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Assessment of White Yam Tuber Rot Disease and *in vitro* Management of *Aspergillus niger* in Ebonyi State, Nigeria

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Abstract

White yam (*Dioscorea rotundata* Poir) is an important tuber crop cultivated for consumption and income generation in Nigeria. Its production is severely affected by the rot disease, which causes a reduction in tuber yield. A study was carried out in Abakiliki and Ikwo Local Government Areas of Ebonyi State to assess the level of infection of tubers in the major markets; the pathogenicity of the isolates on tubers *in vivo*; the effect of aqueous extracts of five plants (leaves of *Eucalyptus globulus*, *Melaleuca cajuputi*, *Andrographis paniculata* and *Azadirachta indica*, and shoots of *Euphorbia hirta)* at 50 and 100% concentrations, the synthetic fungicides Mancozeb® and Tandem® (at the half and full recommended rates) for management of *Aspergillus niger in vitro*. A negative control without the fungicide or plant extract was included. The mean percentage of infection of tubers in the markets was 13.5%. All the pathogens isolated were fungi (*Fusarium anthophilum*, *Rhizopus stolonifer, Aspergillus niger, A. fumigatus, A. flavius, Pythium ultimum, Mucor* sp., and *Penicillum oxalicum*). *Aspergillus niger* had the highest frequency (21.4%). All plant extracts inhibited the growth of *A. niger* significantly compared to the negative control and the level was higher at 100% concentration than at 50%. The synthetic fungicides gave higher inhibition of the fungus than the plant extracts; the level of inhibition was more with Mancozeb® than with Tandem®. In conclusion, the aqueous extracts of all the plants, especially 100% *E. globulus*, showed promising activity against *A. niger*, a major pathogen that causes yam tuber rot disease.

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Introduction

Sub-Saharan Africa records more than 54 million metric tons of yam annually, mainly from Nigeria, Benin, Togo, Ghana and Côte d'Ivoire (FAO, 2015). Resource-poor farmers put in a lot of inputs and efforts in yam production, but their difficulties are further complicated by pests and diseases in the field and during storage. This is a daunting task since only crude implements and strategies are available to them. For instance, in Anambra State, Nigeria, yam farmers face the following constraints: inadequate extension services, severe pest and disease attack, high cost of hired labour, high cost of seeds, lack of improved seeds, unfavourable climatic conditions and bad road network (Udemezue and Nnabuife, 2017). These problems are further compounded by inadequate storage facilities, as reported by 50% of the farmers in a survey carried out by Adejo (2017). Dry rot is considered the most devastating of all the diseases of yam tubers in storage. Farmers are familiar with the disease, although they are unable to manage it adequately. This is complicated because Scientists have associated several organisms with it (Table 1). Other pathogens include *Colletotrichum sp.* and *Curvularia eragrostide* (Ekefan *et al.,* 2018), *Sclerotium rolfsii (*Ezeonu *et al.,* 2018) *and Candida albicans (*Hamzat, 2014). In the study by Ogbo and Agu (2015), the authors also listed *Exophiala* sp., *Fonsecaea* sp., *Graphium* sp., *Mucor circinelloides, Phialophora* sp. and *Saccharomyces* sp. Based on frequency of occurrence / reported cases, *Aspergillus* spp are associated with the disease more than the other pathogens listed.

Given that several fungi and a few bacteria are associated with the disease and farmers have very few options to tackle it, yam production seems to be a gamble. The use of thiabendazole, locally made dry gin and wood ash applied before storage were options found to protect the tubers against fungal infection in storage (Amusa *et al*., 2003). Aqueous and ethanol extracts of *Azadirachta indica* A. Juss. seeds, leaves and stem bark were found to be more effective than the synthetic fungicide Ketoconazole, against the growth of *L. theobromae (*Ezeonu *et al.,* 2018). The aqueous extract of *Oxalis corniculata* was active against *A. niger* while *Phyllanthus debilis* suppressed the growth of *P. theae* (Iqbal *et al*., 2001). Similarly, aqueous and ethanol extracts of *Cassia alata* were effective against *A. niger* (Aji *et al.,* 2018). Mycelial growth of *B. theobromae* was significantly ($P<0.05$) reduced by ethanol extracts of *C. aurantifolia*, *A. indica* and *C. alata* during the period of incubation (Nweke, 2015).

Piper guineense, *Zingiber officinale*, *A. indica, Carica papaya* and *Nicotiana tabacum* were toxic against *F. solani;* however, the first two botanicals were more potent than the others (Ekefan *et al.,* 2018). The extracts of *Parkia biglobosa, Moringa oleifera* and *Daniellia oliveri* effectively inhibited the growth of *A. niger* and *A. flavus* ($P\leq0.05$) (Liamngee *et al.,* 2015). Aqueous extracts of *M. oleifera* and *Vernonia amygdalina* inhibited mycelial growth of *A. flavus* and *B. theobromae in vitro* (Mamkaa and Gwa, 2018). The results obtained by the authors showed that *the botanicals* possess antifungal compounds that inhibit the growth of *both pathogens*. However, the extract of *V. amygdalina* was more effective than that of *M. oleifera* in managing *A. flavus*. Otegwu (2011) investigated the antifungal activity of five commonly used antidermatophytic pharmaceutical agents against spores of some phytopathogenic fungi (*A. flavus, A. niger, P. citrinum and R. stolonifer*) and found that they were effective in the order Terbinafine HCl > Fluconazole > Ketoconazole > Sodium propionate > Griseofulvin.

In Nigeria, the cultivation of yam is constrained by diseases, pests, poor storage facilities, intensive labour demand and the high cost of inputs. Production is mostly by resource-poor farmers who are ignorant about effective pest and disease management methods, and crop yields are low. Research results showing appropriate disease management techniques would be of immense benefit. There is inadequate information on the causal complex and management of white yam tuber rot disease. Research work has been carried out on the use of synthetic chemicals and botanicals to manage plant diseases. However, synthetic chemicals are effective but costly and issues of toxicity have been raised. On the other hand, botanicals are easy to use and non-toxic; many of them have inhibitory effects on pathogens *in vitro* and should be adequately tested *in vivo*. This research was conceived to contribute to the limited information that exists on the tuber rot disease complex of yam and its management. Therefore, the objectives of this study were to identify the fungal pathogens associated with yam tuber rot disease in Ebonyi State and determine the effects of aqueous extracts of selected plants on the major pathogen associated with the disease.

Materials and methods

Site of the experiment

A survey was carried out in two main markets in Ikwo and Abakaliki Local Government Areas (LGAs) of Ebonyi State, Nigeria. The preparation of plant extracts, isolation of fungi, and studies on the effects of pathogens on yam tubers in storage and effects of the extracts on *A. niger* were done at the Faculty of Agriculture Laboratories, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo.

Ebonyi State is located in the southeast of the country and the inhabitants are mostly farmers or petty traders. It shares a boundary with Cross River State and experiences two seasons: rainy from April to November and dry from December to March.

Survey

A survey was carried out in April 2020 to assess the incidence of yam tuber rot disease in the study area. Ten heaps of tubers (10 per heap) were randomly selected in each market and disease incidence was assessed as the ratio of the number of tubers with the disease symptom to the total number sampled, multiplied by 100 (%).

Preparation of plant extracts

Fresh leaves of *Eucalyptus globulus* Labill. (eucalyptus), *A. indica* (neem), *Andrographis paniculata* (Burm.f.) Wall. ex Nees (chuan xin lian) and *Melaleuca cajuputi* Powell (gelam), as well as shoots of *Euphorbia hirta* (asthma weed) were collected from trees in Abakiliki. Each plant material (50 g) was washed with tap water, surface sterilized with 1% sodium hypochlorite for one hour and sun-dried in a dust-free environment for 14 days. Each material was surface sterilized with 1% sodium hypochlorite for 15 minutes, ground using a mortar and pestle, and used to prepare its extract per 100 ml of sterile distilled water (100% concentration). The extracts were filtered into separate beakers using sterile muslin cloths and used for the *in vitro* trial.

Effects of plant pathogens on yam tubers in storage

Three tubers were surface sterilized using 3.5% sodium hypochlorite and allowed to dry. A flamed cork borer (5 mm in diameter) was used to make a shallow hole in each tuber; a 5 mm disk of the inoculum from diseased samples collected in the markets was inserted in the hole. The holes were sealed with petroleum jelly and the tubers were stored in a cool, dry place in the Laboratory (Fig. 1).

Two sub-trials were set up using the tubers. One had fast-growing fungi, while the other had those that were slow-growing. The tubers were cut at the point of inoculation for data collection at 28 days after storage (DAS) for the set with fast-growing fungi and at 42 DAS for the second set. Data were collected on the length of the rot along the surface of the tubers, the depth of penetration, and the width at the point of inoculation, using a transparent ruler.

The front edge of the advancing rot on the tuber was cut with some adjoining healthy tissue using a cork borer and placed on solidified potato dextrose agar (PDA). Three portions were placed equidistant to each other on a plate and replicated three times.

The PDA was prepared according to the manufacturer's instructions and the plates were incubated at room temperature for 14 days. The pathogens were sub-cultured on PDA to obtain pure isolates for identification. The fungal colonies were identified microscopically and macroscopically with the aid of the literature.

Effects of plant extracts on A. niger in vitro

The experiment was laid out using a completely randomized design with 15 treatments replicated three times. The treatments consisted of 50 and 100% concentrations of aqueous extracts of the five botanicals, two synthetic chemical checks (Tandem® and Mancozeb) and control (0%) without the extract or chemical. Tandem® is a systemic and contact fungicide; Mancozeb is a contact fungicide. The effect of the extracts on the fungus was determined using the poisoned food technique, as reported by Lum *et al.* (2019). The radii of the mycelia were obtained using a transparent ruler at an interval of 24 hours from two to eight days after inoculation. The percentage inhibition of the pathogen was calculated using the following formula: $I = ((Rc–Rt)/Rc) \times 100\%$

Where

I = Percentage inhibition of fungus growth.

Rc= Radius of fungus growth in the control plate.

Rt= Radius of the fungus in the treated plate.

Data analyses

All the data collected were subjected to analysis of variance and the means were separated using Fisher's least significant difference (LSD) at $P \leq 0.05$. The Statistical Package used was GenStat 2nd Discovery Edition.

Results

Survey of the Incidence of Yam Tuber Rot Disease in Abakiliki and Ikwo LGAs

Results of the survey revealed that the disease incidence was 14% in Abakiliki LGA and 13% in Ikwo. The rates of infection were similar in both locations (mean=13.5%). This was based on tubers deemed marketable by the farmers.

Table 1. Organisms Associated with Yam Tuber Rot Disease in Africa (1978–2020).

No	Key causal organisms	Sources
1	*Aspergillus* spp. (*A. ochraceus, A. tamarii, A. flavus, A. niger and A. fumigatus)*	Nwankiti and Arene (1978), Nweke and Enujeke (2006), Otegwu (2011), Nwawuisi *et al.* (2012), Hamzat (2014), Ogbo and Agu (2015), Liamngee *et al.* (2015), Ekefan *et al.* (2018), Ezeonu *et al.* (2018), Aji *et al.* (2018) and Mamkaa and Gwa (2018).
2	*Fusarium* spp. (*F. moniliforme, F. oxysporum* and *F. solani)*	Nwankiti and Arene (1978), Nweke and Enujeke (2006), Nwawuisi *et al.* (2012), Ogbo and Agu (2015) and Ekefan *et al.* (2018).
3	*Penicillium spp*. (*P. citrinum, P. sclerotigenum* and *P. oxalicum)*	Nwankiti and Arene (1978), Otegwu (2011), Nwawuisi *et al.* (2012), Hamzat (2014) and Nweke and Enujeke (2006).
4	*Botryodiplodia theobromae*	Nwankiti and Arene (1978), Liamngee *et al.* (2015), Ekefan *et al.* (2018) and Mamkaa and Gwa (2018).
5	*Lasiodiplodia theobromae*	Ogbo and Agu (2015) and Ezeonu *et al.* (2018).
6	*Rhizopus* spp. (*R. stolonifer* and *R. nigricans)*	Otegwu (2011), Nweke and Enujeke (2006) and Aji *et al.* (2018).

Isolation of pathogens from rotten yam tubers

The organisms isolated and identified on the rotting yam tubers were *Fusarium anthophilum*, *Rhizopus stolonifer*, *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Pythium ultimum*, *Mucor* sp. and *Penicilium oxalicum* (Fig. 2). Among the fungal isolates, *R. stolonifer* and *A. niger* had the highest frequency of occurrence and were followed by *P. ultimum* and *Mucor* sp. The other four species (*F. anthophilum*, *A. fumigatus*, *A. flavus*, *Penicilium oxalicum*) had the lowest frequency.

In general, the genus *Aspergillus* occurred most frequently and *A. niger* was the most common species. It is a filamentous fungus that forms hyphae that make them appear like small plants. The macroscopic observation revealed that its growth is initially white but changes to black after a few days, and it produces conidial spores. The edges of the colonies appear pale yellow and produce radial fissures. The microscopic view revealed that it has smooth coloured conidiophores and conidia. The conidiophores are protrusions from septate and hyaline hyphae. The conidial heads appear radial and split into columns (biseriate). The characteristic heads of the fungus are globose and large.

Fig. 1. Section of yam tubers inoculated with rot fungi (Pathogenicity Test).

Effects of fungi pathogens on yam tubers in storage

Results of the study on effects of the pathogens on the yam tubers in storage revealed that they caused rot (Figs 3 and 4). The nature of the rot was different for the various pathogens; *A. niger* had the highest frequency and the rot it caused covered a larger area of the tubers than those from other organisms since it was longer, wider and deeper than the others. The pattern of infection was in the following order: *R. stolonifer* > *Mucor* sp for the fast growing isolates; *A. niger* > *F. anthophilum* > *A. flavius* = *P. ultimum* > *A. fumigatus* for the slow growing ones.

Antifungal activity of plant extracts on *A. niger*

There were significant differences (P≤0.05) among the treatments throughout the sampling period (Fig. 5). In general, the aqueous extract of all the plants at both tested concentrations significantly inhibited the growth of *A. niger* compared to the control with no plant extract or synthetic chemical.

The inhibitory effect was more at 100% concentration than at 50% for the different plant extracts but none of them completely inhibited the growth of the fungus. The inhibitory effect of all the plants on the pathogen was less than 50% throughout the period of sampling. The recommended rate of Mancozeb caused complete inhibition of the fungus; 50% of the recommended rate resulted in more inhibition of the fungus than both concentrations of Tandem and all the plant extracts. However, the synthetic fungicides at both rates significantly inhibited the growth of the fungus compared to the plant extracts.

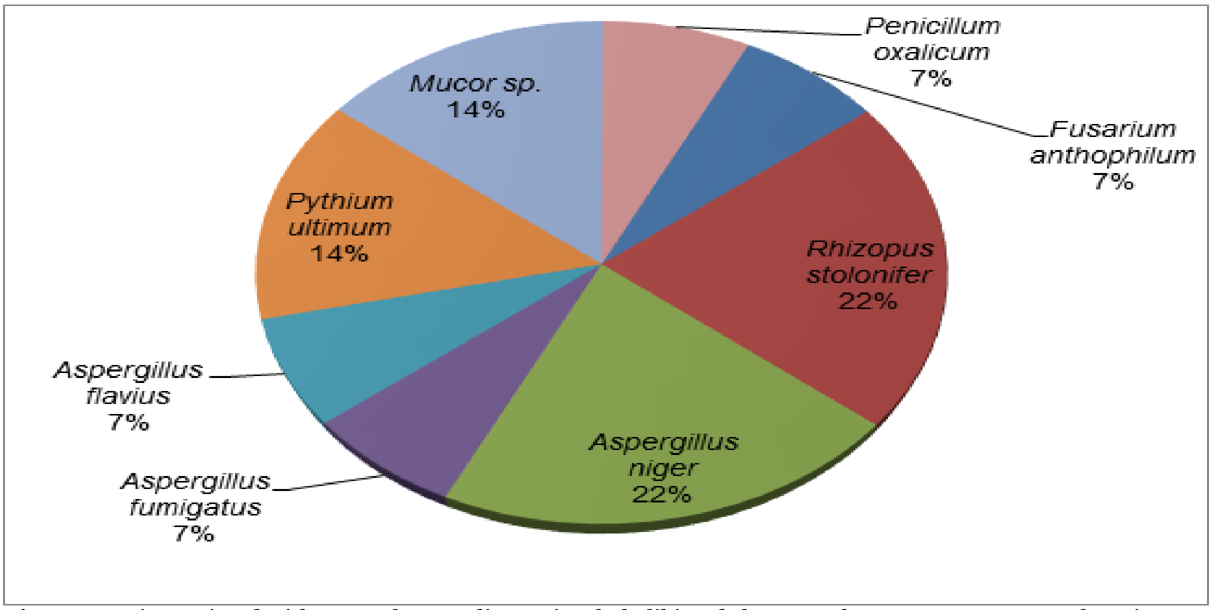

Fig. 2. Fungi associated with yam tuber rot disease in Abakaliki and Ikwo Local Government Areas, Ebonyi State, Nigeria.

At 72 hours after inoculation (HAI), the highest level of fungal inhibition was recorded for the different plant extracts. Among them, *E. globulus* at 100% concentration had the highest inhibitory effect (48%), followed by 100% *A. indica* (41%).

The general pattern observed among the treatments was Mancozeb at recommended rate > 50% Mancozeb > Tandem at recommended rate > 50% Tandem > 100% *E. globulus* > 100% *A. indica* > 50% *A. indica* = 50% *E. globulus* > 100% *E. hirta* = 50% *E. hirta* > 100% *A. paniculata* > 50% *A. paniculata* > 100% *M. cajuputi* = 50% *M. cajuputi*. From 96 to 144 HAI, the antifungal activity of the plant extracts decreased and 100% *E. globulus* consistently inhibited the growth of the fungus better than all the other extracts.

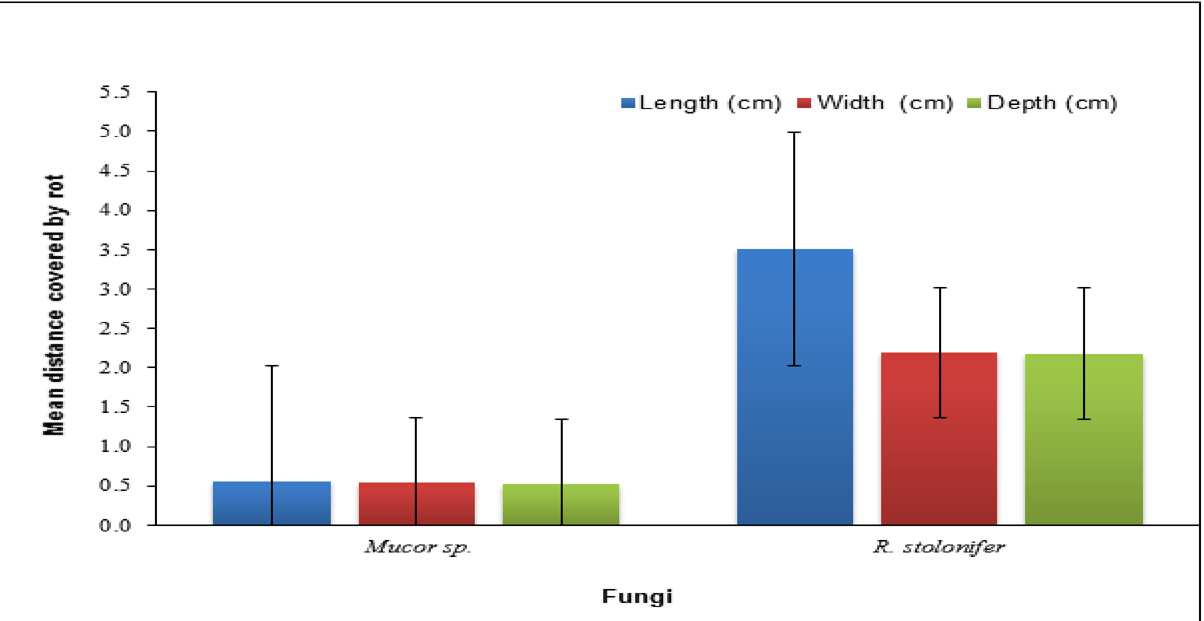

Fig. 3. Effects of rot pathogens on yam tubers at 28 days after inoculation.

Discussion

The results of the survey indicated that the disease was present in tubers obtained from Abakiliki and Ikwo LGAs. They provided information on the type of tubers planted and the pathogens that infect them during storage. All the pathogens identified were fungi (*Fusarium anthophilum*, *Rhizopus stolonifer*, *A. niger*, *A. fumigatus*, *A. flavus*, *Pythium ultimum*, *Mucor* sp. and *Penicilium oxalicum*). With the exception of *F. anthophilum, P. ultimum* and *Mucor* sp. the organisms had been previously associated with dry rot of yam in Nigeria (Aji *et al.*, 2018; Ekefan *et al.*, 2018; Gwa and Ekefan, 2018). Among the pathogens, *A. niger* was the most frequently isolated. *Aspergillus niger* had been previously reported as a major pathogen that causes rot in yam (Gwa and Ekefan, 2018). Several pathogenic fungi have been associated with the disease. In an earlier study carried out in Abuja, Nigeria, the authors reported that fungi were among the organisms with the highest incidence of dry rot disease on white yam tubers in storage (Anjorin *et al.*, 2014). These pathogens cause rot in stored tubers and this could lead to rotting of setts after planting.

The results revealed that the synthetic fungicides and aqueous extracts of all the plants tested inhibited the mycelial growth of *A. niger* significantly ($P<0.05$) compared to the control. This suggests the presence of fungicidal properties that were potent enough to inhibit the mycelial growth of the fungus. Among the plants, *E. globulus* and *A. indica* had better activity against the fungus than the others; this may be due to the availability of a higher concentration of the active compounds in these plants. Also, the activity of these plant extracts was more at 100% than at 50%; therefore, the treatments were more potent at a higher concentration than when low. These observations also indicate that the inhibitory effect of the aqueous extracts on the fungus depends on the type of plant and the concentration. Similar findings were reported from previous studies on the evaluation of plant extracts for management of pathogenic fungi both *in vitro* and *in vivo* (Okigbo and Emeka, 2010; Gwa and Akombo, 2016; Ekefan *et al.*, 2018; Gwa and Ekefan, 2018; Lum *et al.*, 2019).

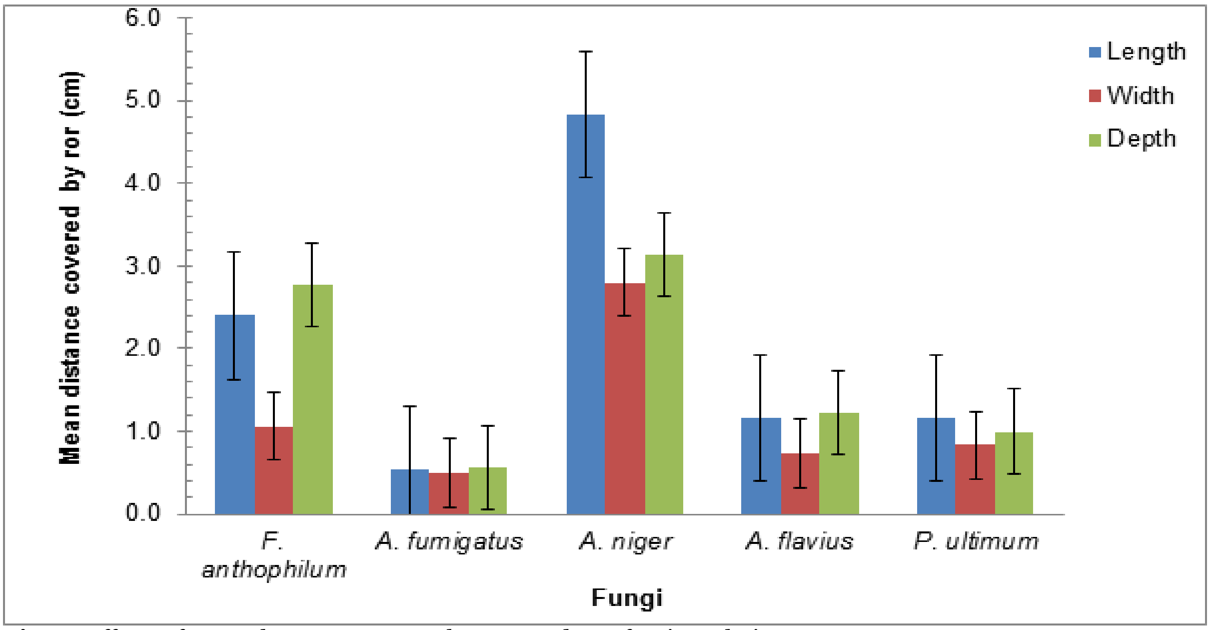

Fig. 4. Effects of rot pathogens on yam tubers at 42 days after inoculation.

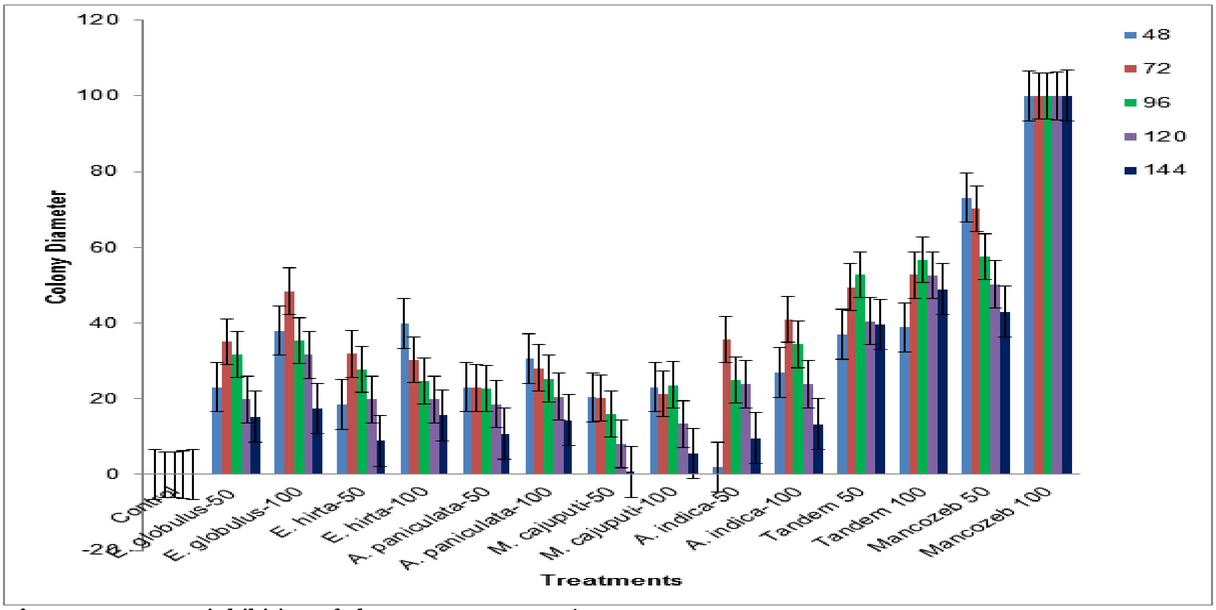

Fig. 5. Percentage inhibition of plant extracts on *A. niger*.

The synthetic fungicides Mancozeb and Tandem had higher inhibitory effects on the fungus than the plant extracts. The fungicide Mancozeb was even more effective than Tandem at both concentrations tested. The recommended rate of Mancozeb gave complete inhibition of the fungal mycelium throughout the test period.

Conclusion

Yam tubers are susceptible to different fungal pathogens in storage: *F. anthophilum*, *R. stolonifer*, *A. niger*, *A. fumigatus*, *A. flavus*, *P. ultimum*, *Mucor* sp. and *P. oxalicum*. The major pathogen identified to cause dry rot of yam tubers in storage was *A. niger*. Aqueous extracts of fresh leaves of *E. globulus*, *A.*

indica, *A. paniculata*, and *M. cajuputi* as well as shoots of *E. hirta* possess antifungal properties that were capable of inhibiting the growth of *A. niger* with the highest rate recorded at 72 HAI. However, the extracts of *E. globulus* and *A. indica* were the most potent. The management of the pathogen was most effective with the synthetic fungicide Mancozeb at the recommended rate. However, the use of synthetic fungicides is being discouraged due to high cost, toxicity to humans and the environment. These plants should therefore be further screened for use as alternatives to synthetic fungicides since they are cheaper and more environmentally friendly.

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