Efficacy of Some Leaf Extracts on Some Seed Borne Fungi of African Yam Bean (*Sphenostylis stenocarpa* Hochest Ex. A. Rich)

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Abstract

The antifungal effect of ethanol leaf extracts of five plants, (viz. Jatrohpa curcas L, Newbouldia laevis (P. Beauv.) seem. Ex Bureau, Ficus exasperate Vahl, Parkia biglobosa (Jacq.) and Cassia alata L.) Seed plus 30 wettable solvent (WS) and Water on some Seed borne fungi: Aspergillus niger and Fusarium oxysporium of African yam bean (Sphenostylis stenocarpa) seeds was studied in vitro using agar plate method of isolation. The efficacy of the treatments was tested separately on the individual fungal isolates. All the plant extracts except Newbouldia laevis (P. Beauv.) seem. Ex Bureau, significantly (P > 0.05) inhibited the radial hyphal growth of Aspergillus niger. Jatropha curcas L extract gave the highest inhibition at all levels of concentrations while Parkia biglobosa (Jacq.) was the least. The positive control; Seed plus 30 wettable solvent (WS) inhibited the growth of A. niger significantly (P > 0.05) while water had no inhibition on the growth of the fungus. On the other hand, Jatropha curcas L., Cassia alata L. and Parkia biglobosa (Jacq.) extracts had inhibitory effects on the hyphal growth of Fusarium oxysporium while Newboudia laevis (P. Beauv.) seem. Ex Bureau and Ficus exasperate Vahl. extracts showed no inhibition on the growth of the fungus at all levels of concentrations. Parkia biglobosa (Jacq.) extract was the most effective while Cassia alata L. extract was the least in the inhibitory efficacy. The positive and negative controls; Seed plus 30WS and Water respectively recorded no significant inhibition on the hyphal growth of Fusarium oxysporium. The ethanolic leaf extracts of Jatopha curcas L., Parkia biglobosa (Jacq.), and Cassia alata L. which are very cheap and environmentally safe were proved promising for protecting African yam bean seeds against Aspergillus niger and Fusarium oxysporium.

Keywords: Leaf Extract, Hyphal Growth, Seed Borne Fungi, African yam bean

Introduction

African yam bean (*Sphenostylis stenocarpa* Hochest ex. A. Rich) is a herbaceous plant occurring throughout tropical Africa (Potter, 1992). It belongs to the family *Fabaceae* and Order *Fabales.*

It is found in open and wooded savannah in forest, on rocks and both as a weed and a crop in cultivated fields (potter, 1992). Sometimes, it may grow in marshy and disturbed places at altitudes of 200 to 1950m. It grows well on a range of soils, not necessarily producing the best on rich garden soils (Okigbo, 1973). According to Zohary (1972), African yam bean can tolerate annual precipitation of 870 to 1300mm, annual temperature of 19.7 to 26.2^{o}C and pH of 5.0 to 6.5.

According to Ene-Obong and Okoye (1992; 1993), African yam bean has a total seed protein value of about 19.5 – 29% on a dry weight basis. The value is similar to that reported for cowpea but not comparable to that of soybean which is at about 35%. Though the amino acid composition is however comparable with that of soybean, the lysine and methionine levels in the protein are equals to or better than those of soybean.

African yam bean is used for feeds and human consumption. It is among the most important source of plant protein in human food due to its content of essential amino acids which may or may not be obtained in other legumes (Zohary, 1972; Evans *et al.*, 1997; Quenton *et al*., 2003).

However, for Africans to meet both animal and human consumption needs of African yam bean, the health of the seed must be considered. Most fungi are serious parasites of seed primordia, maturing and stored seeds and their invasion can result to various damage including reduction of seed yields (qualitatively and quantitatively), discolorations, decrease germinability, mycotoxin production and total decay (Quenton *et al.*, 2003 and Castillo *et al*., 2004). Embaby and Abdel (2006) reported 260 pathogen isolates from legume seeds in which African yam bean were one. According to him, 200 belong to fungi while 60 were bacteria. The most common in the fungi isolates belonged to five genus namely; *Alternaria, Aspergillus, Epicoccum, Fusarium* and *Trichoderma*. Nwachukwu and Umechuruba (2001) reported the isolation of *Aspergillus niger, A. flavus, Fusarium moniliforme* and *Botryodiplodia theobroma* from African yam bean seeds. Apart from other damages causes by seed-borne pathogens, some like *Aspergillus niger* and other *Fusarium* species causes several diseases to an immunocompromised persons after consuming their infected seeds (Nwachukwu and Umechuruba 1997). However, Neergaard (1971) reported that both chemical and biological means have been used to combat the ravages caused by various pathogens. Lister (1990) treated seeds infected with *Alternaria spp; Rhizopus spp.* and *Aspergillus spp* with thiram (fernasan) which is a fungicidal seed-dressing chemical and found that it was very effective in reducing the fungal growth considerably.

In spite of the success made with other systemic fungicides, seed plus® 30ws (a.i : 10% imidacloprid + 10% metalaxyl + 10% carbendazim WS) was introduced as seed-dressing chemical. Seed plus 30ws was very effective in curing and protecting many grains and vegetables seeds. Though seed plus and other chemicals are effective, Amadioha (1998) reported them hazardous to human and environment.

Due to these problems associated with seed-dressing chemicals, there is a need to develop alternative fungicides of plant origin to control seed-borne fungi. This study was designed to evaluate the effects of selected medicinal plants extracts on fungi isolates of African yam bean seed.

Materials and Methods

The study was conducted at the pathology laboratory of the Department of Crop Science, University of Nigeria, Nsukka, located on the latitude of 06^{o}C 52'' North, longitude of 06^{o}C 21'' and altitude of about 400 meters above sea level. The experimental layout was a 7x4 factorial in completely randomized design (CRD) with five plant extracts, seed dressing chemical (seed plus 30ws) and distilled water at four levels of concentration. The study lasted for eight months (January to August 2009).

Source of plant materials, seeds and seed dressing chemical (seed plus ® 30ws): The plant used were; *Jatropha curcas, Parkia biglobosa, Newbouldia laevis, Ficus exasperata* and *Cassia alata.* These plants were collected in the province of Nsukka in January 2009. The plants were obtained locally from their wild growing stands. The seed sample of African yam bean (*Sphenostylis stenocarpa* Hachest ex. A. Rich) was obtained from Orie Orba main market, in Ude-Enu Local Government Area, Enugu state. The seed dressing chemical (seed plus 30ws) was obtained from Crop protection unit, Department of Crop Science, University of Nigeria, Nsukka.

Preparation of extracts: Fresh leaves of the test plants were collected and identified botanically at the Department of Botany, Faculty of Biological Science, University of Nigeria, Nsukka, after which they were sun dried separately for five days. The drying was timed for nine hours daily.

Each of the samples was then ground to powder using laboratory grinding machine, after which it was sieved to pass through 0.02mm diameter sieve. Powders of the above size were obtained from each sample.

Powdered leaves 115g each were soaked in 600ml of analytical ethanol for 24 hours. The solution (each) was filtered through sterile cheese cloth and the supernatant collected and concentrated to dryness in an oven (100 to 105^{o}C). The dry supernatant of each was used as the crude leaf extracts (Adedunle and Uma, 2000).

Culture media source: Potato dextrose agar (PDA) was used as the main medium throughout the study. It comprised the following; 200g potato infusion, 20g of dextrose and 15g of agar. Ready made PDA of micromaster laboratories PVT Ltd was used during the study. To prepare a liter of the media, 39g of the PDA was mixed thoroughly in 1000ml volume of pyrex-England flask and autoclaved at 15 pounds pressure at a temperature of 121^{o}C for 20 minutes in order to dissolve it. Later, it was then poured into Petri dishes, 20ml per one and allowed to solidify.

Isolation and purification of pathogens: Agar plant method was used (Neergaard, 1977). Physically healthy seeds, white accession of African yam bean (*Sphenostylis stenocarpa)* were selected and surface sterilized in 0.1% mercuric chloride. The sterilized seeds were rinsed in three changes of sterile distilled water and plated on potato dextrose agar medium in the presence of antibiotic (streptomycin).

The antibiotic was assed in the concentration of 3g per 500ml of PDA or 0.12g/20ml of PDA in each Petri dish in order to inhibit the growth of bacteria. Five seeds were plated per dish and ten dishes were used. All the dishes were incubated at 25$\pm$2^{o}C on the laboratory benches for seven days.

Seeds were examined every day after which any fungal growth was transferred and purified using hyphal tip and or single spore techniques onto PDA medium in the presence of streptomycin and incubated for another four days. Subsequent sub-culturing was made from the inoculated PDA plates unto clean PDA plates, until pure culture was obtained.

Isolation of pathogens: Two organisms were isolated from the seeds of African yam bean. The pathogens were designated isolate A and B.

Assay of plant extracts: The efficacy of *Jatropha curcas, Ficus exasperata, Newbouldia laevis, Parkia biglobosa* and *Cassia alata* leaf extracts were tested in vitro, against the fungi; *Aspergillus niger* and *Fusarium oxysporium,* flowing the hyphal growth inhibition technique (Picman *et al.,* 1990).

Here, 2g (2000mg) of test extracts each was dissolved in 5ml of dimethysulfoxide (DMSO) and mixed thoroughly to obtain the test solution of concentration 400mg/ml per each extracts. From the solution, serial 2-fold test tube dilution was made using three different test tubes and concentration of 0.05, 0.1, 0.2, and 0.4g/ml were obtained from each extracts respectively.

Fungal inoculation was done using fungal solution method. Fungal solution of pathogens; *Aspergillus niger* and *Fusariun oxysporium,* were made each by dissolving a scope of fungal mycelium cut from the edge of active growing colony in a test tube containing 2ml of sterile distilled water. It was thoroughly mixed and the resultant solution of each organism was used as standard innocula during the study.

Inoculation procedure followed thus; two to three drops of the fungal solution of one organism was mixed gently with 20ml of sterile melted PDA medium at 40^{o}C in a sterilized glass Petri dish after which it was allowed to gel. This was repeated in three other Petri dishes and then four plates were used per extracts. Fungal solution of the second organisms was prepared in the same way.

Moreover, treatment was carried immediately after inoculation using agar-well diffusion method (Irobi *et al.,* 1994).
During treatment, plate of solidified or gelled inoculated medium was divided into four portions externally using black permanent marker and then numbered 1 to 4 respectively.

Well of 5mm in diameter was made in each portions using sterile standard cork borer.

The four wells in the plate were filled with various concentrations of test solution (extract dissolved in DMSO) in respect to the numbering using plastic injection syringe. Number one well contains test solution of concentration 0.4 g/ml, number two has 0.2 g/ml, and number three has 0.1g/ml while number four has that of 0.05g/ml respectively.

The test solution was allowed to diffuse into the medium for one hour at room temperature after then the set up was incubated at 28 $\pm$ 2^{o}C on the laboratory chamber for four days, and then the inhibition zones were measured and recorded in millimeters.

The procedures were repeated in the other Petri dishes and the whole plant extracts were tested in a similar manner with four replicates for each concentration per extract using individual organisms respectively.

The negative control was set up in same ways except that the extracts were replaced with sterile distilled water.

Assay of seed plus ® 30ws: The same procedures and set ups employed in the plant extracts assay were used here except that the DMSO used in dissolving the crude plant extracts was replaced by distilled water. Therefore, sterile distilled water of same miles of DMSO was used to dissolve same 2g of (2000mg) of seed plus 30ws and concentration of 400mg/ml was obtained.
2 fold test tube dilutions were also carried and concentration of 0.05, 0.1, 0.2 and 0.4g/ml were obtained respectively. Every other procedure remains same as that of plant extracts assay.

Identification of pathogens: The fungal isolates were identified using Riddle's classic culture method (Riddle, 1950).

Statistical analysis: Genstat software was used as the statistical tool. The data were transformed using squaring method of transformation during analysis ($\sqrt{(X + C)}$). X is the inhibitory zone diameter measured while C is constant (0.5). The means separation was done using Least Significant Difference (LSD)

Results

Pathogens: The isolates were *Aspergillus niger* and *Fusarium oxysporium*. *Aspergillus niger* on the PDA plates was black when observed with ordinary eyes and *Fusarium axysporium* was whitish initially and becoming tinged with salmon at maturity. *Fusarium oxysporium* grows more rapidly than *Aspergillus niger* on PDA medium.

Under the microscope, *Aspergillus niger* had profusely branched septate, hyaline and well developed hyphae with an abundance of conidiophores. The conidiophores were long and erect each terminating in a bulbous head called vesicle (Fig. 1). The conidia were borne in chains. Combining these microscopic and macroscopic features, the organism was confirmed and identified as *Aspergillus niger*.

Fusarium oxysporium as seen under light microscope has long, crescent shaped, multiseptate macroconidia and very small spherical oval microconidia. The microcondia usually produced abundantly, slightly sickle-shaped, thin-walled, with an attenuated apical cell and a foot-shaped basal cell (Fig. 2). The organism has hyphae that are septate and hyaline and thus was identified as *Fusarium oxysporium*.

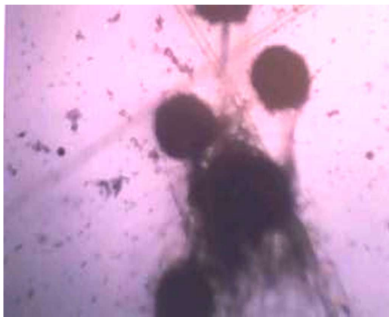

Fig. 1: Photomicrograph of *Aspergillus niger*

Fig. 2: Photomicrograph of *F. oxysporium*

Effects of plant extracts, seed plus® 30ws and water on in-vitro inhibition of hyphal growth of *Aspergillus niger:* *Jatropha curcas*, *Cassia alata*, *Ficus exassperata* and *Parkia biglobosa* leaf extracts significantly ($p<0.05$) inhibited the radial hyphal growth of *Aspergillus niger* with inhibition varying from one extract to another, *Newbouldia laevis* leaf extract have no inhibition on the growth of *Aspergillus niger* (Table 1). The positive control; Seed plus® 30ws also significantly ($p<0.05$) inhibited the growth of *Aspergillus niger* while the negative control; water had no inhibition on the growth of the organism.

The highest concentration 0.4 gave the highest inhibition. Seed plus® 30ws gave the highest inhibition (6.24), but among the extracts, the highest inhibition was recorded on J. curcas (5.02).
The inhibition rate of the hyphal growth of *A. niger* varied with the concentrations of the extracts. The main effect of the concentrations; 0.05g/ml, 0.10g/ml, 0.20g/ml, 0.40g/ml were significantly different ($p<0.05$). The inhibition rate increases with increase in concentration. The highest inhibition on the growth of *A. niger* was on the concentration of 0.40 while the least was recorded on the concentration of 0.05g/ml respectively (Table 1).

Table1: Effects of plant leaf extracts, seed plus® 30ws and water on *Aspergillus niger*

Extracts	Concentration (g/ml)				Means
	0.05	0.10	0.20	0.40	
CAT	3.56	3.98	4.22	4.49	**4.06**
FET	3.49	3.93	4.09	4.61	**4.03**
JUC	3.93	4.48	4.79	5.02	**4.56**
NLV	0.00	0.00	0.00	0.00	**0.00**
PBG	2.73	3.45	3.70	3.99	**3.49**
SPL	5.16	5.48	5.74	6.24	**5.66**
WTA	0.00	0.00	0.00	0.00	**0.00**
Means	**2.70**	**305**	**3.22**	**3.48**	

LSD (P=0.05) for Extracts =0.16; LSD (P=0.05) for Concentrations =0.12; LSD (P=0.05) for Interactions (Concentrations x Extracts) =0.31; ***Note:*** *CAT Cassia alata, FET Ficus exasperate, JUC Jatropha curcas, NLV Newbouldia laevis, PBG Parkia biglobosa, SPL Seed plus 30ws, WTA Water.*

Table 2: Effects of plant extracts, seed plus 30ws and water on *Fusarium oxysporium*

Extracts	Concentrations (g/ml)				Means
	0.05	0.10	0.20	0.40	
CAT	3.26	3.74	3.97	4.18	**3.79**
FET	0.00	0.00	0.00	0.00	**0.00**
JCU	3.45	3.84	4.08	4.52	**3.97**
NLV	0.00	0.00	0.00	0.00	**0.00**
PBG	3.38	3.95	4.49	5.10	**4.23**
SPL	0.00	0.00	0.00	0.00	**0.00**
WTA	0.00	0.00	0.00	0.00	**0.00**
Means	**1.44**	**1.65**	**1.79**	**1.97**	

LSD (P=0.05) for Extracts=0.11; LSD (P=0.05) for Concentrations=0.09; LSD (P=0.05) for (Extracts x Concentrations) =0.23; Note; CAT Cassia alata, FET Ficus exasperata, JCU Jatropha curcas, NLV Newbouldia laevis, PBG Parkia biglobosa, SPL Seed plus 30ws, WTA Water

The interaction of concentrations and extracts has effect in the inhibition of the hyphal growth of *A. niger*. The interaction effect of the positive control; seed plus 30ws at concentration of 0.05(5.16) was different with various extracts at concentration of 0.20($p<0.05$).

Seed plus 30ws at concentration of 0.05 (5.16) and *J. curcas* at 0.40g/ml (5.02) has no significant different ($p<0.05$). J. curcas and *F. exasperata* at concentration of 0.05g/ml (3.93) and 0.20g/ml (4.09) respectively have the same effect on *A. niger*. It was also found that the inhibitory effect *J. curcas* at concentration of 0.05 (3.93) and *P. biglobosa* at concentration of 0.40 (3.99) have no significant difference ($p<0.05$). There was no significant difference in the inhibition effect of *J. curcas* and *C. alata* at concentrations of 0.10g/ml (4.48) and 0.40g/ml (4.49) respectively ($p<0.05$).

Effects of plant extracts, seed plus® 30ws and water on in-vitro inhibition of hyphal growth of *Fusarium oxysporium*: *Jatropha curcas*, *Cassia alata* and *Parkia biglobosa* leaf extracts significantly ($p<0.05$) inhibited the radial hyphal growth of the fungus with inhibition varying from one extract to another while *Newboudia laevis* and *Ficus exasperata* leaf extracts were found to have no inhibition on the growth of *Fusarium oxysporium* (Table 2).

The positive and negative controls; Seed plus and Water respectively have no inhibitions on the growth of *Fusarium oxysporium* at the concentration used. The leaf extract of P. biglobosa gave the highest inhibition (5.10) at concentration of 0.4g/ml on the *F. oxysporium* ($p<0.05$).

The inhibition rate of the hyphal growth of *F. oxysporium* varied with the concentrations of the extracts. The inhibition rate increases with increase in concentration. The highest inhibition on the growth of *F. oxysporium* was at the concentration of 0.40 while the least was recorded on the concentration of 0.05 respectively (Table 2).

The interaction of extracts and their concentrations affected the inhibition rate of the *F. oxysporium* growth. The inhibitory activity of P. biglobosa and *C. alata* at 0.05 concentration (3.38 and 3.26 respectively) has no statistical difference while at concentration of 0.20 (4.49 and 3.97 respectively), the inhibition was significantly different ($p<0.05$). At concentrations of 0.05 and 0.10, *P. biglobosa* and *J. curcas* have the same inhibitory activity on the growth of *F. oxysporium* while at 0.20 and 0.40; the two extracts inhibited the growth of the organism differently.

Comparative effects of plant extracts and seed plus® 30ws on *F. oxysporium* and *Aspergillus niger:* The result of comparative activities of extracts and seed plus 30ws was presented in histogram form on Figure 3. At concentration of 0.05, *C. alata* and *J. curcas* inhibited the hyphal growth of *A. niger* more than that of *F. oxysporium*. Moreover, *Ficus exasperata* and the positive control; seed plus 30ws have inhibitory activity on *A. niger* only. Parkia biglobosa has higher activity on the *F. oxysporium* than *A. niger*. The result remained virtually the same in other concentrations except that the diameter of inhibitions increases from low to highest concentration. This is observed in the height of bars which increase from 0.05g/ml to 0.40g/ml of concentration respectively (Fig. 3).

Phytochemical screening: The results of the phytochemical screening are presented in the Table 3. The leaf extracts contained alkaloids, though it was much more positive test for that of *Parkia*

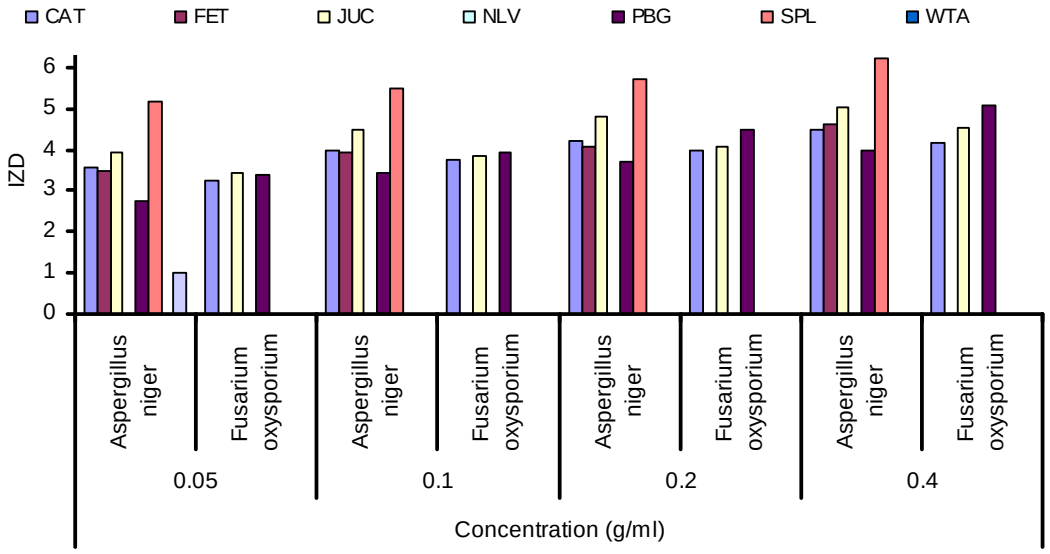

Fig. 3: Interactive effects of plant extracts and seed plus®30ws on Fuasriun oxysporium and Aspergillus niger

Note; CAT *Cassia alata*, FET *Ficus exasperata*, JCU *Jatropha curcas*, NLV *Newbouldia laevis*, PBG *Parkia biglobosa*, SPL Seed plus 30ws, WTA Water, IZD Inhibition Zone Diameters

Table 3**: Phytochemical Analysis**

Extracts	Phytochemical Compounds(mg/100g)				
	Alkaloids	Saponins	Tannins	Cynogenic glycosides	Soluble carbohydrate
F. exasperata	22.50	0.39	8.40	1.87	2.28
C. alata	34.17	1.40	9.02	2.85	1.91
N. laevis	21.18	0.08	8.24	2.69	2.91
J. curcas	32.08	1.49	8.11	7.08	3.99
P. biglobosa	56.67	0.04	9.40	6.99	1.96

biglobosa, followed by *Cassia alata* and the least is *Newbouldia laevis*. Tannins were also indicated in the entire extracts but in different proportions. *P. biglobosa* records the highest amount of tannins followed by *C. alata*. However, saponins was much more on *J. curcas, C. alata* and least on *P. biglobosa*. Cynogenic glycoside was present in all the extracts but appears more in *J. curcas* and *P. biglobosa* while *F. exasperata* had the least. Soluble carbohydrate also contained in the whole extracts but was much on *J. curcas* and least in *C. alata.*

Discussion

The efficacy of different plant leaf extracts (Jatropha *curcas*, *Ficus exasperata*, *Newbouldia laevis*, *Parkia biglobosa*, and *Cassia alata*) on the control of seed borne fungi (*Aspergillus niger* and *Fusarium oxysporium*) of African yam bean seeds was tested in vitro. It was found that four extracts (*J. curcas, F. exasperata, C. alata* and *P. biglobosa)* significantly ($p<0.05$) inhibited the hyphal growth of *A. niger* with inhibition varying from one extract to another (Table 1). Variation of microorganisms' inhibition according to extracts in this work agrees with the report of Nwachukwu and Umechuruba (2001). They reported that the inhibition of growth of the tested fungi varies from Neem (*Azadirachta indica*) extract to Lemon grass (*Cymbopogen citrates)* which was the least among the tested plant extracts.

On the other hand, *P. biglobosa, C. alata* and *J. curcas* leaf extracts significantly ($p<0.05$) inhibited the growth of *Fusarium oxysporium*, with inhibition varying from one extract to another (Table 2).

The inhibitory efficacy of plant extracts on this study conformed with earlier reports by researchers on the fungitoxic effect of some plant extracts (Areo, 1989), and the report by Onyeke and Maduewesi (2006), where plant extracts from *Azadirachit indica*, *Moringa lucisa*, and *Acalypha ciliate* were used to inhibit the hyphal growth of fungal isolates of Banana (*Musa sapientum*), *Botryodiplodia theobromae* and *Colletotrichum musae*. It is also in line with the report of Nwachukwu and Umechuruba (2001), where leaf extracts of Neem, Biter leaf, Pawpaw, Lemon grass and Basil significantly inhibited the growth of *A. niger, F. moliforme* and *Botryodiplodia theobromae* of African yam bean seeds. It concured with the finding of Phongpaichit *et al*. (2004), that *C. alata*

inhibited the growth of *Microsporum gypseum*, *Trichophyton rubrum* and *Penicillum mornefei*.

The positive control; seed plus® 30ws was effective in controlling the growth of *A. niger* and have no effect on the growth of *F. oxysporium* at the concentration used. This may be as result of the selectivity in action of the active ingredients; matalaxyl and carbendazim. According to Lyr (1977), metalaxyl amd carbendazim when mixed has stronge influence on the growth and development of *Pythium spp*., *Rhizoctonia spp*., *Aspergillus spp*., and some species of *Fusarium.* Following the fact, *F, oxysporium* is one of the *Fusarium* species that resist the activity of metalaxyl and carbendazim mixture. Wada (2002), also reported that the combination of metalaxyl and carbendazim was very effective against *U. scitaminea* of sugar cane smut disease but some fungi pathogens resist it in in-vitro test. Consequent to that, *F. oxysporium* is one of those fungi that resist the activity of the chemical in in-vitro test. Moreover, according to the manufacturer of the chemical (Seed plus), some strains of fungi can prove resistant to the chemical and as such, *F. oxysporium* is one of the strains that resist the effect of the chemical. These and other factors are the reasons why Seed plus 30ws could not inhibit the hyphal growth of *F. oxysporium* in this study.

Jatropha curcas was the most effective among the extracts in the control of *A. niger* growth. It had inhibitory activity on both *A. niger* and *F. oxysporium* but was more effective on *A. niger* than *F. oxysporium*. This result agreed with finding of Akinpelu and Kolawole (2004), that *J. curcas* leaf extract among all the tested extracts was more active against isolated microbes and in particular *Pseudomonas aeruginosa* known to be resistant to most synthetic antibiotics. The higher microbial activity of *J. curcas* may be due to its pytochemical compositions. According to the pytochemical screening conducted during this study, J. curcas leaf extract has higher component of saponin (1.49mg/100g) than other extracts. It also has moderate amount of tannins and alkaloids.

Jun *et al*. (1989), reported that saponin has antifungal properties. Its mode of action is by killing and inhibiting the excessive division of cells and therefore is anticancer (Koratkar and Rao 1996; 1997). Also, Agarwal and Rastogi (1974), reported that saponin has a cytotoxic potency and is structural dependant in function.

For the fact that saponin has all these properties and J. curcas has much of it, its inhibitory activity is due to high component of saponin and other pytochemicals. Also, because the structure of *A. niger* and that of *F.oxysporium* are not the same and saponin, the major component of *J. curcas* is structural dependent in function therefore, the differential inhibitory activity of *J. curcas* on the two fungal organisms is justify by saponin.

However*, Cassia alata* was found seconding *J. curcas* in controlling the growth of *A. niger*. It has inhibitory activity on the two isolates but more on the *A. niger* than that of *F. oxysporium*. Its efficacy may also be phytochemical dependent. Just like *J. curcas*, *C. alata* has high component of saponin (1.40mg/100g) as the major phytochemical compounds and therefore observed the structural differential activity on the pathogens. The inhibitory activity of *C. alata* in this study agreed with the report of Farnsworth and Bunyaprapatasara (1992), that *C. alata* inhibited the growth of *Trichophyton rubrum*, *Microsporum gypseum* and *Pinicillium marneffei*.

Parkia biglobosa was found effective in controlling the growth of the two fungal isolates but in contrary to other extracts, it was more effective on *F. oxysporium* than *A. niger*. This may be either it bas some pytochemical not present in other plants or in lower or higher proportion than others. According to Ajaiyeoba (2002), ethanol extract of P. biglobosa was fiund to be very effective in controlling growth of *Bacillus cereus* with inhibition zone diameter of 20 mm and above. She confirmed the present cardiac glycosides, tannins, alkaloids and steroids pytochemical as the base of its antimicrobial action. The phytochemical screening of *P. biglobos*a reveals the present of 56.67mg/100g of alkaloids, 9.40mm/100g of tannins and negligible amount of saponins.

Tannin has been reported to play major roles in plant defense against fungi and insects (Butler,1989). Mehansho *et al* (1987), reported that tannin is a special antifungal agent that inhibits the germination or growth of some fungal spores. They noted that tannin ihibited the growth of *Colletorichum germinicol*a and *A. niger.*

On the other hand, alkaloid has been noted to possess antifungal property; it inhibited the growth activity of *F. oxysporium, F. solani,* and *Aphanomycetes eutaches* (Van Ettern, 1976). He also added that Fusarium species were found more susceptible to inhibition by alkaloids and isoflavonoids. Therefore, Parkia biglobosa having higher proportion of alkaloids followed by other phytochemical, its higher activity on *F. oxysporium* is justified.

Moreover, *Newbouldia laevis* was the only extract neither that inhibited *A. niger* and *F. oxysporium*. This may be attributed to the fact that it is lacking some phytochemicala that can inhibit the growth of the isolate or it has the phytochemical in inappropriate concentration that can not initiate inhibition of the fungal isolates. The phytochemical screening of *N. laevis* reveals that it contains the lowest alkaloid content among others (21.18), the tannin content is moderately low (8.24) and negligible amount of saponin. These three phytochemical compounds; tannin, saponin and alkaloid, are the major antifungal plant secondary metabolites analyzed in this study. Therefore, consequent to the fact that *N. laevis* has the three compounds in little or low proportion, its no inhibitory activity on the two fungal isolates is justified. Other phytochemical unscreened in the *N. laevis* may be responsible for its activity on other pathogens order than *F. oxysporum* and *A. niger.*

Antimicrobial activity of *N. laev*is has been reported by many researchers but commonly on antibacterial than antifungal. According to Eyong *et al.* (2005), *N. laevis* is widely used as folklore remedies for treatment of bacteria related diseases. Gafner *et al* .(1998) also reported that isolation of naphthaquinon a phytochemical compound from the

extract of *N. laevis* was strongly antibacterial on some tested bacteria isolates. Also, following the above reports, it is clear that *N. laevis* is more antibacterial than antifungal in activity.

The satisfactory minimum inhibition concentration (MIC) of the plant extracts and seed plus®30ws on *Aspergillus niger* were 0.05g/ml, 0.06g/ml, 0.06g/ml, 0.07g/ml and 0.07g/ml for seed plus, *J .curcas, F. exasperata, Cassia alata* and *P. biglobosa* respectively while that on *Fusarium oxysporiun* were 0.05g/ml, 0.07g/ml and 0.1g/ml for *P. biglobosa, C. alata* and *J. curcas* respectively. The MIC values of the extracts on both fungal organisms were rather low and indicate that the crude extracts would be good enough and economically advantageous to treat any infection caused by the organisms accordingly.

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