

## **Antifungal Activity of Leaf Extract of *Alchornea cordifolia* Against *Aspergillus flavus* the causal Agent of Yam Tuber Rot**

### **Aktivitas Anticendawan Ekstrak Daun *Alchornea cordifolia* terhadap *Aspergillus flavus* Penyebab Busuk Umbi Ubi Jalar**

**David Nwazuo Enyiukwu<sup>1\*</sup>, Anderson Chidi Amadioha<sup>1</sup>, Inemesit Ndarake Bassey<sup>2</sup>**

<sup>1</sup>Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike,  
KM 10 Umuahia-Ikot Ekpene Road, PMB 7267 Umuahia, Abia State, Nigeria.

<sup>2</sup>Department of Botany and Ecological Studies, University of Uyo,  
Ikpa Road, PMB 1017 Uyo, Nigeria

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#### **ABSTRACT**

Rots and mycotoxin contamination of agro-produce are prevalent in tropical world. *Aspergillus flavus* is a major mycotoxigenic rot contaminant of tubers in Abia state, Nigeria. Consumption of mycotoxin contaminated foodstuffs accounts for 25% of medically important human diseases in Nigeria. The objective of this study was to assess antifungal activity of *Alchornea cordifolia* against *A. flavus* a mycotoxigenic rot pathogen of stored tuber produce both *in vitro* and *in vivo*. Isolates were made from rotted yam tubers and pathogenicity test carried out to confirm *A. flavus* as pathogen. Five concentrations (100-500 mg mL<sup>-1</sup>) of methanol leaf extracts of the plant and thiophanate-methyl—a standard fungicide—were evaluated against the growth of the fungus in culture and pathogen-induced rot development and spread *in vivo*. The experiment was made up of 7 treatments and 3 replicates laid out in CRD. The plant leaf extract demonstrated varying levels of inhibition of *A. flavus* *in vitro* and rot development and spread in living tissues of yam. About 56.38%–68.22% and 67.245–80.01% inhibition was recorded by 100 mg mL<sup>-1</sup> and 500 mg mL<sup>-1</sup> of *A. cordifolia* extract for spore germination respectively whereas the same concentrations reduced rot development from 60% in the control experiment to 21.80% and 12.10% respectively which compared favorably (P<0.05) with standard fungicide, thiophanate-methyl. The plant demonstrated strong antifungal activity *in vitro* and minimized *A. flavus* induced rot advancement in living yam tissues.

**Keywords:** *Alchornea cordifolia*, *Aspergillus flavus*, *in vitro*, *in vivo*, fungitoxicity,  
leaf extract, thiophanate-methyl

#### **ABSTRAK**

Kejadian busuk dan kontaminasi mikotoksin pada produk pertanian banyak terjadi di negara tropis. *Aspergillus flavus* adalah cendawan penghasil mikotoksin utama penyebab busuk pada umbi-umbian di negara bagian Abia, Nigeria. Konsumsi bahan makanan yang terkontaminasi mikotoksin menyumbang 25% penyakit manusia yang penting secara medis di Nigeria. Penelitian dilakukan untuk menilai aktivitas anticendawan ekstrak daun *Alchornea cordifolia* terhadap *A. flavus*—cendawan patogen penyebab busuk pada umbi ubi jalar yang disimpan—baik secara *in vitro* maupun *in vivo*. Isolat *A. flavus* dari umbi

\*Alamat penulis korespondensi: Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike. KM 10 Umuahia-Ikot Ekpene Road, PMB 7267 Umuahia, Abia State, Nigeria.  
Tel: +234(0)09092309790, Surel: enyidave2003@gmail.com

ubi jalar dengan gejala busuk digunakan pada uji patogenisitas untuk memastikan perannya sebagai patogen. Lima konsentrasi (100–500 mg mL<sup>-1</sup>) ekstrak daun *A. cordifolia* dengan pelarut metanol dan tiofanat-metil—fungisida standar—dievaluasi terhadap pertumbuhan cendawan dalam kultur *in vitro* dan perkembangan busuk yang disebabkan oleh patogen serta penyebarannya secara *in vivo*. Percobaan terdiri atas 7 perlakuan dan 3 ulangan yang disusun dalam rancangan acak lengkap. Penghambatan sebesar 56.38%–68.22% dan 67.245–80.01% ditunjukkan pada konsentrasi ekstrak daun *A. cordifolia* 100 mg mL<sup>-1</sup> dan 500 mg mL<sup>-1</sup> terhadap perkecambahan spora, sedangkan konsentrasi yang sama mengurangi perkembangan busuk sebesar 60% pada perlakuan kontrol menjadi berturut-turut 21.80% dan 12.10% ( $P < 0.05$ ) dibandingkan dengan fungisida standar, tiofanat-metil. Tanaman ini menunjukkan aktivitas antifungal yang kuat secara *in vitro* dan meminimalkan perkembangan pembusukan yang disebabkan oleh *A. flavus* secara *in vivo*.

Kata kunci: *Alchornea cordifolia*, *Aspergillus flavus*, fungitoksisitas, ekstrak daun, *in vitro*, *in vivo*, tiofanat-metil

## INTRODUCTION

*Aspergillus flavus* (Ascomycotes) is a soil-borne fungal pathogen of field crops, causing ear and kernel rot of cereals. The fungus is associated with postharvest spoilage and deterioration of basic tuber staples such as cassava, sweet potato, cocoyam and yams in particular in Nigeria (Molotja *et al.* 2011; Enyiukwu *et al.* 2014a; Ano 2019; Paul *et al.* 2021). Nigeria accounts for 70% of annual production of yams worldwide (Ano 2019). Postharvest rots in yams due to *A. flavus* amongst other mycotic pathogens are severe in tropical locations ranging between 25%–60% (Adeniji 2019; Ano 2019; Wumbei *et al.* 2022) and constitute a significant impediment to yam production and food security in Nigeria (Ogaraku and Usman 2008; Gwa and Nwankiti 2018).

Also, *A. flavus* is toxigenic and contaminates stored tubers and food products with noxious aflatoxins in many tropical countries where poor traditional storage systems abound (Enyiukwu *et al.* 2014a; Kinyungu *et al.* 2019). Postharvest contamination of yam tubers and flours with rot causing *Aspergillus* spp. and their aflatoxins have been variously reported in Nigeria (Odoemelam and Osu 2009; Enyiukwu *et al.* 2014a; Salisu *et al.* 2020).

Control of rots of yams and their associated mycotoxins have been carried using good agronomic practices such as field and store hygiene (Amadioha 2004; Enyiukwu *et al.*

2014a; Enyiukwu 2014b). Chemical interventions in storage using fungicides like captan, mancozeb, and thiabendazole are age-long and have proven very effective fast-acting strategies for control of several types of rots (Zhao *et al.* 2023). However, fungicide residues in tubers and associated produce remains a major drawback to their continued adoption and use (Adeniji 2019), necessitating search for botanicals as cheap, eco-friendly and readily available alternative control strategy (Amadioha 2004). For instance, extracts of neem, tobacco, black pepper and ginger were reported to demonstrate strong rot inhibition against *A. niger* and *A. flavus* in yams (Gwa and Nwankiti 2018; Adeniji 2019).

Ethno-pharmacological potentials of *Alchornea cordifolia* (Christmas bush) for prevention and therapy of human diseases including antimicrobial, antiretroviral and antitoxin activities have been reported (Okeke *et al.* 1999; Farombi 2004; Noundou *et al.* 2014; Kadija *et al.* 2021). In crop protection, Enyiukwu *et al.* (2021) reported that aqueous and methanol extracts of *A. cordifolia* amongst others significantly impeded the growth and spread of anthracnose caused by *Colletotrichum destructivum* *in vitro* and *in vivo*. Similarly, methanol extracts of *A. cordifolia* demonstrated *in vitro* inhibition of *Botrydiploia theobromae*—a rot causing fungal agent (Amienyo and Ataga 2007).

The objectives of this paper therefore were to assess antifungal activity of methanol leaf extract of *A. cordifolia* against *A. flavus*

a mycotoxigenic fungus causing rot of yam tuber staples in storage both *in vitro* and *in vivo*.

## MATERIALS AND METHODS

### Experimental site and location

The experiment was conducted at the Plant Health Management Laboratory of the Michael Okpara University of Agriculture, Umudike, and characterized at the Botany and Ecological Laboratory Department University of Uyo. Umudike is located on latitude 5° 28' 0"N, longitude 7° 33' 0"E and altitude of 121.65 meters above sea level. Uyo on the other hand lies on 45 meters above sea level, latitude of 5° 2' 20"N and longitude 7° 54' 34"E.

### Isolation of Pathogen

The pathogen was isolated from infected yam tubers using classical procedures of Amadioha (2004) and Enyiukwu *et al.* (2021). Rotted yam tubers were collected from grocers at Ariam market in Ikwuano LGA, Abia State, Nigeria. Advancing edges of rot lesions on the tubers were cut in bits (5mm diameter), sterilized in 70% ethanol for 1 minute, washed in several changes of distilled water, blotter-plated on humid Petri dishes and then incubated in the incubation chamber for 5 days. Bits of the organisms that grew out of the plated yam specimens were repeatedly sub-cultured on potato dextrose agar (PDA) until pure cultures of the organisms were obtained.

### Pathogenicity Test and Identification of Pathogen

All the isolates were tested individually for their ability to initiate rot disease symptoms on relatively healthy (uninfected) yam tubers. The healthy yam tubers were surface sterilized with 10% sodium hypochlorite and bored on the surface with a 3 mm cork borer.

Disc (3 mm) of the pure culture isolate was separately inserted into the hole and plugged with the yam tuber tissue previously removed from the tuber after 1 mm had been cut to compensate for the thickness of the disc.

The inoculated points were sealed with Vaseline to prevent contamination. All the inoculated yam tubers containing 6 different fungal isolates were incubated at room temperature (27–30 °C) over a period of 10 days and examined daily for rot symptoms such as wetting, softening, discoloration (darkening) and emission of offensive odor. Those that showed rot symptoms at the end of the incubation period were each cut longitudinally through the uninfected portion to expose the rotted regions. The rotted regions were re-planted on PDA and re-isolated (Amadioha 2004). The isolate that caused rot and bore true morphological and cultural resemblance to the original fungal culture was regarded as pathogen whereas those isolates that did not cause any significant rot were regarded as saprophytes and discarded.

Slides of the pathogen were then prepared, mounted and examined on under a low-high power Olympus digital compound microscope fitted with the software Scopevision version 9.0, and its identity obtained by comparing colony and spores characteristics of the fungus with standard monographs. The organism identity tallied with the descriptions of *A. flavus* by Barnett and Hunter (1998).

### Methanol Extraction of *Alchornea cordifolia* Leaves

Fresh aerial portions of *A. cordifolia* were collected from bushes in the ancient Kingdom of Ekebedi (Lat. 5° 38' 21"N, Long. 7° 58' 03"E, 112 meters above sea level). The plant sample was authenticated by Prof. M. C. Dike at the Department of Forestry, College of Natural Resources and Environmental Management (CNREM) of the Michael Okpara University of Agriculture, Umudike, Abia State and the plant specimen (Voucher number: AC/7344) was deposited at the Departmental Herbarium.

The fresh leaves of *A. cordifolia* (200 g) were washed in tap water, rinsed in 3 changes of sterile distilled water (400 mL) and before being air-dried on the laboratory bench for 18 days, enveloped and oven-dried at 40 °C for 10 minutes, and then milled into powder using a Thomas Wiley machine (Model: ED-5 USA).

Then 60 g of the milled powder was packed into a 2 L Soxhlet apparatus and extracted exhaustively with 400 mL of analytical grade methanol for 24 h. The methanol leaf extract was concentrated using a rotary evaporator at 45 °C and left on the laboratory bench for two days to afford a light green residue 7.30 g (Okwu and Ukanwa 2010).

### Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

Proportionally decreasing concentrations of the leaf extracts ranging from 40 to 0.625 mg mL<sup>-1</sup> were prepared using double dilution method. Then 40% (400 mg mL<sup>-1</sup>) concentration of the extract was prepared by dissolving 1 g of *A. cordifolia* residue in 10 mL of methanol. Then 1 mL of the test extract so prepared was transferred to 9 mL methanol in another test tube and the process repeated until the sixth test tube when the concentration of 0.625 was obtained. The tubes of different decreasing concentration of *A. cordifolia* were separately inoculated with 0.1 mL of spores of standardized 8-day culture of *A. flavus* and allowed to stand for 30 min. The suspensions were then incubated at 27 °C for 96 h and the lowest concentration of the plant extract in the test tubes that showed no growth after careful examination was considered as the minimum inhibitory concentration (MIC) of the extract against *A. flavus*. The MIC concentration was inoculated onto sterile PDA and then incubated at 27 °C for 96 h after which they were examined for presence or absence of *A. flavus* growth. The concentration of *A. cordifolia* in the suspensions that yielded no visible fungal growth on the fresh PDA were taken as the MFC (Adeshina *et al.* 2010; Akinpelu *et al.* 2015).

### In Vitro Experiment

**Evaluation of Extract on Spore Germination of the Fungus.** Spores suspension of *A. flavus* obtained by irrigating 8-day old culture of the organism with sterile distilled water was standardized to

$1 \times 10^5$  spores mL<sup>-1</sup> of distilled water. Two drops of this suspension were placed separately on five different sterile slides to which equal volume of the proportionally increasing concentrations (0, 100, 200, 300, 400 and 500 mg mL<sup>-1</sup>) of methanol leaf extract of *A. cordifolia* was separately added and incubated in a humid chamber for 24 h. The controls were mixed with no suspension of the bio-fungicide or thiophanate-methyl (0.5 g L<sup>-1</sup>). Further spore germination was stopped by adding 0.05 mL of lactophenol in cotton blue to each of the preparation on the 5 slides. Then 100 randomly selected spores of the pathogen under low-high power of an Olympus compound microscope field ( $\times 100$ -400) were examined on each of the slides. The percentage inhibition of spore germination of the pathogen was determined using the formula:

$$\text{SGI (\%)} = \frac{x - y}{x} \times 100\%, \text{ with}$$

SGI, spore germination inhibition (%); x, average number of germinated spores of the test fungus with control; and y, average number of germinated spores of the test fungus with treatment.

**Evaluation of Extract on Radial Growth of the Pathogen.** A 3 mm disc of 8-day old culture of the organism was planted on the center of solidified PDA smeared with 1 mL of different concentrations (100, 200, 300, 400 or 500 mL) of *A. cordifolia*. The controls were set up with no bio-fungicide or thiophanate-methyl impregnated. The dishes were covered and incubated at 27 °C for 7 days. The experiment consisted of 7 treatments, each was replicated 5 times and laid out in completely randomized design (CRD). At 7 days after incubation, the radial growth of the pathogen was measured along perpendicular lines through the center of the dishes with a meter ruler.

The percentage inhibition of mycelial elongation of the fungus was taken as an indication of mycotoxicity of *A. cordifolia* methanol leaf extract, and calculated using the formula:

$$RGI (\%) = \frac{x - y}{x} \times 100\%, \text{ with}$$

RGI, radial growth inhibition (%); x, average diameter of fungal colony with control; and y, average diameter of fungal colony with treatment.

### In Vivo Experiment

The *in vivo* experiment was conducted based on the protocols adopted by Amadioha (2004) and Chukwuebuka *et al.* (2016). A total of 20 yam tubers weighing about 240 g each were inoculated with 5 mm diameter disc of *A. flavus* and incubated on the laboratory bench for 21 days. The experiment was laid out in CRD made up of 7 treatments (0, 100, 200, 300, 400, 500 mg mL<sup>-1</sup> of *A. cordifolia* methanol extract and thiophanate-methyl 0.5 g L<sup>-1</sup>) and all replicated 3 times. At the end of the study period the yam tubers were cut longitudinally as in the pathogenicity tests. The mycotoxicity of the extracts of *A. cordifolia* was taken as a reduction of rotted area of the tubers per treatment per replicate compared to the control based on a modified 8-point descriptive key adopted by Amadioha (2004) as: 1, No rot; 2, slightly rotted; 4, moderately rotted; 6, heavily rotted; and 8, tuber completely rotted.

Rot initiation and development in the tubers were calculated as difference in weight of area of the treated tubers macerated by the rot pathogen against the final weight of the treated tubers expressed as a percentage using

the formula adopted by Chukwuebuka *et al.* (2016):

$$\text{Rot disease severity} (\%) = \frac{x - y}{x} \times 100\%, \text{ with}$$

x, final weight of the inoculated tubers; and y, weight of rotted regions of the treated tubers.

### Data Analysis

Data generated from this study were analyzed by percentages, and analysis of variance using Genstat computer program version 12.0 (Nwaneri *et al.* 2020). Means were compared using LSD at 5% level of probability.

## RESULTS

### The Identity and Pathogenicity of The Pathogen

Six fungal organisms were isolated from the rotted yam tubers i.e *A. flavus*, *Sclerotium rolfsii*, *Mucor sp.*, *Fusarium oxysporum* and *A. niger*. Amongst these organisms, *A. flavus* and *A. niger* induced substantial rot of the yam tubers. However, *A. flavus* was the most virulent rot inducing agent during the pathogenicity evaluation. Hence, it was used in the study while all others were discarded.

### Fungitoxic Activities of the Plant Extract

Table 1 showed that the zone of inhibition of the extract against *A. flavus* ranged between 12.33–35.33 mm with mean MIC and MFC values of 5 mg mL<sup>-1</sup> and 18.33 mg mL<sup>-1</sup>

Table 1 Zone of inhibition, minimum inhibitory concentration and minimum fungicidal concentration of *Alchornea cordifolia* methanol extract against *Aspergillus flavus*

<i>A. cordifolia</i>	Concentration of <i>Alchornea cordifolia</i> methanol extract against (mg mL <sup>-1</sup> )							MIC	MFC	MFC/MIC
	40	20	10	5.0	2.5	1.25	0.625			
Rep. 1	35	33	28	25	14	5	7	5.0	20	4
Rep. 2	37	32	30	23	11	9	6	5.0	20	4
Rep. 3	34	30	27	24	12	8	5	5.0	15	3
Mean	35.33 ± 0.02	31.67 ± 0.10	28.33 ± 0.07	24.00 ± 0.31	12.33 ± 0.03	7.33 ± 0.16	6.00 ± 0.22	5.0 ± 0.01	18.33 ± 0.03	3.67 ± 0.022

\*Data are means of 3 determinations, MIC: minimum inhibitory concentration; MFC: minimum fungicidal concentration; values of zone of inhibition ranging 5–7.5mm = no inhibition.

respectively, while the ratio of MFC/MIC (R) value was  $\leq 3.65$ . R-value of  $\leq 3.65$  thus confirms that the plant extract was fungicidal against *A. flavus*

### Effects of Plant Extract on Spores Germination and Radial Growth of *Aspergillus Flavus*

Results presented in Figure 1 indicated that incorporation of the respective concentrations of *A. cordifolia* in the culture medium had significant effects on germination of spores, and radial growth of the test pathogen. The fungus was most sensitive to 500 mg mL<sup>-1</sup> concentration of *A. cordifolia* which demonstrated 67.24% and 80.01% inhibitions of spore germination and radial growth of the fungus respectively. In like manner, incorporation of 400 mg mL<sup>-1</sup> concentration of *A. cordifolia* extract in the growth medium impeded germination of spores of the test organism by (66.49%) and radial growth (79.09%). This was closely followed by 64.52% and 75.12% inhibitions obtained for the respective attributes by 300 mg mL<sup>-1</sup> concentration of the extract; while 100 mg mL<sup>-1</sup> extract concentration which had inhibition values of 56.38% and 68.22% for spore germination and radial growth of the pathogen respectively was the least (Fig. 1). Though

inhibition effects recorded from thiophanate-methyl were statistically ( $P < 0.05$ ) superior to values obtained for all levels of *A. cordifolia* used in the study. However, inhibition effects from all the plant extracts compared favorably with it. Also, all the rates of incorporation of the tissue extracts of *A. cordifolia* showed sufficiently ( $P < 0.05$ ) greater potentials than the control in impeding germination and mycelial development of the fungus in culture (Figure 1).

### Effects of Plant Extract on Growth and Development of *Aspergillus Flavus* in Living Yam Tissues

The results presented in Figure 2 indicated that the mean percentage incidence of *A. flavus*-induced rot on the untreated control was 100%. It also showed that the mean percentage rot disease severity recorded on the control experiment (without addition of *A. cordifolia* leaf extract) after 21 days of incubation was approximately 60.00%; suggesting therefore that the fungus was aggressively virulent. The test extract applied at 100, 200, 300, 400, and 500 mg mL<sup>-1</sup> concentrations strongly reduced the mean percentage rot disease severity from 60% recorded on the control tubers to 21.8%, 19.80%, 18.30%, 15.60% and 12.10% respectively on *A. cordifolia* leaf extract treated tubers.

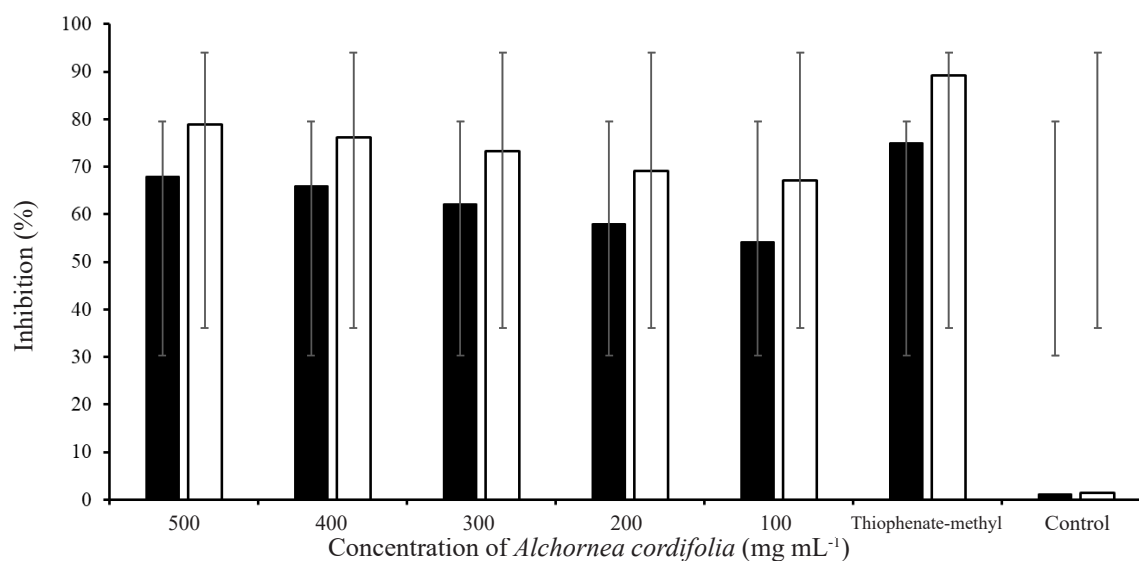


Figure 1 Effects of methanol extracts of *Alchornea cordifolia* on spore germination (■) and radial growth (□) of *Aspergillus flavus* *in vitro*.

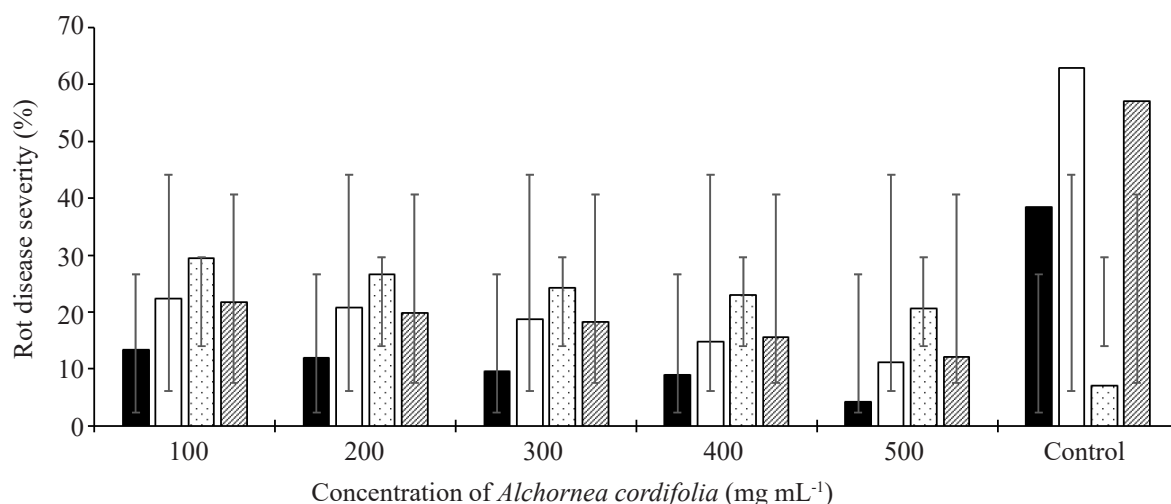


Figure 2 Percentage rot disease severity on treated yam tubers after 21 days of incubation. ■, 7 days after inoculation (DAI); □, 14 DAI; ▨, 21 DAI; and ▩, Mean effect.

## DISCUSSION

Results obtained from pathogenicity assessment in this study showed that *A. flavus* and *A. niger* were associated with rot of yam tubers in storage, however, *A. flavus* was more virulent than *A. niger*. This is in consonance with the report of other workers in Nigeria and elsewhere where these organisms have been implicated with rots of yam and other tuber crops in storage (Ogaraku and Usman 2008; Ano 2019; Gwa and Nwankiti 2018) with *A. flavus* demonstrating strong virulence (John *et al.* 2019).

Results of *in vitro* antifungal activity studies of *A. cordifolia* indicated that *A. cordifolia* exhibited appreciable fungitoxic activity against *A. flavus*. In a similar evaluation, *Shinus mole* extracts significantly retarded sporulation of *Manginiella scaetiae* causal agent of rot of inflorescence of date palm (Bouhlahi *et al.* 2021). Also, a dose-wise inhibition of radial growth, sporulation and stroma production of the rot pathogens *Botryodiplodia theobromae*, *A. niger* and *M. scaetiae* by extracts of *Cassia alata*, *A. indica*, *Cupressus Atlanta*, *Eucalyptus torquata* and *A. cordifolia in vitro* have been variously reported (Wokoma and Anaemena 2003; Tijjani *et al.* 2018; Bouhlahi *et al.* 2021). Findings in this study where different concentrations of methanol leaf extracts of *A. cordifolia*

impeded radial growth and spore germination of *A. flavus* at varying degrees is consistent with many antimicrobial assays as mentioned above. It is also in line with submissions from parallel antifungal trials where aqueous and organic extracts derived from *Piper guineense*, *A. laxiflora* and *A. cordifolia* amongst others impeded development and growth of *B. theobromae*, *Alternaria alternata*, *Rhizopus stolonifer*, *A. niger*, *A. glaucus*, *A. fumigatus* and *A. flavus* in culture (Owoseni *et al.* 2013; Akinpelu *et al.* 2015; Tripathi *et al.* 2017; Agboke *et al.* 2020).

Also, alcohol leaf extracts of *A. indica* and *Cymbopogon citratus* significantly inhibited rot development in living tissues of Irish potato (Amadioha 2004). Molotja *et al.* (2011) in a similar experiment found that *A. flavus* and *A. niger* were sensitive to stem, leaf, bark, root extracts of *A. cordifolia*. These views expressed by the afore-mentioned workers also tally strongly with our findings in this study. The observations in this study where *A. cordifolia* reduced the mean rot percentage on treated yam tubers by frustrating the growth of *A. flavus in vivo* are also consistent with the reports of Okwu and Njoku (2009) and Amienyo and Ataga (2007). However, findings in this work are not consistent with the reports documented by Nwaneri *et al.* (2020) where aqueous extracts of *A. cordifolia* was mildly fungitoxic to *B. theobromae in vivo*.

Differences in solubility of the active principles of *A. cordifolia* in water and methanol or differences in constituents of cell walls of the respective fungus may explain the differential toxicity of the test extract to *A. flavus* as observed in this study.

Our study also showed that percentage rot disease severity increases with duration of incubation and that rot reduction on the *A. cordifolia* treated tubers varies with concentration and duration of exposure of yam tissues to extract treatment. These observations are in accord with the views of several workers on storage health of cassava, sweet potato, cocoyam and yam tubers (Amadioha 2004; Okigbo and Nmeka 2005; Amadioha and Markson 2007a, 2007b; Ugwuoke *et al.* 2008; Nwachukwu and Osuji 2008; Markson 2010; Tijjani *et al.*, 2013; Markson *et al.*, 2014; Nwaneri *et al.* 2020). It also means that the active principles of the test plant are relatively stable and can afford preservation of the living tissues for a long time.

Different classes of phytochemicals including tannins, saponins, alkaloids, flavonoids, phenolic compounds and fatty acids with potent antimicrobial activities have been identified or isolated from extracts of *A. cordifolia* (Nwaoguikpe *et al.* 2014; Boniface *et al.* 2016; Sinan *et al.* 2021). Preparatory thin layer chromatography (PTLC) and column chromatographic fingerprinting on methanol leaf extract of *A. cordifolia* yielded a total of 32 and 104 fractions with 163 compounds respectively which are thought to underscore its potent bioactivity (Ebi 2001). Ellagic acid, lauric acid, methyl gallate, friedelin are reported as the active antimicrobial ingredients from tannins and phenols-rich fractions of leaf and twig tissues of the plant. In crop protection, ellagic acid and fumaric acid amongst other active phenolics from ethanol extracts of *Caryocar brasiliense* (Perqui) peels and *Vitis vinifera* were implicated for mycotoxicity against the phytopathogens *Fusarium* sp., *Alternaria solani*, *A. alternata* and *Venturia pirina* (Breda *et al.* 2016; Fraternali *et al.* 2015). Also, mycelia development, sporangia

formation, spore generation and germination of *Phytophthora sojae* causal agent of rot of soybean, *Rhizoctonia solani*, *Pythium ultimum* and *Blumeria graminis f. sp. hordei* causing powdery mildew of cereals were effectively inhibited on liquid and agar medium as well as retardation of powdery mildew on *planta* by lauric acid and its derivative glycerol monolaurate (GML) (Walters *et al.* 2003; Liang *et al.* 2021). These compounds may be responsible for the mycotoxicity by *A. cordifolia* against *A. flavus* observed in this study.

The mechanism of antimicrobial activity of extracts and compounds from *A. cordifolia* have been suggested to be linked perhaps with its high anti-enzymes activity especially inhibition of cholenesterase activity and subsequent impeding of coordination of electrical impulses amongst microbial cells and tissues leading to atrophy, paralysis and death of the target organism (Enyiukwu *et al.* 2016). Or it may be connected with cell membrane damage, mitochondrial energy disruption, central dogma abnormality in spores, and/or deregulation of key genes associated with aflatoxin B<sub>1</sub> biosynthesis (Zhao *et al.* 2023). Damage of cell membranes and leakage of radicals were reported as the mechanism underpinning lauric acid activity against *P. sojae* (Liang *et al.* 2021) and may be one of the mechanisms underscoring mycotoxicity of *A. cordifolia* against the test fungus in this study.

In this study, *A. cordifolia* demonstrated appreciable *in vitro* antifungal activity against *A. flavus* and minimized pathogen-induced rot lesions in living tissues. Data from this study showed that 400 mg mL<sup>-1</sup> and 500 mg mL<sup>-1</sup> concentrations of *A. cordifolia* gave the highest mean inhibition of spore germination, mycelia elongation and reduction of rot disease severity *in vivo*. As such the fungitoxic activities the test plant at these concentrations (400–500 mg mL<sup>-1</sup>) could be exploited in the effective control of postharvest microbial deterioration of stored tuber products.



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