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Efficacy of Plant Extracts on Management of Anthracnose Disease of Yam (*Dioscorea* spp.) in Bamenda, Cameroon

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Abstract

Anthracnose is a devastating disease in yam (*Dioscorea* spp)-growing regions, causing heavy yield losses. This study evaluated the efficacy of plant extracts as alternatives to chemical fungicides used to control yam anthracnose. Yam varieties in five species were used to

establish an experiment in a split plot randomized block design at the Research Farm of College of Technology, University of Bamenda from March to September 2023. Scent leaf (*Ocimum gratissimum*) infusion, neem (*Azadirachta indica*) seed oil and chemical fungicide (mancozeb) were used to treat yam varieties at 2-weekly interval for four months. Yams were assessed for anthracnose incidence, severity and yield. Fungal isolates from infected plants were identified using morphological traits and pathogenicity tested. Mycelia fragments were inoculated on potato dextrose agar (PDA) media, pre-treated with plants extracts and mancozeb, to test *in-vitro* inhibition of mycelia growth. Incidence of anthracnose was significantly higher ($p \leq 0.05$) on untreated plants (40 to 80%) than treated plants (0 to 50%) among yam varieties. Severity of anthracnose was not significantly different ($p \leq 0.05$) amongst yam varieties treated with *O. gratissimum* infusion (25±0.07%, *D. rotundata*) and mancozeb (20±0.07%, *D. rotundata*). Tuber yield was lowest among untreated plants (1.8±0.8 kg, *D. alata*) and highest among plants treated with *O. gratissimum* infusion (5.3±0.25 kg, *D. alata*) in all yam species. Fungal isolates were identified as *Colletotrichum gloeosporioides* and its pathogenicity for anthracnose confirmed. Plant extracts at 100% concentration inhibited mycelia growth (92%, *D. dumetorum*) with similar ($p \leq 0.05$) efficacy to mancozeb (100%, *D. dumetorum*). This study suggests *O. gratissimum* infusion and *A. indica* seed oil as good alternatives of chemical fungicides in integrated management of yam anthracnose, with broader implications in global yam-growing areas.

Keywords: yam (*Dioscorea* spp.), plant extracts, anthracnose disease, integrated management, yield, Cameroon

1. Introduction

Yam (*Dioscorea* spp.) is a major source of food and income for millions of people in tropical and sub-tropical areas of the world, particularly in West and Central Africa with over 97 % of the world's total production of 88 million metric tons (MT) (FAO, 2022). While Nigeria stands out as the world's highest yam producer with over 61 million MT, Cameroon is at the 6th rank with 543280 MT, cultivated on a land surface area of 72000 ha annually (FAO, 2022). In Cameroon average yield of yams is 7.5 MT/ha – lower than the world's average yield of 8.4 MT/ha and it is ranked third among root and tuber crops after cassava and cocoyam/taro based on production quantities (Ngo-Ngwe *et al*., 2014; FAO, 2022). Main yam species cultivated in Cameroon are *D. rotundata, D. cayenensis, D. dumetorum* and *D. alata*. (Njukeng *et al*., 2014; Azeteh *et al*., 2019).

Yam is important because of its tubers which are a staple food for over 300 million people in the tropics and subtropics (Ngo-Ngwe *et al*., 2015; Siadjeu *et al*., 2018). The tubers are a good source of carbohydrate, protein, fibre, vitamins, minerals and have a low glycemic index which offers consumers protection against obesity and diabetes (Njukeng *et al*., 2014). Furthermore, yam cultivation greatly contributes in food security and poverty alleviation in Cameroon.

Despite its importance, optimum production of yam is hindered by many constraints including pests and diseases. Among these diseases, anthracnose (dieback) caused mainly by the fungi *Colletotrichum spp and Lasiodiplodia theobromae*, is of economic importance as it has been reported to reduce the crop yield by 50 to 90 % under favorable conditions for pathogen infection, establishment, and disease development (Okon *et al*., 2022). Among yam

species, water yam *(D. alata)* is reported to be more susceptible to anthracnose disease than other species (Egesi *et al*., 2007).

Anthracnose affects mostly leaves, but also other parts of the plant where symptoms appear initially as small dark necrotic concentric spots with yellow hallow which expand and coalesce leading to extensive necrosis and chlorosis as the disease progresses (Akem, 1999; Egesi *et al*., 2007). The symptoms vary with yam species and typical on *D. alata* cultivars include circular black spots on leaf surfaces, expanding to leaf edge necrosis and then progressing to vine blackening and tip die-back. On other yam varieties such as *D. rotundata and D.cayenensis* typical symptoms are black circular spots randomly distributed on leaf surfaces with extensive defoliation and vine blackening from severe infection (Okon *et al*., 2022). Anthracnose disease reduces the photosynthetic efficiency of yam plants by destroying the leaves and stems, leading to severe limitation in tuber production. Infected plants produce several smaller size tubers instead of few large ware yams.

Conventional strategy of management of anthracnose on crops is with the use of synthetic fungicides which leave residues on the environment and crops. In addition, some of these fungal strains easily develop resistance to these chemical fungicides when the same formulation is used frequently. Based on the fore-going, there is the need to consider integrated disease management practices with the goal to mitigate the effects of excessive use of synthetic fungicides in agriculture (Richard et al., 2005; Kuberan *et al*., 2012). In integrated disease management, alternatives to synthetic fungicides include botanicals which have antifungal properties and are environmentally friendly, cost effective and readily available for the control of anthracnose (Amadioha, 2000; Agrios, 2005).

There is lack of adequate information on the use of botanicals for the management of yam anthracnose in the field and *in-vitro* in Cameroon; hence there is need to evaluate the effect of plant extracts in the management of this plant disease. This study which has broader implications in sub-Saharan Africa and other yam growing areas in the tropics and sub-tropics, seeks to investigate the efficacy of some common fungitoxic botanicals in the management of yam anthracnose disease in Cameroon.

2. Materials and Methods

2.1 Acquisition of Yam Germplasm and Planting

Tubers of five yams species with a minimum weight of 150 g each, for *D. bulbifera* and 500 g for *D. alata, D. bulbifera, D cayenensis, D. dumetorum and D. rotundata* were bought from the food market in Bamenda. The tubers were stored at ambient temperature of 23±5 ^{o}C on dry shelves under shade for 8 weeks to allow them break dormancy before planting (Figure 1). The yam germplasm collection was used to set up an experiment at the Research Farm of the College of Technology (COLTECH) of the University of Bamenda, Cameroon, located between latitudes 4^{o}50$^{'}$ and 5^{o}20$^{'}$N, and longitudes 10^{o}35$^{'}$ and 11^{o}59$^{'}$E on an altitude of 1600 to 2000 meters above sea level (Ndenecho, 2010).

Figure 1. Seed yams that have broken dormancy prior to planting. (a) *D. rotundara,* (b) *D. alata,* (c) *D. dumetorum,* (d) *D. bulbifera,* and (e) *D. cayenensis*

Yam species in the trial received four treatments including; T_0 (no treatment with pesticides) T_1 (treatment with seed oil of neem – *Azadirachta indica,* family *Meliaceae),* T_2 (Treatment with the synthetic fungicide Mancozeb) and T_3 (treatment with aqueous extract of scent leaf – *Ocimum gratissimum,* family *Lamiaceae*) (Figure 2). The experiment was arranged in a split plot randomized block design with four replications each containing five yam species and four treatments (Figure 2). Each replicate consisted of 20 experimental units measuring 6 m x 50 m giving an area of 300 m^2, with an inter-replicate space of 2 m. All the four replications covered a land surface area of 1500m^2.

Prior to planting, whole bulbils of *D. bulbifera* with a minimum weight of 100 g and setts of 200 to 300 g sliced from large ware yam tubers of other species using a sterilized knife were prepared. The yam setts were pre-treated by immersing in a pesticide solution composed of an insecticide and a fungicide for 5 minutes and air-dried under shade at ambient temperature (23±5 ^oC) for 48 hours before planting. This treatment was used to disinfect and protect the setts from damage by pests. At planting, five setts of each yam species were planted per experimental unit at 1.25 m x 2.5 m spacing along and between units, respectively (Figure 2). Poultry manure was applied at the rate of 1 kg per plant, during planting, to increase soil fertility. Staking was done for each plant and standard agronomic practices such as manual weeding and trailing of vines was carried out as required.

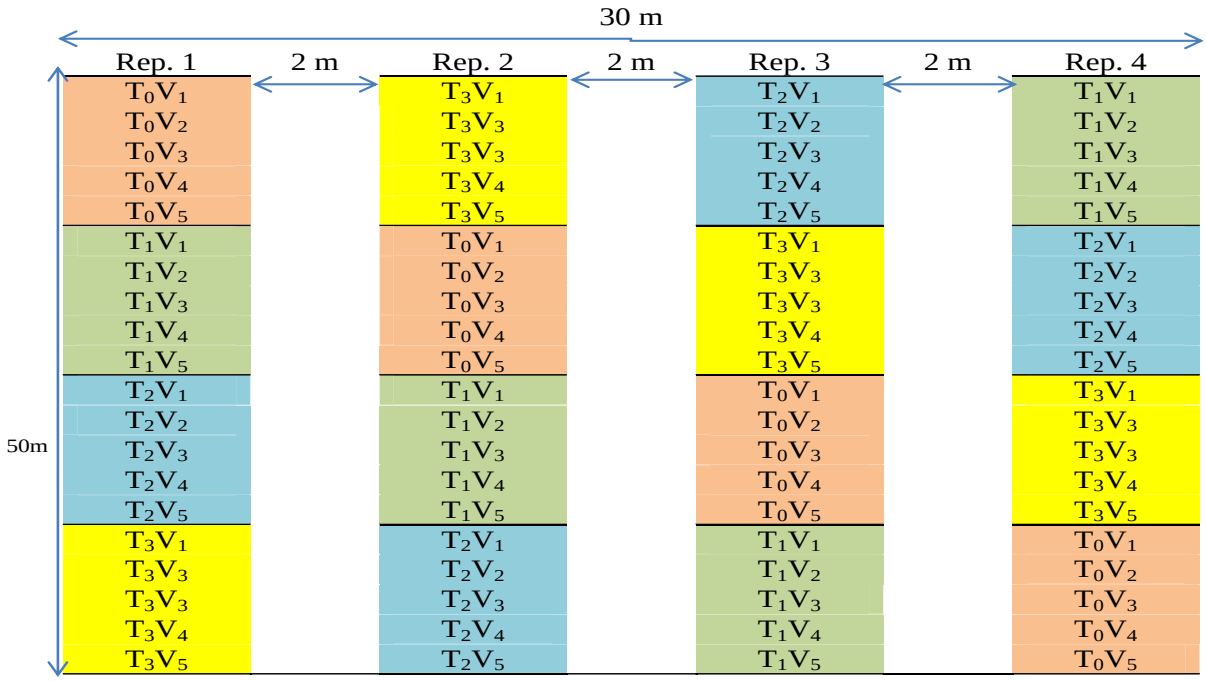

Figure 2. Field layout of the experiment

T_0 represents units with no fungicide treatment (negative contro), T_1, T_2 and T_3 represent treatment with *A. indica seed* oil, Mancozed and *O. gratissimum* extract, respectively. V_1, V_2, V_3, V_4 and V_5 represent *D. rotundata, D. dumetorum, D. cayenensis, D. bulbifera*, and *D. alata*, respectively

2.2 Fungicides Preparation and Application

Seed oil from *A. indica* was diluted by dispensing 240 ml of the oil (Figure 3) and 20 mL of tween-20 in water and mixing thoroughly to obtain 16 L of spray mix solution, which was stored at ambient temperature (23±5 ^{o}C) under shade. The detergent helped to emulsify the seed oil in water for a homogeneous spray mixture to be obtained. Fresh leaves (1.2 Kg) of *O. gratissimum* were dusted by washing under running tape water, and air-dried at room temperature (23±5 ^{o}C) under shade for 72 hours before crushing into powder using a blender (Figure 3). Eight hundred grams of the powder was added into 16 L of water, stirred thoroughly and allowed to infuse for 48 hours. The aqueous infusion obtained was then filtered by passing through a strainer and preserved at 10^{o}C in a refrigerator pending usage. The infusion from *O. gratissimum* and diluted seed oil of *A. indica* were used to drench yam leaves and vines at their designated experimental units. Following the manufacturer's instructions, 100 g of Mancozeb (WP) was dissolved in water to make a 16 L spray mixture and used to spray the yam plants on designated experimental units (Figure 3). This treatment (with botanicals and mancozeb) was carried out simultaneously, immediately the first symptoms of anthracnose appeared at one month after planting (MAP), and continued at a 2-weekly interval for a period of four months.

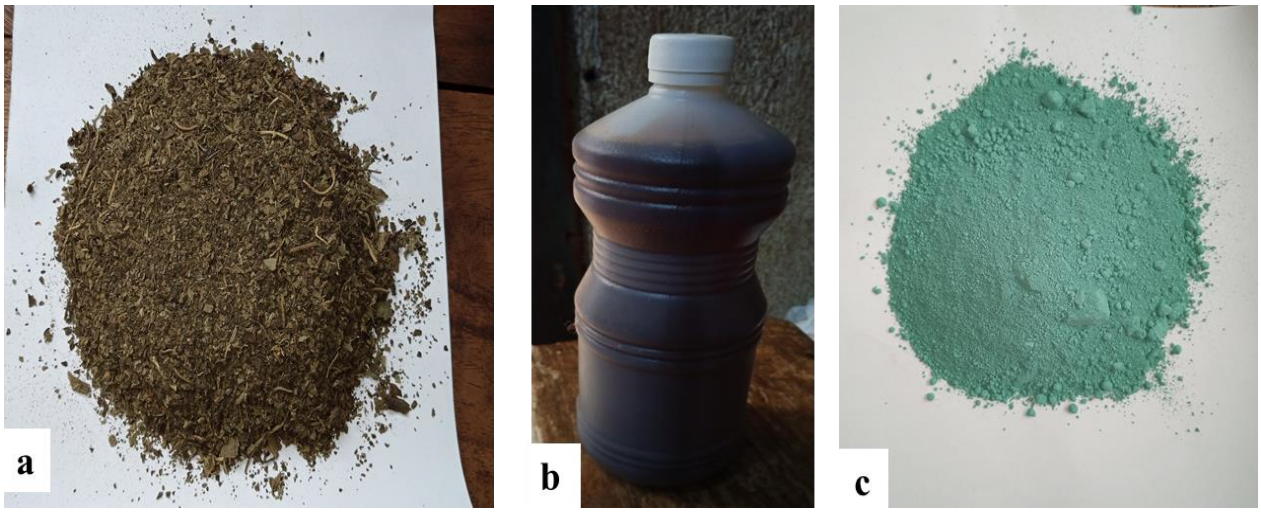

Figure 3. Botanicals and chemical fungicides used in the experiment

(a) Ground dry leaves of *O. gratissimum* (b) Undiluted seed oil of *A. indica* and (c) Mancozeb powder (synthetic fungicide)

2.3 Assessment of Anthracnose Disease and Crop Yield

At four month after planting yam plants in the trial were assessed for incidence and severity of anthracnose disease symptoms in the different treatments. The disease incidence was determined as percentage of yam plants with anthracnose disease symptoms relative to the total number of plants assessed using the formula;

$$Incidence(\%) = \frac{number\ of\ plants\ with\ disease\ symptoms}{Total\ number\ of\ plants\ assessed} x \frac{100}{1}$$

Disease severity was also evaluated using ordinal rating scale of 1 – 5 (Yousaf *et al*., 2018), where: 1 = No symptoms, 2 = leaf lesions covering 1 % – 25 % of total leaf surface, 3 = leaf lesions covering 26 % – 50 % of total leaf area, 4 = lesions covering 51 % – 75 % of total leaf area and 5 = lesions covering 76 % – 100 % of total leaf area. Cooke's formula (2006), was used to calculated percentage diseases severity as:

$$Disease\ severity\ (\%) = \frac{Area\ of\ plant\ leaf\ tissue\ affected}{Total\ leaf\ area} x \frac{100}{1}$$

At maturity, ware yam tubers per treatment were carefully dug up, cleaned, weighed using a scale balance and weight recorded. Bulbils of *D. bulbifera* were also gathered per treatment weighed and weight recorded. Yield was assessed as average weight of tubers per treatment per species.

2.4 In-vitro Evaluation of the Effects of A. indica Seed Oil and O. gratissimum Infusion on Fungal Pathogen of Yam Anthracnose

2.4.1 Preparation of Potato Dextrose Agar (PDA) Medium

Potato dextrose agar (PDA) culture medium used for the experiment was prepared by cooking 200 g of peeled, washed and diced potato in 200 ml of sterile distilled water (sdH_2O) for 20 min. Cheese cloth was used to strain the potato infusion. To prepare a 1 L of PDA, 500 mL of sdH_2O was poured into a conical flask and stirred using a magnetic stirrer. Pre-prepared potato infusion was added, followed by 20 g of dextrose sugar and 20 g of agar while stirring until a homogenous mixture was obtained. Furthermore, 200 mg of Calcium carbonate was added to produce a clear growth medium and more sdH_2O added and stirred to make up the volume to 1000 mL of a homogeneous mixture. The final pH was set at 5.6 at a temperature of 25 ^oC, and the culture medium obtained was sterilized by autoclaving at 121^o C and pressure of 115PSI for 20 min and allowed to cool down to 50^o C. Nystatine (20 mg), Penicillin (250 mg) and Ampicillin (250 mg) were further added in the medium to deter bacterial growth. Finally, 20 mL of PDA produced was dispensed into each sterile petri dish (Bush *et al*., 2006), covered and allowed to cool in an inverted position in the Laminar flow chamber prior to inoculation.

2.4.2 Isolation and Identification of Fungal Pathogens of Anthracnose from Infected Plants

Yam leaves with necrotic lesions were collected from the trial farm on all the five yam species, labeled and surface-sterilized using 0.1 % sodium hypochlorite for 3 min and 70 % alcohol for 1 min. The leaves were rinsed thrice using sterile distilled water and moisture eliminated from the surface by blotting with dry sterile tissue paper. Flame-sterilized surgical blades were used to cut two 5 mm^2 sections from each infected leaf sample (including infected portions) and placed on pre-prepared solidified PDA medium in petri dishes. Leaf samples from each species were inoculated separately, petri dishes covered, sealed with tape and labeled. Inoculated petri dishes were incubated at room temperature (23±5 °C) in growth chamber and examined daily for five days to record emergence of colonies. Sub-culturing was carried out three consecutive times at four days interval in PDA to obtain pure strains of the pathogen (Fokunang *et al*., 1995; Barnett & Hunter, 1998). Morphological characteristics of mycelia fragments teased in lactophenol cotton blue on a slide were observed under a compound microscope to identify the fungal isolates. The identity of the fungal isolates was based on growth rate, mycelia pattern and colour, and form of conidia (Ramirez, 1982).

2.4.3 Inhibitory Effect of Plant Extracts on Mycelia Growth of Fungal Isolates

Pre-prepared infusion from *O. gratissimum* and seed oil solution from *A. indica* (serving as mother solutions) were further separately diluted using sdH_2O into sub-solutions of 25%, 50 % 75 % and 100 % concentration. Each of the four concentration levels of these plant extracts and mancozeb (6.7 g per litre of sdH_2O) were separately dispensed at the rate of 5 mL per petri dish into five separate petri dishes (corresponding to five yam species in the study), mixed with melted PDA and allowed to solidify (Okigbo & Emoghene, 2003). Mycelia discs (6 mm in diameter) obtained from 4 days old pure culture were inoculated into

the petri dishes and incubated at 27 ^{o}C. Petri dishes containing only PDA and fungal culture served as the control. For each petri dish, measurement of the zone of inhibition on colony diameter was recorded for five consecutive days. Percentage inhibition of fungal growth was determined using Whipps (1987) method, as:

$$Inhibition\ (\%) = \frac{R1 - R2}{R1} x \frac{100}{1}$$

Where R1 = Furthest radial distance of fungus in control plates (PDA only) and R2= Furthest radial distance of fungus mycelium in treated dishes.

2.5 Pathogenicity Test

In the screen house, isolates of the identified fungus were tested for pathogenicity of anthracnose on five yam species (*Dioscorea alata*, *Dioscorea rotundata*, *Dioscorea cayenensis*, *Dioscorea dumetorum* and *Dioscorea bulbifera*). Four out of five healthy two months old plants of each yam species planted in polyethene pots were inoculated with conidial suspension prepared from 10 days old culture of the fungus isolated from yams in the trial. The concentration of spores in the conidial suspension was adjusted to 4×10^{4} spores /mL in distilled water using a microscope and haemocytometer. Inoculation was done by spraying the spore suspension on the healthy plants using a hand atomizer. One among the five plants per yam species was sprayed with distilled water to serve as the negative control. Inoculated plants and the control were covered with transparent polyethene bags and examined daily to record the appearance of symptoms of anthracnose. Lesion diameter (LD) was measured using a transparent plastic ruler and disease incidence was expressed as the percentage of infected plants relative to the total number of plants inoculated (Doungous *et al*., 2022). Fungal isolates from infected yams in the screen house were further identified using morphological traits

2.6 Data Analysis

Data recorded on disease incidence, disease severity, inhibition of mycelia growth and yield was subjected to analysis of variance (ANOVA) using statistical software package JMP 11. The mean values were separated using Tukey's least significant difference (LSD) pot-hoc test at 95 % confidence interval. The results were presented in tables and figures.

3. Results

3.1 Effect of Plant Extracts on Anthracnose Disease of Yam

On stems, leaves and petioles of infected yam plants in the trial farm early symptoms of anthracnose disease observed included small round dark or brown spots (2 to 20 mm in diameter) with slight depression in the center, which later turned grey-white, with a yellow halo surrounding them (Figure 4b). As the disease progressed the spots merge to form patches leading to extensive necrosis (Figure 4e & f). These symptoms were first observed on *D. alata* and *D. rotundata*, followed by the other species. At harvest heavily infected plants produced many small-sized tubers instead of a few larger ones (Figure 4c).

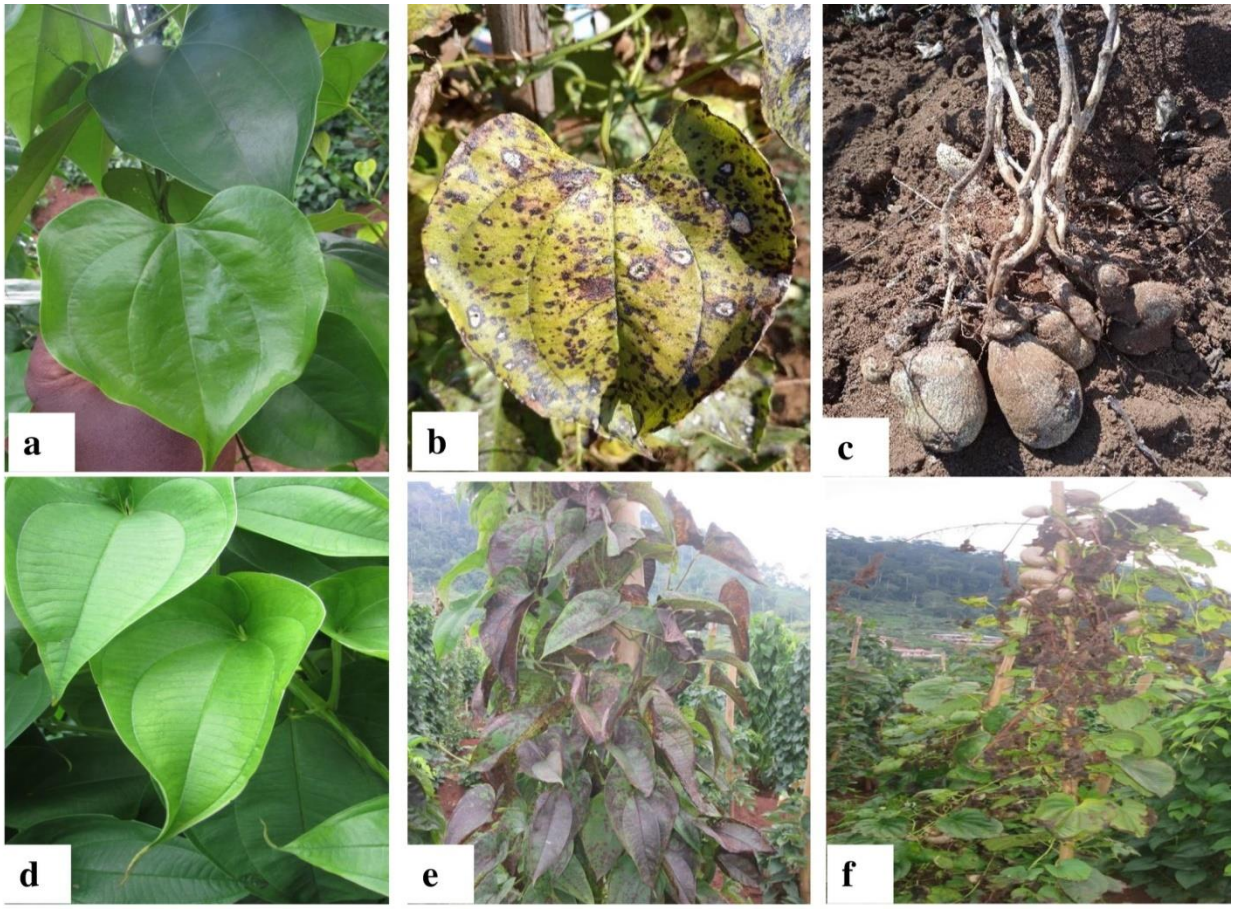

Figure 4. Presentation of anthracnose symptoms on yam plants

(a) Healthy *D. cayenensis* (b) Brown to dark concentric blotches with chlorotic hallows on leaves of *D. cayenensis*, (c) Small sized tubers in *D. cayenensis*, (d) Healthy *D. alata*, (e) Extensive necrosis on *D. alata*, (f) necrotic leaves and vine of *D. bulbifera*

The incidence of anthracnose symptoms was significantly higher ($P \leq 0.05$) among untreated yams (80±0.12%, in *D. rotundata*) than all those treated (0 %, in *D. dumetorum* & *D. bulbifera*) with plants extracts or mancozeb (Table 1). Amongst treated yams the incidence of anthracnose symptoms was least (0 %, $P \leq 0.05$) on mancozeb-treated plants as compared with those treated with *A. indica* seed oil (20 %) and *O. gratissimum* extract (20 %), particularly for *D. dumetorum* and *D. bulbifera.* There was no significant difference ($P \leq 0.05$) in the incidence of anthracnose symptoms on *D. cayenensis, D. rotundata and D. alata* treated with mancozeb (40±0.75%, 20±0.07% and 40±0.25%) and extract of *O. gratissimum* (40±0.75%, 20±0.75, and 40±0.68%), respectively. The incidence of anthracnose disease on plants treated with *A. indica* seed oil was similar to that of plants treated with mancozeb and *O. gratissimum* extract among plants of *D. rotundata* (50±0.09 %) and *D. alata* (40±0.68 %). There was no significant difference ($P \leq 0.05$) in the severity of anthracnose disease symptoms observed on four yam species (*D. rotundata, D. cayenensis, D. dumetorum* and *D. bulbifera*) treated with mancozeb, *O. gratissimum* extract and *A. indica* seed oil (Table 1). However, the disease severity was significantly higher in all untreated units compared to fungicide-treated

units (Table 1). Among untreated units the highest disease incidence was recorded on *D. rotundata* (80±0.12 %) while the highest severity was recorded on *D. alata* (80.3±0.09%).

Table 1. Incidence and severity of anthracnose on five yam species treated with plant extracts and chemical fungicide (mancozeb)

Incidence (%)*	**Yam species**				
Treatments	*D. rotundata*	*D. cayenensis*	*D. alata*	*D. dumetorum*	*D. bulbifera*
T_0(No treatment)	80±0.12^{a}	50±0.25^{a}	75±0.34^{a}	40±0.13^{a}	40±0.68^{a}
T_1(*A. indica* seed oil)	55±0.09^{b}	35±0.09^{b}	40±0.68^{b}	20±0.09^{b}	20±0.68^{b}
T_2(Mancozeb)	40±0.75^{bc}	20±0.07^{c}	40±0.25^{b}	00±00^{c}	00±00^{c}
T_3(*O. gratissimum* infusion)	40±0.75^{bc}	20±0.75^{c}	40±0.68^{b}	20±0.60^{b}	10±0.22^{b}
Severity (%)*	**Yam species**				
Treatments	*D. rotundata*	*D. cayenensis*	*D. alata*	*D. dumetorum*	*D. bulbifera*
T_0 (No treatment)	75±0.09^{a}	75±0.09^{a}	81.25± 0.09^{a}	62.5±0.08^{a}	62.5±0.08^{a}
T_1(*A. indica* seed oil)	32.5±0.08^{ab}	40±0.07^{b}	42.5±0.08^{b}	30±0.07^{b}	43.75±0.07^{b}
T_2(Mancozeb)	20±0.07^{b}	20±0.07^{b}	20±0.07^{c}	15±0.07^{b}	10.75±0.07^{b}
T_3(*O. gratissimum* infusion)	25±0.07^{b}	25±0.07^{b}	30±0.07^{c}	20±0.07^{b}	20.75±.07^{b}

*For each parameter (incidence and severity) means with different superscripts in the same column are significantly different ($p<0.05$) statistically

3.2 Effect of Plant Extracts on Yield of Yams

The lowest mean yield ($P\leq0.05$) per treatment per yam species was recorded on units with no fungicide treatment (T_0) in all yam species in the trial, except *D. bulbifera* (Table 2). The highest ($P\leq0.05$) mean yield was recorded on yams treated with leaf infusion of *O. gratissimum* (5.3±0.25 kg, *D. alata*) compared with yields of plants treated with *A. indica* seed oil (3±0.19 kg, *D. alata)* and chemical fungicide (Mancozeb) (2.5±0.05kg) which showed a similar mean yield in nearly all five yam species. There was no significant difference ($P\leq0.05$) in yield amongst all the plants treated with chemical fungicide (mancozeb) and *A. indica* seed oil (Table 2).

Table 2. Yield of yam species treated with plant extracts and chemical fungicide (mancozeb)

Treatment	**Yam species:** Average weight of tubers per plant and species (Kg)*				
	D. rotundata	*D. cayenensis*	*D. alata*	*D. dumetorum*	*D. bulbifera*
T_0 (No treatment)	0.8±0.01^{c}	1±00^{c}	1.8±0.17^{c}	0.5±0.01^{c}	2.1±0.13^{a}
T_1(*A. indica* seed oil)	1.6±0.10^{b}	1.9±0.11^{b}	3±0.19^{b}	1±0.12^{b}	2.3±0.21^{a}
T_2(Mancozeb)	1.8±0.12^{b}	2±0.18^{b}	2.5±0.05^{b}	1±0.21^{b}	2.2±0.11^{a}
T_3(*O. gratissimum* infusion)	3±0.22^{a}	3.2±0.16^{a}	5.3±0.25^{a}	2.1±0.14^{a}	2.4±0.08^{a}

*Means with different superscripts in the same column are significantly different ($p<0.05$) statistically

3.3 Identification of Fungal Isolates from Anthracnose-Infected Yams

Based on morphological characteristics of mycelia fragment (growth rate, mycelia pattern and

colour, and form of conidia), the fungus was identified as *Colletotrichum gloeosporioides* (Figure 5).

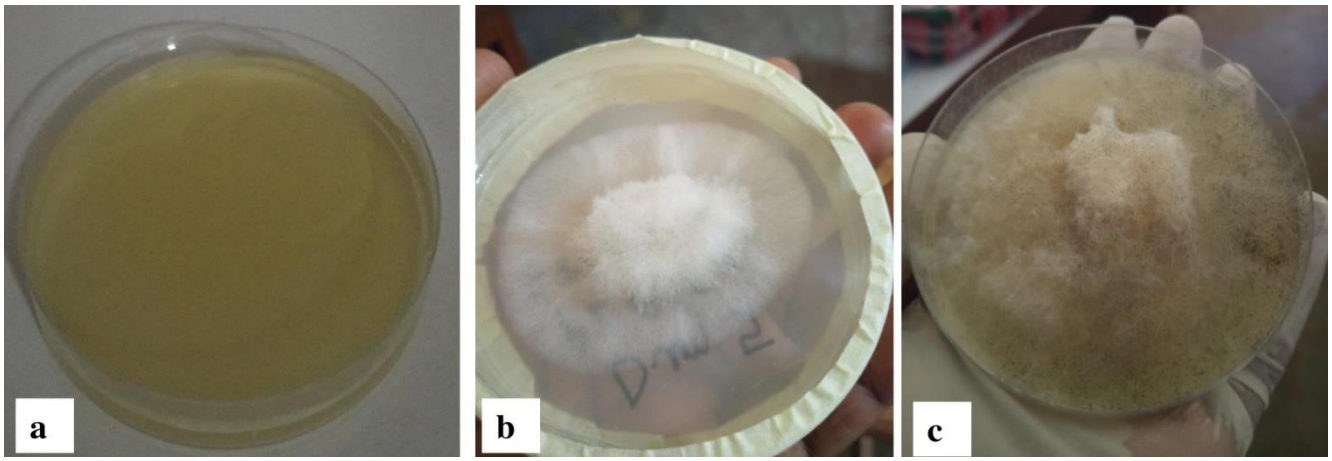

Figure 5. Fungal mycelia growth on PDA medium

(a) PDA media in petri dish, (b & c) 3rd and 4th sub-culture of *C. gloeosporioides*

Three strains of the fungus were identified including; fast-growing grey (FGG), fast-growing salmon (FGS) and fast-growing olive (FGO) on all yam species in the experiment.

3.4 In-vitro Evaluation of the Efficacy of Plant Extracts to Inhibit Fungal Mycelia Growth

Seed oil from *A. indica* and infusion from *O. gratissimum* inhibited growth of *C. gloeosporioides* mycelia at all the concentration levels (25, 50, 75 and 100 %) applied. However, the antifungal activity of both plant extracts was found to increase with increased concentration in all the yam species studied (*D. rotundata, D. dumetorum, D. cayenensis, D. bulbifera,* and *D. alata*) (Table 3). Using seed oil of *A. indica* or *O. gratissimum* infusion, the highest ($p \leq 0.05$) percentage inhibition of fungal mycelia growth was recorded at 100 % concentration (Table 3). Mancozeb (6.7g/L), a standard fungicide used in the study, showed 100 % inhibition of *C. gloeosporioides* mycelia growth in all yam species studied (Table 3), meanwhile petri dishes with no application of pesticides showed no inhibition of fungus mycelia growth in all yam species.

Table 3. Percentage inhibition of *C. gloeosporioides* mycelial growth by plant extracts and chemical fungicide (mancozeb)

Treatment	Concentration (%)	*Mycelia growth inhibition (%)				
		D. rotundata	*D. dumetorum*	*D. cayenensis*	*D. bulbifera*	*D. alata*
O. gratissimum leaves infusion	25	52.38±0.95^{c}	54.76±1.90^{c}	52.38±0.95^{c}	66.66±0.67^{c}	52.38±0.95^{c}
	50	64.28±1.48^{bc}	76.19±0.47^{b}	59.52±0.95^{c}	74.19±0.47^{c}	59.52±0.95^{c}
	75	73.80±2.38^{b}	83.33±3.33^{b}	69.04±1.90^{b}	80.95±2.38^{b}	73.80±2.38^{b}
	100	85.71±2.85^{a}	92.85±1.42^{a}	88.09±2.38^{a}	92.85±1.42^{a}	83.33±3.33^{a}
A. indica seed oil	25	52.38±0.95^{c}	57.14±2.85^{c}	50.00±0.90^{c}	59.52±3.80^{c}	52.38±0.95^{c}
	50	54.77±1.90^{c}	61.90±4.76^{bc}	59.52±3.80^{bc}	71.42±1.43^{b}	57.14±2.85^{bc}
	75	64.29±0.71^{b}	71.42±1.43^{b}	69.04±1.90^{b}	76.19±0.47^{b}	69.04±1.90^{b}
	100	80.95±2.38^{a}	85.71±4.28^{a}	83.33±3.33^{a}	90.47±1.90^{a}	80.95±2.38^{a}
Mancozeb	6.7 g/L	100.00±0.0^{a}	100.00±0.0^{a}	100.00±0.0^{a}	100.00±0.0^{a}	100.00±0.0^{a}

*For each treatment (*O. gratissimum* leaf infusion and *A. indica* seed oil) means with different superscripts in the same column are significantly different (P≤0.05) statistically

At 25 % concentration of both plant extracts (seed oil of *A. indica* and infusion of *O. gratissimum*), there was no significant difference (P≤0.05) in the percentage inhibition of *C. gloeosporioides* mycelia growth which ranged from 50±0.9 % (in *D. cayenensis*) to 66.7±0.67 % (in *D. bulbifera*) in all the five yam species studied (Table 4). At 50 % concentration of both seed oil of *A. indica* and infusion of *O. gratissimum*, the percentage inhibition of *C. gloeosporioides* mycelia growth ranged from 54.8±1.9 % in *D. rotundata* to 76.19±0.47 % in *D. dumetorum*. The efficacy of *O. gratissimum* infusion to inhibit mycelia growth (64.28±1.48 % and 76.19±0.47 %) was significantly higher (P≤0.05) than that of *A. indica* seed oil (54.77±1.90 % and 61.90±4.76 %) in *D. rotundata* and *D. dumetorum*, respectively (Table 4). At 100 % concentration of both seed oil of *A. indica* and infusion of *O. gratissimum*, the percentage inhibition of *C. gloeosporioides* mycelia growth ranged from 80.95±2.34% in *D. cayenensis* to 92.85±1.42 % in *D. dumetorum* and *D. bulbifera*. Also, at 100% concentration of plant extracts there was no significant difference (P≤0.05) in the efficacy of *O. gratissimum* infusion and mancozeb to inhibit mycelia growth in *D. rotundata*, *D. dumetorum*, *D. cayenensis* and *D. bulbifera*, and *D. alata* (Table 4). The efficacy of *A. indica* seed oil to inhibit mycelia growth was significantly lower (P≤0.05) than that of *O. gratissimum* infusion and mancozeb in *D. rotundata* and *D. cayenensis* (Table 4).

Table 4. Comparison of percentage inhibition of *C. gloeosporioides* mycelia growth by different concentration of plant extracts

Treatment (%)	*Mycelia growth inhibition (%)				
	D. rotundata	*D. dumetorum*	*D. cayenensis*	*D. bulbifera*	*D. alata*
O. gratissimum (25%)	52.38±0.95^{b}	54.76±1.90^{b}	52.38±0.95^{b}	66.66±0.67^{b}	52.38±0.95^{b}
A. indica (25%)	52.38±0.95^{b}	57.14±2.85^{b}	50.00±0.90^{b}	59.52±3.80^{b}	52.38±0.95^{b}
Mancozeb (6.7 gL^{-1})	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}
O. gratissimum (50%)	64.28±1.48^{b}	76.19±0.47^{b}	59.52±0.95^{b}	74.19±0.47^{b}	59.52±0.95^{b}
A. indica (50%)	54.77±1.90^{c}	61.90±4.76^{c}	59.52±3.80^{b}	71.42±1.43^{b}	57.14±2.85^{b}
Mancozeb (6.7 gL^{-1})	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}
O. gratissimum (75%)	73.80±2.38^{b}	83.33±3.33^{a}	69.04±1.90^{b}	80.95±2.38^{a}	73.80±2.38^{b}
A. indica (75%)	64.29±0.71^{c}	71.42±1.43^{b}	69.04±1.90^{b}	76.19±0.47^{b}	69.04±1.90^{b}
Mancozeb (6.7 gL^{-1})	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}
O. gratissimum (100%)	85.71±2.85^{a}	92.85±1.40^{a}	88.09±2.38^{a}	92.85±1.42^{a}	83.33±3.33^{b}
A. indica (100 %)	80.95±2.34^{b}	85.71±4.28^{a}	83.33±3.33^{b}	90.47±1.90^{a}	80.95±2.38^{b}
Mancozeb (6.7 gL^{-1})	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}

*Means with different superscripts in the same column are significantly different (P≤0.05) statistically

3.5 Pathogenicity Test

When healthy plants in the screen house were inoculated with fungus isolates from yams in the trial farm, anthracnose symptoms soon develop on the plants (Figure 6). The fungus re-isolated from infected plants in the screen house was further identified to be *C. gloeosporioides.* Three strains identified were the fast-growing grey (FGG), the fast-growing salmon (FGS) and the fast-growing olive (FGO), similar to isolates form the farm whose spores were inoculated on plants in the screen house. The three strains were detected in all the five yam species studied.

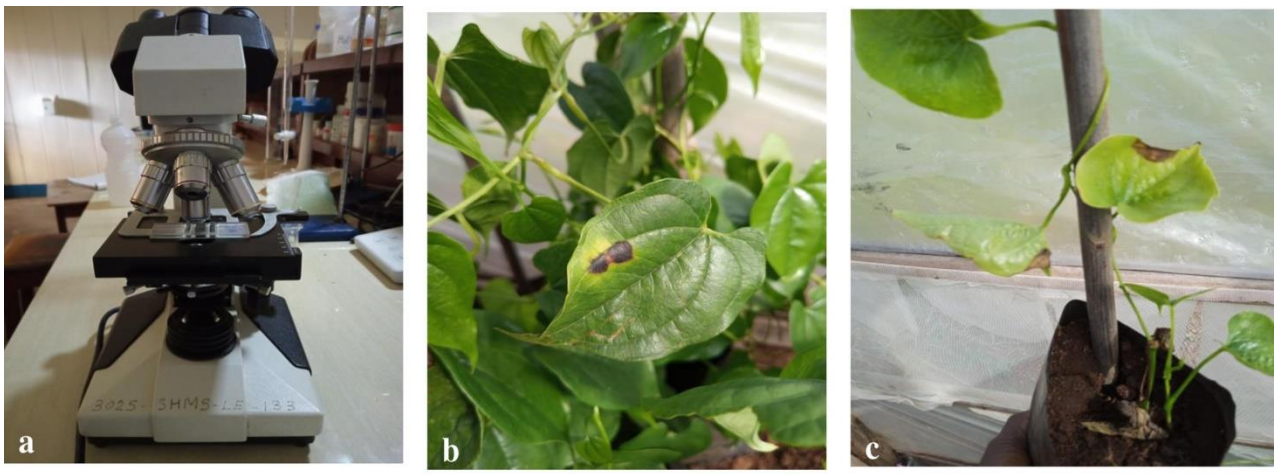

Figure 6. Some yams used for pathogenicity test in screen house

(a) Haemocytometer mounted on microscope for counting spores, (b) Anthracnose-infected leaf, (c) Lower part of stem showing more infection

4. Discussion

A high incidence of symptoms of anthracnose was detected on all yam species, indicating that this disease is widespread and a major challenge to yam production in Cameroon. This observation is in line with previous reports by Tariq *et al.* (2024), in which anthracnose is cited amongst prominent diseases that pose a serious threat to yam in terms of economic losses and quality degradation (Amusa *et al*., 2003). The fungus *Collectotrichum gloeosporioides* also called *C. alatae* (Weir *et al*., 2012) was isolated in yam varieties showing symptoms of anthracnose. Pathogenicity test confirmed *C. gloeosporioides* as the cause of anthracnose disease in all yam species as previously reported in west Africa (Egesi *et al*., 2007) and India (Achar *et al*., 2013). Three strains (fast-growing grey -FGG, the fast-growing salmon -FGS and the fast-growing olive -FGO) of the fungus also identified in this study are among four forms that have previously been described (Abang *et al*., 2002; Ntui *et al*., 2021). The slow-growing grey (SGG) which is reported as the most aggressive and virulent strain in terms of spread across the west Africa yam belt, causing 100% defoliation and premature death of plants (Mignouna et al., 2001; Abang *et al*., 2003) was not diagnosed. The *Collectotrichum* genus is rated as the eighth most important plant pathogenic fungus affecting major economic crops worldwide with C. *gloeosporioides* being the primary cause of anthracnose on yams (Achar *et al*., 2013; Ntui *et al*., 2021) with yield loss of over 90 % reported (Tariq et al., 2024). The fungus attacks all plant parts but leaves and vines are usually the most affected, with symptoms including many black or brown spots, defoliation, scorched vines and dry stems (Egesi *et al*., 2007; Redy, 2015) as observed in this study. Symptoms observed in the field and screen house started with small round black or brown spots with slight depression in the center which later turn grey and surrounded by a yellow halo as previously reported (Ntui *et al*., 2021; Tariq *et al*., 2024). Anthracnose spreads in the form of spores (conidia) dispersal by wind, farm tools, insects and rain-splashed soil containing spores on plants, on which they germinate through intact cuticles and natural openings (Milgroom, 2001; Ntui *et al*., 2021).

Many strategies have been employed to manage and mitigate the impact of anthracnose disease on crops with the use of chemical fungicides such as mancozeb, maneb and benomyl playing a central role. However, hazards linked to the use of chemical fungicides such as pollution and development of fungicide-resistant strains (Onyeka *et al*., 2006) have prompted serious consideration of the use of integrated management which is more environmentally friendly, to manage anthracnose on major crops including yams. Important components of integrated disease management include cultural methods, breeding for resistance and biological control such as the use of plant extracts (Ntui *et al*., 2021; Tariq *et al*., 2024). Extracts from neem (*A. indica*), African basil or scent leaf (*Ocimum gratissimum*), tobacco (*Nicotiana tabaccum*), garlic (*Allium sativum*), and ginger (*Zingiber officinale*), and have been used to control anthracnose on pepper (Islam *et al*., 2010; Alves *et al*., 2015), beans (Silva *et al*., 2022), yam (Pwakem *et al*., 2020).

Field efficacy of extracts of *A. indica* and *O. gratissimum* to control anthracnose on yams was largely comparable with that of chemical fungicide (mancozeb) with more than 50 % reduction in disease incidence. Similarly untreated plants showed the highest incidence of

anthracnose diseases and the lowest yield compared to those that were treated with *A. indica* seed oil and *O. gratissimum* infusion. This observation suggests that the application of these plant extracts significantly reduced the incidence of anthracnose and consequently contributed to better yields in pesticide-treated yams, and further supports previous reports that anthracnose greatly contributes to yield loss in yams (Egesi *et al*., 2007; Ntui *et al*., 2021).

In-vitro, extracts of *A. indica* and *O. gratssimum* inhibited C. *gloeosporioides* mycelia growth by over 80 % with no significant difference between their efficacy and that of mancozeb. These observations indicates extracts of *A. indica* and *O. gratssimum* as veritable alternatives to chemical fungicides used to control anthracnose disease on yams as previously reported (Pwakem *et al.,* 2020; Tariq *et al*., 2024). Plant extracts contain bioactive compounds such as tannins, terpenoids, flavonoids, alkaloids, phenols, and anthraquinones which disrupt fungal cell membranes, inhibit spore germination and interfere with essential metabolic pathways by inhibiting essential enzymes leading to cell death (Islam *et al*., 2010; Fatima *et al.*, 2023; Ali *et al*., 2024). Antifungal activity of aqueous extract of *O. gratssimum* observed in this study has been reported to be mostly associated to monoterpenes, sesquiterpenes with phenolic groups which can establish hydrogen bonds with active sites of target fungal enzymes (Akpo *et al*., 2023). For neem seed oil, azadirachtin is the most well-known antifungal agent that has been reported to act by inhibiting enzymes in biosynthetic pathways (Salazar *et al*., 2015). Although essential oils of *O. gratissimum* have previously been reported to be more effective against anthracnose and other field and storage diseases of crops (Okigbo & Ogbonnaya, 2006; Akpo *et al*., 2023), its crude infusion used in this study proved to have better antifungal activity and more effective in controlling *C. gloeosporioides* on yams in the field and *in-vitro*. These findings agree with previous reports on the inhibitory effect of plant extract on the mycelia and spore germination of some pathogenic fungi (Ajayi & Olufolaji, 2008; Okoi & Afuo, 2009).

5. Conclusion

This study has contributed in bringing out the importance of plant extracts as alternatives to chemical pesticides, which, although very effective are generally not eco-friendly. Plants extracts are non-phytotoxic, readily biodegradable, cheap, and readily available, have strong fungicidal activity and can serve as important component in integrated management of anthracnose in yam cultivation to improve production. Chemical constituents from neem (*A. indica*) seeds oil and Scent leaf (*O. gratissimum*) leaves may be formulated into bio–fungicides against anthracnose.

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Authors contributions

All authors collaborated in carrying out this research. Dr. MEB designed the study, wrote the

protocol, corrects the first draft of manuscript, performed the statistical analysis and revised the manuscript. Dr. INA managed the research design, statistics, and literature and wrote the first draft of the manuscript. Dr. KDN and Miss TF managed the study design, statistics and literature searches. All authors read and approved the final manuscript.

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Authors declare that there if no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work reported in this paper.

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Obtained.

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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No additional data are available.

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