Antifungal Efficacy of Plant Extracts against Fungal Pathogens Associated with Postharvest Rot in Cocoyam

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ABSTRACT

Fungal pathogens affecting stored cocoyam are estimated to result in over 40% post-harvest and financial losses for cocoyam farmers in Sub-Saharan Africa. A study was conducted to isolate and identify fungal organisms responsible for postharvest rot of cocoyam corms during storage, as well as to assess the fungicidal efficacy and mycelia growth inhibitory effects in vitro of plant extracts from Black pepper (*Piper nigrum*), Neem (*Azadirachta indica*), and Alligator pepper (*Aframomum melegueta*) against fungi associated with cocoyam rot in storage. Cocoyam corms exhibiting signs of rot were retrieved from storage to isolate fungal pathogens. Fresh, healthy cocoyam corms were also used to assess the pathogenicity of the isolated fungi. The fungicidal capacity of the aqueous plant extracts was assessed using three extract concentrations (10% w/v, 20% w/v, and 30% w/v). Means were separated using Duncan Multiple Range Test, and analysis of variance was utilized for analysing the data at the 95% confidence level. All fungal pathogens isolated caused rot in both varieties of healthy cocoyam corms following a 14-day inoculation period. *Rhizopus stolonifer* and *Bipolaris* sp caused 73.33% rot severity each, followed by *Aspergillus flavus* (60.0%), *Colletotrichum gloeosporioides* (33.33%) and *Botryodiplodia theobromae* (26.67%). The highest radial growth inhibition (90.0%) was recorded for 30% w/v Alligator pepper on *Rhizopus stolonifer* and 30% w/v black pepper on *Bipolaris spp*. Alkaloids, tannins, phenols, saponins, flavonoids, cardiac glycosides terpenoids, and Phytosterols were found in the crude aqueous plant extracts. Aqueous extracts of Alligator pepper, Black pepper, and Neem significantly ($P \leq 0.05$) inhibited the mycelia growth of fungal pathogens in vitro and can also be used as a substitute for conventional fungicides.

Keywords: Cocoyam varieties, Fungi pathogens, Pathogenicity, Plant extracts, Antifungal

INTRODUCTION

Cocoyam, a perennial herbaceous monocotyledonous plant belongs to the Araceae family. *Xanthosoma sagittifolium* (the white type or tannia) and *Colocasia esculenta* (the red type or taro) are the two most widely grown species (Onyeka, 2014; Bartholomew et al. 2017; Fru and Vange, 2023). The production of cocoyam in Sub-Saharan Africa is primarily carried out by resource-poor, small-scale farmers with limited agricultural input (Bartholomew et al. 2017). In Nigeria, cocoyam is the third most important root and tuber crop that is grown and consumed after cassava and yam, and it is superior in terms of nutrition to cassava and yam regarding minerals and digestible crude protein (Ca, Mg, and P) contents (Green, 2003; Chukwu et al. 2008; Ezeonu et al. 2018). Nutritionally, it has high carbohydrate content (13 - 29%), proteins (1.4 - 3.0%), vitamins, and minerals. More than 60% of the world's cocoyam production comes from Cameroon, Ghana, and Nigeria (Onyeka, 2014; Fru et al. 2024).

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Cocoyam corm decay and loss during storage in Nigeria is primarily as a result of microbial action with an estimated 40 – 50% loss (Eze and Maduewesi, 1990). According to Chukwu et al. (2008), sprouting, rots and other physiological changes during cocoyam storage resulted in roughly 50% economic losses after two months and after five months, approximately 95%. Rot is the softening of the plant parts brought on by a proteolytic enzyme that the pathogen secretes into the plant tissues, dissolving the plant parts or fruits (Akueshi et al. 2002). Rot pathogens that have been isolated from corms of *C. antiquorum, C. esculenta,* and *X. sagittifolium* during storage in Nigeria include *F. salani, B. theobromae, F. oxysporium, S. rolfsii, Fusarium sp,* and *R. stolonifer*. According to reports, these fungal pathogens are also the main causes behind storage rots in other root and tuber crops, including sweet potatoes, yams, and cassava (Amienyo & Ataga, 2006; Banito et al. 2010; Okigbo et al. 2010; Eze & Ameh, 2011). Rapid and pervasive host tissue degradation leads to quantitative pathogenic losses of stored cocoyam. The pattern of attack generally consists of one or a few peculiar pathogenic or saprophytic organisms growing on the decomposing moribund tissues left over from the initial infection, which results in wounds from harvest bruises and the places where the corms have detached (Eze and Ameh, 2011).

To combat the possible risks and pollution issues related to the use of synthetic chemicals, the use of biopesticides derived from plants has been proposed as an alternative to chemical use (Amadioha and Obi, 1998; Amadioha, 2000). According to Iwuagwu et al. (2018), fungicidal plants are highly effective at preventing fungal growth both in vitro and in vivo. Neem (*Azadirachta indica*), black pepper (*Piper nigrum*), and alligator pepper (*Afromomum meleguata*) are among plants with anti-fungi properties and potential alternatives to control and prevent cocoyam decay during storage. This study aimed to isolate and identify fungi associated with the postharvest decay of cocoyam corms in storage, evaluate the pathogenicity of the isolated pathogenic fungi, and assess the efficacy of organic plant extracts of Neem (*Azadirachta indica*), Black pepper (*Piper nigrum*), and Alligator pepper (*Afromomum melegueta*) in inhibiting the radial growth of fungi pathogens in vitro and identifying the phytochemicals found in the plant extracts.

MATERIALS AND METHODS

Sources of Cocoyam Corm

A total of 50 rotten cocoyam corms were randomly collected from a cocoyam storage study conducted at Benue State University, Makurdi, Nigeria. The collected diseased corms were taken to the Biological Sciences Laboratory in the University for further Studies. Twenty-four (24) freshly harvested corms of *Colocasia esculenta* (taro) and *Xanthosoma sagittifolium* (tannia) were each obtained from local farms

Sterilization

Petri dishes, beakers, volumetric flasks, cork borer, test tubes, and other glass wares were sterilized for an hour at 160^0C in a hot air oven. Sterilisation of the wire loops was done by burning them until red hot using a Bunsen burner and letting them cool. The work surfaces were cleaned with 70% alcohol to avoid contamination.

Media Preparation

The isolation media utilised was potato dextrose agar (PDA). The culture medium was prepared according to the manufacturer's instructions by dissolving 39g of media in 1L of sterile distilled water. The solution was heated using a heating mantle and aseptically sterilized using an autoclaved at 121^0C at 15 psi for 15 minutes, and then let to cool (42 – 45^0C). The antibiotic chloramphenicol was added to the culture medium and mixed thoroughly before it was poured into sterilized Petri dishes to prevent bacteria growth.

Isolation of Fungal Pathogens from Cocoyam Corms

Mahmoud and Al-Ani (2016) isolation methods were applied. Using a sterile knife, pieces of diseased tissues (3 x 2 mm) were cut off the outside of decaying cocoyam corms. They were then surface-sterilized for one minute in a 5% sodium hypochlorite (NaOCl) solution. After being surface sterilised, they were rinsed three times with distilled sterile water and dried in a sterile Lamina flow chamber before being plated on a PDA medium that had hardened and been modified with chloramphenicol. The inoculated plates were incubated at room temperature

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and observed for microbial growth. Mycelia growth was observed for 3 - 5 days and pure cultures were obtained through sub-culturing. Pure cultures were obtained by several transfers of each growth colony from PDA plates containing previously cultured fungi (Liamngee et al. 2015). Using the formula used by Fayinminu et al. (2025), the occurrence of each isolated fungal pathogen was calculated by dividing the frequency of each fungus by the total number of fungi on each plate and the result was then reported as a percentage.

$$\text{Percentage of Occurrence} = \frac{\text{Total number of each fungal pathogen in all the corms}}{\text{Total number of fungal pathogens in all the corm screened}} \text{ x } 100$$

Identification of Fungal Isolates

Macroscopic and microscopic analyses of the growth morphology of the fungi were used to identify pure cultures (Terna et al. 2019). On the Petri plates, colony traits like appearance, change in medium colour, and growth rate were noted for macroscopic identification. Lactophenol cotton blue dye was used to produce slide mounts of the isolates for microscopic inspection, which was then conducted under a microscope. Barnett and Hunters (1985) and Marthur and Kongsdal (2003) Standard Fungi Manuals were consulted in order to compare the isolates that were observed.

Pathogenicity Test

The pathogenicity test was conducted using the Okigbo and Ikediugwu (2000) approach. Soil and debris were removed from healthy cocoyam corms by washing them with tap water. The corms were rinsed with sterile distilled water after being surface sterilised for 2 minutes with 1% sodium hypochlorite, and air dried. 1cm deep holes were bored from the tip of healthy cocoyam corms using a 5mm sterile cork borer. 7 - days - old cultures of each fungus were inserted into the holes in the corms, and the cocoyam cores from the corm were reinstalled after parts had been removed. A sterile PDA disc was used in place of the culture discs to serve as the control. No fungus was placed in the control. Each fungus was replicated four times in a completely randomized design. The inoculated corms were incubated for 14 days at room temperature (28 ± 2^{o}C). The same procedure was used for the control except that discs of un-inoculated PDA were placed in the holes created in the corms (Amienyo and Ataga, 2006).

Assessment of Cocoyam Corm Tissue Rot

Using a sterile knife, the inoculated corms were cut open at right angles along the inoculation points at the conclusion of the 14-day incubation period to yield identical halves. Morphological characteristics and growth patterns were observed in each case and compared with those of the original isolates for infection and disease development (Fatimoh et al. 2017; Liamngee et al. 2018).

Disease incidence

Disease incidence in corms was calculated as shown by Liamngee et al. (2015).

$$\text{Disease incidence \% (DI)} = \frac{\text{number of infected corms}}{\text{total number of corms sampled}} \times 100$$

Disease severity

The corm disease severity was assessed on a scale of 0 – 5 as described by Bdliya and Langerfeld, (2005), where;

0 = no symptom of rot

1 = 1–15% of corm rotten

2 = 16–30% of corm rotten

3 = 31–45% of corm rotten

4 = 46–60% of corm rotten

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5 ≥ 61% of corm rotten

The disease severity was computed using the formula

Disease severity % (DS) $= \frac{\sum(a+b)}{N.Z} X100$

Where: Σ (a + b) = Sum of symptomatic corms and their corresponding score scale

N = Total number of cocoyam corms assessed

Z = highest score scale on the severity scale.

Measurement of rot

The extent of the rot was determined by calculating the area of rot using the formula adopted by Ezeibekwe and Ibe (2010). The diameter and depth of the rot were measured using a transparent ruler that had been sterilised. By deducting the initial depth (1 cm) from the end depth, the true depth was got.

Area of Rot $= \pi dl$

Where: d = diameter

L = depth

$\prod$ = 22/7 (Constant)

Preparation of Aqueous Plant Extracts

Seeds of *Aframomum melegueta* (Alligator pepper) and *Piper nigrum* (Black pepper) were sourced from Wadata Market, Makurdi, and fresh leaves of *Azadirachta indica* (Neem) were harvested from Benue State University campus. The fresh neem leaves were washed separately under a gentle stream of tap water to remove surface dirt, then in sterile distilled water containing 1% sodium hypochlorite for 2 minutes and air dried for 7 days before milling.

The seeds of *A. melegueta*, *P. nigrum*, and *A. indica* leaves were finely ground into powder using a blender. Extracts of *A. melegueta*, *P. nigrum*, and *A. indica* were obtained by adding each powder (100g, 200g, and 300g) to 1000 ml of sterile distilled water in 1000 ml conical flasks using the cold solvent extraction method as described by Nweke (2015) and Fru & Vange (2023). Each suspension was manually shaken for 2 minutes and allowed to stand for 24 hours before being filtered into a fresh flask using a four-fold sterile muslin cloth.

In Vitro Efficacy of Plant Extracts on the Radial Growth of Isolated Fungi Pathogens

The antifungal activities of the different plant extracts were evaluated using the poisoned food method described by Lum et al. (2019) with slight modifications in which 2 ml of each plant extract was added to 15 ml of PDA on Petri dishes and each plate was gently swirled on the laboratory bench to ensure even dispersion of extracts to give a PDA-extract mixture. The mixture was permitted to solidify prior to inoculation at the center of each plate with 4 mm diameter mycelia taken from the colony edge of pure cultures the isolated fungi (7 days old). Three replicates of each treatment were used in the control experiment, which involved inoculating sterile PDA with sterile distilled water containing the identified fungal pathogen.

Using a transparent meter rule, the radial growth diameters of the test fungi were measured in centimetres (cm) at 3, 5, and 7 days after the inoculated plates had been incubated at ambient temperature. The mean growth in two directions along two perpendicular lines drawn on the back of the plates was used to calculate the colony diameter. With minor adjustments, the formula of Ndifon and Lum (2021) was used to determine the inhibition of fungal growth by plant extracts.

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Percentage Inhibition $= \frac{Rc-Rt}{Rc} \times 100$

Where Rc = Radial growth diameter of the pathogen in control

Rt = Radial diameter of the pathogen in PDA-Extract plates

The scale outlined by Okigbo et al. (2015) was adopted to rate the extracts inhibitory effects;

0 % inhibition = Not effective

1 – 19 % inhibition = Slightly effective

20 – 49% inhibition = Moderately effective

50 – 99% inhibition = Effective

100 % inhibition = Highly effective

Phytochemical Analysis of Plant Extracts

Phytochemical analysis was carried out on part of the pulverized plant materials to reveal the presence of secondary metabolites in them using the method of Fru and Vange, (2023).

Data Analysis

Analysis of variance (ANOVA) was performed on the data collected using SPSS. Using Duncan Multiple Range Test (DMRT), treatment means were separated at a 5% probability level.

RESULTS AND DISCUSSIONS

Isolation, Identification, and Pathogenicity of the Fungi Causing Decay During Storage

The fungi pathogens isolated from the stored rotten cocoyam corms and their percentages of occurrence were *Colletotrichum gloeosporioides* (22.45%), *Rhizopus stolonifer* (11.57%), *Bipolaris* sp (33.33%), *Botryodiplodia theobromae* (17.69) and *Aspergillus flavus* (14.97%) as shown in Tables 1 and 3. This agrees with Anukworji et al. (2012) who identified most of these species in a study on isolation of fungi causing rot of cocoyam. Other fungi pathogens associated with cocoyam rot in Nigeria include *Aspergillus flavus, Penicillium digitatum, Botryodiplodia theobromae, Sclerotium rolfsii, Fusarium solani, F. oxysporium, Botrytis spp, Pithium spp, Phytophthora colocasia, Rhizoctonia bunoides* and *Erwinia carotovora* (Brunt et al. 2001). Some of these pathogens have also been isolated from yam (Okigbo et al. 2015; Ndifon and Lum, 2021; Ndifon and Lum, 2023).

The pathogenicity test revealed that all the isolated pathogens could induce rot in healthy cocoyam corms though, at various percentages of severity, mean area of rot caused after 14 days of inoculation as shown in Tables 2 and 4. The most virulent fungi pathogens were *Rhizopus stolonifer* and *Bipolaris* sp causing 73.33% rot each followed by *Aspergillus flavus* (60.0%) and *Colletotrichum gloeosporioides* (33.33%). The least virulent was *Botryodiplodia theobromae* inducing 26.67% rot in infected tissue. The un-inoculated cocoyam corms in the control units showed no signs of decay after 14 days. This is in agreement with the reports of many researchers on cocoyam corms (Offei, 1999; Eze and Ameh, 2011; Anukworji et al. 2012; Ezeonu et al. 2018). The isolation of more than one fungal pathogen from a particular corm affirms the possibility of multiple infections whose combined effect may cause rapid rotting of the cocoyam corms and this agrees with the reports of Anukworji et al. (2012) and Okigbo et al. (2015) on cocoyam and yam respectively. The ability of the fungal isolates to cause infection in healthy cocoyam corms was because the pathogens can utilize the nutrients from the corms as a substrate for growth and development (Liamngee et al. 2015). Thus, it is recommended to use the area method to measure the extent of pathogenic fungal damage to cocoyam corms and other root and tuber crops. The degree

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of severity of the mean area rot of *Colocasia esculenta* was significantly higher than the mean area rot of *Xanthosoma sagittifolium*.

Table 1: Percentage Occurrence of the Fungi Isolates from the Rotten Cocoyam Corms

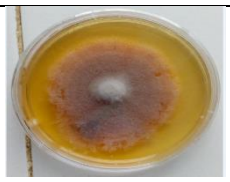
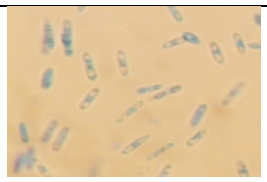
Fungi Species	Number of Isolates	Percentage Occurrence (%)
Colletotrichum gloeosporioides	33	22.45
Rhizopus stolonifer	17	11.57
Bipolaris sp	49	33.33
Botryodiplodia theobromae	26	17.69
Aspergillus flavus	22	14.97
Total	**147**	**100.00**

Table 2: Incidence of decay and rot severity on healthy cocoyam corms inoculated with the test fungi

Fungi	***Colletotrichum gloeosporioides***	***Rhizopus stolonifer***	***Bipolaris* sp**	***Botryodiplodia theobromae***	***Aspergillus flavus***
Incidence					
Inoculated	100.0	100.0	100.0	100.0	100.0
Control	0.0	0.0	0.0	0.0	0.0
T-test	**39.74****	**19.49****	**57.74****	**64.81****	**75.59****
Severity					
Inoculated	33.33	73.33	73.33	26.67	60.0
Control	0.00	0.00	0.00	0.00	0.00
T-test	**7.56***	**11.0****	**16.63****	**6.05***	**10.39****

***significant at* 1% *level of probability and *significant at* 5% *level of probability*

Table 3: Characterization of fungal isolates from decaying cocoyam corms during storage

Macroscopic characteristics	**Microscopic characteristics**	**Appearance on PDA**	**Photomicrograph**	**Probable organisms**
Pinkish mycelia colour with a cottony-like structure and traces of whitish cream colouration around the edges	Cylindrical-shaped conidia with a septum, single cell, hyaline, and a smooth round end			*Colletotrichum gloeosporioides*

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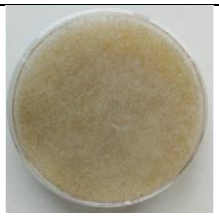
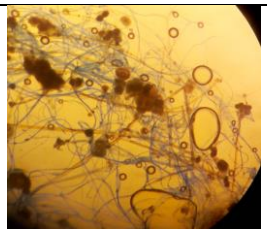
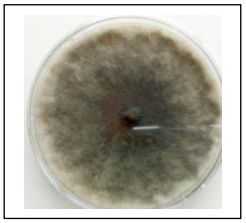
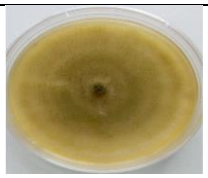
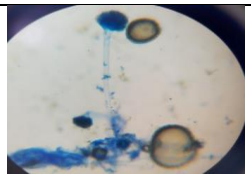
Fast-growing colonies with cotton fluffy colouration becoming greyish-brown with time.	Non-septate sporangiospores are hyaline, smooth-walled, simple, or branched forming large terminal globose sporangium			*Rhizopus stolonifer*
The colony colour started as an effuse white cottony-like structure and the colour later turned to grey and finally turned to blackish brown.	Curved conidia with septate hyphae			*Bipolaris* sp
Growth began as white aerial filamentous mycelia with a grey centre. Colony turned grey and then dark grey to black as days progressed.	Conidia were ellipsoid in shape, thick-walled, and hyaline. Spores were aseptate when immature but matured into 2-celled dark-brown spores			*Botryodiplodia theobromae*
The colony was a dense felt yellowish-green colouration. Mycelia growth was usually in concentric rings.	Conidia bluish green in nature with smooth long and hyaline conidiophores.			*Aspergillus flavus*

Table 4: Rot Types and Mean Area (cm^2) of rot caused by each pathogenic fungus after 14 days of inoculation on cocoyam varieties

Pathogenic Fungi	Rot Types	Area of Rot (cm^2)	
		Colocasia esculenta	***Xanthosoma sagittifolium***
Colletotrichum gloeosporioides	Dry	44.40a	24.69ab
Rhizopus stolonifer	Soft	44.60a	44.49a
Bipolaris sp	Dry	53.79a	41.14a
Botryodiplodia theobromae	Dry	19.07b	12.98b
Aspergillus flavus	Soft	40.19a	42.06a
Control	None	0.00c	0.00c
($P \leq 0.05$)		**0.001**	**0.001**

In Vitro Efficacy of Plant Extracts on the Radial Growth of Isolated Fungi Pathogens

The results of this study demonstrated the presence of fungi-toxic substances in the seeds of alligator pepper, black pepper, and neem leaves since they were capable of inhibiting the mycelia growth of the pathogenic fungi of cocoyam. The findings are consistent with previous reports of other studies, but with different fungal diseases and crops (Okigbo et al. 2009; Doherty et al. 2010; Nweke, 2015; Ezeonu et al. 2018; Gwa and Ekefan, 2018;

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Bamidele, 2019; Lum et al. 2019). However, the efficiency of the extracts differed with plant material, concentration, and each test fungus. The difference observed in fungi-toxic activity of the extracts is likely due to the solubility of the active compound(s) in water or the presence of inhibitors to the fungi-toxic principle. This also agrees with the report of Amadioha (2001) and Okigbo and Ogbonnaya (2006).

Mycelia growth inhibition of *Colletotrichum gloeosporioides*

The radial growth of *C. gloeosporioides* in vitro was significantly suppressed by the synthetic fungicide (Mancozeb) and the different aqueous plant extracts used in the study at different concentrations. 30% w/v extract concentration (85.39%) proved to be the most fungi-toxic on *C. gloeosporioides* while the least inhibitory effect was observed at 10% w/v extract concentration (82.20%). This agrees with Anukworji et al. (2012) who stated a significant difference between mycelia growth values recorded on the various plant extract concentrations. *Colletotrichum gloeosporioides* was significantly susceptible to the synthetic fungicide (Mancozeb) followed by Alligator pepper, Neem, and Black pepper with the least radial growth inhibition as shown in Table 5. This result agreed with the findings of Amienyo and Pandukur (2016) on the isolation of post-harvest fungi of cocoyam. Mancozeb at 8g/L (88.53%) significantly had the highest inhibitory effect on *C. gloeosporioides* in vitro compared to Neem at 10% w/v (79.67%) with the least inhibitory effect.

Table 5: Main effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Colletotrichum gloeosporioides* after inoculation

	Mean Radial Growth Inhibition Days After Inoculation (%)		
Concentration (w/v)	**Day 3**	**Day 5**	**Day 7**
10 %	60.61	80.27b	82.20b
20 %	63.69	81.42ab	83.79b
30 %	66.51	83.01a	85.39a
($P \leq 0.05$)	**NS**	**0.046**	**0.016**
Plant Extracts			
Alligator Pepper	64.68	81.88ab	85.07ab
Black Pepper	64.92	83.11ab	82.91b
Neem	61.21	79.71b	83.40b
Mancozeb	69.53	84.90a	87.80a
($P \leq 0.05$)	**NS**	**0.042**	**0.033**

Table 6: Interaction effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Colletotrichum gloeosporioides* after inoculation

Plant Extracts	**Concentration (w/v)**	**Mean Radial Growth Inhibition Days After Inoculation (%)**		
		Day 3	**Day 5**	**Day 7**
Alligator Pepper	**10 %**	64.93ab	82.33a	84.67abc
	20 %	61.60ab	80.86a	84.67abc
	30 %	67.50ab	82.43a	85.87abc

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Black Pepper	**10 %**	65.30ab	84.17a	82.27bc
	20 %	65.30ab	82.73a	82.03bc
	30 %	64.17ab	82.43a	84.43abc
Neem	**10 %**	51.60b	74.30b	79.67c
	20 %	64.17ab	80.67a	84.67abc
	30 %	67.87ab	84.17a	85.87abc
Mancozeb	**4 g/L**	67.87ab	84.17a	87.07ab
	8 g/L	71.20a	85.63a	88.53a
(P ≤ 0.05)		**0.049**	**0.046**	**0.045**

Mycelia growth inhibition of *Rhizopus stolonifer*

Mancozeb and the different plant extracts used in the study at different concentrations were very effective in the inhibition of mycelia growth of *R. stolonifer* in vitro. Mancozeb gave the highest radial growth inhibitory effect on *Rhizopus stolonifer* by 91.53%, followed by the aqueous extract of Alligator pepper with 89.23% while aqueous extracts of Black pepper and Neem showed the least mycelia growth inhibition of 88.21% and 86.69% respectively as shown in Table 7. Similar results with Bibah (2014) and Ezeonu et al. (2018) showed that plant extracts were very effective in inhibiting the mycelia growth of *R. stolonifer*. The efficacy of the interaction of aqueous plant extracts and concentration in inhibiting *R. Stolonifer* was significantly different from Mancozeb at 8g/L (93.07%) with the highest inhibitory effect and Neem at 30% w/v (84.63%) with the least inhibitory effect as shown in Table 8.

Table 7: Main effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Rhizopus stolonifer* after inoculation

	Mean Radial Growth Inhibition Days After Inoculation (%)		
Concentration (w/v)	**Day 3**	**Day 5**	**Day 7**
10 %	89.64a	88.48	88.47
20 %	88.27ab	87.98	87.71
30 %	87.72c	87.98	87.96
(P ≤ 0.05)	**0.031**	**NS**	**NS**
Plant Extracts			
Alligator Pepper	89.37b	89.26ab	89.23b
Black Pepper	88.81bc	88.49ab	88.21bc
Neem	87.44c	86.69b	86.69c
Mancozeb	91.42a	91.53a	91.53a
(P ≤ 0.05)	**0.032**	**0.005**	**0.011**

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Table 8: Interaction effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Rhizopus stolonifer* after inoculation

Plant Extracts	**Concentration**	**Mean Radial Growth Inhibition Days After Inoculation (%)**		
	(w/v)	**Day 3**	**Day 5**	**Day 7**
Alligator Pepper	**10 %**	90.20ab	89.23bc	89.23bc
	20 %	88.53b	88.50bcd	88.47bcd
	30 %	89.37b	90.03b	90.00b
Black Pepper	**10 %**	88.53b	86.97cde	86.93cde
	20 %	88.53b	89.23bc	88.47bcd
	30 %	89.37b	89.27bc	89.23bc
Neem	**10 %**	90.20ab	89.23bc	89.23bc
	20 %	87.70b	86.20de	86.20de
	30 %	84.43c	84.63e	84.63e
Mancozeb	**4 g/L**	90.20ab	90.0b	90.00b
	8 g/L	92.63a	93.07a	93.07a
(P ≤ 0.05)		**0.009**	**0.005**	**0.014**

Mycelia Growth Inhibition of *Bipolaris* sp

The radial growth of *Bipolaris* sp in vitro was significantly ($P \leq 0.05$) suppressed by Mancozeb and the different aqueous plant extracts used in the study at different concentrations. 30% w/v concentration (88.05%) was fungi-toxic on *Bipolaris* sp while the least inhibitory effect was observed at 10% w/v concentration (81.11%) as shown in Table 9. This agrees with Anukworji et al. (2012) and Hasan et al. (2012) who observed significant differences between mycelia growth values recorded for the various plant extract concentrations. *Bipolaris* sp was more susceptible to Mancozeb (100.00%) followed by Black pepper (86.96%), Neem (84.71%), and Alligator pepper (81.91%) with the least radial growth inhibition. Similar results with Hasan et al. (2012); Prashith Kekuda et al. (2016), and Elsherbiny et al. (2017) showed that plant extracts were very effective in inhibiting mycelia growth of *Bipolaris* sp. Mancozeb at 8g/L (100.00%) and Mancozeb at 4g/L (100.00%) had the highest mycelia growth inhibitory effect of *Bipolaris* sp compared to Alligator pepper at 10% w/v (75.83%) with the least inhibitory effect as shown in Table 10.

Table 9: Main effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Bipolaris* sp after inoculation

	Mean Radial Growth Inhibition Days After Inoculation (%)		
Concentration (w/v)	**Day 3**	**Day 5**	**Day 7**
10 %	75.70b	80.98c	81.11c
20 %	80.28a	84.40b	84.42b
30 %	84.38a	87.36a	88.05a

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(P ≤ 0.05)	**0.003**	**0.001**	**0.001**
Plant Extracts			
Alligator Pepper	73.68c	80.49c	81.91c
Black Pepper	83.96b	85.82b	86.96b
Neem	82.72b	86.42b	84.71ab
Mancozeb	100.00a	100.00a	100.00a
(P ≤ 0.05)	**0.001**	**0.001**	**0.003**

Table 10: Interaction effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Bipolaris* sp after inoculation

Plant Extracts	**Concentration**	**Mean Radial Growth Inhibition Days After Inoculation (%)**		
	(w/v)	**Day 3**	**Day 5**	**Day 7**
Alligator Pepper	**10 %**	65.37d	74.64e	75.83d
	20 %	75.40c	82.17d	83.23c
	30 %	80.27bc	84.70bcd	86.6bc
Black Pepper	**10 %**	82.73bc	83.73cd	85.00bc
	20 %	82.67bc	84.60bcd	85.87bc
	30 %	86.47b	89.13b	90.00b
Neem	**10 %**	79.00bc	84.60bcd	82.50bc
	20 %	82.77bc	86.43bcd	84.17c
	30 %	86.40b	88.23bc	87.47bc
Mancozeb	**4 g/L**	100.00a	100.00a	100.00a
	8 g/L	100.00a	100.00a	100.00a
(P ≤ 0.05)		**0.001**	**0.001**	**0.001**

Mycelia Growth Inhibition of *Botryodiplodia theobromae*

Fungi-toxic activities of the aqueous plant extracts increased with an increase in concentration against *B. theobromae*. 30% w/v (82.53%) proved to be the most effective while the least inhibitory effect was observed at 10% w/v (75.92%) on *B. theobromae*. This supports previous studies by Anukworji et al. (2012), Amienyo and Pandukur (2016), and Gwa and Ekefan (2018). The study showed that Mancozeb (100.00%) was highly effective against mycelia growth of *B. theobromae* while extracts of Alligator pepper (71.30%) were least effective among the extracts in vitro as shown in Table 11. This is similar to the results obtained by Anukworji et al. (2012) who reported a highly effective inhibition of *A. niger*, *F. solani*, *S. rolfsii*, and *B. theobromae* with aqueous plant extracts of *C. papaya*, *G. kola*, *A. sativum*, and *A. indica*. This study showed that *B. theobromae* is more susceptible to Mancozeb at 8g/L (100.0%) and Mancozeb at 4g/L (100.0%), but less susceptible to aqueous extracts of Neem at 10% w/v (70.50%), Alligator pepper at 10% w/v (70.43%), and Alligator pepper at 20% w/v (68.87%) as shown in Table 12.

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Table 11: Main effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Botryodiplodia theobromae* after inoculation

	Mean Radial Growth Inhibition Days After Inoculation (%)		
Concentration (w/v)	**Day 3**	**Day 5**	**Day 7**
10 %	61.12c	77.72c	75.92c
20 %	66.40b	80.59b	78.97b
30 %	71.34a	83.43a	82.53a
(P ≤ 0.05)	**0.001**	**0.001**	**0.001**
Plant Extracts			
Alligator Pepper	50.21d	71.67d	71.30d
Black Pepper	78.42b	86.82b	87.15b
Neem	70.23c	83.76c	78.98c
Mancozeb	100.00a	100.00a	100.00a
(P ≤ 0.05)	**0.001**	**0.001**	**0.002**

Table 12: Interaction effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Botryodiplodia theobromae* after inoculation

Plant Extracts	**Concentration**	**Mean Radial Growth Inhibition Days After Inoculation (%)**		
	(w/v)	**Day 3**	**Day 5**	**Day 7**
Alligator Pepper	**10 %**	49.77cd	70.83ef	70.43f
	20 %	44.77d	69.27f	68.87f
	30 %	56.10c	73.40de	74.60e
Black Pepper	**10 %**	77.43b	86.27bc	86.83bc
	20 %	75.57b	84.47c	85.20cd
	30 %	82.27b	89.73b	89.40b
Neem	**10 %**	56.17c	76.07d	70.50f
	20 %	78.87b	88.03bc	82.83d
	30 %	75.67b	87.17bc	83.60cd
Mancozeb	**4 g/L**	100.00a	100.00a	100.00a
	8 g/L	100.00a	100.00a	100.00a
(P ≤ 0.05)		**0.001**	**0.001**	**0.001**

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Mycelia Growth Inhibition of *Aspergillus flavus*

The radial growth of *A. flavus* in vitro was significantly suppressed by Mancozeb and the different aqueous plant extracts used in the study at different concentrations. 30% w/v (85.22%) extract concentration proved to be the most fungi-toxic on *A. flavus* while the least inhibitory effect was observed at 10% w/v (82.09%) extract concentration after 7 days of incubation as shown in Table 13. The results are in agreement with the reports of many researchers like Anukworji et al. (2012) and Sulaiman et al. (2019) who stated a significant difference between mycelia growth values recorded in the various concentrations. *Aspergillus flavus* was more susceptible to the Mancozeb (87.62%) and Alligator pepper (85.59%), with Neem (82.91%) and Black pepper (82.79%) having the least fungi-toxic effect. These results agree with the findings of Okigbo and Nmeka, (2005) on the control of yam tuber rot with leaf extracts. The most significant fungi-toxic effects on the interaction between concentration and extracts were observed with Mancozeb at 4g/L (88.20%) compared to Neem at 10% w/v (78.97%) with the least fungi-toxic effect on *A. flavus* as shown on Table 14. The presence of antifungal substances in the different aqueous plant extracts which caused mycelia growth inhibition of fungi pathogens in vitro agrees with reports of other researchers (Okigbo and Nmeka, 2005; Anukworji et al. 2012; Okigbo et al. 2015; Amienyo and Pandukur, 2016; Sulaiman et al. 2019).

Table 13: Main effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Aspergillus flavus* after inoculation

	Mean Radial Growth Inhibition Days After Inoculation (%)		
Concentration (w/v)	**Day 3**	**Day 5**	**Day 7**
10 %	69.08c	76.99b	82.09b
20 %	73.99b	78.83b	83.99ab
30 %	76.17a	81.15a	85.22b
(P ≤ 0.05)	**0.009**	**0.047**	**0.045**
Plant Extracts			
Alligator Pepper	75.31	81.60ab	85.59ab
Black Pepper	70.52	77.36c	82.79b
Neem	73.40	78.01bc	82.91b
Mancozeb	80.50	85.53a	87.62a
(P ≤ 0.05)	**NS**	**0.046**	**0.049**

Table 14: Interaction effects of concentrations and plant extracts on percentage mean radial growth inhibition on *Aspergillus flavus* after inoculation

Plant Extracts	**Concentration**	**Mean Radial Growth Inhibition Days After Inoculation (%)**		
	(w/v)	**Day 3**	**Day 5**	**Day 7**
Alligator Pepper	**10 %**	76.10a	82.93abc	85.97ab
	20 %	75.83a	81.60abc	85.97ab
	30 %	74.00ab	80.27abc	84.83abc

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Black Pepper	**10 %**	65.43b	75.67cd	81.33cd
	20 %	72.13ab	76.57cd	82.33bcd
	30 %	74.00ab	79.83abc	84.70abc
Neem	**10 %**	65.70b	72.37d	78.97d
	20 %	74.00ab	78.33bcd	83.63bc
	30 %	80.50a	83.33abc	86.13ab
Mancozeb	**4 g/L**	80.50a	84.87ab	87.03ab
	8 g/L	80.50a	86.20a	88.20a
(P ≤ 0.05)		**0.004**	**0.008**	**0.022**

Phytochemical Analysis of Plant Extracts

Results of the qualitative phytochemical screening of aqueous plant extracts of Alligator pepper (*Aframomum melegueta*), Black pepper (*Piper nigrum*), and Neem (*Azadirachta indica*) are shown in Table 15. Phytochemical analysis of aqueous plant extracts revealed the presence of alkaloids, tannins, phenols, saponins, flavonoids, cardiac glycosides terpenoids, and Phytosterols. The fungicidal and pharmacological potential of all these phytochemicals were proven by the report of several works (Okigbo et al. 2009; Ezeonu et al. 2019; Fru and Vange, 2023). Phytochemical screening of Black pepper extract by Aghale et al. (2017) indicated the presence of alkaloids, flavonoids, saponins, tannins, and phenols. The phytochemical analysis of Alligator pepper by Doherty et al. (2010) revealed the presence of tannin, saponin, flavonoid, steroid, terpenoids, cardiac glycoside, alkaloid, and phenols.

Table 15: Phytochemicals present in the plant extracts

Phytochemicals	**Plant Extracts**		
	Aframomum melegueta	***Piper nigrum***	***Azadirachta indica***
Alkaloids	++	++	++
Tannins	-	+	++
Saponins	++	++	++
Flavonoid	+	+	++
Phenols	-	+	+
Cardiac Glycosides	+	++	++
Terpenoids	+	+	+
Phytosterols	+	+	+

- = Absent + = Present ++ = Highly present

CONCLUSION

This study identified *Colletotrichum gloeosporioides*, *Rhizopus stolonifer*, *Bipolaris* sp, *Botryodiplodia theobromae*, and *Aspergillus flavus* as the main fungi pathogens responsible for causing postharvest losses to

cocoyam varieties during storage in Makurdi. Pathogenicity test and mean area of rot confirmed the ability of the isolated pathogens to induce rot, with *Rhizopus stolonifer* and *Bipolaris* sp causing the highest severity. Results obtained from phytochemical screening of the aqueous extracts of Alligator pepper, Black pepper, and Neem confirmed their anti-microbial potency and their use in disease control. Due to their ability to considerably limit the growth of fungal pathogens in vitro, this study demonstrated that the various aqueous plant extracts had fungi-toxic compounds. Notably, the plant extracts were shown to be just as effective as the conventional synthetic fungicide, and more effective in many instances. This indicates that the plant extracts possess naturally active antimicrobial components. Considering matrices of performance parameters it can be concluded that black pepper extract is the most effective of the three plant extracts and 30% w/v was the most effective concentration of the different plant extracts. The botanicals can be used commercially as an alternative to synthetic fungicide due to their availability, accessibility, and affordability as safe treatment in a sustainable organic post-harvest management system.

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