

**MANAGEMENT OF WHITE MOLD FUNGUS (*Sclerotinia spp.*) USING JUICE
AND LEAF EXTRACT OF BUSH CANE (*Costus afer*): AN IN-VITRO
ASSESSMENT**

A SEMINAR PRESENTATION

BY:

**MOSES, ESTHER PETER
AK16/AGR/CRS/035**

SUPERVISED BY:

**MR. ISREAL ENO
DEPARTMENT OF CROP SCIENCE
FACULTY OF AGRICULTURE
AKWA IBOM STATE UNIVERSITY
OBIO AKPA CAMPUS.**

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The attempt to improve crop yield in order to produce enough food for consumption in the face of increasing population is a decision in the right direction although it is being hampered by many constraints. One of the most important and interesting problem encountered by scientists is how to drastically reduce or wholly prevent plant diseases (Akinbode and Ikotun, 2008; Singh *et al.*, 2000). Several attempts have been made to control diseases through cultural, physical, chemical, and biological methods (Adekunle *et al.*, 2001). Even though, there is increasing control methods such as resistant varieties to control the diseases, the control has become a continual battle because the pathogens normally attack the crops suddenly; hence ineffective (Akinbode and Ikotun, 2008). In the recent past, control of plant-parasites, essentially, involves the use of synthetic pesticides. However, apart from its very high cost, indiscriminate and unsafe use, increased concern for the environment and the inherent danger pose to man and his livestock calls for caution in its utilization (Adegbite and Adesiyun, 2005; Alabi *et al.*, 2005). Furthermore, it has been reported that more than one hundred species of plant pathogens have become resistant to fungicides while some resistant varieties have become susceptible (Zitter *et al.*, 2005). Efforts have been made by researchers to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants and one of such resources is botanicals (plant extracts) (Okereke and Wokocha, 2007). Using systematic screening, novel effective compounds have been discovered (Tomoko *et al.*, 2002).

In view of the foregoing, it becomes necessary to use plant extracts to ascertain their effectiveness in the control of white mold fungal disease. White mold, caused by the fungus *Sclerotinia sclerotiorum*, causes wilt, rot, or blight diseases on more than 370 ornamental plant species, field crops, weeds, and vegetables in 64 plant families (Cheryl and Martins, 2001). These plant species differ greatly in their susceptibility to the white mold fungus. Levels of infection can range from hardly noticeable to complete destruction of the plant (Cheryl and Martins, 2001). Unfortunately, even those plants which are only slightly susceptible may help build up levels of the fungus in the soil. Then when a very susceptible crop such as cabbage or petunia is planted and conditions are favorable, severe disease losses result can be recorded, hence there is need for it control or whole eradication and *costus afer* considered (Fatahalla *et al.*, 2019). *Costus afer* is also known as “bush cane” belonging to family *Coastaceae*, is a substantial medicinal and ornamental plant used traditionally to cure different diseases (Fatahalla *et al.*, 2019). The phytochemical analysis of *Costus afer* parts has revealed its richness in carbohydrate, starch, amylase, protein, lipid

and vitamin A (Karthikeyan *et al.*, 2012). Moreover, the rhizomes are wealthy in bioactive substances as quercetin, rutin, luteolin, kaempferol and coumarin (Thabit, 2018). Also it contains antioxidant components like β -carotene, Vitamin C, Vitamin E and trace elements as nitrogen, calcium, potassium, sodium and magnesium (Thabit, 2018). Aside, this plant has been investigated to exhibit antifungal and antimicrobial potential, hence there is need for evaluation in its capability to control white mold fungus.

1.2 Justification of the Study

Application of chemical fungicides to protect plants from the attack of the pathogenic fungi was the primary means for controlling diseases. In recent years, chemical fungicides have become hazardous to human, plants and other beneficial organisms (Singh *et al.*, 2000). Biological control methods could be considered as an alternative of chemical control. Successful biological control of some fungal diseases has been achieved by a number of researchers under greenhouses, field and in-vitro trials using fungal and bacterial antagonists (Singh *et al.*, 2000; Abd El-Moneim, 2001). Plant extracts were recently used to control many pathogens and diseases (El-Kazzaz *et al.*, 2003). Previous report has indicated that *Costus afer* extracts contain some bioactive ingredient that has inhibitory effects on some microorganisms (Anyasor *et al.*, 2010). One of the most important diseases affecting some crops is white mold caused by the fungus *Sclerotinia spp.* Losses from this disease have averaged as high as 20 percent, with a few individual field losses exceeding 65 percent. Based on these evident data, it is concluded that there is need for white mold fungus control using plant extract such as; *Costus afer* as an alternative and complementary therapy but further scientific studies on the toxicological and pharmacological evaluation need to be carried out (Schwartz *et al.*, 2011).

1.3 Objectives of the Study

The objective of this study is;

- To evaluate the effect of the plant extract (*costus afer*) in the control white mold fungus pathogen (*Sclerotinia spp.*).

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical and Ecological Description of *Costus afer*

Costus afer (Linn) is an ornamental and important medicinal plant, belonging to family *Coastaceae* (*Zingiberaceae*) which is often called bush cane or spiral ginger (Srivastava *et al.*, 2011). Family *Zingiberaceae* is a family of about 52 genera and more than 1,300 species (Robinson *et al.*, 2009). The genus *Costus* comprises 175 species like: *Costus afer*, *Costus arabicus*, *Costus speciosus*, etc. *Costus afer* is widely distributed throughout the world, it occurs in the humid tropics area (EL-far *et al.*, 2013). *Costus afer* is a succulent, upstanding, perennial, ornamental, herbaceous, tuberous stem, sub-woody at the base usually an unbranched tropical plant of ten seen as herb with a creeping rhizome (Srivastava *et al.*, 2011). It is a relatively small monocot shrub which is commonly found in humid and monstrous forests and riverside (Ekpo *et al.*, 2008). It is a perennial plant which can grow as tall as 4m and bears white and yellow flowers (Ekpo *et al.*, 2008). Its inflorescence is a highly compact, terminal, conical spike of about 2.5cm to 7.5cm long, sessile; bracts are oblong, convex, 3.5cm long, densely imbricate, upper ones usually smaller; apex is truncate to rounded, green with purple markings, each subtending two flowers; bracteoles are boat-shaped, 2.5cm×1cm; and keel is thick and ridged, pale green with pink markings and thin pink papery margin (Omokhua, 2011). *Costus afer* has simple leaves, which are arranged spirally. The sheath is lobular, closed, and green with purple blotches. The ligule is about 4 to 8mm long, which is leathery and glabrous. The leaf blade is elliptical to obovate of about 15cm to 35cm×3.5 to 9.5cm with culminate apex (Aweke, 2008). The margin is sparsely hairy with a bisexual and zygomorphic flower. *Costus afer* is pan tropical with about seventy species, of which forty are found in tropical America, twenty-five in West tropical Africa, and five in South-East Asia (Atere, 2018). In Africa, the plant is found in the forest belt from Senegal to Ethiopia and in the East to Tanzania. In tropical, West Africa, it is found in the rain forest and riverbanks of countries including Ghana, Sierra Leone, Senegal, Guinea, Togo, Cameroon, and Nigeria (Omokhua, 2011; Ekpo *et al.*, 2008). The rhizomes have brownish colour with incense odor. The rhizome and aerial parts of the plant are edible (Choudhury *et al.*, 2012). Traditionally, the plant parts (stem, leaves and rhizome) has several medicinal and pharmacological significance that have led to numerous researches (Karthikeyan *et al.*, 2012). Previous report has indicated that *Costus afer* extracts contains some bioactive ingredient that has inhibitory effects on some microorganisms (Anyasor *et.al.*, 2010). The stem is crew to reliance cough and sores, among other diseases (Edeoga *et. al.*, 2000)

2.2 Classification of *Costus afer*

Kingdom	<i>Plantae</i>
Domain	<i>Eukaryota</i>
Family	<i>Zingiberaceae</i> or <i>Costaceae</i>
Genus	<i>Costus</i>
Species	<i>Costus afer</i>

Karthikeyan *et al.*, 2012

2.3 Medicinal Importance of *Costus afer*

Costus afer is a plant commonly known as ginger lily, spiral ginger, or bush cane. It is reportedly used in traditional medicine practice to treat and manage many ailments including diabetes mellitus, stomach ache, arthritis, inflammation, and goitre (Dike, 2009). These purported ethnomedicinal uses have triggered many research studies on the plant to a mass scientific evidence. It is revealed that the stem and leaves of the plant (*Costus afer*) contain substantial amounts of micronutrients and macronutrients (Anyasor *et al.*, 2014). The leaves, stem, rhizomes, and roots of *Costus afer* contain several steroidal sapogenins, aferosides, dioscin, and paryphyllin and flavonoid glycoside kaempferol-3-O- α -L-rhamnopyranoside (Anyasor *et al.*, 2014). Experimental studies on various parts of the plant showed bioactivities such as antihyperglycemic, hepatocellular protection, cardioprotection, nephroprotection, testicular protection, central nervous system depressant, analgesic, antiarthritis, antibacterial, and antioxidant (Akpan *et al.*, 2012).

2.4 Nutritional and Phytochemical of Composition of *Costus afer*

Costus afer is used by the local folks, due to its nutritional and medicinal properties. This involves the use of the plant parts such as leaf, stem, and the rhizome in preparation of food (Ukpabi *et al.*, 2012). The proximate analysis of different parts of *Costus afer* shows the presence of both macro- and micronutrients (Dike, 2009; Anyasor *et al.*, 2014). Both the leaves and stem are rich in macronutrients such as carbohydrate, crudeprotein, fat, ash, moisture, and a good source of fiber. There are also reports of the presence of certain vital nutrients such as vitamins B (1,2,3,6, and 12), E, and C in the leaves (Ekpe *et al.*, 2018). The oil extracted from the plant is made up of 78% saturated fatty acids and 22% unsaturated fatty acids (Ekpe *et al.*, 2018). The phytochemical analysis of the leaves, stem, and the rhizome of this plant in different solvents shows the presence of alkaloids, phenols, saponins, triterpenes, tannins, and glycosides (Akpan *et al.*, 2012; Jesus *et al.*, 2016). These phytochemicals and nutrients may justify the nutraceutical use of the plant (Anyasor *et al.*, 2014). Research on the chemical identification and isolation of bioactive compounds from *Costus afer* has been carried

out, and this has led to the elucidation of structures from different parts of the plant (Akpan *et al.*, 2012; Jesus *et al.*, 2016). For instance, the rhizome is reported to contain steroidal saponins such as diocin, paryphyllin C, aferoside B, and aferoside C. Kaempferol-3-O-R-L-rhamnopyranoside, which is a flavonoid glycoside, has also been identified from the aerial part of the plant (Anyasor *et al.*, 2014). Additional aferoside A and aferosides B and C have been isolated from the roots of *Costus afer* which are essential for pharmaceutical usage (Uwah *et al.*, 2015).

2.5 Experimental Trial with *Costus afer*

2.5.1 Antifungal Activity

Several studies have been conducted to proof the use of *Costus afer* extracts in remediating several diseases and one of such include; the trial of the antifungal activity of *Costus afer*. According to a study carried out by Mary *et al.*, (2016) to determine the effect of *Costus afer* on fungal pathogens causing yam rot. Two fungi pathogen isolated and identified were: *Aspergillus niger* and *Aspergillus flavus*. Three different concentrations of *Costus afer* (10, 20 and 30%) was used and phytochemical constituents of *Costus afer* extracts was also evaluated. According to their results, the extracts of *Costus afer* was efficient in inhibiting the growth of *Aspergillus niger* and *Aspergillus flavus*. The extracts showed significant difference at 5% probability level. The highest antifungal activity was observed with 30% *Costus afer* extract and ethanol had the highest inhibition 89% and 90%, when compared to aquae's 81 and 82% and crude 85 and 87% respectively. The result of this study shows the possible use of plant extracts in the management and control of yam rot, hence, *Costus afer* has antifungal capacity.

2.5.2 Antidiabetic Property of *Costus afer*

Diabetes mellitus is a chronic hormonal and metabolic disorder that is characterized by a persistent increase in blood glucose levels. In an induced rat model, there was a significant reduction in blood glucose level when *Costus afer* leaf extract at concentrations of 375, 750, and 1125mg/kg and the control drug (glibenclamide) (5mg/kg) were orally given (Uwah *et al.*, 2015). A study by Ezejiofor and colleagues reported in (2015) showed that *Costus afer* leaf and stem extracts are able to reverse histopathological damage of pancreatic β -cells in allox an-induced diabetes mellitus (Ezejiofor *et al.*, 2017). According to a report by Ezejiofor and colleagues published in 2015, oral administration of 750 and 1125mg/kg of *Costus afer* leaf extract produced a more prominent regeneration and repopulation of islet cells. The same research group in 2017 in a histopathological study of *Costus afer* stem extract on alloxan-induced damaged pancreatic cells noticed an organ protective effect (Ezejiofor *et al.*, 2017). This therefore indicates that *Costus afer* leaf and stem extracts have pancreatic islet cell

protective and regenerative effect that could be explored in managing type I diabetes mellitus.

2.5.3 Antioxidative and nephroprotective properties of *Costus afer*

Medicinal plants contain bioactive compounds which are capable of preventing and fighting these diseases. In the study of Anthonet *et al.*, (2016), the nephroprotective and antioxidant properties of the aqueous leaf extract of *Costus afer* against cyclosporine A CsA - induced nephrotoxicity in Wistar albino rats were investigated. The treatment with *Costus afer* extract at doses of 375, 750 and 1125 mg/kg prevented the CsA induced nephrotoxicity and oxidative impairments of the kidney, as evidenced by a significantly ($p < 0.05$) reduced plasma creatinine, BUN, K⁺ and renal malondialdehyde (MDA). Furthermore, all the doses were able to induce a significant increment ($P < 0.05$) of renal levels of glutathione (GSH) and plasma superoxide dismutase (SOD) activity, catalase (CAT), glutathione-S-transferase (GST), serum electrolytes (Na⁺ and HCO₃⁻), body and kidney weight. The nephroprotective effects of *Costus afer* extract were confirmed by a reduced severity of renal cellular damage.

2.6 White Mold Fungus (*Sclerotinia spp.*)

One of the most important disease affecting some crops (especially, vegetables and legumes) is white mold caused by the fungus *Sclerotinia sclerotiorum*. Losses from this disease have averaged as high as 20 percent, with a few individual field losses exceeding 65 percent (Schwartz *et al.*, 2011). White mold is first observed as wet, soft spots or lesions on infected leaves, branches, stems and pods. These lesions enlarge into a watery, rotten mass of tissue that is covered by a white moldy growth. Infection of stems and branches will cause affected plant parts to wilt and later die, taking on a bleached and dried appearance. This bleaching symptom is characteristic of white mold infected pinto and great northern types and differs from the normal tan color resulting from senescence (aging) or other diseases (Rahman *et al.*, 2015). Black and irregularly shaped sclerotia (resting structures of the fungus) form on and within infected plant parts. Spread and development of white mold is greatly influenced by the prevailing weather conditions and certain agronomic practices. The disease may cause serious yield losses during wet, cool periods near the end of the growing season of crops (El-Kazzaz *et al.*, 2003). Agronomic practices, such as irrigation management, plant density and varietal growth characteristics, are all closely linked with the life cycle of the pathogen. Recent research has shown how management of these factors, in addition to the use of chemical control, can be most effectively combined to reduce disease severity and minimize yield losses to crops (Zitter *et al.*, 2005).

2.7 Survival of the Fungus

Survival of the Fungus The most important survival mechanism of *Sclerotinia sclerotiorum* is the formation of sclerotia. A sclerotium is a compact mass of hardened

mycelium (cobweb-like fungal threads) that contains reserve food materials. These structures are produced in abundance on infected plant tissue, allowing the fungus to survive during periods of unfavorable environmental conditions (Zitter *et al.*, 2005). Sclerotia usually are dark colored, circular to irregular in shape, and range in size from less than 1/8 inch in diameter up to the size of a large bean seed. The center of a sclerotium usually is cream or white. Studies have shown that up to 75 per cent of sclerotia buried 12 inches deep in a bean field remain alive and infective three years or longer. *Sclerotinia sclerotiorum* can survive from one sea son to the next as mycelium in infected bean straw and seeds that have been scattered over the field during harvest (Schwartz *et al.*, 2011). Post-harvest tillage reduces the potential for infection since diseased tissue is turned under and generally decomposes in the soil before the next bean growing season. However, sclerotial survival still can be a problem in subsequent seasons. The fungus can be transported within infected seeds or in sclerotia-contaminated seed lots to be planted during the next growing season (Rahman *et al.*, 2015).

2.8 Effect of Botanicals in Control of Plant Diseases

The use of botanicals is now popular in the control of disease to ameliorate the effect of chemical disease control measures or fungicides.

Tabil (1995) reported that the leaf extract of *L. inermis* was effective against mycelial growth of cumin wilt caused by *Fusarium oxysporum* f. sp. Similarly, antifungal activity of *V. amygdallina* leaf extract was reported by Wedge *et al.* (2000) and Alabi *et al.* (2005). Fresh leaf extract of *M. lanceolata* was identified as an effective botanical plant for the control of covered smut of sorghum. Its performance was similar to the standard fungicide Tiram (Aschalew *et al.*, 2012).

According to the study of Mihiret *et al.*, 2014, *Cymbopogon citratus* (lemon grass) is severely infected by a rust disease caused by *Puccinia nakanishikii* L. which is often responsible for drying of the leaves. The study was undertaken to investigate the potential efficacy of various botanicals for the control of the disease. Crude acetone extract of *Lantana camara* L., *Milletia ferruginea* L., *Eucalyptus globules* L., *Maesa lanceolata* L, *Ruta chalapensis* L., *Vernonia amygdalina* L., *Datura stramonium* L., and *Artemisia annua* A. were investigated in greenhouse and field conditions. Plants sprayed with Impuls EC were used as standard check and untreated plants as control for both in vivo and field experiments. For field experiment, botanicals were sprayed on well-established plants before disease occurrence and continued at 15 day interval for four rounds. Data was recorded on fresh leaf yield (kg/ha), dry leaf yield (kg/ha), fresh stem wt (kg/plot), essential oil content, essential oil yield, disease severity and disease control. *D. stramonium*, *V. amygdalina* and *A. annua* were effective botanicals against *P. nakanishikii* both in the green house and field experiments.

Furthermore, in the study of Nahunnaro and Ibayaso (2011), valuated the effect of two plant extracts, *Ricinus communis* and *Chromolaena odorata* on the control of the early blight pathogen, *Alternaria solani*. Three concentrations (25%, 50%, and 100%) of each plant extract were determined for inhibitory activity of *A. solani* growth. From the radial growth results, it revealed that *Ricinus communis* at 100 % concentration was recorded for the lowest radial growth rates of 1.43 cm, 2.00 cm and 2.72 cm at 24, 48 and 72 hours were recorded, respectively. It was concluded that the plant extracts used at different concentrations showed promising prospects for control *Alternaria solani* growth in vitro. However, he was recommended that there is a need to evaluate the inhibitory function of the plant extracts in the field to ascertain their effectiveness.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study will be carried out at Crop Science Laboratory, Faculty of Agriculture, Akwa Ibom State University. Obio Akpa Campus, Oruk Anam Local Government Area, Akwa Ibom State. The area lies between latitude 4⁰30'N and 5⁰ 00'N and longitudes 70⁰ 30'E and 80⁰ 00'E (SLUS-AK, 1989).

3.2 Plant Materials

Fresh leaves, stem and roots of *Costus afer* will be collected from the farm along Obio Akpa, Oruk Anam, Akwa Ibom State for the study and identified by a Botanist in the Department of Crop Science, Akwa Ibom State University.

3.3 Media Preparation

Sabouraud Dextrose Agar (PDA), will be used and prepared according to the manufacturer's instructions and will be dispensed into 3 sterile petri dishes for culture. The plates will be streaked with the selected organisms and then covered to prevent contamination, examined for growth and pure cultures will be used for identification. The isolates will be observed according to the methods (Barnett and Hunters, 1998).

3.4 Source of Test Organisms

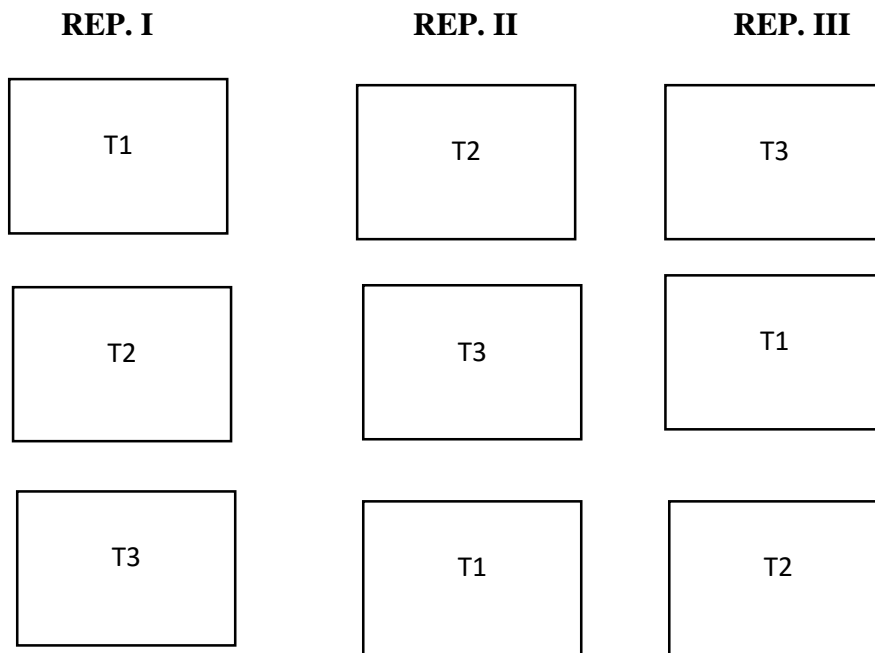
Clinical isolates of white mold fungus (*Sclerotinia spp.*) will be obtained and confirmed using Gram staining and Biochemical test in the Crop Science Department, Akwa Ibom State University. The isolates will be maintained on the sabouraud dextrose agar and stored until required.

3.5 Extraction of *Costus afer*

The extraction will be carried out according to the method described by Harborne (1973). Some of the plant parts collected will be air dried or sun-dried for 7 days. The plant materials will be then ground into powder form using a mechanical grinder. 10g of the ground leaves will be weighed out and 10g of the ground root will be also weighed out. The extraction will be done using the Soxhlet method after which the extracts will be stored at 4°C until required. All extracts will be filtered through Whatman (No.1) filter paper and concentrated over a water bath using Soxhlet to recover the solvent.

3.6 Experimental Design

The experiment will be laid out in a randomized complete block design (RCBD) with treatments and three replications. The treatments to use include; plant extract from *Costus afer* extract at different concentrations and the control unit will be standard fungicides.



T1 – Standard Fungicides; T2 – 5% of *Costus afer* Extract; T3 – 10% of *Costus afer* Extract

Figure 1: Experimental Layout

3.7 Phytochemical Screening

The extract will be subjected to phytochemical screening using standard procedure as described by Trease *et al.* (1989).

3.8 Antifungal Bioassay

The antifungal activity test of the plant extracts will be carried out using the agar well diffusion method (Irobi *et al.*, 1994). Clinical isolates of *Sclerotinia spp.* will be inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and each fungus spread over the entire surface of agar plate to obtain a uniform inoculum. Two (2) agar wells of 6mm diameter and 5mm depth will be made on the solid agar on each plate using a sterile glass borer and then labeled. Each extract of 0.02 ml will be then dispensed into their respective wells on the inoculated plates. After which the set up will be allowed to stay for 24 hours. After 24 hours of incubation, the zones of inhibition will be measured in millimeters (mm) using a transparent ruler. Oxoid (1985) standard susceptibility range will be used to classify zones of inhibition as either sensitive (>10 mm) or resistant (≤ 10 mm).

3.9 Statistical Analysis and Interpretation of Data

The data will be subjected to Fischer's method of analysis of variance and interpretation of data will be done as described by Gomez and Gomez (1984). The level of significance used in F^o and t-test will be 0.05. Critical difference (CD) values will be calculated whenever the F-test is significant.

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