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Full Length Research Paper

Isolation of fungi causing rot of cocoyam (*colocasia esculenta* (l.) schott) and control with plant extracts: (*allium sativum*, l., *garcinia kola*, heckel., *azadirachta indica*, l. and *carica papaya*, l.)

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Antifungal effect of aqueous and ethanol extracts of *Allium sativum, Azadirachta indica, Carica papaya and Garcinia kola* on the growth of fungal pathogen of stored cocoyam cormels were investigated invitro. Four different extract concentrations were obtained from each plant parts used by blending 25g, 50g, 75g and 100g in 100ml of sterile distilled water (SDW). Phytochemical screening of the plants was conducted using different standard methods; this revealed the presence of alkaloid, saponin, tannins, flavonoid, phytate, oxalate and phenol in all the plants but at different concentrations. Effect of standard antibiotics (Grisovid) comparative to the plant extracts was determined. Pathogenicity test revealed that *Aspergillus niger, Fusarium solani, Sclerotia rolfsii* and *Botryodiplodia theobromae* induced rot in healthy cocoyam cormels after 6 days of inoculation with *Botryodiplodia theobromae* being the most virulent. Even though all the extracts showed varying degrees of antifungal efficacy, ethanol extract proved to be more potent. The efficacy of the extract varied with the solvent of extraction, extract concentration and the test pathogens. Inhibition of fungal growth increased with a corresponding increase in extract concentration. *Allium sativum and Azadirachta indica* depicted an effective/high rate of inhibition on the mycelia growth of all the test fungi ranging from 40.57% to 92.40% whereas, extract of *Carica papaya* and *Garcinia kola* showed a lower inhibition rate ranging from 0.0% to 60.17%. 10% extract concentration of *Allium sativum* and *Azadirachta indica* being the most fungitoxic 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/cormels recommends their use to farmers as alternative to commercial / synthetic fungicides.

Key words: Fungi, Isolation, Antifungal effect, Cocoyam.

INTRODUCTION

Cocoyam (*Colocasia esculenta* Lin) of Araceae family, is a perennial monocotyledonous herb, it grows to a height of 1-2 metres, the plant consist of central corn (lying just below the soil surface) from which leaves grow upward, roots grow downwards, while cormels, daughter corms and runners (stolons) grows laterally, the root systems is fibrous and lies mainly in the top one metre of soil (Onwueme, 1978). *Colocasia esculenta* is known as food crop which provides high yield of roots (or corms) and foliage, it is a tropical food crop that can be grown under flooded or upland conditions (Chayty *etal*., 2007).

However, various lines of ethnobotanical evidence suggest that *Colocasia esculenta* originated from south central Asia, probably in India of the Malay peninsula (Kolchaar, 2006). Wild forms occurs in various parts of south eastern Asia (Purseglove, 1972), hence south

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east Asia is said to be an important region for ethnobotanical and genetic diversity of *Colocasia esculenta* Lin . From its centre of origin, it spread east ward to the rest of South –East Asia and to China, Japan and the Pacific Islands. From Asia it spread west ward to Arabia and the Mediterranean region. It arrived on the east coast of Africa over 2,000 years ago. It was taken by voyagers, first across the continent of Africa, and later on slave trade to the Caribbean. Today *Colocasia esculenta* lin is pan-tropical in its distribution and cultivation. The largest area of cultivation is in West Africa, which therefore account for the greatest quantity of production. Significant quantities of taro are also grown in the Caribbean and virtually in all humid and sub-humid parts of Asia (Purseglove, 1972).

More so, *Colocasia esculenta* lin is grown in about 30 countries, either in flooded wetland or in uplands. The bulk of its production is in Africa (spore 2003). The need to achieve food security in Nigeria has generated increased interest in research, production and consumption of cocoyam. According to FAO (2006) Nigeria is the largest producer of cocoyam in the world, accounting for about 37% of the world's output estimated to annual production of 5.49 million metric tones, followed by Ghana which producers 31%, conversely Eze and Okorji (2003) documented that Nigeria accounted for about 40% of total world's output of cocoyam, thus from the evidence, the cultivation of cocoyam in Nigeria is declining (Onyenweaku and Eze, 1987; Zuhair and Hunter, 2000). Production of cocoyam has not been given priority attention in many countries, probably because of its inability to earn foreign exchange, as well as its unacceptability by the high income countries for both consumption and other purposes (Onyenweaku and Eze, 1987). However, 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 declining yields, low storability and the socio – cultural perception of the crop as women's crop, as women do not have control over land, labour and capital in some parts especially South –eastern Nigeria (Coursey, 1984; Spore, 2003), this is worsened by the devastating disease, cocoyam root rot blight complex (CRRBC) which is a major threat to cocoyam production.

Post-harvest loss of root and tuber crops has been a very serious problem to farmers as more than 40% of their harvest may be lost because of decay (Olurinola *et al*., 1992). Studies have shown that fungal rot is the greatest cause of root and tuber loss in storage (IITA, 1985). The principal species of microorganisms associated with cocoyam rot in Nigeria include *Aspergillus flavus, Penicillium digitatum, Botryodiplodia theobromae, Sclerotia rolfsii, Fusarium solani and Erwinia carotovora,* these fungi were reported to be pathogenic to four cultivars of *Colocasia esculenta*, causing rot of cocoyam in several parts of southern Nigeria (Onuegbu, 1999).

The use of chemicals has helped in control of rot but due to the identifiable problems (eg. chemical residues, biodegradation, phytotoxicity, pollution, development of resistance in target organism, high cost, atimes non availability and hazard to man and his environment) renders them either slow to adopt by farmers or farmers have totally failed to adopt them, for one cultural reasons or the other (Okigbo and Odurukwe,2009), hence alternative control methods are employed. Presently considerable efforts are directed at exploring the potentials of botanicals (plant extracts) as alternatives or complimentary to synthetic chemicals. Botanicals have the advantage of not only being readily available and affordable but are also sources of non-phytotoxic and easily biodegradable alternative fungicides and antibiotics, hence environment friendly (Akuesh *et al*., 2002; Okigbo and Nmeka, 2005; Okigbo and Omodamiro, 2006).

More so, many plants are extensively used locally in traditional medicine for the treatment and control of disease (Okigbo and Igwe, 2007), these plants posses effective broad – spectrum antifungal activities in laboratory studies over the years (Wee yeow Chin, 1992). Their effectiveness has been confirmed by modern scientific studies. Among such plants are *Carica papaya* (L.) of the family Caricaceae, it is a small unbranched tree, about 5-10m tall with edible fruits, it originated from Southern Mexico, but it is now cultivated in most tropical countries, it is highly frost sensitive, limiting its production to tropical lands. However *Carica papaya* is used as food and in medicine. It's main biological active agent (papain) is also applied topically for the treatment of cuts, rashes, stings and burns.

Allium sativum Linn of the family Alliaceae is an aromatic perennial herb found in tropical and temperate regions, it contains allicin as the main biological active compound, allicin has been reported to have anti-microbial properties (Stephen, 2005). In invitro studies, *Allium sativum* has been found to have antibacterial, antiviral and antifungal activity, it is also claimed to prevent heart diseases and cancer.

Neem (*Azadirachta indica* A Juss) is a tree in the Mahogany family Meliance. It is one of two species in the genus Azadirachta, it is a common tropical tree widely distributed in Africa and Asia but it is a native of Bangladesh, India, Myanmar and Pakistan, growing in tropical and semi-tropical regions. Other vernacular names include: Azad Dirkht (Persian), Dogon yaro (Nigerian), Neeb (Arabic). In East Africa it is also known as Mwarobaini (Kiswahili), which means the

trees of the 40, it is said to treat 40 different diseases. However, *Azadirachta indica* A Juss has been under intensive study for the past decades (Schmutterer *et al*., 1981). Its medicinal uses have been known for several countries, fungicidal properties of neem seed has been recorded to significantly reduce conidial germination in several fungi (Lal *et al*., 1980). Neem can reach a height of 15-20m, rarely to 35-40m. It is ever green, but under sever drought it may shed most or nearly all of its leaves. The branches are widespread. The fairly dense crown is roundish or oval and may reach the diameter of 15-20m in old (Ganguli, 2002).

Garcinia kola, commonly called bitter kola belongs to the family Cluisiaceae formerly Guttiferae, It is mostly in central and Western Africa. It is a non timber forest tree that is mostly utilized in Africa (Adebisi, 2004). Virtually all the parts can be used for medicinal purposes, the sap from *Garcinia kola* is used for the treatment of parasitic skin diseases, while the latex is orally ingested for the treatment of gonorrhea. It is also useful in the eradication of guinea worm infestation (Ofakansi *et al.,* 2008). The pharmacological and medicinal values of *Garcinia kola* are numerous, hence because of its high interest resulting in its over exploitation, *Garcinia kola* is extinction- threatened in several west African and central Africa countries (Eyog-Matig *et al*., 2007).

However, the benefits of these natural plant products over synthetic chemicals which cannot be over emphasized necessitated the need for undertaking this study to ascertain the antimicrobial potentials / efficacy of these plant extracts at different concentrations under different extraction medium in controlling post harvest rot of *Colocasia esculenta* (cocoyam).

MATERIALS AND METHODS

Sources of Plant Materials.

Cocoyam (*Colocasia esculenta* linn) cormels with symptoms of post harvest rot were obtained from cocoyam barn of National Root Crops Research Institute Umudike. Fresh healthy Cocoyam cormels were also collected from the same barn. Based on previous biological activities, leaves of *A. indica*, *C. papaya,* bulbs of *A. sativum* and *G. kola* seeds were used. Cloves of *Allium sativum* Linn, and *Garcinia kola* seeds were purchased from umuahia main market, while fully expanded leaves of *Carica papaya* and *Azadiratcha indica* were collected along the road side of Umudike. The botanical identities of the plants were authenticated by the Horticuture Unit of National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria.

Isolation of Fungal Pathogens from Rotten Cocoyam Cormels.

The dishes were inoculated with cocoyam samples by cutting sections of approximately 2mm cubes from the tissue at the junction between healthy and infested portion of the cocoyam cormels with surface sterilized blade (Forcep). They were surface sterilized (to remove surface contaminants) in 70% ethanol and then rinsed twice (one minute each wash) in sterile distilled water (SDW) (Ritchie, 1991). The cormel piece were placed on sterile paper towels in a Laminar Air flow Hood chamber for 10 minutes to dry and then placed on to PDA. The plates were incubated at 27^0C for four days and then examined daily for the development of fungi growth.

Subculturing /Purification and Identification of Test Fungi Pathogens.

When growth has established, subcultures were prepared using inocula from the different organisms in the mixed cultures to obtain a pure culture, this was done by transferring hyphal tips from the colony edge of the mixed cultures to fresh plates of PDA using flame sterilized blades. After sub-culturing the plates were incubated at 27^0C until pure cultures were obtained. The Petri dishes of pure cultures of the test fungi were then sealed with parafin to prevent 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.

This test was carried out using four test organisms (*Sclerotia rolfsii, Aspergillus niger, Fusarium solani and Botryodiplodia theobromae*) from the rotten samples. Fresh health 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 12minutes in a Laminar Air flow hood. Sterile cork borer (5mm diameter) was used to bore holes in the cocoyam cormels. The parts of the cormels which were bored out at each point were kept in sterile petri dishes.

An agar block measuring 4mm by 4mm from growing cultures of each test isolates (pure cultures) was inoculated into the hole made with the aid of another

cork borer (4mm diameter), after the inoculation the parts of the cormel bore out were carefully replaced and sealed with sterile vaseline to prevent contamination and labeled accordingly. A control experiment which bore no isolate was also set-up (inoculated with 1ml of sterile distilled water).

After inoculating the entire test isolates into their respective healthy cormels, all the cormels were incubated for 6 days in a humidity chamber. The cormels were examined daily for evidence of rot such as softening, discoloration and offensive odour. At the end of the 6 days 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. Where positive the length and girth of the rot area and those of the entire cormels as shown were measured and recorded.

Preparation of Plant Extracts

The seeds of *Garcinia kola*, bulbs of *Allium sativum* and fresh leaves of *Carica papaya* and *Azadiratcha indica* (Neem) were thoroughly washed with tap water and then with sterile distilled water (SDW) and were sun dried for 5 days, at a point they were dry enough for milling. The dried samples were separately grinded in a laboratory Mill (Thomas, Wiley, model ED-5 made in USA) after which the grinded samples were sieved to obtain powdered processed sample used for the extraction. Using cold solvent extraction method (Harbone, 1973; Junaid *et al*., 2006; Doughari *et al*., 2007). 25g, 50g, 75g and 100g portion of each processed sample were mixed with 100ml of each solvent (Aqueous and ethanol) separately in a bottle to produce 25%, 50%, 75% and 100% extract concentrations respectively. The extracts were sieved through four layers of sterile cheese cloth and stored in sterile cornical flask which was later used for mycelia growth inhibition.

Test for purity

Each of the extracts obtained was tested to ensure its purity by streaking it separately onto sterile plates of the test media. The plates were incubated at 37^0C for 24hours (cheesbrough, 2000) and was examined for possible growth of contaminants. The absence of which confirms the purity of the test extracts.

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 (Sangoyomi, 2004). One milliliter of each plant extract concentrations (25%, 50%, 75% and 100%) was dispensed per petri dishes 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 2.5%, 5.0% 7.5% 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 consist of three replicates. The negative control set up consist of blank agar plate (no extract) inoculated with the test fungi as described above. Petri-dishes dispensed with molten PDA and one ml of grisovid solution (0.5g in 100ml of sterilized distilled water) inoculated with each test fungus served as the commercial fungicides.

All the plates were incubated at 28 ± 2^0C for 5days and examined daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two direction on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Whips (1987).

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_2} \times \frac{100}{I}$$

Where R_1 is the farthest radial distance of Pathogen in control plate while R_2 is the farthest radial distance of Pathogen in extract incorporated agar plates.

Quantitative Photochemical Screening

To ascertain the presence of phytochemical of interest in the plant extract, quantitative and qualitative tests were conducted using different standard methods, the presence of Phytate, Flavonoid, Alkaloid, Saponin, Tannins, Oxalate and Phenol was determined.

Experimental Design

The experimental design used was 6 x 4 factorial laid in a complete Randomized Design (CRD). The data collected were subjected to analysis of variance (ANOVA) and means were separated using Least significant difference (LSD) at 0.05 probability level.

RESULT

Table 1: Frequency of Occurrence (%) of Isolated Fungi on Rotten Cocoyam Samples

Fungal Isolates	% Occurrence
Botryodiplodia theobromae	20.7
Aspergillus niger	13.8
Aspergillus flavus	6.9
Fusarium solani	17.2
Sclerotia rolfsii	27.6
Rhizopus stoloifer	10.3
Penicillium spp	3.4

Table 2: Pathogenicity Test/Mean Percentage of Rot by Test Isolates on Healthy Cocoyam Cormels.

Isolates/Inoculated Fungus	Percentage Rot
Sclerotia rolfsii	56
Botryodiplodia theobromae	80
Fusarium solani	20
Aspergillus niger	71

Occurrence of Fungal Pathogens Isolated from Samples of Cocoyam Cormels.

The fungi pathogens that were constantly isolated from the rot-infested tissues of the cocoyam cormels includes *Scle*rotia *rolfsii, Botryodiplodia theobromae, Aspergillus niger, Fusarium solani, Aspergillus flavus, Rhizopus stolonifer and Penicillium digitatum.* The frequency of occurrence varied with different fungi associated with the rotten cocoyam cormels. The most frequently occurred were *Sclerotia rolfsii, Botryodiplodia theobromae, Fusarium solani and Aspergillus niger* with 27.6, 20.7, 17.2 and 13.8 percentages of occurrence respectively, while others had lower frequencies of occurrence from 3.4 to 10 3% (Table 1).

Pathogenicity Test

The pathogenicity test showed that all the four test fungi (*Sclerotia rolfsii*, *Botryodiplodia theobromae*, *Fusarium solani* and *Aspergillus niger*) were pathogenic, hence causes rot in healthy cocoyam cormels after six (6) days of inoculation. The most virulent among the four test fungi was *Botryodiplodia theobromae,* with rot incidence of 80%, followed by *Aspergillus* niger (71%) while the least virulent was *Fusarium solani* with rot incidence of 20% (Table 2).

Effect of Extracts, Extraction Medium and Grisovid on the Mycelia Growth of the Four Test Fungi.

The effect of concentrations of extracts on the test organisms was significant ($P < 0.05$). Colony diameter of the inhibition increased as the concentration of the extract increased as follows (25%> 5.0%> 7.5%> 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 (*Aspergillus niger, Botryodiplodia theobromae, Fusarium solani and Sclerotia rolfsii).*

Aqeous extracts of *Allium sativum* gave the highest inhibitory effect of *Aspergillus niger* by 69.51%, followed by *Azadirachta indica* with (62.84%), while *Carica papaya* and *Garcinia kola* showed the least inhibition of 23.74% and 24.85% respectively (Table.3). The inhibitory effect of *Allium sativum* was significantly ($P< 0.05$) higher than that of other extracts, while the inhibitory effect of *Azadirachta indica* was significantly greater than that of *Carica papaya* and *Garcinia kola*. The inhibitory effect of *Carica papaya* and *Garcinia kola* did not show any significant difference at ($P>0.05$), mean while grisovid showed 100% inhibition on the growth of the fungus, whereas the control did not show any inhibition. For plant extract concentration, there is significant ($P<0.05$) difference between all the levels/rates .10% showed the highest inhibitory effect of (62.97%), followed by 7.5% which gave (50.34%), the least inhibition was recorded by 5.0% and 2.5% which gave inhibition percentages of 41.25% and 26.12% respectively, however, there was significant ($P<0.05$) different among all the values recorded by the different concentrations (Table 3.) .For their interactions *Allium sativum* at 10% extract

Table 3: Effect of Grisovid and Plant Extract Extracted with Water on the Inhibition of *Aspergillus niger* 7 days After Inoculation

Extract, Grisovid and Control	Conc. (%) 0	0.5	2.5	5.0	7.5	10	$\bar{x}$ botanical
Water	0.00						0.00
C. papaya			0.90	22.50	27.43	44.13	23.74
G. kola			0.00	21.13	35.97	42.30	24.85
A. sativum			55.83	63.10	72.93	86.17	69.51
A. indica			47.73	59.33	65.03	79.27	62.84
Grisovid		100					100.00
$\bar{x}$ Conc.	0.00	100.00	26.12	41.52	50.34	62.97	

$LSD_{0.05}$ for comparing the means of botanicals = 2.247
$LSD_{0.05}$ for comparing the means of concentration = 2.247
$LSD_{0.05}$ for comparing the means of botanical x concentration interaction mean = 4.493

Table 4: Effect of Grisovid and Plant Extract Extracted with Ethanol on the Inhibition of *Aspergillus niger* 7 days After Inoculation

Extract, Grisovid And Control	Conc. (%) 0	0.5	2.5	5.0	7.5	10	$\bar{x}$ botanical
Water	0.00						0.00
C. papaya			7.03	26.00	32.13	44.70	27.47
G. kola			7.03	29.80	36.77	42.03	28.91
A. sativum			55.23	70.17	78.83	90.33	73.64
A. indica			47.37	66.67	77.07	85.07	69.04
Grisovid		84.22					84.22
$\bar{x}$ Conc.	0.00	84.22	29.17	48.16	56.20	65.53	

$LSD_{0.05}$ for comparing the means of botanicals = 3.890
$LSD_{0.05}$ for comparing the means of concentration = 3.890
$LSD_{0.05}$ for comparing the means of botanical x concentration interaction mean = 7.780

concentration gave the highest inhibitory effect of (92.40%), this was significantly (P<0.05) different from the inhibitory effect of (83.07%) recoded by *Azadiratcha indica* at 10.0% extract concentration, it was also significantly (P<0.05) greater than the inhibitory effect shown by *Carica papaya* and *Garcinia kola* at all concentrations (Table 3).

The ethanol extract of *Allium sativum* gave the highest inhibition on *Aspergillus niger* (72.43%), which is significantly (P<0.05) greater than 59.42% recorded by *A. indica*. *Carica papaya* and *Garcinia kola* which gave an inhibition percentages of 25.43% and 27.58% did not shown any significant difference among themselves but were significantly (p<0.05) lower than *A. indica* and *A. sativum,* meanwhile, grisovid showed effective inhibition of (84.22%) on the growth of the fungus (Table, 4) .For concentration, 10.0% gave the highest inhibitory effect of (62.42%) and this is significantly (P<0.05) different from (51.28%) recorded by 7.5% concentration, the least inhibitory effect was observed in 5.0% and 2.5% extract concentration which gave inhibition percentages of (48.16% and 29.17%) .For their interactions *Allium sativum* and *Azadiratcha indica* at 10% extract concentration each

Table 5: Effect of Grisovid and Plant Extract Extracted with Aqueous on the Inhibition of *Botryodiplodia theobromae* 7 days After Inoculation

Extract, Grisovid and Control	Conc. (%) 0	0.5	2.5	5.0	7.5	10	$\bar{x}$ botanical
Water	0.00						0.00
C. papaya			4.26	20.53	34.17	42.77	25.43
G. kola			7.70	21.40	36.77	44.47	27.58
A. sativum			59.80	69.23	74.37	86.33	72.43
A. indica			48.73	53.00	59.83	76.10	59.42
Grisovid		79.46					79.46
$\bar{x}$ Conc.	0.00	79.46	30.12	41.04	51.28	62.42	

$LSD_{0.05}$ for comparing the means of botanicals = 6.054
$LSD_{0.05}$ for comparing the means of concentration = 6.054
$LSD_{0.05}$ for comparing the means of botanical x concentration interaction mean = 12.107

gave inhibition of 90.33% and 85.07% which were significantly (P<0.05) greater than other interactions, the least inhibition was observed in *Carica papaya* and *Garcinia kola* at 2.5% extract concentration each, they gave inhibition of (7.03%) each which was significantly (P<0.05) lower than other interactions. The inhibitory effect of other interactions ranges from (26.00% to 78.83%).

The aqueous extracts of *Allium sativum* had the highest inhibitory effect on *Botryodiplodia theobromae* by (72.43%), followed by *Azadirachta indica* (59.42%) and the least were *Carica Papaya* (25.43%) and *Garcinia kola* (27.5%) (Table 5). The inhibitory effect of *Allium sativum* was significantly (P<0.05) greater than that of *Azadirachta indica,* which were on the same hand significantly (P<0.05) greater than the inhibition showed by *Carica papaya* and *Garcinia kola.* The control showed an uninhibited growth of the fungus while Grisovid showed an effective inhibition of 79.46% but not significantly (P<0.05) higher than *Allium sativum* .10% extract concentration gave the highest inhibitory effect of (62.42%), followed by 7.5% which gave an inhibition percentage of (51.28%), they were significantly (P<0.05) different form each other and also significantly (P<0.05) greater than (41.04% and 30.12%) recorded by 5.0% and 2.5% extract concentration respectively. For their interaction *Allium sativum* at 10% gave the highest inhibition of (86.33%) which is significantly (P<0.05) greater than other interactions with the exception of *Allium sativum* at 7.5% and *A. indica* at 10% extract concentration which gave (74.37% and 76.10%) respectively. The least inhibitory effect was observed in *Carica papaya* and *Garcinia kola* at 2.5% extract concentration each whereas, the inhibitory effect of other interactions ranges from (20.53% to 69.23%) (Table.5).

The ethanol extracts of *Allium sativum* with percentage inhibition of (77.53%) had the highest inhibitory effect on *Botryodiplodia theobromae*, followed by *A. indica* (68.91%), the least inhibitory effect was observed in *Carica papaya* and *Garcinia kola* which gave percentage inhibition of (26.64% and 27.48%) respectively, hence not significantly different from each other. Grisovid showed an effective inhibitory effect of (94.10%), while the negative control showed uninhibited grow of *Botryodiplodia theobromae* (Table 6). For extract concentration, 10% had the highest inhibitory effect of (67.09%) on *Botryodiplodia theobromae* and this was significantly different from (53.77%) recorded by 7.5% extract concentration, with the least being (44.87% and 34.83%) observed in 5.0% and 2.5% extract concentration respectively. For their interaction, *Allium sativum* at 10.0% extract concentration exhibited the highest inhibitory effect of (92.40%) which was significantly greater than other interactions, followed by *A indica* at 10% (83.07%) though not significantly (P<0.05) greater than A. *sativum* at 7.5% extract concentration which gave (77.10%) inhibition *Carica Papaya* and *Garcinia kola* at 2.5% extract concentration each showed the least inhibitory effect of (8.40% and 6.95%) respectively, which are significantly (P<0.05) smaller than other interactions. The inhibitory effect of other interactions ranged from (21.13% to 75.30%) (table 6).

Aqueous extract of *Allium sativum* with inhibition of (61.56%) had the highest inhibitory effect on *Fusarium*

Table 6: Effect of Grisovid and Plan Extract Extracted with ethanol on the Inhibition of *Botryodiplodia theobromae* 7 days After Inoculation

Extract, Grisovid and Control	Conc. (%) 0	0.5	2.5	5.0	7.5	10	$\bar{x}$ botanical
Water	0.00						0.00
C. papaya			8.40	21.13	34.70	42.33	26.64
G. kola			6.95	21.13	31.27	50.57	27.48
A. sativum			65.30	75.30	77.10	92.40	77.53
A. indica			58.67	61.90	72.00	83.07	68.91
Grisovid		94.10					
$\bar{x}$ Conc.	0.00	94.10	34.83	44.87	53.77	67.09	

$LSD_{0.05}$ for comparing the means of botanicals = 3.871
$LSD_{0.05}$ for comparing the means of concentration = 3.871
$LSD_{0.05}$ for comparing the means of botanical x concentration interaction mean = 7.741

Table 7: Effect of Grisovid and Plant Extract, Extracted with Aqueous on the Inhibition of *Fusarium solani* 7 days After Inoculation

Extract, Grisovid and Control	Conc. (%) 0	0.5	2.5	5.0	7.5	10	$\bar{x}$ botanical
Water	0.00						0.00
C. papaya			18.83	35.90	47.93	49.57	38.06
G. kola			12.80	27.33	35.07	37.60	28.20
A. sativum			46.17	56.40	65.83	75.20	61.56
A. indica			44.46	49.57	67.53	77.80	60.90
Grisovid		92.30					92.30
$\bar{x}$ Conc.		92.30	30.57	44.02	54.09	60.04	

$LSD_{0.05}$ for comparing the means of botanicals = 2.413
$LSD_{0.05}$ for comparing the means of concentration = 2.413
$LSD_{0.05}$ for comparing the means of botanical x concentration interaction mean = 4.827

solani although not significantly (P>0.05) different from (60.90%) exhibited by *A.indica.* *Carica papaya* and *Garcinia kola* showed a significantly (P<0.05) smaller inhibitory effect of (38.06% and 28.20%) respectively than *A. indica* and *A.sativum*. Grisovid had an inhibitory effect of (92.30%), whereas the negative control did not show any inhibition on the growth of *Fusarium solani* (Table 7) .Concentrations of 10% gave the highest inhibition of (60.04%) which was significantly (P<0.05) greater than other concentrations, whereas the least inhibitory effect was observed in 2.5% extract concentration (30.5%) also the effect of 5.0% and 7.5% extract concentration which gave an inhibitory effect of (44.02% and 54.09%) was significant (P<0.05) (table 7). For their interactions, *Allium sativum and Azadiratcha indica* at 10% extract concentration each exhibited the highest inhibitory effect of (75.20% and 77.80%) respectively, this is significantly (P<0.05) higher than *A. sativum and A. indica* at 7.5% extract conc. which gave (65.83%

Table 8: Effect of Grisovid and Plant Extract Extracted with Ethanol on the Inhibition of *Fusarium salani* 7 days After Inoculation

Extract, Grisovid and Control	Conc. (%) 0	0.5	2.5	5.0	7.5	10	$\bar{x}$ botanical
Water	0.00						0.00
C. papaya			27.10	44.10	51.50	61.00	45.93
G. kola			20.27	32.93	42.53	60.17	38.98
A. sativum			40.57	56.77	65.30	78.83	60.37
A. indica			49.90	60.17	73.57	79.63	65.82
Grisovid		80.47					80.47
$\bar{x}$ Conc.	0.00	80.47	34.46	48.49	58.23	69.91	

$LSD_{0.05}$ for comparing the means of botanicals = 2.852
$LSD_{0.05}$ for comparing the means of concentration = 2.852
$LSD_{0.05}$ for comparing the means of botanical x concentration interaction mean = 5.703

Table 9: Effect of Grisovid and Plant Extract, Extracted with Aqueous on the Inhibition of *Sclerotia rolfsii* 7 days After Inoculation

Extract, Grisovid and Control	Conc. (%) 0	0.5	2.5	5.0	7.5	10	$\bar{x}$ botanical
Water	0.00						0.00
C. papaya			5.13	17.97	39.33	52.13	28.64
G. kola			2.53	23.87	32.47	46.16	26.26
A. sativum			55.57	62.40	67.50	77.77	65.81
A. indica			46.17	53.83	64.93	70.10	58.76
Grisovid		98.28					98.28
$\bar{x}$ Conc.	0.00	98.28	27.35	39.52	51.06	61.54	

$LSD_{0.05}$ for comparing the means of botanicals = 3.067
$LSD_{0.05}$ for comparing the means of concentration = 3.067
$LSD_{0.05}$ for comparing the means of botanical x concentration interaction mean = 6.134

and 67.53%) respectively, this is on the same hand significantly ($P \leq 0.05$) different from other interactions. The least inhibitory effect of (12.80% and 18.83%) was observed in *Carica papaya* and *Garcinia Kola* respectively at 2.5% extract conc. the inhibitory effect of other interactions ranged from 27.33% to 56.40%) (Table7).

The Ethanol extract of *A. india* had the highest inhibitory effect on *Fusarium solani* by (65.83%) followed by A.*sativum* with percentage inhibition of (60.37%) the least inhibitory effect was recorded by *Carica papaya* and *G. kola* which gave inhibition percentages of (45.93% and 38.98%) respectively. Grisovid showed (80.47%) inhibition on the growth of the fungus (table 8). 10% extract concentration gave the highest inhibitory effect of (69.91%) which was significantly ($P<0.05$) greater than (58.23%) recorded by 7.5% extract concentration. 2.5% and 5.0% extract concentration were their least effective, they gave percentage inhibition of (48.49% and 34.46%) respectively, which were significantly ($P<0.05$) different from other levels of concentration. For the interaction, *A. sativum* and *A. indica* at 10% extract conc. gave the highest inhibitory effect of (78.83% and 79.63%)

Table 10: Effect of Grisovid and Plant Extract Extracted with Ethanol on the Inhibition of *Sclerotia rolfsii* 7 days After Inoculation

Extract, Grisovid and Control	Conc. (%) 0	0.5	2.5	5.0	7.5	10	$\bar{x}$ botanical
Water	0.00						0.00
C. papaya			15.37	33.97	43.73	58.97	38.01
G. kola			14.43	29.00	35.06	52.20	32.68
A. sativum			55.43	65.80	67.53	79.30	67.02
A. indica			51.27	62.40	68.30	82.90	66.22
Grisovid		99.16					99.16
$\bar{x}$ Conc.	0.00	99.16	34.13	47.79	53.66	68.34	

$LSD_{0.05}$ for comparing the means of botanicals = 2.713
$LSD_{0.05}$ for comparing the means of concentration = 2.713
$LSD_{0.05}$ for comparing the means of botanical x concentration interaction mean = 5.426

respectively although *A. indica* at 10% extract conc was not significantly (P>0.05) different from *A. india* at 7.5% extract conc. *Carica papaya* and *Garcinia kola* at 5.0% extract conc. showed the least inhibitory effect of (27.10%, 20.27% and 32.93%) respectively, they were significantly (P>0.05) smaller than other interactions. The inhibitory effect of other interaction ranged from (40.57% to 65.30%) (Table 8).

Aqueous extract of *A. sativum* gave the highest inhibitory effect of *Sclerotia rolfsii* of (65.81%), followed by *A. indica* with (58.76%), while *C.papaya* and G.*kola* showed the least inhibition of (28.64% and 26.26%) respectively. The inhibitory effect of *A. sativum* was significantly (P<0.05) different from that of other extracts, while *A. indica* was significantly (P<0.05) greater than that of *Carica papaya* and *G. kola.* The inhibitory effect of *Carica papaya* and *G.kola* did not show any significance (P>0.05) difference, meanwhile, grisovid showed an effective inhibitory effect of (98.28%) on the growth of the fungus, while control did not show any inhibition. For plant extract concentration, there is a significant (P<0.05) difference across all the levels concentration, there is a significant (P<0.05) difference across al the levels. 10% showed the highest inhibitory effect of (61. 54%) followed by 7.5% which gave (51.06%), the least inhibitory effect was recorded by 5.0% an 2.5% extract conc. Which gave (39.52% and 27.35%) respectively. For their interactions, *A. sativum* at 10% extract conc. gave the highest inhibitory effect of (77.77%), this was significantly (P<0.05) different from the inhibitory effect of (70.10%) recorded by *A. indica* at 10% extract conc. this is significantly (P<0.05) different from other interactions with the exception of *Allium sativum* at 7.5% extract conc. which gave percentage inhibition of (67.50%). The least inhibitory effect was shown by *Carica papaya and Garcinia kola* at 2.5% extract conc. each , they were significantly (P<0.05) lower than other interactions. The inhibitory effect of other interactions ranged from (17.94% to 64.93%) (table 9).

Ethanol extract of *A. sativum* with percentage inhibition of (67.02%) had the highest inhibitory effect on *Sclerotia rolfsii,* although it was not significantly (P>0.05) different from (66.22%) observed in *A. indica. Carica papaya* and *Garcinia kola* exhibited a significantly (P<0.05) lower inhibitory effect of (38.01% and 32.68%) to *A. indica* and A. *sativum.* Grisovid gave an inhibitory effect of (99.16%), while the control did not show any inhibition on the growth of *Sclerotia rolfsii* (Table 10). For extract concentration, 10% gave the highest inhibitory effect of (68.34%) on *Sclerotia rolfsii,* this was significantly (P<0.05) different from (53.66%) observed in 7.5% extract conc. with the least being (47.79% and 34.13%) exhibited by 5.0% and 2.5% extract concentration respectively. Their interaction depicted that *Allium sativum* and *Azadiratcha indica* each which gave (79.30% and 82.90%) inhibitory effect respectively were significantly (P<0.05) different from other interactions, followed by *A. sativum* at 5.0% and 7.5% and *A. indica* at 5.0% and 7.5%) with inhibitory effect of (65.80%, 67,53%, 62.40% and 68.30%) respectively. These were significantly (P<0.05) different from other interactions. The least inhibitory effect was recorded by *C. Papaya* and *G. kola* at 2.5% extract conc. each, they recorded inhibitory effect of (15.37% and 14.43%) respectively. The inhibitory

Table 11.T-test Comparison Between Aqueous and Ethanol Extracts

Plant Extracts	Group		T-Statistics	Prob. > +
	Aqueous	Ethanol		
Carica papya	28.96854± 2.4357	34.51042± 2.3543	-5.837	<0.001
Garcinia kola	26.72292± 2.1462	32.00958± 2.2818	-4.376	<0.001
Allium sativum	67.1625± 1.6418	69.6375± 1.9796	-2.968	<0.0047
A. indica	60.64375± 1.6688	67.49583± 1.8079	-7.583	<0.001

effect of other interactions ranged from 29.00% to 58.97% (Table, 10).

T-test Comparison Between Aqueous and Ethanol Extracts.

Comparison between aqueous and ethanol extract of *Carica papaya* indicated that ethanol extract give a mean inhibition percentage of 34.51042 ± 2.3543, this is highly significant (P<0.05) different from the mean inhibition of 28.96854± 2.4357 observed in aqueous medium (Table, 11).

On the same hand, ethanol extract of *Garcinia kola* which gave a mean inhibition percentage of 32.00958± 2.2818 showed a highly significant (P<0.05) difference from 26.72292±2.1462 recorded in aqueous medium (Table 11).

Aqueous and ethanol extract of *Allium sativum* showed a little change from the trend observed in *Garcinia kola* and *Carica papaya*, although ethanol extract which gave a mean inhibition of 69.6375± 1.9796 was significantly (P<0.05) different from 67.1625±1.6418 observed in aqueous medium (Table 11), but the significance was very slight.

The test reveled a very high significance (P<0.05) between ethanol and aqueous extract of *A. indica.* The ethanol extract which gave mean inhibition of 67.49583±1.8079 was significantly (P<0.05) different from 60.64375± 1.6688 mean inhibition observed in aqueous medium (Table11) .

Phytochemical Screening of Test Plants

Qualitative Test

Screening of all the test plants(*Allium sativum, Garcinia kola, Azadirachta indica and Carica papaya)* for phytochemicals of interest (Saponin, Phytate, flavonoid, Alkaloid, Tannis, Oxalate and Phenol) revealed that all the plants test positive to these phytochemicals. (Table 12).

Quantitative Test

The quantitative test revealed that the quality of phytochemical in these plants ranged from 0.024% to 6.450% (Table12). The highest quantitative yield of flavonoid was obtained in *A. sativum* (4.850%), followed by *A. indica* which gave a quantitative yield of 3.860%, the least in flavonoid was *Carica papaya* (0.280%). On the same hand, *A. sativum,* also recorded the highest quantitative yield of phytate (2.800%), followed by *Garcinia kola* (2.25%) while the least was *Carica papaya.* For Oxalate, *Allium sativum* was also the highest, with a quantitative yield of 0.940%, next to it was *A .indica* (0.905%) and the least in Oxalate was *Garcinia kola* with a yield of 0.840%. The quantity of Alkaloids in *A. indica* is 6.450% this is the highest followed by *Carica papaya (*3.440%) while the least quantity of *alkaloid* was observed in *Garcinia kola* (0.685%). More Saponin was detected in *A. indica (*2.280%), preceded by C. *papaya* (1.260%) the least in Saponin being *Garcinia kola* (0.460%). Test for Tannins showed that *A. indica* with a yield of 1.240% was highest, next to it being *Carica papaya (*0.880%) while the least was *A. sativum* (0.560%). *A. indica*

Table 12: Quantitative Phytochemical Screening

	Phytochemical %						
Plant Extracts	**Phytate**	**Flavonoid**	**Alkaloid**	**Saponin**	**Tannins**	**Oxalate**	**Phenol**
A. indica	0.650	3.860	6.450	2.280	1.240	0.905	0.540
C. papaya	0.250	0.280	3.440	1.260	0.880	0.840	0.045
G. kola	2.250	1.120	0.685	0.460	0.840	0.702	0.024
A. sativum	2.800	4.850	1.560	0.620	0.560	0.940	0.480

also gave the highest quantitative phenol yield of 0.540%, followed by *Allium sativum* (0.480%), 0.024% observed in *Garcinia kola* was the least (Table12).

DISCUSSION

The organisms associated with post-harvest rot of cocoyam cormels in this study where (*Aspergillus niger*, *Botryodiploidia theobromae*, *Fusarium solani*, *Aspergillus flavus*, *Penicillium digitatum, Rhizopus stolonifer and Sclerotia rolfsii.* These were frequently isolated from rotten cocoyam cormels. These organisms have been reported to cause extensive rot of cocoyam cormels in storage. (D' Souza and Moniz, 1968; Gollifer and Booth, 1973; Onwueme 1978; Ugwuanyi and Obeta 1996; Eze and maduewesi 1990) also the involvement of the test fungi (*B. theobromae, A.niger, F.solani and Sclerotia rolfsii)* in pathogenesis were also confirmed. This result is in agreement with the reports of many workers on other root and tuber crops (Okigbo *et al.,*2009b; Ugwuanyi and Obeta, 1996). The isolation of more than one pathogenic organisms from a particular cormel confirms the possibility of multiple infections whose cumulative effect may cause rapid rottening of root and tuber crops this agrees with the reports of Sangayomi, (2004) on yam. In most cases fungi gain entrance into cocoyam cormels though natural opening and wounds created during harvesting, transportation, handling and marketing. However, Okigbo and Nmeka (2005) noted that root and tuber crops at time of harvest may already be infested by pathogens derived from disease foliage, roots or mother tubers/cormels.

This study revealed that fungitoxic compounds were present in *A. sativum, A. indica, C. papaya and G. kola,* since they were able to inhibit the growth of the test fungi, this result is in consonance with the earlier reports of several researches but on different fungal organisms (Ameinyo and Ataga, 2007; Sangoyomi *et al.*, 2009; Okigbo *et al.*,2009b; Okigbo *et al.*, 2009c; Suleiman, 2010), hence the four plant extracts used have the potential application in the protection of mechanically injured cocoyam corms/cormels against rot fungi. However, the efficacy of the extracts differed with the plant material, concentration, solvent of extraction and with each test fungus.

Ethanol extracts were more effective than aqeous extract, this suggests that water used in the extraction process was probably not able to dissolve all the principles compounds present in the plants, which are contained in the ethanol extract. The ethanol extract gave higher yield in all the plants, this agrees 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. The difference in the fungitoxic between the extraction medium can also be as a result of the different susceptibility of each of the test isolates to different concentrations of the extracts, this also agrees with the findings of some workers (Amadioha, 2000; Onifade, 2002; Okigbo and Nmeka, 2005; Okigbo and Odurukwu, 2009; Okigbo *et al.*, 2009a).

The present observations showed that *A .sativum and A. indica* are highly effective against mycelia growth of almost all the test fungi with inhibition ranging from 40.57% to 79.63% while extract of *C. papaya* ranged from slightly to moderately effective inhibition (0.90% to 61.00%), whereas *G. kola* showed between non effective (Uninhibited) to moderately effective inhibition (0.00% to 61.17%). This is similar to the results obtained by Sangoyomi, (2004) on yam rot

and that of Suleiman (2010) on post harvest rot of yam, who reported a highly effective inhibition with *A. sativum and Azadirachta indica* respectively, but differs with the results of Okigbo *et al*., (2009) who reported a moderately effective inhibition by *A. sativum.* The commercial fungicides (Grisovid) showed a very significant effective inhibition on the radial mycelia growth of the fungi tested (79.10-100%) meanwhile, *Sclerotia rolfsii and A.niger* showed the highest percentage of inhibition with Grisovid. There was a similar trend in the fungitoxic effect of all the plant extracts with respect to concentration. 10.0% extract concentration proved to be the most fungitoxic on all the test organisms, followed by 7.5% extract concentration, while the least inhibitory effect was observed at 2.5% extract concentration. This agrees with the observations of Suleiman (2010) who stated a significant difference between mycelia growth value recorded on the various plant extract concentration, this suggests that there is difference in the solvent soluble antifungal element in the respective leaves extracts as reported by Iwu (1993) and Sofowora (1997).

The presence of bioactive substance have been reported to confer resistance to plants against bacterial, fungi and pest (Srinwasan *et al*., 2001), this therefore explains the demonstration of antifungal activity by the plant extracts used in this study, hence the antifungal properties of these plant extracts is probably due to the presence of phytochemicals which are anti microbial agents (Okwu and Joshia, 2006), that are inhibitory to the growth of these pathogens (Okigbo and Ajalie, 2005). Phytochemical screening of the plants showed positive for all the phytochemicals tested (Alkaloid. Flavonoid, Phytate, Saponin, Tannins, Oxalate and Phenols). Medicinal and pharmacological potential of all these phytochemicals was proved by the report of several workers (Okwu, 2004, Okigbo *et al*.,; 2009; Caragay, 1992).

This study have revealed the potentials of botanicals (*A. sativum, A. indica, C. papaya* and *G .kola*) in the control of cocoyam rot in storage, with *A. sativum and A .indica* exhibiting the most fungitoxic activity, this study also depicted that ethanol extracts demonstrated a higher antifungal activity over aqeous extract, indicating that ethanol extract of *A. sativum and A. indica* could be an alternative or complimentary to synthetic chemicals in controlling cocoyam rot, where, *A. sativum* and *A .Indica* are not available, *Carica papaya* and *Garcinia kola* can also be used as a second option because they exhibit a moderate fungitoxic activity on the test organisms. However, the result of this study has gone a long way in providing better alternative to the over dependence on synthetic fungicides, the use of plant extracts in controlling rot causing organisms and pests could reduce over reliance on one source of agricultural chemicals to the farmers, that are reported to predicate long term harmful consequences on environment, Man and wildlife, as well as reduce production cost, hence the antimicrobial activity of the extracts was comparable to those of the antibiotics, the demonstration of activity against the test fungi produces scientific bases for the local usage of these plants in controlling microbial rot, since these plants are locally available, less expensive, environment friendly with easy extraction method, it can be confidently exploited in the control of cocoyam rot. Therefore the cogent data on the antimicrobial potentials of plant extracts deserve multi-institutional attention as early as possible. The prospects of relatively cheaper means of controlling rot inducing organisms could then be brighter, particularly for the numerous peasant farmers across the globe and in Nigeria in particulars.

From the results obtained in this study, it is obvious that *C. papaya, G. kola, A. sativum and A. indica* possess potential inhibitory activity against rot-inducing fungi to varying degrees; hence the demonstrated antifungal potential of these plant extracts on cocoyam rot causing fungi recommends their use as natural fungicide on cocoyam corms and cormels in storage.

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 crops of economic importance after yam and cassava in Nigeria and the queen consort of yam in South-eastern Nigeria must not be pushed further down the ladder to the status of a minor vanishing crop. With respect to the plants used, further pharmacological evaluation, toxicological studies and possible isolation of the therapeutic antifungal from these plants are the future challenges, hence it is recommended that further investigations should be done on the chemical nature of the active principles of the plants, also further investigations can combine the plant extracts for possible synergistic effect, further research involving invivo assay would be needed to investigate the fungistatic effects of these botanicals on the fungal inducing rot of cocoyam corms and cormels that are not included among the test fungi in this research work. Also, very essential is the need to devise good storage facilities to prolong the shelf life of cocoyam after harvest.

However, the future of cocoyam an age old crop in Nigeria is tied to the solutions to the above problems, to enlarge the potential uses of the crops as an incentive to increased production. This is the only path to greater expectations of substantial contribution of cocoyam to food supply and thereby to the economy of Nigeria. In short run and at the existing level of technology, cocoyam production/availability can be

increased by merely containing the 40% or more pre- and post harvest spoilage (rot) losses.

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