,30,31]. Confirmed that the soil adhering to the harvested tubers contain many microorganisms that could be pathogenic to the tubers. The findings also demonstrated that *P. nigrum, Z. officinale, A. indica, C. papaya* and *N. tabacum* and the synthetic chemical; mancozeb all possess fungitoxic substances and potentials to protect yam tubers against mycelia growth of *Colletorichum* sp. The susceptibility of this pathogen to the tested plant extracts varied with duration of incubation, type of plant extract used as well as the concentrations of the extracts. This is in agreement with earlier work done by

[32,33]. Results of the findings revealed that the isolate was more susceptible to *P. nigrum, Z. officinale, A. indica* and the synthetic chemical, mancozeb; compared with *C. papaya* and *N. tabacum*. The variation noted in their potencies may be as a result of solubility of the active substances in water or the presence of inhibitor against fungicidal principles [34]. It may also depends on time of collection of plant extract, the type of extract, age of the plant and the method used in the extraction [35]. It has been reported that plant extracts contain phytochemical compounds such as carbohydrates, proteins, anthraquinones, flavonoids, saponins, tannin, glycosides, phenols and alkaloids in the leave, seeds and bark [36,37] These alkaloids play a significant role in plant physiology, agriculture, host-plant resistance, entomology, the diet and medicine [38].

N. tabacum contain nicotine which inhibits the growth of pathogens which is dose dependent [39,40]. It has been found that *P. nigrum* leaf extract inhibits the growth of *Pseudomonas aeruginosa* [41] describes the antimicrobial activity of volatile oils of black pepper against *Bacillus subtili*s, *Pseudomonas aeruginosa, Aspergillus niger, Candida albicans* and *Saccharomyces cervisisae.* Papaya leaves which contain papain were also used in inhibiting the mycelia growth of *Rhizopus nigricans* and *Mucor circinelloides* which are responsible for soft rot disease of post-harvest yam.

Earlier demonstrated the fungitoxic activity of seed extract of *Azadirachta indica* (neem) and *Xylopia aethiopica* against the anthraconose fungus (*Colletotrichum lindemuthianum*) of cowpea [42]. The control of *C. lindemuthianum* using neem seed, leaf, bark and root extract, recording a 100% inhibition of spore germination and mycelia growth [43]. Crude extracts from stem bark and root bark of *Azadirachta indica*, *Vernonia amygdalina* and *Cochlosspermum planchonii* exhibited strong fungitoxicity against *Colletotrichum capsici* and have potential of being formulated into products for the control of anthracnose of sweet pepper [44]. Biu et al. [36] in his investigation observed the presence of anti-nutrients like saponins, tannins, glycosides, alkaloids, terpenes and flavenoids in the aqueous extracts of the leaves of *Azadirachta indica* to be responsible in inhibiting mycelia growth of pathogens. The activity of plant extracts on mycelia growth of pathogens could produce no effect, could result in inhibition of pathogen or stimulate the growth of pathogen; this result confirms his observation as the inhibition of *Colletotrichum* sp. decreased when the concentration of *A. indica* was increased beyond 60 g/l [45]. Taiga et al. showed that *N. tabacum* cold extract inhibited the mycelia of *F. oxysporum* yam rot organism [46]. According to Wang et al. and Suresh et al. the more the active compound in *N. tabacum* (nicotine) the more the inhibition of mycelia growth of the pathogens. The finding is in agreement with the work of Krishnapillai, [47] who evaluated the fungicidal properties of ginger rhizome extract and found out that the growth inhibition on *Fusarium* spp*., Colletotrichum* spp. and *Curvularia* spp. by ginger rhizome extract were 70.0%, 71.0% and 64.2%, respectively. In the present study piperine an alkaloid which is the major constituent of piperamides present in the skin and seed of the black pepper is responsible for the antimicrobial activity which confirms report by Shiva et al., [16]. The susceptibility of *Colletotrichum* sp. by leaf extract of *C. papaya* and other plant extracts was in agreement with the work of Bautista-Banos et al. [48] who reported that aqueous extracts of leaves and seeds of *C. papaya* are known to have antifungal activity against *C. gloeosporioides*. The use of these plant extracts also agreed with the work of Gwa and Akombo [49] who inhibited the mycelia growth of *Aspergillus flavus in vitro* with seed extracts of *P. nigrum*, rhizomes of *Z. officinale*, leaves of *A. indica*, leaves of *N. tabacum* and leaves of *C. papaya* at 30 g/l, 60 g/l and 90 g/l. At 30 g/l, they recorded mean percentage growth inhibition of 69.06%, 75.75%, 57.89%, 64.13% and 53.83% respectively. At 60 g/l, the authors recorded mean percentage growth inhibition of 73.43%, 75.74%, 70.31%, 64.13% and 59.98%, respectively while at 90 g/l, the inhibition of *A. flavus* by these plant extracts were 80.76%, 83.84%, 74.73%, 67.91% and 71.64%, respectively. The mechanism of action of these extracts could probably be due to the degradation of plant cell wall and lyses as well as enzyme production of the fungus (Khan and Nasreen). The result therefore shows that the inhibition of the mycelia growth of *Colletotrichum* sp. is dose and duration dependent. However, *P. nigrum, Z. officinale* and *A. indica* were generally more fungitoxic than *C. papaya* and *N. tabacum* at the same concentrations and durations. This could probably be due to higher concentration of the active principles in the extracts [16]. It is possible that an increase in the concentrations will eventually result to 100% inhibition of mycelia growth but that will not be cost effective in the management of the pathogen and also combining two or more extracts will more probably result in higher efficacy of the extract. Although mancozeb consistently gave 100% inhibition, it is advice that its use can only be when other methods prove ineffective due to its toxic effect on the environment [8].

CONCLUSION

Results from the present study, established the fact that all the five plant extracts possess antifungal substances significantly toxic to *Colletotrichum* sp. isolated from rotted yam tubers. However, *P. nigrum*, *Z. officinale* and *A. indica* were considered better extracts than *C. papaya* and *N. tabacum* at all the levels of concentrations and durations. The test plants therefore, have potentials to control post-harvest yam rot. It is therefore, suggested that combining the

plant extracts even at lower concentration will help in achieving a better result in managing the fungus. The extracts could hence, be used as protective pesticide, since mycelia inhibitions of the pathogen was effective and also plant extracts are easily biodegradable and eco-friendly compared to chemicals.

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