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Effect of Three Tropical African Plants on some Fungal Rot of Stored Cocoyam (*Colocasia Esculenta* L)

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Antifungal effects of aqueous and ethanol extracts of *Chromolaena odorata, Azadirachta indica, and Vernonia amygdalina* on the growth of fungal pathogen of stored cocoyam cormels were investigated *in-vitro*. Pathogenicity test revealed that *Botryodiplodia theobromae, Aspergillus niger ,Rhizopus stolonifer* and *Trichoderma viride* induced rot in healthy cocoyam cormels after 9 days of inoculation, and *Botryodiplodia theobromae* is the most virulent with the highest percentage occurrence of 39.1%, followed by *Aspergillus niger* (26.0%). Although all the extract showed varying degrees of antifungal efficacy, ethanol extract proved to be more potent. *Chromolaena odorata* and *Vernonia amygdalina* showed a complete inhibition of *Botryodiplodia theobromae.* The effect of the extracts varied with the solvent of extraction, extract concentration and the test pathogens. The inhibitory effect of *Chromolaena odorata* extract is higher than other extracts in all concentrations and showed a significant ($P<0.05$) inhibition on all the test fungi pathogens. The fungitoxic potential of these plant extracts on rot inducing fungi of stored cocoyam corms encourages more research on the active ingredient of these plants for easy use by farmers as alternative to commercial/ synthetic fungicides.

Keywords : *Chromolaena odorata* L., *Vernonia amygdalina* Del, *Azadirachta indica* (A. Juss) Antifungal, Africa

Introduction

Cocoyam (*Colocasia esculenta* L) is a herbaceous perennial monocotyledonous herb which belongs to the genus *colocasia* and family of Araceae (Onwueme, 1999), originated from South Central Asia, (Kolchaar, 2006). It occurs wild in tropical Asia extending as far as New Guinea and was spread by human settlers East ward over 2000 years ago, where it became one of the most important food plants economically and culturally (Purseglove, 1992; Anukwuorji *et al.*, 2012). According to FAO (2006), the world largest producer of cocoyam is Nigeria with a production figure of about 5.49m tones

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per annum, followed by Ghana. C*olocasia esculenta* (L) is an important crop in many parts of the world. The crop plays a major role in the lives of many as a food security crop and has rich economic and socio-cultural connotation (Mwenye, 2009). Cocoyam products have shown considerable potential in different industries. Recent studies revealed that cocoyam starch is fine and contains small granules, a property required in many industries (Perez *et al*., 2005; Rodriguez *et al.*, 2006).

However, Eze and Okorji (2003) documented that Nigeria accounted for about 40% of total world's output of cocoyam, but from evidence, the cultivation of cocoyam in Nigeria is declining (Onyenweaku and Eze, 1987; Zuhair and Hunter, 2000), which storage rot is one of the most important. Moreso, it is widely perceived that cocoyam production and processing in the country does not keep pace with other major root and tuber crops (Asumugha and Mbanaso, 2002), this is believed to be attributed to its low storability and diseases that affect stored cocoyam. In storage, serious losses due to rotting of the corms and cormels are the major factors affecting quantity and quality of cormels for consumption and planting (Eunice and Osuji, 2008). Fungi reported as important rot pathogens include *Fusarium solani, Botryodiplodia theobromae, Rhizopus stolanifer, Aspergillus niger and Sclerotium rolfsii* (Madukwesi and Onyeka, 1981). These fungi create local discoloration of the surrounding tissues of the infected tubers resulting to deterioration of texture and taste. Rot fungi causes post-harvest losses, reduction of market value and misfortune to farmers (Tijjani *et al*., 2013). The implications are therefore serious particularly in relation to the availability of sufficient planting materials to sustain yearly cropping (Janseen, 2001). In Nigeria, the rapid decline in *Colocasia* production is threatening the survival and existence of the crop and extinction is eminent (Ugbajah and Uzuegbuna, 2012) thereby making the nation's poverty reduction plan through sustainable agriculture to be weaker and less effective.

Nevertheless, some plants traditionally, are used as medicine for the treatment of diseases. Medicinal plants have been extensively studied as an alternative treatment for diseases in order to overcome the problem of antibiotic resistance by pathogenic organisms (Timothy *et al*., 2008; Phillip *et al*., 2005). And many researchers have used plant extracts to control fungal diseases of plants in Nigeria such as black Sigatoka of banana (Okigbo and Emoghene, 2004), Yam rot tuber (Okigbo and Nmeka, 2005), Pea root rot (Abdulaziz and Younes, 2010) and Paw-paw fruit rot (Ebele, 2011). Numerous plants and their phytochemical constituents have been proven by many researchers to have medicinal value of which *Azadirachta indica* (A. juss), *Vernonia amygdalina* (Del), *and Chromolaena odorata* (L) are inclusive.

Veronia amygdalina (Del) known as bitter leaf belong to the family Asteraceae, it is a valuable medicinal plant that is widespread in West Africa (Udochukwu, *et al*. 2015), originated in Nigeria. Traditionally, the plant is used as tonic and remedy against constipation, worms, fever and high blood pressure. (Iwalokun, *et al*., 2006). According to (Udochukwu, *et al*. 2015), the phytochemical components of *V. amygdalina* includes; oxalate, phytate, tannins, saponins, flavonoids, cyanogenic glycosides, alkaloids, anthraquinone, steroid and phenol.

Chromolaena odorata (L) is a tropical species of flowering shrub in the sun flower family Asteraceae, a native of North-America. Common names include Siam weed, Christmas bush; Devil weed and floss flower (Schmidt and Schilling, 2000). It thrives in most soils and is a prolific weed found in abundance on open wasteland and along roadsides (Ling *et al*., 2007). It is used as an antibacterial, antiplasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypentensive, antiinflammatory, astringent, deuretic and hepatotropic agent (Phan *et al*., 2001; Akinmoladun *et al*., 2007). It is sometimes grown as a medicinal and ornamental plant. The young leaves contain carcinogenic pyrrolizidine alkaloids and can be used to treat skin wounds. (Lu, *et al*., 2002).

Azadirachta indica (A.Juss) belongs to the family Meliance, it is one of the two species of the genus Azadiratchta, a native of Bangladesh and India. It is used in traditional medicine as a source of many therapeutic agents in Indian culture and can be used for the treatment of diabetes (Maragathavalli *et al.*, 2012). *Azadirachta indica* (A.Juss) leaves have antibacterial properties and could be used for controlling airborne bacterial, contamination in the residential premise (Mahmood *et al*., 2010). Earlier studies on *Azadirachta indica* (A.Juss) showed that it contains active substances with multiple medicinal properties (Maragathavalli *et al.,* 2012). Although the effectiveness of these plants has been confirmed scientifically based on their antifungal activities and have variously been reported as possessing antimicrobial and antisecticidal properties against wide range of micro-organisms, however, documented reports of the effect of *Vernonia amygdalina* (Del), *Chromolaena odorata* (L) *and Azadirachta indica* (A. Juss) leaves on control of fungal rot of stored cocoyam are very scanty.

Thus, the aim of this research is to determine the effect of Ethanol and Aqueous extract of *Azadirachta indica* (A.Juss), *Chromolaena odorata* (L) and *Veronia amygdalina* (Del) on some fungal organisms causing rot on stored cocoyam.

Materials and Methods

Study area

This experiment was conducted at the Pathology laboratory of National Root Crops Research Institute Umudike (NRCRI) located between the Latitude 5˚28˙North , Longitude 7˚55˙South and an Altitude of 122m above sea level in rain forest agro-ecological zone of Southeastern Nigeria.

Source of plants and preparation of Extracts

The leaves of *Chromoleana odorata* were procured from an open field fallow of Nnamdi Azikiwe University, while the leaves of *Azadirachta indica* and *Vernonia amygdalina* were obtained from National Root Crop Research Institute staff quarters. These plants were verified and authenticated in the horticulture unit of NRCRI Umudike, Abia State Nigeria. All the leaves were thoroughly washed and shade dried in dust free environment. The dried leaves were milled into powder. Rotten and healthy cocoyam cormels (*Colocasia esculenta* L) were collected from cocoyam barn of National Root Crop Research Institute Umudike.

Subculturing/purification and characterization of test fungi pathogens

Subcultures of different mycelia colonies from the inoculated plates were made to obtain pure culture of the rot causing organism and were identified with reference to (Sulton 1980; Barnett and Hunter, 1987). This was done by transferring hyphal tips from the colony edge of the mixed cultures to fresh plates of PDA using flame sterilized blades and incubated at 27^0C. This was done repeatedly until pure cultures were obtained and were sealed with paper tape to avoid contamination. The resulting pure cultures were used for characterization and subsequent identification of the fungi isolates with the aid of a compound microscope and identification guides (Sulton, 1980).

Pathogenicity Test.

Four (4) test organisms (*Aspergillus niger, Trichoderma viride Rhizopus stolonifers and Botryodiplodia theobromae*) from the rotten samples were used. Fresh healthy cocoyam cormels were first washed with tap water and then surface sterilized with 70% ethanol solution. The cormels were placed on sterile paper towels and allowed to dry for 15minutes in a Laminar Air flow hood.

Holes were bored on the healthy cocoyam with sterile cork borer (5mm diameter). The bored out parts were replaced after inoculating a 5 day old culture of the isolated fungus (Pure culture). They were sealed with sterile Vaseline to prevent contamination and were labeled accordingly. A control was set up in reference to (Amienyo and Ataga, 2006). The inoculated cormels were placed in micro humidity chamber at 28±2˚C. All the cormels were incubated and were examined daily for evidence of rot for 9 days in a humidity chamber (incubation period). The cormels were carefully cut open along the line of inoculation to expose the regions of the cormels which were then examined for rot and the length and girth of the rot area and those of the entire cormels were measured and recorded.

Preparation of Plant Extracts

The leaves of *Chromolaena odorata* L. (Siam weed), *Vernonia amygdalina* Del. (Bitter leaf) and *Azadiratcha indica* A.Joss (Neem) were thoroughly washed with tap water and then with sterile distilled water (SDW) and were sun dried for 5 days. The dried samples were separately ground in a laboratory Mill (Thomas, Wiley, model ED-5 made in USA) after which the ground samples were sieved to obtain powdered processed sample used for the extraction. Cold solvent extraction method (Harbone, 1973; Junaid *et al*., 2006; Doughari *et al*., 2007) was used. Four different aqueous and ethanol extract concentration were prepared by weighing 30g, 50g, 70g and 100g of leaf powder of each of the plants and were dissolved into 100ml of each solvent separately in round bottom flasks and allowed to stand for 24hrs, sieved with fine cheese cloth, thus concentration was gotten by dividing each gram separately by 100ml i.e.

conc. = $\frac{g}{100}$ x 100. Where g is the gram

Thus having 30%, 50%, 70% and 100% concentration respectively and were used for the inhibition of mycelia growth.

Effect of Plant Extracts on Fungal Growth

Effect of plant extract on mycelia growth of the four test fungi was studied using the food poisoning techniques (Songoyomi, 2004). One milliliter of each plant extract concentrations (30%, 50%, 70% and 100%) was dispensed per Petri dish and 9ml of the media (molten PDA) was added to each of the Petri dishes containing extract and carefully spread evenly over the plate, this gave rise to PDA -extract mixture with corresponding 3.0%, 5.0% 7.0% and 10%

extract concentration. This was used for the inhibition of mycelia growth. The plates were gently rotated to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the center with a 4mm diameter mycelia dish obtained from the colony edge of 7-day old pure cultures of each of the four test fungi. Each treatment had three replicates. The negative control set up was blank agar plate (no extract) inoculated with the test fungi as described above.

All the plates were incubated at 28 ± 2^0C for 6days and examined daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of diameter of inhibition in millimeters.

Quantitative Yield of extract for Test Plants

Twenty grams (20g) portion of each processed sample was mixed with 200ml of each solvent in a plastic sample bottle. The mixture was shaken in a mechanical shaker (Gallen kamp) for 1hr and then allowed to stand for six hours at room temperature, thereafter; it was filtered through whatman No 42 grade of filter paper. The filtrates were transferred into separates evaporation dish and evaporated over a steam bath at 90^0C while the solvent was lost thus leaving the pasty extract. The dish was weighed while empty and its weight recorded. The weight of the dish and its content after evaporation in the water bath were recorded. The yields of the extract was calculated using the formular

$$\% \text{ yield} = \frac{W_1 - W_2}{W} \times 100$$

Where W = weight of sample used ; W1 = weight of empty dish; W2 = weight of dish + pasty extract

Experimental Design

The experimental design used was 3 x 4 x 2 factorial laid in a Completely Randomized Design. Treatment means were separated using Duncan's Multiple Range Test (DMRT)

Result

Percentage Occurrence of isolated fungi on rotten Cocoyam samples

Percentage occurrence of isolated fungi on rotten cocoyam samples depicted that of the four fungi isolated from rotten cocoyam samples, *Botryodiplodia theobromae* showed the highest percentage occurrence 39.1%

while *Trichoderma viride* showed the least percentage occurrence 13.0%. (figure 1)

Pathogenicity Test

The pathogenicity test showed that all the four test fungi (*Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Aspergillus niger and Trichoderma viride*) were pathogenic, hence cause rot in healthy cocoyam cormels after nine (9) days of inoculation. The most virulent among the four test fungi was *Botryodiplodia theobromae* (Table 1)

Quantitative Yield of the Extract

Result of the quantitative yield of the extract revealed that in ethanol and aqueous solvent, *Chromolaena odorata* gave higher percentage yield (65.05±0.07% and 50.00±0.07%), followed by *Vernonia amygdalina*. Thus, between the two solvents, the yields of the extract were higher in ethanol than aqueous and was significant ($p<0.05$) (Table 2).

Inhibitory Effect of Aqueous Extract of Chromolaena odorata, Azadirachta indica and Vernonia amygdalina on the growth of isolated fungi

The effect of different concentrations of aqueous extracts on the test organisms was significant ($P < 0.05$). Diameter of the inhibition increased as the concentration of the extract increased (3.0%>, 5.0%>, 7.0%>, 10.0%). The interaction of extraction medium and concentration of extract was also significant ($P<0.05$) on the inhibition of all the four test fungi while control did not show any inhibition. Inhibitory effect of aqueous extract of test plant (*Chromolaena odorata* (L) *Azadirachta indica* (A.Joss) and *Vernonia amygdalina* (Del) on the growth of fungi isolates at 3.0% concentration demonstrated inhibition. *Azadirachta indica* extract showed highest inhibition of *Botryodiplodia theobromae* (3.00±0.001mm) and lowest on *Aspergillus niger* (3.50±0.141mm). The inhibition of the four test fungi showed significant difference ($p<0.05$) between the plant extract (Table 3). The inhibitory effect of *Vernonia amygdalina* and *Chromolaena odorata* did not show any significant difference ($P>0.05$) on *Botryodiplodia theobromae* and *Aspergillus niger* at 5.0% concentration (Table 4). At 7.0%, the inhibitory effect of *Azadirachta indica* was significantly ($P<0.05$) higher than that of other extracts, (Table 5). Interaction of the extracts at 10% concentration showed that *Vernonia amygdalina* gave the highest inhibitory effect of (1.50±0.001mm) on

Trichoderma viride, this was significantly (P<0.05) different from the inhibitory effect of *Azadirachta indica* (2.00±0.001mm) and *Chromolaena odorata* (1.80±0.001mm), there is no significant difference(P>0.05) between the test plants on *Rhizopus stolonifer* (Table 6). The inhibitory effect of the plant extract at various concentrations depicted that *Azadirachta indica* and *Vernonia amygdalina* increases with concentration of the extract while *Chromolaena odorata* showed highest inhibition at 7.0% and 10% concentration (Figure 2). Meanwhile, the susceptibility of the test fungi increases with concentration of plant extract and there is significant difference (p<0.05) in the susceptibility of fungi between concentrations of the plant extracts (Figure 3).

Effect of Ethanol Extract of Chromolaena odorata, Azadirachta indica andVernonia amygdalina on the Growth of Isolated Fungi

Ethanol extract of *Chromolaena odorata* and *Vernonia amygdalina* at 3.0%, showed a complete inhibition of *Botryodiplodia theobromae*. Thus, the test plants showed no significant difference (P>0.05) on *Trichoderma viride* inhibition (Table 7). At 5.0% concentration, *Chromolaena odorata* gave the highest inhibition on all the test fungi *(Botryodiplodia theobromae, Aspergillus niger, Rhizopus stolonifer* and *Trichoderma viride).* Inhibition of *Trichoderma viride* however did not show any significant difference (p>0.05) between the ethanol extract of *Azadirachta indica* and *Vernonia amygdalina* (0.90±0.001mm and 0.50±0.001mm) on *Trichoderma viride, Azadirachta indica and Chromolaena odorata* was not significant (p>0.05) on *Aspergillus niger* growth (0.70±0.001mm and 0.50±0.001mm) while *Vernonia amygdalina and Chromolaena odorata* showed no significant difference (p>0.05) on *Rhizopus stolonifer* (0.40±0.001mm and 0.40±0.001mm) respectively (Table 8). At 7.0%, growth of *Botryodiplodia theobromae* was completely inhibited by extracts of *Vernonia amygdalina, Chromolaena odorata* and *Azadirachta indica* while *Trichoderma viride* was completely inhibited by *Chromolaena odorata* and *Vernonia amygdalina* (Table 9). At 10%, ethanol extract showed complete inhibition on *Botryodiplodia theobromae* while the inhibition of *Aspergillus niger and Rhizopus stolonifer* showed a significant difference (p<0.05) between the ethanol extract of *Vernonia amygdalina, Chromolaena odorata* and *Azadirachta indica* (Table 10).

The inhibitory effect of the test plants (*Vernonia amygdalina, Chromolaena odorata* and *Azadirachta indica*) increased with concentration of the extract. In comparison, the inhibitory effect of *Chromolaena odorata* extract is higher than other extracts in all concentration. Analysis of variance

($p>0.05$) showed no significant between plant extracts and concentrations of extract (Figure 4). The susceptibility of fungi Isolates in various concentrations of ethanol plant extracts showed significant difference ($p<0.05$) in the susceptibility of fungi between plant extracts concentrations (Figure 5). Inhibitory effect of ethanol and aqueous extract of test plants depicted that the test plant showed greater inhibition in ethanol when compared to water. Analysis of variance showed a significant difference ($p<0.05$) in the inhibitory effect between ethanol and aqueous (Figure 6). The susceptibility of isolated fungi (*Botryodiplodia theobromae*, *Aspergillus niger*, *Trichoderma viride* and *Rhizopus solonifer*) are higher in ethanol extract of the test plants than in aqueous extract (Figure 7)

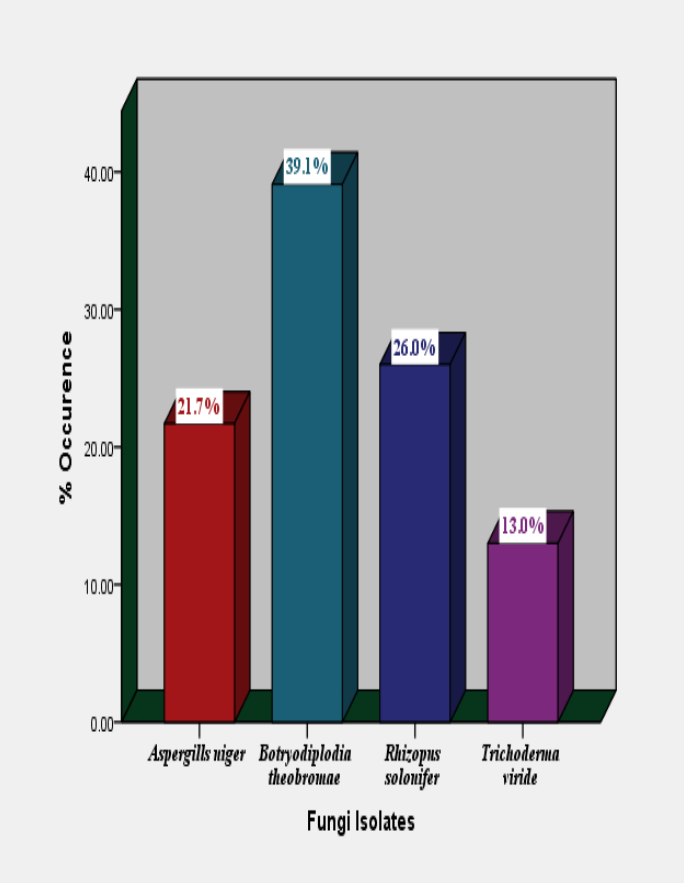

Figure 1: Percentage Occurrence of isolated fungi on rotten Cocoyam samples

Table 1: Pathogenicity Test of Isolated Fungi

Isolates Inoculated	Diameter of Rot (mm)*
B. theobromae	2.0 ±0.03
T. viride	1.5 ±0.01
A. niger	1.0 ±0.03
R. stolonifer	1.5 ±0.02
Control	0.00

*p<0.05

Table 2: Quantitative Yield of the Extract

	Extraction Medium		p-value
Plant Extract	**Ethanol (%)**	**Aqueous (%)**	
A. indica	40.02 ± 0.03^{b}	30.05 ± 0.07^{b}	**
V. amygdalina	20.02 ± 0.07^{c}	10.03 ± 0.07^{c}	**
C. odorata,	65.05 ± 0.07^{a}	50.00 ± 0.01^{a}	**
p-value	**	**	

**p<0.05

Table 3: Effect of Aqueous Extract of test plants on test fungi at 3.0% after 6 days

	Growth of Isolates (mm)*			
Extract	*B. theobromae*	*T. viride*	*A. niger*	*R. stolonifer*
Control	3.97 ± 0.058^{a}	3.87 ± 0.115^{b}	3.87 ± 0.115^{a}	3.97 ± 0.058^{a}
A. indica	3.00 ± 0.001^{c}	2.70 ± 0.141^{a}	3.50 ± 0.141^{b}	3.20 ± 0.001^{b}
V.amygdalina	3.90 ± 0.001^{a}	3.80 ± 0.141^{b}	2.20 ± 0.141^{c}	3.90 ± 0.001^{a}
C. odorata,	3.80 ± 0.001^{a}	2.40 ± 0.141^{a}	2.30 ± 0.141^{c}	3.20 ± 0.001^{b}
p-value	**	**	**	**

Results are in mean ± standard deviation
*columns with similar alphabets are not significantly different
**p<0.05,

Table 4: Effect of Aqueous extract of test plants on isolated fungi at 5.0% after 6 days

	Inhibition Zone of Pathogens(mm) in Aqueous Extract*			
Extract	*B. theobromae*	*T. viride*	*A. niger*	*R. stolonifer*
Control	3.97 ±0.056[a]	3.87 ±0.115[a]	3.87 ±0.115[a]	3.97 ±0.058[a]
A. indica	2.80 ±0.424[b]	2.50 ±0.001[b]	3.40 ±0.283[b]	3.00 ±0.001[b]
V.amygdalina	3.80 ±0.001[a]	3.70 ±0.001[a]	2.00 ±0.141[c]	3.90 ±0.001[a]
C. odorata,	3.80 ±0.283[a]	2.20 ±0.001[c]	2.00 ±0.001[c]	2.90 ±0.283[b]
p-value	**	**	**	**

Results are in mean ± standard deviation
*columns with similar alphabets are not significantly different
**P<0.05

Table 5: Effect of Aqueous Extract of test plants on the isolated fungi at 7.0% after 6 days

	Growth of Isolates (mm) *			
Extract	*B. theobromae*	*T.viride*	*A.niger*	*R. stolonifer*
Control	3.97 ±0.058[a]	3.87 ±0.115[a]	3.87 ±0.115[a]	3.97 ±0.058[a]
A. indica	2.50 ±0.283[c]	2.20 ±0.001[c]	3.20 ±0.141[b]	2.80 ±0.001[b]
V. amygdalina	3.70 ±0.001[b]	3.60 ±0.001[b]	1.80 ±0.141[c]	3.80 ±0.001[a]
C. odorata,	3.60 ±0.001[b]	2.00 ±0.001[d]	1.80 ±0.001[c]	2.70 ±0.283[b]
p-value	**	**	**	**

Results are in mean ± standard deviation
*columns with similar alphabets are not significantly different
**p<0.05,

Table 6: Effect of Aqueous Extract of test plants on the isolated fungi at 10.0% at 6 days

	Growth of Isolates (mm) *			
Extract	*B. theobromae*	*T. viride*	*A. niger*	*R. stolonifer*
Control	3.97 ±0.058[a]	3.87 ±0.115[a]	3.87 ±0.115[a]	3.97 ±0.058[a]
A. indica	2.00 ±0.141[c]	2.00 ±0.001[b]	3.00 ±0.283[b]	3.50 ±0.001[a]
V. amygdalina	3.50 ±0.141[b]	1.50 ±0.001[c]	2.50 ±0.000[c]	3.70 ±0.424[a]
C. odorata,	3.80 ±0.001[a]	1.80 ±0.001[d]	1.50 ±0.001[d]	3.50 ±0.001[a]
p-value	**	**	**	ns

Results are in mean ± standard deviation
*columns with similar alphabets are not significantly different
**p<0.05, ns: not significant

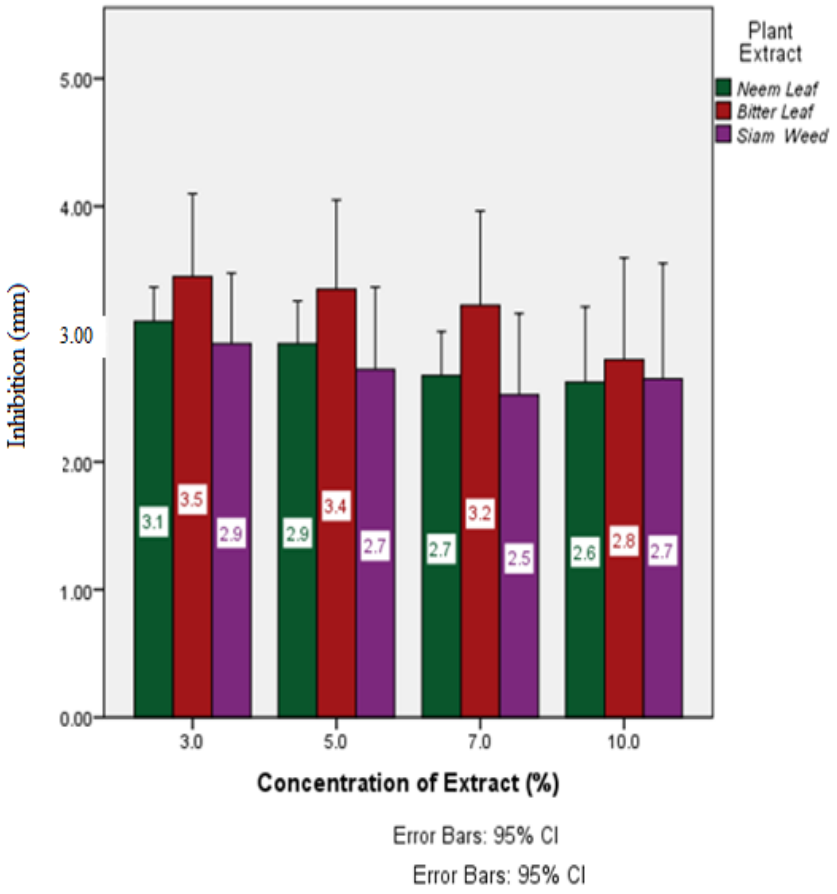

Figure 2: Inhibitory effect of plant extract at various concentrations

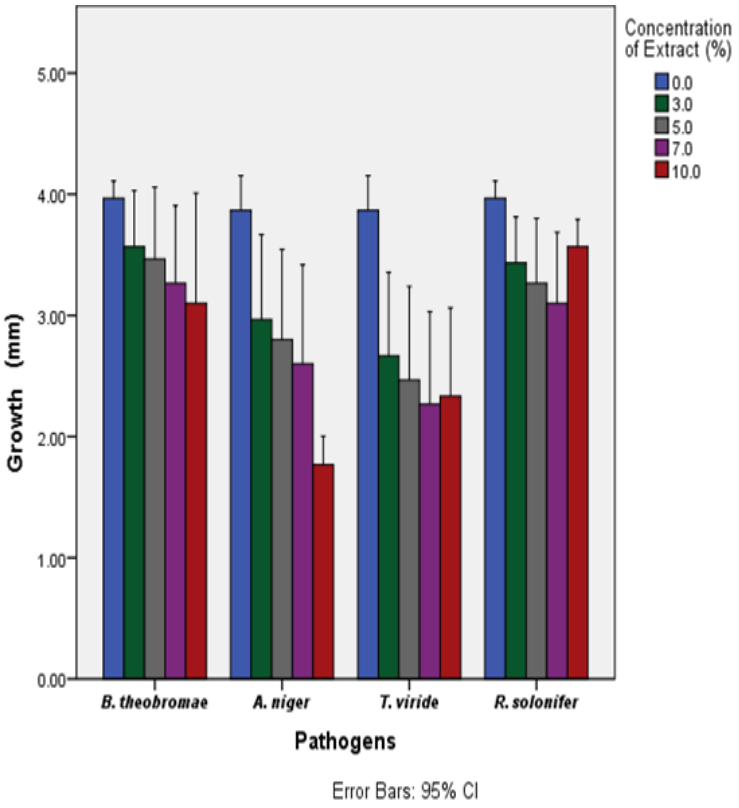

Figure 3: Susceptibility of fungi Isolates in various Concentrations of Aqueous plant extracts

Table 7: Effect of Ethanol Extract of test plants on isolated fungi at 3.0% after 6 days

	Growth of Isolates (mm)*			
Extract	*B. theobromae*	*T. viride*	*A. niger*	*R.stolonifer*
Control	3.97 ±0.058 [a]	2.13 ±1.904[a]	3.63 ±0.231[a]	3.97 ±0.058[a]
A. indica	1.80 ±0.141 [b]	1.20 ±0.001[a]	1.00 ±0.001[c]	1.70 ±0.001[b]
V. amygdalina	-	1.00 ±0.001[a]	2.50 ±0.001[b]	0.80 ±0.001[c]
C. odorata	-	0.20 ±0.001[a]	0.90 ±0.001[c]	0.80 ±0.001[c]
p-value	**	ns	**	**

Results are in mean ± standard deviation
*columns with similar alphabets are not significantly different
**p<0.05, Ns: Not significant
— Complete inhibition

Table 8: Effect of Ethanol Extract of test plants on isolated Fungi at 5.0% after 6 days

	Growth of Isolates (mm)*			
Extract	*B. theobromae*	*T, viride*	*A.niger*	*R. stolonifer*
Control	3.97 ±0.058	2.13 ±1.904[a]	3.63 ±0.231[a]	3.97 ±0.058[a]
A. indica	1.80 ±0.001[b]	1.00 ±0.283[a]	0.90 ±0.001[c]	1.60 ±0.001[b]
V. amygdalina	-	0.90 ±0.001[a]	2.20 ±0.001[b]	0.60 ±0.141[c]
C. odorata,	-	-	0.70 ±0.001[c]	0.60 ±0.001[c]
p-value	**	ns	**	**

Results are in mean ± standard deviation
*columns with similar alphabets are not significantly different
**p<0.05, Ns: Not significant
— Complete inhibition

Table 9: Effect of Ethanol Extract of test plants on isolated fungi at 7.0% after 6 days

	Growth of Isolates (mm) *			
Extract	*B. theobromae*	*T. viride*	*A.niger*	*R. stolonifer*
Control	3.97 ±0.058[a]	2.13 ±1.904[a]	3.63 ±0.231[a]	3.97 ±0.058[a]
A. indica	0.90 ±0.001[c]	0.90 ±0.001[a]	0.70 ±0.001[c]	1.50 ±0.001[b]
V. amygdalina	-	0.50 ±0.001[a]	2.00 ±0.001[b]	0.40 ±0.001[c]
C. odorata,	-	-	0.50 ±0.001[c]	0.40 ±0.001[c]
p-value	**	ns	**	**

Results are in mean ± standard deviation
*columns with similar alphabets are not significantly different **p<0.05, Ns: Not significant
**p<0.05, Ns: Not significant - Complete inhibition

Table 10: Effect of Ethanol Extract of test plants on isolated fungi at 10.0% after 6 days

	Growth of Isolates (mm) *			
Extract	*B. theobromae*	*T. viride*	*A. niger*	*R. stolonifer*
Control	3.97 ±0.09 [a]	2.13 ±1.904 [a]	3.63 ±0.231[a]	3.97 ±0.058[a]
A. indica	-	0.50 ±0.001 [c]	0.50 ±0.001[c]	1.20 ±0.001[b]
V. amygdalina	-	-	1.00 ±0.001[b]	0.20 ±0.001[c]
C. odorata,	-	-	0.20 ±0.001[c]	-
p-value	NA	ns	**	**

Results are in mean ± standard deviation
*columns with similar alphabets are not significantly different
**p<0.05, NA: Not Applicable, Ns: Not significant
— Complete inhibition

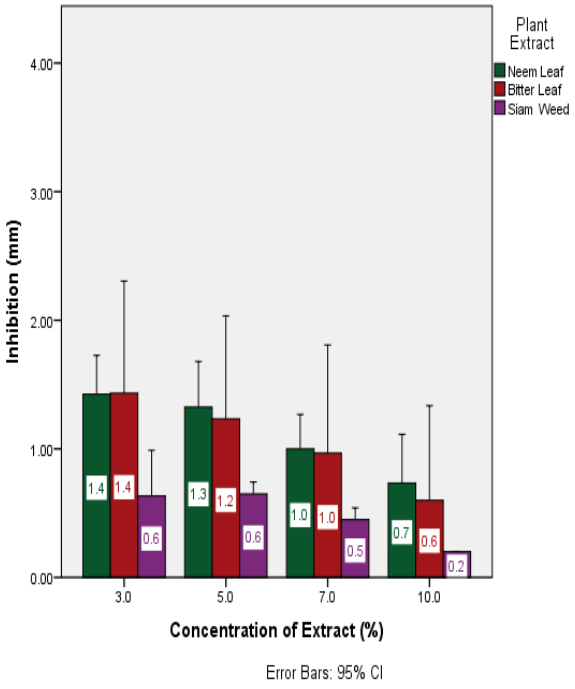

Figure 4: Inhibitory effect of ethanol plant extract at various concentrations

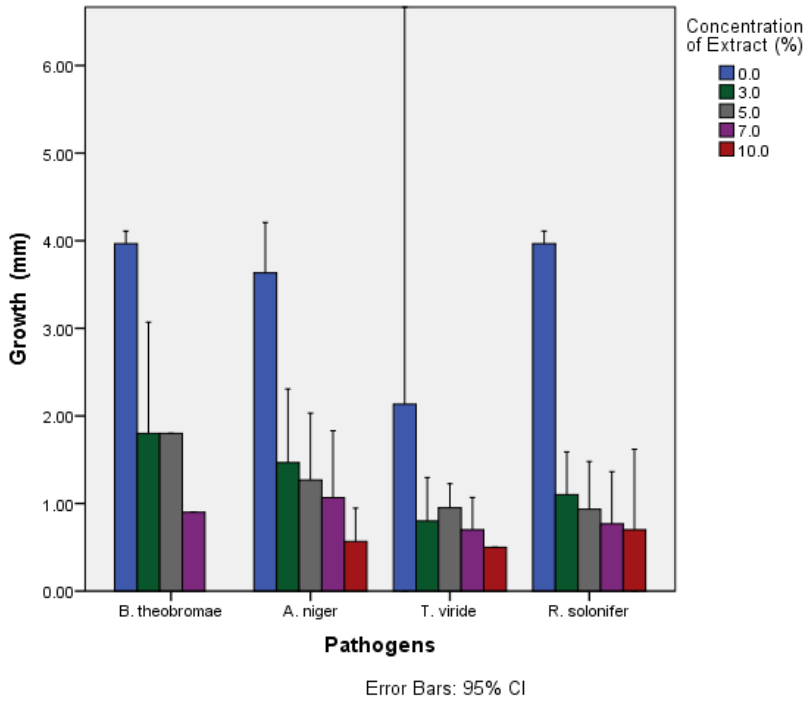

Figure 5: Susceptibility of fungi Isolates in various Concentrations of ethanol plant extracts

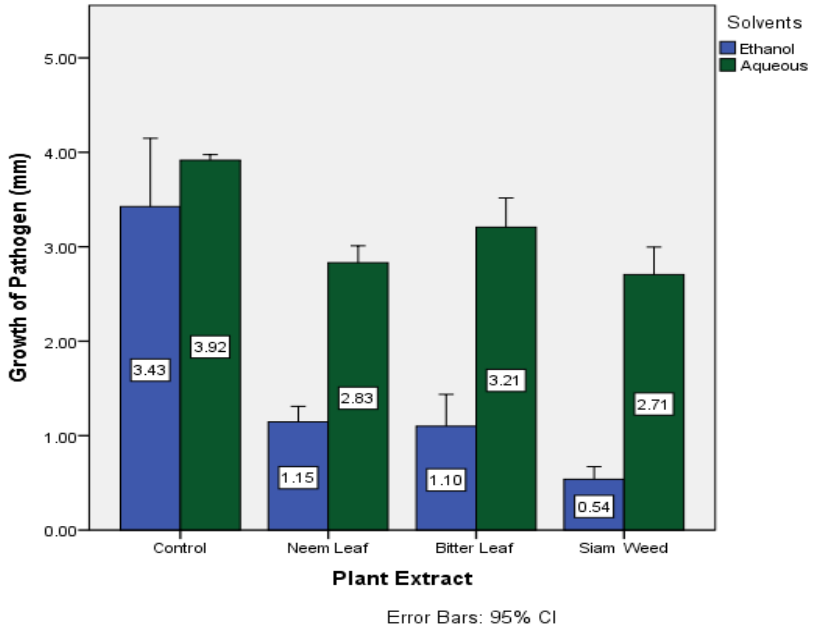

Figure 6: Effect of Ethanol and Aqueous Extract on the Growth of Isolated Fungi

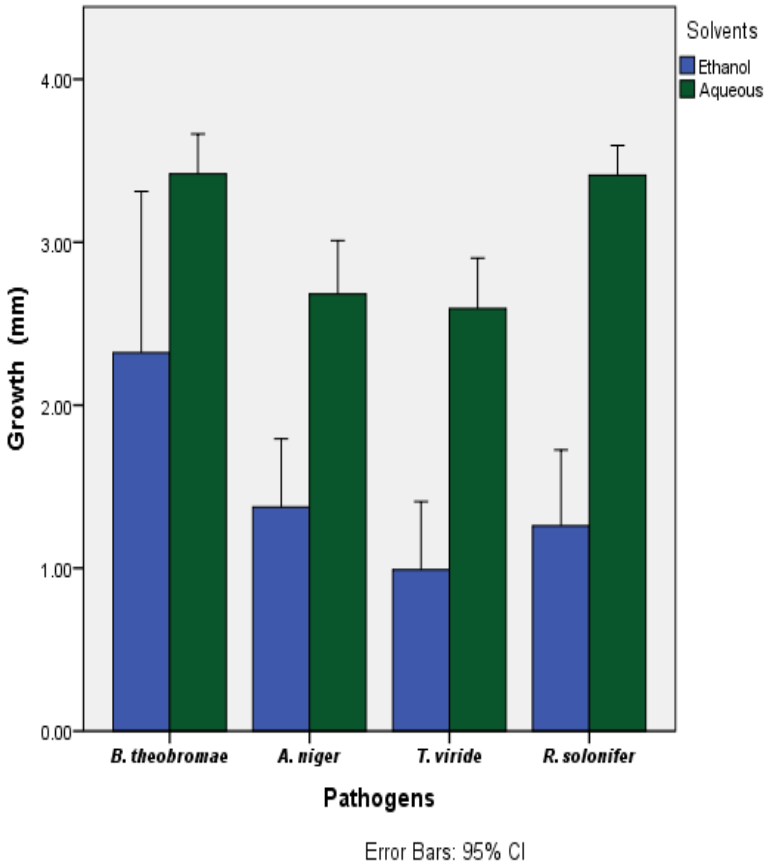

Figure 7: Susceptibility of fungi Isolates in ethanol and aqueous plant extracts

Discussion

Organisms responsible for post-harvest rot of cocoyam cormels in this study were (*Aspergillus niger*, *Botryodiploidia theobromae*, *Rhizopus stolonifer and Trichoderma virides).* These have been reported to cause extensive rot of cocoyam cormels in storage. This result is in agreement with the reports of many workers on other root and tuber crops (Okigbo *et al.,* 2009; Anukwuorji *et al.,* 2012). Investigation on the antifungal properties of these plant extracts on fungal growth showed that plant extracts possess some inhibitory components which caused significant reduction in mycelia growth of the pathogen. This agrees with earlier reports of Udo *et al.,* (2001) on the inhibition of growth and sporulation of fungal pathogens on *Ipomea batatas and Dioscorea sp*, with garlic extract; Okigbo and Nmeka, (2005) on the use of *Xylopia aethiopica and Zingiber officinale* to control yam tuber rot caused by *F. oxysporum, A. niger and A.flavus* , Amienyo *et al.,* (2007) on the use of *Z. officinale, Annona*

muricata, Gacinia cola, Alchorniea cordifolia, Allium sativum to control wet rot on sweet potato caused rot fungal pathogen, Ebele, (2011) on the use of *Carica papaya, Chromolaena odorota and Acalypha ciliate* on the control of pawpaw fruit rot fungi. Tijjani *et al*., (2013) on efficacy of *Azadirachta indica* and *Moringa oleifera* on the control of wet rot disease on mechanically injured sweet potato. Result showed that the effectiveness of the used plants (*Chromolaena odorota, Azadrachta indica and Vernonia. amygdalina*) depended on the extraction medium (Ethanol and aqueous). The ethanol plant extracts were more effective than Aqueous plant extracts, this may be as a result of some active compounds that may be absent in aqueous plant extracts. This is in conformity with the reports of Ekwenye and Elegalam (2005) on garlic who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds (phytochemical) required for antifungal activity; Anukwuorji *et al*., (2012) on *Allium sativum*, *Garcinia kola, Azadirachta indica*, and *Carica papaya* who suggested that water used in the extraction process was probably not able to dissolve all the phytochemical compounds present in the plants.

However, all the test plant extracts at varying concentrations inhibited mycelia growth. The inhibitory effect of the test plants (*Chromolaena odorota, Azadirachta indica and Vernonia amygdalina*) increased with concentration of the extract. The degree of control of cocoyam rot by different plant extracts varied and was significant ($P < 0.05$). This agrees with the report of Suleiman (2010) who stated the significant difference between mycelia growth value recorded on the various concentrations of *Azadirachta indica* and *Carica papaya* extract. The study depicted that, ethanol extract of *Chromolaena odorota, Vernonia amygdalina* could be an alternative to synthetic chemicals in controlling cocoyam rot and when not available, *Azadirachta indica* could be an alternative.

Conclusion

In conclusion, this study has shown that the test plant extracts (*Chromolaena odorota, Azadirachta indica and Vernonia amygdalina*), have the potentials in the protection of cocoyam corms and cormels against postharvest rot. Therefore, the use of chemical in the control of plant disease may result to food poisoning thereby reducing the quality and quantity of cocoyam production because most farmers have little or no knowledge of chemical application and this could affect the crops. The challenges of cocoyam as a result of post-harvest rot is readily obvious and as such requires urgent

attention if cocoyam, the third ranked root and tuber crop of economic importance after yam and cassava in Nigeria must not decline.

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