UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF *Colletotrichum gloeosporioides* CAUSING ANTHRACNOSE DISEASE OF WHITE YAM (*Dioscorea rotundata* Poir) IN THE TOLON DISTRICT OF NORTHERN GHANA AND ITS MANAGEMENT WITH PLANT EXTRACTS

JOSEPH KWOWURA KWODAGA

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BY

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OCTOBER, 2018

DECLARATION

Student

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere.

Joseph Kwowura Kwodaga ---------- ----------

(Name of Student) Signature Date

Supervisors'

We, hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

The anthracnose disease caused by *Colletotrichum gloeosporioides* is a major constraint to yam production worldwide. The harmful effects of synthetic fungicides on the environment have necessitated the use of eco-friendly alternatives. This study investigated the incidence and severity of anthracnose disease of laribako and pona yam varieties in the Tolon District of Ghana, and also characterised *C. gloeosporioides* isolates associated with infected laribako and pona crops. Phytochemical analysis was conducted on *Azadiratcha indica*, *Balanites aegyptiaca*, *Jatropha curcas* and *Khaya senegalensis* seeds, *Icacina oliviformis* leaves and *Capsicum* spp. (Legon 18 variety) fruit extracts which were tested *in vitro* and *in vivo* for their potency in managing yam anthracnose disease. The *C. gloeosporioides* isolates were characterised using PCR techniques with the primer pairs ITS1/ITS4, NS1/NS2, CgInt/ITS4, CgLac-f/CgLac-r, CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r2. The antifungal activities of 10, 25, 50, 75 and 100% concentrations of each of the plant extracts against *C. gloeosporioides* were assessed *in vitro* using the food poison technique. The fungitoxicity of the plant extracts against yam anthracnose disease was assessed under field conditions through foliar application of 75 and 100% concentrations of each plant extract. The anthracnose disease incidence and severity of *D. rotundata* crops recorded for the 2016 and 2017 cropping seasons in the Tolon District increased significantly ($P \leq 0.05$) from July to September. Generally, the 2016 cropping season recorded higher disease incidence and severity than 2017. The *C. gloeosporioides* isolates produced characteristic band sizes on ITS1/ITS4, NS1/NS2, CgInt/ITS4 and CgLac-f/CgLac-r. None of the isolates produced a band on CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r2. The phytochemical analysis revealed alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatinnins, saponins, steroids, tannins and terpenoids. Contents of total phenols, flavonoids, tannins and alkaloids varied among the plant extracts. Each of the plant extracts significantly ($P \leq 0.05$) inhibited the

mycelia growth and spore germination of *C. gloeosporioides*. The plant extracts also reduced the incidence and severity of anthracnose disease and enhanced vine length, shoot and root dry weights, and tuber yield. This showed that potentially these extracts can be used to produce formulations for the management of anthracnose disease of yam.

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DEDICATION

To my dear wife Dr. Hamdiyah Alhassan for her love and support, and my children Anulana, Awetogah and Wemanga.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Yam (*Dioscorea* sp.) is an important cash crop that constitutes about 53% of total root and tuber consumption in West Africa and also serves as a cheap source of carbohydrate in the diets of millions of people worldwide and in tropical West Africa (Asiedu *et al*., 2014). The crop contains an appreciable amount of proteins, vitamins and minerals (Babaleye, 2003). Globally, Ghana is the second largest producer of yam after Nigeria (FAOSTAT, 2017) and also the largest yam exporter in the world (Anaadumba, 2013). The cultivation of yam cuts across the Forest, Coastal Savannah, Forest Transition and the Guinea Savannah agroecological zones of Ghana (Osei-Adu *et al*., 2016). The handling of yam through the value chain provides employment and income for rural smallholder farmers, especially women, who play a critical role as marketers of yam, and as such contribute significantly to the rural economy in major producing areas in Ghana (IITA, 2013).

The yam anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is a major threat to yam production in all yam growing regions of the world, and can result in yield losses of 80 - 90% when infections commence prior to or during tuber formation (Agrios, 2005; O'Sullivan, 2010). The disease is ubiquitous in all yam growing regions of Ghana, with a 96.8% incidence and an estimated yield loss of 50% or more (Peters, 2000). It attacks several species of *Dioscorea*, particularly *D. alata*, *D. cayenensis*, *D. rotundata* and *D. trifida*. Various methods employed in managing the disease include the use of clean pathogen-free planting setts, crop rotation, spraying yam foliage with recommended fungicides and cultivating disease resistant varieties (Agrios, 2005; Offei *et al*., 2008; Reddy, 2015). Fungicides usage which is the most popular means of control

comes with certain challenges. Natural based pesticides such as plant extracts, which are biodegradable but can be used for the control of plant diseases (Kwodaga *et al.*, 2017) such as the anthracnose, should therefore be exploited as alternatives.

1.2 Problem statement

In spite of the negative effects of anthracnose disease on yield, majority of yam farmers in Ghana do not take any control measures against it; due to lack of knowledge of the causes of the disease (Appiah-Kubi *et al*., 2015). This is not surprising since Andrade *et al.* (2009) reported that yam is a low-input crop which is usually grown without many inputs. Yam farmers who try to manage the disease usually resort to the use of synthetic fungicides (Palaniyandi *et al*., 2011; Reddy, 2015) which usage usually comes with some challenges (Newton *et al*., 2010). Some of these challenges are impacts of pesticides on the environment and human health.

Harmful effects of chemical fungicides usage on farmers' health, other non-target organisms and pollution of the environment have been reported (Voorrips, 2004; Newton *et al*., 2010). Three million cases of pesticide poisoning with nearly 220,000 fatalities occur world-wide every year (Bolognesi, 2003; Cooper and Dobson, 2007). Also, excessive use of chemical fungicides usually leads to development of resistance by plant pathogens which results in reduction of the effectiveness of chemical fungicides on the pathogens (Conway, 2004).

The adverse effects of synthetic fungicides on non-target important soil organisms (macro-organisms such as earthworm and micro-organisms like saprophytic fungi) pose a potential risk to the long-term fertility of the soil (Wightwick *et al*., 2008; Komarek *et al.*, 2010). This situation could contribute to decreasing yam yield, since it has been established that,

soil infertility is a key constraint in yam production (Lebot, 2009; CSIR/SARI, 2013). Agbaje *et al.* (2005) observed that yam yields decreased by 50% in 5 – 6 years because of declining soil fertility.

Farmers in most yam growing areas of the world no longer rely on the effectiveness of synthetic fungicides for the control of yam anthracnose due to the development of fungicide resistant *C. gloesporioides* strains (McDonald and Linde, 2002) and also high fungicides cost or its unavailability to farmers (Kutama *et al*., 2011).

1.3 Justification

There is no doubt that, yam cultivation if given the necessary attention, could contribute to the nutritional needs and also alleviate poverty among rural farmers whose livelihood depends on yam production. However, yam production comes with constraints such as pests and diseases infestation, storage losses and high cost of labour, which limit the potential income of yam farmers and also threaten food security.

There is a potential for Ghana to take advantage of the rising global demand for yam to increase production (IITA, 2010) and foreign exchange earnings.

There can be a tremendous increase in yam production in Ghana if serious attention is given to the management of anthracnose disease of yam caused by *C. gloeosporioides*. Since the use of synthetic fungicides comes with certain challenges, there is the need to find environmentally-friendly alternatives for the control of plant pathogenic fungi such as *C. gloeosporioides* which causes anthracnose disease of yam. Developing appropriate control measures to reduce losses due to anthracnose could go a long way to increase yield of farmers and also eliminate mechanically or chemically expenses of controlling the disease (Agrios, 2005). Since the attention of the world is currently focused on sustainable

agriculture, there is the need to adopt strategies that can manage yam anthracnose disease without adverse effect on the environment.

The adoption of environmentally-friendly methods of managing the disease will go a long way to enhance organic farming which is currently being embraced by the world. This may also increase the market value of yam cultivated under such conditions; since organic food products have a higher market value compared to those cultivated with synthetic inputs (Sgroi *et al*., 2015). This has necessitated the need to explore other eco-friendly alternatives such as plant extracts for plant diseases control. Plant materials used as fungicides usually have a narrow target range with specific mode of action; hence, their uses are directed towards specific targets and as such have limited effect on non-target organisms (Zaker, 2016). These products also have a shorter shelf-life limiting their persistence on fields and as such posing no residual threat (Zaker, 2016). Biofungicides obtained from plant extracts also biodegrade quickly and usually have multiple mode of action that makes pathogens less resistant (Quarles, 2009). Nuzhat and Vidyasagar (2013**)** advocated that, since some farmers in developing countries use plant extracts as traditional medicine in the treatment of human diseases, it will be easy for them to adopt these extracts as bio-fungicides for the management of plant diseases. The successful exploitation of local plants within farmers' localities as bio-fungicides in managing anthracnose disease of yam will ensure ready availability of these materials to farmers. This will not only enhance yam yield, improve food security and increase farmers' incomes, but will also ensure sustainable and cost effective yam production. The focus on one or two plant species used as pesticides could be expanded since there are diverse plant species that possess pesticidal properties (Isaac and Khan, 2015).

1.4 Objectives

The objectives for this study were to:

- determine the incidence and severity of anthracnose disease of yam on farmers' and experimental yam fields in the Tolon District.
- characterise *C. gloeosporioides* isolates obtained from infected yam plants.
- determine the phytochemical constituents of *Azadiratcha indica*, *Balanites aegyptiaca*, *Jatropha curcas*, *Khaya senegalensis* seeds, *Icacina oliviformis* leaves and *Capsicum* spp. (hot pepper, Legon 18 variety) fruit extracts.
- evaluate the efficacy of *Azadiratcha indica*, *Balanites aegyptiaca*, *Jatropha curcas*, *Khaya senegalensis* seeds, *Icacina oliviformis* leaves and *Capsicum* spp. (hot pepper, Legon 18 variety) fruit extracts *in vitro* and *in vivo* for the management of anthracnose disease of yam.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of yam

2.1.1 Taxonomy of yam

Yams are monocotyledons and belong to the family *Dioscoreaceae* and the genus *Dioscorea*, with about 644 species (Govaerts *et al.*, 2007; Ngo-Ngwe *et al*., 2014; Reddy, 2015). The genus *Dioscorea* is subdidived into five sections namely; Enantiophyllum, Lasiophyton, Opsophyton, Macrogynodium and Combilium (Lebot, 2009; Lopez-Montes and Edemodu, 2015). The direction of twining of the crop's vines, anticlockwise or clockwise, is a characteristic of each taxonomic section (Lebot, 2009). Enantiophyllum consist of yam species which vines twine to the right, and these are *D. rotundata*, *D. alata*, *D. cayenensis*, *D. opposite*, and *D. japonica*; whiles species sections Lasiophyton (*D. dumetorum* and *D. hispida*), Opsophyton (*D. bulbifera*), Macrogynodium (*D. trtfida*) and Combilium (*D. esculenta*) vines twine to the left (Behera *et al*., 2009; Lebot, 2009; Lopez-Montes and Edemodu, 2015). Yam species belonging to the Enantiophyllum section usually produce one to three large tubers, while that of Combilium and Macrogynodium produce a greater number of smaller tubers (Lebot, 2009).

2.1.2 Morphological description of yam

Yams are annual or perennial tuber–bearing and twining climbers (IITA, 2006; Lebot, 2009). The plant is a multi-species, polyploid and vegetatively propagated tuber crop that is cultivated widely in the tropics and subtropics for its starchy tubers (Mignouna *et al*., 2007; Sartie and Asiedu, 2011). The above-ground vegetative mass of the crop includes stem (rope-like structure), branches and leaves (Behera *et al*., 2009). Vines of yam plants are either rhizomatous or tuberous, with the latter containing all the horticulturally important

food species (Lebot, 2009; Mignouna *et al*., 2009). Even though the vines are unable to support the weight of the leaves and have to climb by twining, they have no specialized organs such as tendrils (Lebot, 2009). The crop has a spiral leaf arrangement on the stem, which may either be opposite or alternate depending on species, and quite often can also be alternate on the lower part of the stem and opposite on the upper part (Lebot, 2009). Yam plants are dioecious, with separate pollen- and pistil-bearing flowers borne on different individuals (Mignouna *et al*., 2009). The root system which provides anchorage for the plant is superficial, consisting of several thick and long roots which usually develop rapidly after sprouting, reaching considerable distances of 3 to 4 m in radius around the plant (Lebot, 2009). The development of the root system is however restricted when the crop is cultivated in mounds (Lebot, 2009). *Dioscorea rotundata* which is the major yam species cultivated in West Africa has a glabrous, fistulose and streaked stem, bearing leaves (oval or almost round in shape) which are opposite with a long petiole (Lebot, 2009; Reddy, 2015). In Ghana, the most cultivated varieties of *D. rotundata* are laribako and pona (Demuyakor *et al*., 2013; Otoo *et al*., 2015) with distinctive morphological characteristics (Table 2.1).

Table 2.1: Range of morphological variability in *D. rotundata* cultivars (laribako and pona)

Parameter	*D. rotundata* cultivar	
	Laribako	**True Pona**
Leaves	Small and saggitate light green shiny smooth surface already formed at emergence of sprout	Large and cordate Deep green Rough and dull Develops long after emergence of sprout
Vines	Spineless	Vary from long slender to long big, with or without spines
Sprout	sole sprouting	Sole sprouting (true pona) to multiple sprouting (Kulunku)
Tuber Shape	Cylindrical	Oval-oblong
Tuber flesh colour	White	White with yellowish tint
Seed yam crown colour	White	Purple
Cooked tuber	very soft and very mealy	Soft and mealy

Source: Otoo *et al*. (2009)

2.2 Origin and distribution of yam

2.2.1 Origin

Various species of edible yam have been domesticated independently in America, Africa, Madagascar, South and Southeast Asia, Australia and Melanesia (Lebot, 2009). Out of the over 600 *Dioscorea* species only 10 are considered as economically important edible yams; and are regularly cultivated for food (Lebot, 2009; Nyaboga *et al*., 2014). The major cultivated species are *Dioscorea alata, D. cayenensis* and *D. Rotundata*, while minor ones are *D. bulbifera, D. esculenta, D. opposita-japonica, D. nummularia, D. pentaphylla, D. transversa* and *D. trifida* (Lebot, 2009). The 10 most important cultivated yams and their geographical origin are shown in Table 2.2.

Table 2.2: Geographic origin of the ten most important cultivated *Dioscorea* spp.

***Dioscorea* spp.**	**Common names**	**Geographical origin**
Dioscorea alata	Greater, water, winged yam	Southeast Asia, Melanesia
D. bulbifera	Aerial-, bulbil-bearing yam	South America, Africa, Asia, Melanesia
D. cayenensis	Yellow guinea yam	West Africa
D. esculenta	Lesser yam, Asiatic yam	Southeast Asia, Melanesia
D. opposita-japonica	Chinese, Japanese yam	Japan, China
D. nummularia	Spiny yam, wild yam	Melanesia
D. pentaphylla	Five-leaved yam	Southeast Asia, Melanesia
D. rotundata	White guinea yam	West Africa
D. transversa	Marou, waël	Australia, Melanesia
D. trifida	Aja, aje, cush-cush, yampi	South America

Source: Lebot (2009)

2.2.2 Distribution

Yam is cultivated in over 50 tropical countries in the world (Lebot, 2009). Yam production areas in the world are represented in Figure 2.1. *Dioscorea alata* is the most widely distributed species globally whiles *D. rotundata* is indigenous and economically important yam in West Africa (Quain *et al*., 2011; Nyaboga *et al*., 2014). *Dioscorea alata* is cultivated in Asia, the Pacific Islands, Africa, and the West Indies making the species the most widely distributed yam species cultivated in the world (Mignouna *et al*., 2003; Lebot, 2009). The wide cultivation of *D. alata* is attributed to its ease of cultivation, and long postharvest life, but in West Africa it is considered as inferior to *D. rotundata* (Lebot, 2009). It is therefore not surprising that in West Africa; the popularity of water yam is second to white yam (Reddy, 2015).

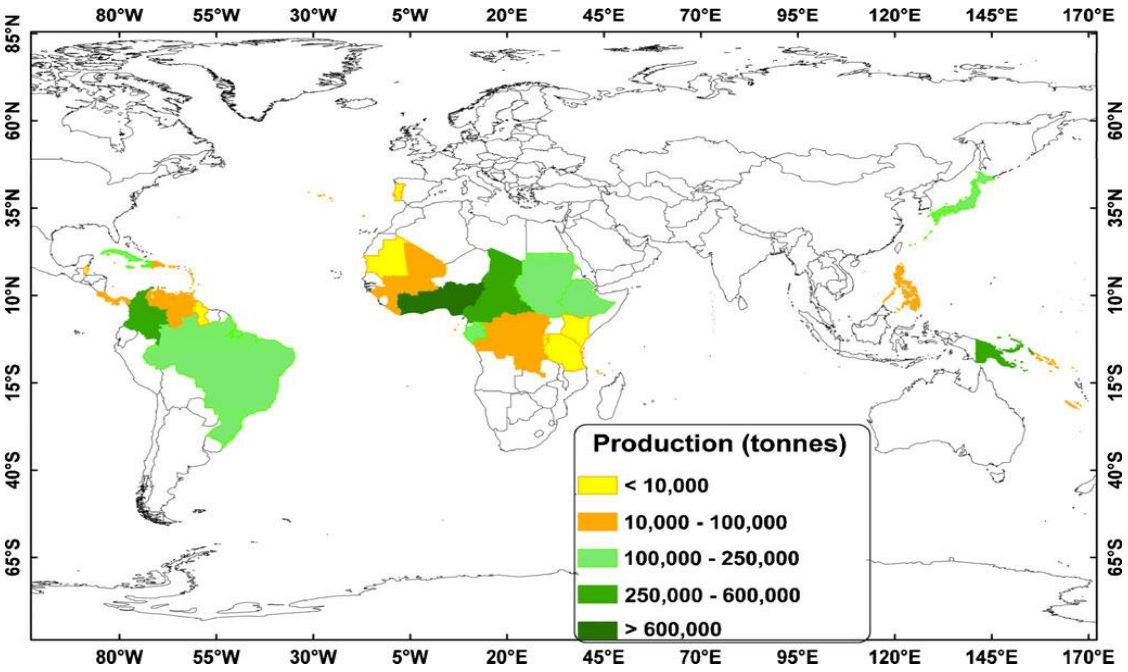

Figure 2.1: World map showing yam production areas

Source: Asiedu and Sartie (2010)

During the 19th and 20th centuries, *D. alata*, *D. cayenensis* and *D. rotundata* which are the most important cultivated species were distributed widely in all tropical countries by colonial powers and missionaries (Lebot, 2009). Table 2.3 shows some of the most important yam species and their distribution in major yam producing areas. The most important areas of yam cultivation and use in Africa are Ivory Coast through Ghana, Nigeria, Togo, Cameroon, Gabon, Central African Republic and the Western part of the Democratic Republic of Congo (Okigbo *et al*., 2015). In West Africa *D. rotundata* is the most preferred cultivated species, accounting for a large proportion of yam production in the sub-region (FAO, 2013). The distribution of *D. rotundata* throughout the West African sub region is illustrated in Figure 2.2.

Table 2.3: Yam species and their main areas of cultivation

Yam species	Main areas of cultivation
D. rotundata	West Africa, Latin America, the Caribbean
D. alata	Oceania, Southeast Asia, Africa, the Caribbean, Latin America
D. cayenensis	West Africa, Latin America, the Caribbean
D. trifida	Guyana, Brazil, Central America, the Caribbean
D. esculenta	Southeast Asia
D. opposita	China, Temperate Asia, France

Source: Reddy (2015)

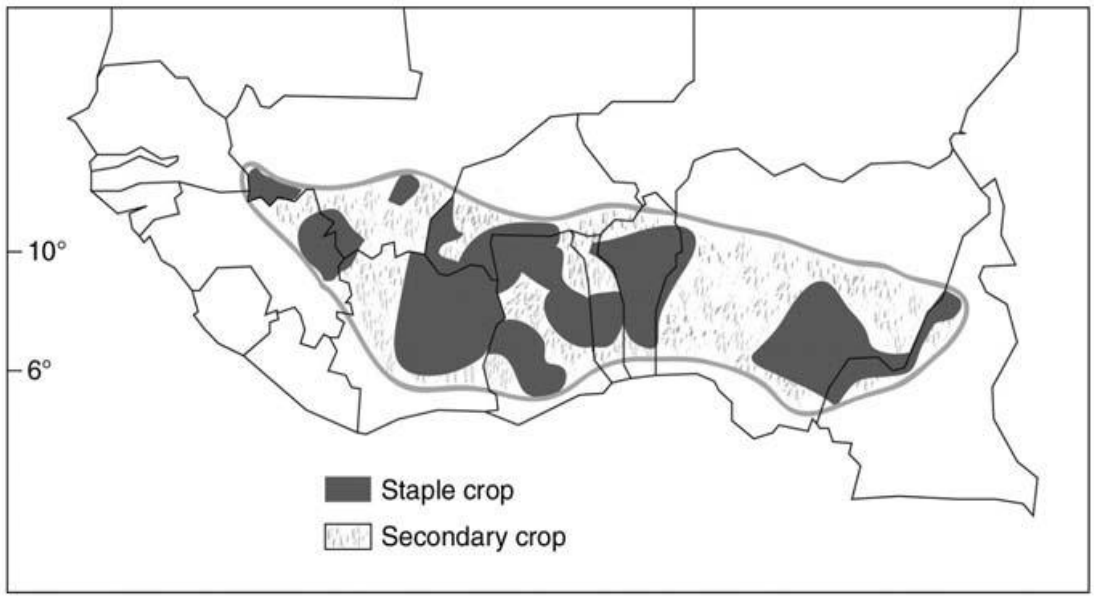

Figure 2.2: Area of distribution of *Dioscorea rotundata* in West Africa

Source: Dumont *et al*. (2006)

2.3 Production estimates of yam

Yam is an economically important crop cultivated in Africa, Asia, Australia, the Caribbean, India, and in South Pacific (IITA, 2010; Nyaboga *et al*., 2014). Table 2.4 shows the regions of cultivation of the most important species of yam staples in the world.

Table 2.4: Major food yam species in different regions of the world

Region of the world	Major economic species.
Africa	*D. rotundata*
	D. cayenensis
Asia	*D. alata*
	D. esculenta
America	*D. trifida*

Source: Reddy (2015)

In West African yam-producing countries such as Nigeria, Ghana, Cote d' Ivoire, Cameroon, Benin and Togo, yam is considered an important food crop (Izekor and Olumese, 2010). The annual estimated yam production in the world for 2014 was 68,132,131 tonnes (FAOSTAT, 2017). West Africa yam producing countries account for about 95% of the annual global yam production (Fu *et al*., 2011; Demuyakor *et al*., 2013). In the West Africa sub region, yam is the second most important tuber crop after cassava (*Manihot utilisima*) in terms of tonnage of production and in food security terms (Osunde, 2008) with *D. rotundata* and *D. alata* as the most important yam species in the region (Ile *et al*., 2006). The top 10 yam-producing countries in the world for the 2014 cropping season are presented in Table 2.5.

In West Africa, the increase in yam production over the past two decades was mainly due to increases in cultivated area rather than yield per hectare (Lebot, 2009). This trend still persists in Ghana where the general increase in quantity of yam produced over the years seems to be directly proportional to the land area harvested (Table 2.6).

Table 2.5: Top ten yam producing countries in the world

Rank	Country	Production in tonnes (2014)	% of World Total
1	Nigeria	45,004,340	66.05
2	Ghana	7,119,000	10.45
3	Cote d' Ivoire	5,808,972	8.53
4	Benin	3,220,654	4.73
5	Ethiopia	1,448,835	2.13
6	Togo	786,394	1.15
7	Cameroon	579,326	0.85
8	Central African Republic	478,764	0.70
9	Haiti	476,667	0.70
10	Chad	443,558	0.65

Source: FAOSTAT data (2017)

Table 2.6: Annual production and cultivated land area of yam in Ghana (from 2006-2015)

Year	Area harvested (Ha)	Production (tonnes)
2006	325,000	4,288,000
2007	324,000	4,376,000
2008	348,000	4,895,000
2009	379,000	5,778,000
2010	385,000	5,960,000
2011	204,000	5,855,000
2012	426,000	6,639,000
2013	422,000	7,075,000
2014	428,000	7,119,000
2015	430,000	7,296,000

Source: MOFA/SRID (2016)

In Ghana, yams are produced in the Northern, Volta, Upper West, Brong-Ahafo, Eastern, Ashanti, Western and Central regions (Demuyakor *et al*., 2013; MOFA/SRID, 2016). Major cultivations are however concentrated in the Brong-Ahafo and Northern Regions constituting about 37% and 34 % of total yam production respectively (MoFA, 2012). The top 5 yam-producing regions in Ghana are presented in Table 2.7.

Table 2.7: Top 5 yam producing regions in Ghana (three years averages, 2013-2015)

Region	Average yield (tonnes)	Share of sub total	Share of overall total
Brong Ahafo	2,397,838.46	36.53%	33.47%
Northern	2,261,468.72	34.45%	31.57%
Eastern	811,492.73	12.36%	11.33%
Upper West	573,107.22	8.73%	8.00%
Ashanti	520,516.64	7.93%	7.27%
Sub total	6,564,423.77		91.64%
Total average production (all regions)	7,163,198.58		

Source: MOFA/SRID 2016

2.4 Yam cultivated in Ghana

Yam such as *D. rotundata*, *D. alata*, *D. bulbifera*, *D. cayenensis* and D. *dumetorum* are cultivated in Ghana (CSIR-SARI, 2011; Otoo *et al*., 2015). The varieties of yam cultivated include laribako, pona, dente, yesumogya, muchumudu, karangba, asobayere, enoti, didi, akaaba, mpuano, kparinjo, akomiya, matches and tilla (Demuyakor *et al*., 2013; Otoo *et al*., 2015). The majority of cultivated yams in Ghana are varieties of *D. rotundata* and *D. alata* (Demuyakor *et al*., 2013) with *D. rotundata* being the most preferred (Otoo *et al*., 2015). A survey carried out to assess yam diversity cultivated across the northern yam belt and the Forest-Savannah Transition agroecology of Ghana revealed that *D. rotundata* is the most cultivated species followed by *D. alata* (CSIR-SARI, 2011; Otoo *et al*., 2015). In Ghana among the many cultivated varieties of *D. rotundata;* in the order of importance, pona, laribako and dente are the main cultivars of preference; due to their sweet and floury characteristics (Otoo and Asiedu, 2009).

2.5 Economic importance of yam

Globally, yam serves as staple food for over one hundred million (100, 000 000) people, and also provides food security and livelihood for more than 60 million people in West Africa (CSIR/SARI, 2013; CSIR/SARI, 2014; Maroya *et al*, 2014). The tubers produced by the yam crop serve as a source of food, feed and drugs or medicines (Demuyakor *et al*., 2013).

Yam is consumed by majority of Ghanaians in both rural and urban areas, with higher consumption in the urban areas and contributing about 13% of household food budget in urban centres (Aidoo *et al*., 2009). Yam tubers may be eaten in boiled, roasted, fried or pounded form (Aidoo *et al*., 2012). The tubers contribute more than 200 calories per person per day for more than 150 million people in West Africa (FAO, 2006). Although the main constituent of the yam tuber is carbohydrate, it is also a major source of dietary fibre, proteins, vitamins (thiamine, riboflavin and vitamins B and C) and essential minerals (phosphorus, calcium and iron) (Babaleye, 2003; Polycarp *et al*., 2012; Okigbo, 2015). The yam tuber is regarded as the most nutritious of the tropical root crops (Wanasundera and Ravindran, 1994). Bradbury and Holloway (1988) reported that yam contains approximately four times as much protein as cassava and is the only major root crop that exceeds rice in protein content in proportion to digestible energy. Yams therefore, provide a good proportion of protein requirement of man when consumed in large quantities (Odurukwe, 1980).

The crop also possesses some pharmacological properties and is used as a raw material in pharmaceutical companies (Amanze *et al*., 2011). Yam contains secondary metabolites such as steroidal saponins, diterpenoids, and alkaloids which have been exploited for pharmaceutical products (Mignouna *et al*., 2008). Yam is also used as a raw material in the

starch industries, and provides employment for a great number of people (Amanze *et* al., 2011). Lebot (2009) reported that, yam starches have good potential as substitutes for modified starches in acid solutions such as tomato products, dressings and sauces; and are also useful for product development of noodles, snacks and baby food products. Yam tubers can be processed into flour, packaged and sold commercially in supermarkets, urban food stores and in foreign markets. Yam flour has a prolonged shelf life and can be stirred into a thick viscous food called *fufu* (Lebot, 2009).

Yam is important in the local commerce in West Africa and accounts for about 32% of farm income (Chukwu and Ikwelle, 2000). The crop also serves as a major source of foreign exchange earnings and as the leading exporter of yam in the world, yam exports contribute significant foreign exchange earning to the Ghanaian economy (Ohene-Yankyera *et al*., 2011). In 2011, yam production in Ghana was over six million metric tonnes, accounting for over 1.6 million US dollars; making the crop the leading foreign exchange earner in terms of agricultural commodities (FAO, 2013).

Yam also has considerable socio-cultural significance, especially in West Africa and the South Pacific islands, where it is central to important annual ceremonies (Sartie and Asiedu, 2014). Besides the nutritional and economic values of yam, it also has cultural values in Ghana. For instance, the annual celebration of new yam festivals in some yam producing regions such as Ashanti, Volta and Brong-Ahafo to usher in newly harvested yams is an indication of the significance of yam in the socio-cultural lives of the people (Narula *et al*., 2007; Tortoe, 2015).

Yam contributes significantly to the national Gross Domestic Product (GDP), generates income and provides employment to small scale farmers and value chain service providers in terms of processing as well as local and international service providers in yam industry

(CSIR/SARI, 2014). It can be stored longer than most other tropical fresh products, and therefore stored yam represents stored wealth and food security. Yam has been identified as a crop with potential for increased commercial exploitation and processing (O' Hair, 1990).

Besides the nutritional and economic importance of yams, the cultivation of the crop is confronted with certain constraints which affect yield output. Despite increasing human population, the quantity of yam production has either remained stagnant or is declining (Maroya *et al*., 2014).

2.6 Constraints in yam production

Yam production in Ghana is affected negatively by several factors. The major constraints to yam production include inadequate capital, non-availability and high cost of quality planting materials, pests and diseases infestation, weeds, inadequate stalking materials, low soil fertility, low yield, high cost of labour and storage losses (Izekor and Olumese, 2010; Omojola, 2014; CSIR/SARI, 2014). Losses associated with the crop limit the potential income of the farmers, threaten food security and exacerbate conditions of poverty among rural households, whose livelihood depends on yam production (Thamaga- Chitia *et al.*, 2004).

2.6.1 Pest and diseases of yam

Pest and diseases are important constraints to yam production; having deleterious effects on tuber yield and quality, with these constraints becoming more severe over time (Aidoo *et al.*, 2011; Nyaboga *et al*., 2014). The yam crop is prone to attack by pest such as millipedes, mealybugs, scale insect, nematodes and yam beetle (CSIR/SARI, 2012). Yam yield losses also result from diseases caused by viruses, fungi and bacteria (Nyaboga *et al*., 2014). Each pest and disease of yam has a different impact on yam production.

2.6.1.1 Insect pests

Beetle species that cause damage in yam include: *Heterolligus meles*, *H. appius*, *Heteronychus licas*, *Prionoryctes rufopiceus*, *P. caniculus* and *Lepidiotica reichei* (Lebot, 2009). These beetles are capable of causing damage to yam plants on the field and tubers in storage. *Heterolligus meles* usually cause damage by feeding on planting setts resulting in wilting and death of the plant and also feed on young developing tubers leading to tuber damage (Lebot, 2009; Reddy, 2015). The scale insect *Aspidiella hartii* can infest tubers in storage reducing their economic value and also resulting in poor germination and growth when used as yam setts (Reddy, 2015). Pest such as mealybugs (*Gonococcus coffeae*, *Phenacoccus gossypii* and *Planococcus citri*) and aphids (*Aphis gossypii*) can cause damage to yam plants by feeding on the sap of leaves and stems of yam plants; causing plant damage (Reddy, 2015).

2.6.1.2 Nematodes

Nematodes are capable of damaging yam plants by puncturing plant cells causing cell breakdown and malfunction in the plant tissues (Lebot, 2009). The most important nematode on yams all over the tropics, especially in the West African yam producing belt is *Scutellonema bradys*, which damage results in 11 million tonnes loss of storage yams in West Africa (Reddy, 2015). *Scutellonema bradys* causes a tuber disease known as dry rot by feeding within the tuber causing tissue breakdown and producing cavities (Reddy, 2015). The nematode *Pratylenchus coffeae* causes damage similar to that of *S. bradys* (Reddy, 2015). Nematodes belonging to *Meloidogyne* spp. are capable of causing both pre- and post-harvest losses in yam (Bridge *et al.*, 2005).

2.6.1.3 Viral diseases

Viral diseases also constitute a factor militating against yam production. Symptoms exhibited by yam plants affected by viral diseases include severe leaf chlorosis, green vein banding, curling, mottling, green spotting, flecking, mosaic, shoestringing, interveinal chlorosis, stunting, and distortion (Reddy, 2015). These result in reduction of photosynthetic area of plant with deleterious effects on the tuber yield and in some cases causing the death of the plant (Reddy, 2015). Although about 15 different viruses are pathogenic on yam plants, the most economically important viral diseases are those caused by the yam mosaic potyvirus (Lebot, 2009; Reddy, 2015). The disease is transmitted by aphids and affects particularly specie of *Dioscorea* such as *D. alata*, *D. cayenensis* , *D. esculenta* and *D. rotundata* (Reddy, 2015).

2.6.1.4 Fungal diseases

Several fungi including *Phyllosticta* spp., *Rhizoctonia solani*, *Corticium rolfsii* and *Fusarium oxysporum* have been isolated from yam plants (Lebot, 2009). These fungi have different impact on yam plants. For instance, *Phyllosticta* spp. infection of yam plants usually results in the development of large necrotic spots on the lamina, which enlarge and spread to all the foliage (Lebot, 2009). *Rhizoctonia solani* infection on yam plants is characterised by browning of the leaves lamina (Lebot, 2009). *Corticium rolfsii* attacks the stem base of the plant causing the entire plant to wilt (Lebot, 2009). Despite the symptoms exhibited by these fungal pathogens, none of them has an economic importance on yam production compared to *Colletotrichum gloeosporioides* which causes anthracnose disease of yam (Lebot, 2009).

2.6.2 Anthracnose disease of yam

The anthracnose disease of yam is one of the most important constraints to yam production world-wide (Abang *et al*, 2002; Reddy, 2015). *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. has been documented as the major causal agent of the disease and is present in all yam growing regions, and by far the most important foliar disease of yams (O'Sullivan, 2010; Reddy, 2015). The disease affects not only the leaves and vines, but also the tubers; severe infection results in yam plant defoliation and vine dieback (Ayodele *et al*., 2000). According to Amusa *et al*. (1996). *Colletotrichum gloeosporioides* is a major necrotic lesion pathogen on yam leaves.

2.6.2.1 Symptoms of yam anthracnose disease

Yam anthracnose disease symptoms vary according to the age of the leaf, the amount of rain, and the variety of yam (Reddy, 2015). Anthracnose infected yam plant possesses symptoms which first appear as small dark brown or black lesion on the leaves, petioles and stems (Amusa *et al*., 2003). The lesion is often surrounded by a chlorotic halo which enlarges and coalesces, resulting in extensive necrosis of the leaves and die-back of the stem (Plate 2.1) (Amusa, 1997). The leaf spots can also run together to form large irregular blotches, the centres of which may fall out giving a "shot hole" (Reddy, 2015).

The necrotic lesions on infected leaves are caused by phytotoxic compounds produced by *C. gloeosporoides* during attack (Alleyne, 1997; Reddy, 2015). The withered leaves and stem dieback give infected plants a scorched appearance (IITA, 1993); converting a field of initially healthy yam plants from 'green' to 'black' within a few weeks (Green and Simons, 1994).

The leaf necrosis and stem dieback of anthracnose infected yam plant results in reduction of photosynthetic surface area of the crop with a concomitant reduction in ability of the yam tuber to store food reserve, which can cause yield loss in excess of 90% (Egesi *et al*., 2007; FAO, 2012).

Colletotrichum gloeosporioides has also been reported to cause an orange-brown tuber rot known as "dead skin" (Green and Simons, 1994; Reddy, 2015). "Dead skin" starts as small blisters on yam tuber surface, making it easy to remove the skin from the layer beneath; and later resulting into a deeper rot development with a winkled shell surrounding a rotting core (Reddy, 2015) (Appendix 3C).

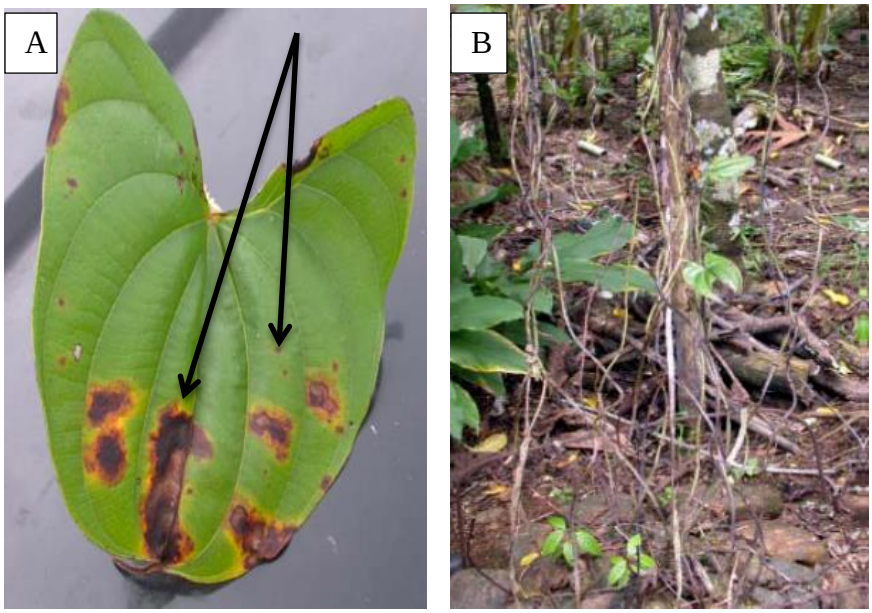

Plate 2.1: Yam Anthracnose lesions on leaf (A) Black lesions on leaves (arrowed) and stems and shoots die (B)

Source: Reddy (2015).

2.6.2.2 Disease cycle and epidemiology of anthracnose of yam

2.6.2.2.1 Disease cycle

Colletotrichum gloeosporioides survives between yam cropping seasons as mycelium or conidia in infected yam setts/tubers or in the soil in infected yam crop debris; serving as a source of primary infection (Agrios, 2005; Chaube and Pundhir, 2009; Lebot, 2009). Other sources of primary infection are alternate hosts on the yam field (Kutama *et al.*, 2013). When the primary inoculum comes into contact with a susceptible yam leaf under favourable conditions, the fungus acervuli produces conidia which germinate and directly penetrate the leaf tissues with the aid of germ tubes (Agrios, 2005). The mycelium grows intercellularly resulting in early infection and invasion of tissues, leading to death and collapse of the tissues (Agrios, 2005). The mycelium colonizes subcuticular tissues and produce acervuli which produce conidia (Agrios, 2005; Than *et al*., 2008). When the acervuli are wet, the cuticle breaks releasing the conidia which are spread by agents such as splashing and blowing rain, wind, insects, other animals and tools (Agrios, 2005; Chaube and Pundhir, 2009). These spread conidia, under favourable conditions of temperature and humidity serve as a source of secondary infection of the disease (Agrios, 2005; Chaube and Pundhir, 2009). The dispersed conidia only germinate in the presence of water and also penetrate the host tissue directly (Agrios, 2005). The hyphae initially grow rapidly intercellularly and intracullarly causing little or no visible anthracnose symptom (Agrios, 2005). Then suddenly the fungus becomes more aggressive; revealing the symptoms of the disease (Agrios, 2005).

2.6.2.2.2 Epidemiology

Colletotrichum gloeosporioides can only survive in soils for a few weeks, but has the capacity to survive between growing seasons on crop debris (Kutama *et al.*, 2013; Reddy, 2015). The pathogen can infect and survive in yam tubers (Green and Simons, 1994) and in decomposing yam residues in soil, which allows it to survive between growing seasons (Ripoche *et al*., 2008).

The pathogen has been reported to be tuber-borne in the Caribbean, West Africa and West Indies (Green and Simons, 1994; Reddy, 2015). Anthracnose tuber-borne infection could have an effect on the disease epidemiology, since infected tubers could be a primary source of inoculum and a means of dispersal (Lebot, 2009).

Colletotrichum gloeosporioides is not host specific, so infects many other crops and weeds and as such spores from these alternate host can be a source of infection of yam crops on the field (Kutama *et al.*, 2013). Also small immature tubers derived from early shoot death, may be a major source of infection of the shoots as they develop (Abang *et al*., 2002; 2004). Planting disease-infected seed yams is a major constraint to yam production because farmers lack appreciation of the consequences of the disease on yield (Nweke *et al*., 2011).

Anthracnose is established on yam plants from one of the various sources of inoculum. The established disease can affect all parts of the yam plant and at all stages of crop development (Akem, 1999). Although *C. gloeosporioides* infects mainly leaves, it causes anthracnose symptoms on all plant parts, which appear initially as small brown spots that expand and cause extensive blackening of the leaves as the disease progress (Akem, 1999).

The severity of the disease may differ from year to year in a particular field since it is influenced by environmental factors (Akem and Asiedu, 1994) and the variety of yam

cultivated. According to Kutama *et al*. (2013), severe outbreak will depend on susceptible varieties following rainstorms or cyclones. This is because spores are found in large numbers on the leaf spots and are splashed in rain and or carried by dripping dew to adjacent and lower leaves and stems (Reddy, 2015). Other factors can also influence the severity of the disease. For example, crop nutrition is widely recognised to affect severity of a range of pests and diseases (Engelhard 1989). Yam crops under nutritional stress are likely to have increased susceptibility to anthracnose infection (O'Sullivan, (2010).

Epidemics that commence prior to or during tuber formation can have a tremendous effect on tuber yield (Abang *et al*., 2003). Anthracnose infected yam plants produce several smaller tubers instead of the normally larger tuber (Palaniyandi *et al*., 2016) (Plate 2.2).

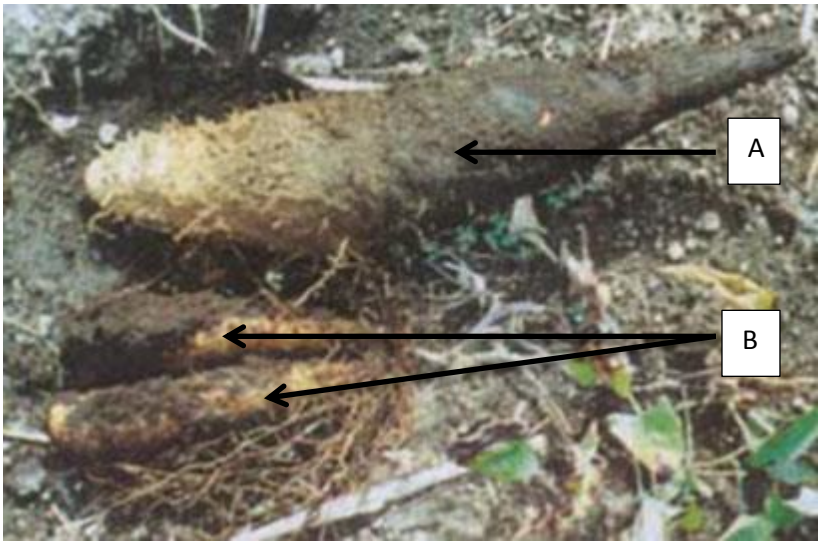

Plate 2.2: Big tuber produced by healthy plants (A) compared to small tubers produced by yam anthracnose infected plants (B).

Source: Reddy (2015)

The onset of the disease before or during tuber formation can result in yield loss exceeding 80% (Green and Simons, 1994; Sweetmore *et al*., 1994 and Agrios, 2005). Yam varieties

such as *D. alata, D. cayenensis* and *D. rotundata* are susceptible to anthracnose disease, with *D. alata* being the most susceptible (Lebot, 2009). Ayodele *et al*. (2000) also reported that anthracnose is one of the most serious leaf and vine epiphytotic diseases of yams, especially *D. rotundata* and *D. alata,* in yam-growing countries of West Africa.

2.7 The pathogen *Colletotrichum gloeosporioides*

Colletotrichum is a major genus of pathogen that causes anthracnose disease among a wide range of plant host ranging from gymnosperms, angiosperms, ornamental and fruit plants, vegetables, crops or even grasses (Dean *et al*., 2012). Species of *Colletotrichum* associated with anthracnose disease include *C. gloeosporioides, C. capsici, C. falcatum, C. truncatum, C. sansevieriae, C. acutatum,* and *C. coccode* (Gautam *et al*., 2012). Majority of crops cultivated in the world are susceptible to one or multiple species of *Colletotrichum*; hence the pathogen could be a major constraint to crop production worldwide (Dean *et al*., 2012; Weir *et al*., 2012; Gautam, 2014). Warm and humid environments are favourable conditions for the pathogen to establish infection and spreading the anthracnose disease uniformly and effectively (Farr *et al*., 2006; Gautam, 2014).

Colletotrichum gloeosporioides is a ubiquitous, proliferate and economically important pathogen causing substantial yield losses due to fruit decay and damage to vegetative parts in a variety of plant species (Freeman and Shabi, 1996). Diseases symptoms such as anthracnose, dieback, and root rot, leaf spot, blossom rot and seedling blight are among many others caused by the pathogen on a wide range of crops (Sanders and Korsten, 2003).

Colletotrichum gloeosporioides is the anamorph (imperfect) or asexual state while *Glomerella cingulate* is the sexual (perfect) stage or teleomorph state of the pathogen (Gautam, 2014). Studies have revealed that the teleomorph state causes severe disease

symptoms comparatively (Cannon *et al*., 2012). During interaction with a susceptible host, *C. gloeosporioides* produces various specialized structures: conidia, acervulli, setae and appressoria which aid in establishing an infection resulting in tissue necrosis of infected plants (Gautam, 2014).

The fungus generally produces circular, woolly or cottony colonies on culture media with characteristic colour (ie pale brown or greyish white) and with a hyaline, septate and branched mycelium (Hiremath *et al.*, 1993; Prabakar *et al.*, 2005; Vidyalakshni and Divya, 2013). The growth and sporulation of *C. gloeosporioides* is influenced by nutritional component of culture media, temperature, moisture and pH; with an ideal pH range of 6-7, temperature 25-30 ^{o}C (Rani and Murthy, 2004; Singh *et al*., 2006; Sangeetha and Rawal, 2009; Hubballi *et al*., 2011; Pandey *et al*., 2012). An exposure of the fungus to alternate cycles of 12 hours light and 12 hours darkness shows maximum mycelia growth in comparison to 24 hours exposure to continuous light or darkness (Hubballi *et al*., 2011).

2.8 Identification of *Colletotrichum gloeosporioides*

The proper identification of a plant pathogen is important because it allows for the development of an effective management strategy to combat the disease. Morpho-physiological methods such as conidia morphology, appressoria formation, presence or absence of setae, presence or absence of telemorph, colour of colonies, mycelia growth rate and sensitivity to fungicides have been used for differentiating *C. gloeosporioides* isolates (Freeman, 2000; Serra and Silva, 2004). Conidia shape and dimension have been used as basic morphological criteria for identification of *C. gloeosporioides* and separation within species (Simmond, 1965; Cox and Irwin, 1988). However, the enormous variation in the morphology accepted for the different species of *Colletotrichum* can make the identification of the species or biotypes difficult and extremely variable (Sutton 1980; Serra *et al*., 2011).

For instance, the identification of *C. gloeosporioides* can be confused with other *Colletotrihum* species, especially *C. acutatum* and as such older records of *C. gloeosporioides* must be re-examined for its correct identification (Brown *et al*., 1996; Cannon *et al*., 2008; Chowdappa and Kumar, 2012). These challenges of identification have paved the way for the successful development of various reliable molecular methods used in the differentiation of *Colletotrichum* species and their isolates. The analysis of the nucleotide sequence of the internal transcribed spacing (ITS) of the ribosomal DNA (rDNA) from genes of ι-tubulin 2 (tub2), histone 4 (his4), glutamine synthase (GS), glyceraldehyde-3-phosphate dehydrogenase (GPDH), mytocondrial DNA (mtDNA), RAPD, RFLP and AFLP markers and isozyme analysis have demonstrated the genetic complexity of *Colletotrichum* isolates obtained from diverse tropical and temperate plants (Freeman, 2000; Talhinhas *et al*., 2005; Serra *et al*., 2011). The use of molecular techniques such as polymerase chain reaction (PCR) with species-specific primers has been employed (Adaskaveg and Hartin, 1997).

2.9 Control of anthracnose disease of yam

Plant diseases such as yam anthracnose may be minimized by the reduction of the causative agent's inoculum, inhibition of its virulence mechanisms and promotion of genetic diversity in the crop (Strange and Scott, 2005). Successful control of yam anthracnose disease would encourage greater wide spread cultivation and significant increase in overall production to meet the high local and overseas demand for yam (FAO, 2002). Developing appropriate control measures to reduce losses due to anthracnose could go a long way to increase yield of farmers and also eliminate expenses associated with chemical and mechanical methods of controlling the disease (Agrios, 2005). For a control measure to be economically effective, it must impede the development of yam anthracnose until the phase of root bulking is

complete (Green, 1994). The major control methods employed in the management of yam anthracnose disease include biological, cultural, use of anthracnose resistant cultivars and application of chemicals.

2.9.1 Biological control

Biological control methods of managing plant diseases through the use of antagonistic microorganisms have been reported as an environmentally-friendly alternative to chemical usage (Bhattacharjee and Dey, 2014). Microbes such as *Trichoderma* spp., *Bacillus subtilis*, *Pseudomonas* and *Streptomyces* spp. have been documented as having antagonistic properties against certain phytopathogenic pathogens (Zhang and Xue, 2010; Palaniyandi *et al*., 2011; Bhattacharjee and Dey, 2014); hence have the potential to be used as biofungicides against a wide range of plant diseases.

The mechanism of operation of these microbes include antibiosis, secretion of volatile toxic metabolites, mycolytic enzymes, parasitism and their ability to compete for space and nutrients (Bhattacharjee and Dey, 2014). For instance, the volatile toxic metabolite chitinases secreted by certain microbes possess antifungal properties which weaken and degrade the cell walls of pathogenic fungi (Edreva, 2005).

Reports have been made about the successful control of *C. gloeosporioides* by bioagents such as *Bacillus licheniformis* (Govender *et al*., 2005), *Brevundimonas diminuta* (Kefialew and Ayalew, 2008) and *Streptomyces cavourensis* (Lee *et al*., 2012). The antifungal activities of certain species of *Trichoderma*, *Chaetomium*, and *Penicillium* against *C. gloeosporioide* have also been established (Soytong *et al*., 2005). *In vivo* antagonistic activity of *Streptomyces* spp. against the yam pathogens *Curvularia eragrostides* and *C. gloeosporioides* has been reported (Soares *et al*., 2006). Palaniyandi *et al*. (2016) reported

the potential use of culture filtrate extract of azalomycin-producing *Streptomyces malaysiensis* strain MJM1968 as a biofungicide for both *in vitro* and *in vivo* management of yam anthracnose disease.

Despite the potential use of microbes as biocontrol agents, certain challenges may be associated with their use. According to Bhattacharjee and Dey (2014), the use of *Trichoderma* spp. as biofungicide may adversely affect arbuscular mycorrhizal fungi which are associated with root of herbaceous plants. They also reported that *Trichoderma* spp. usually have a low field performance as a biocontrol agent. Development of resistance to *Bacillus thuringiensis* toxin as a biofungicide against pathogenic fungi has been reported (Rajagopal *et al*., 2009) and the need for an alternative has been suggested (Veliz *et al*., 2017).

2.9.2 Cultural control

Several crop hygiene practices can contribute to the management of yam anthracnose disease on yam fields. Kutama *et al.* (2013) reported that early planting can delay development of anthracnose on yam crops. Early planting may also allow more time for yam vines to reach the top of their support ahead of the storm season; hence, tuber formation may start before high incidence and severity of the disease at the peak of the rainy season (Reddy, 2015). Planting yam setts with symptoms of rot should be avoided; this will help reduce anthracnose infection on yam fields (Reddy, 2015).

Regular weeding to remove weeds that serve as alternate hosts to the anthracnose pathogen (*C. gloeosporioides*) could also contribute greatly to minimizing anthracnose infection on yam fields (Offei *et al*., 2008). Offei *et al.* (2008) also recommended that extreme care should be taken in the handling of yam crops on the field to prevent the transmission of

disease from one plant to another. Thus, one should not handle an anthracnose infected yam crop before handling a healthy crop without thorough hand wash. This was supported by Jackson (2014) who recommended that movement through yam crops when the foliage is wet should be avoided since this might spread spores of anthracnose fungus.

Frequent inspection and removal of anthracnose disease yam crop parts such as leaves and vines will minimize the disease spread during the cropping season (Offei *et al*., 2008). After harvesting the yam tubers, yam debris such as leaves and vines should be collected and buried or burnt (Agrios, 2005; Chaube and Pundhir, 2009) to destroy any *C. gloeosporioides* inoculum.

Crop rotation is also a recommended way of minimizing anthracnose infection on yam plants (Agrios, 2005; Offei *et al*., 2008). This can be achieved by alternating in succession the cultivation of yam crops with a crop that is non-susceptible to anthracnose infection on the same plot, at such interval as will allow the pathogen population to decrease (Offei *et al*., 2008). Intercropping yam with maize is also a measure of reducing anthracnose infection (Reddy, 2015).

Yam crops under nutritional stress are likely to have increased susceptibility to anthracnose infection, and so appropriate fertilisation may be an effective disease control measure (O'Sullivan, 2010). Avoiding damage during yam harvest (Jackson, 2014) will minimize infection of tubers or setts which may be used as a planting material. Most cultural control measures are not reliable in controlling yam anthracnose disease (Jackson, 2014).

2.9.3 Use of resistant varieties

McDonald and Linde (2002) advised yam farmers to select tubers from plants showing anthracnose resistance and to use only these for propagation and increase of stock. Agrios

(2005) also advocated that, anthracnose resistant yam varieties should be used for cultivation when available. The use of yam resistant varieties for cultivation has been recommended as an eco-friendly alternative to the use of synthetic fungicides in combating the disease (Abang *et al*., 2002). This has paved the way for various researches in the form of conventional breeding toward the development of disease resistant and high yielding yam varieties (Nyaboga *et al*., 2014). However, the genetic improvement of yam through breeding programs faces challenges such as the long breeding cycle, dioecious, poor flowering nature, polyploidy, vegetative propagation, and heterozygous genetic background (Mignouna *et al*., 2008). Variation within *C. gloeosporioides* is still poorly defined and hinders breeding for resistance (Lebot, 2009). Even though some genetically developed yam varieties may be tolerant to anthracnose disease, none is totally resistant to the disease (Abang *et al*., 2002; Jackson, 2014).

2.9.4 Chemical control

Sweetmore *et al*. (1994) reported that high yam yields can be obtained if recommended fungicides foliar spray is used to delay the onset of anthracnose until after root bulking. This necessitated the use of chemical foliar spray to combat anthracnose disease of yam. Yam anthracnose disease has mainly been managed with chemical fungicides, such as benomyl, maneb, chlorothalonil and mancozeb (Palaniyandi *et al*., 2011). Amusa (2001) reported that before planting, yam setts should be treated with a broad-spectrum fungicide to eliminate surface-borne fungi and cutting knives should be treated frequently with bleach. For instance, yam setts should be subjected to slurry treatment by covering them with recommended chemical dust or concentrate such as captan fungicides if the chemical is affordable and available (Offei *et al*., 2008; Jackson, 2014). Anthracnose severity can be

reduced by spraying yam foliage and vine upon shoot emergence with fungicides such as benomyl or maneb at regular interval of 10 and 7 days respectively (Reddy, 2015).

The use of chemical fungicides in managing plant diseases such as yam anthracnose comes with constraints (Voorrips, 2004; Komarek *et al.*, 2010; Newton *et al.*, 2010). The repeated application (biweekly or monthly) of chemical fungicides to treat phytopathogenic diseases throughout the cropping season could have adverse effect on the environment (Onyeka *et al*. 2006). Some of these fungicides may leach into ground water and other water resources damaging both aquatic and drinking water resources (Jørgensen *et al*., 2012). Pesticide use can potentially cause several health problems in humans (Damalas and Eleftherohorinos, 2011). According to Goldman (2008), most fungicides cause acute toxicity, while some cause chronic toxicity. It is therefore not surprising that the World Health Organization (WHO) and the United Nations Environment Programme estimated that, each year, in the developing world three million agriculture workers experience severe pesticide poisoning out of which 18,000 of them die (Miller, 2004).

Synthetic fungicides usually contain heavy metals which upon their continuous use results in the gradual accumulation of those metals over time in soils beyond acceptable levels. For instance, in Italy, the accumulation of copper in soil was attributed to the repeated application of fungicides to control fungal diseases of pear and grape (Toselli *et al*., 2009). There is also the tendency for these metals to accumulate beyond safety levels in plant organs used as food; raising food safety concerns. Heavy metal accumulation in crops may affect food quality and pose a direct threat to human health (Osma *et al*., 2012). The uptake of these metals in food products like fruits and vegetables have been reported (Bvenura and Afolayan, 2012). Addo-Fordjour *et al.* (2013) observed that, the continuous use of copper based fungicides in managing black pod disease of cocoa over 20-30 years period resulted

in considerable copper contamination of the plantation soils, cocoa leaves and beans. Heavy metals contaminations of tubers of cassava, sweet potato and yam have also been reported (Onyedika and Nwosu, 2008).

Chemical fungicide control of anthracnose disease may be ineffective due to infrequent or poorly timed applications resulting from high cost of chemical fungicides, labour and machinery coupled with the likelihood of the presence of fungicide resistant strains of *C. gloeosporioides* (Kutama *et al.*, 2013). Synthetic fungicide can only delay yam anthracnose epidemic but cannot prevent it from establishing during the raining season. There is therefore the need to find environmentally-friendly alternatives in controlling plant pathogenic fungi instead of the use of chemical fungicides (Kutama *et al.,* 2013). Several authors have therefore suggested the use of plant extracts as an alternative to synthetic pesticides (Amadioha, 2000; Subapriya and Nagini, 2005; Nahunnaro, 2008; Mondall *et al*., 2009).

2.10 Use of botanicals

The hazardous effects of excessive and improper use of chemical fungicides on the health of humans, animals and the environment have been established; hence necessitating the search for environmentally-safe and easily biodegradable bio-fungicides (Zaker, 2016). Bio-fungicides are obtained from natural sources and as such have minimum adverse effects on the physiological processes of plants, and are easily convertible into common eco-friendly organic materials (Gnanamanickam, 2002). The extracts, essential oils, gums and resins of certain plants have exhibited antifungal activities against some phytopathogenic fungi, making them suitable to be used as bio-fungicidal products (Fawzi *et al*., 2009; Jalili *et al*., 2010; Romanazzi *et al*., 2012; Zaker, 2016).

The use of plant materials for plant disease control is on the increase and has drawn attention from plant pathologists all over the world (Rhasid *et al*., 2015). Plant extracts used as fungicides are safer, eco-friendly and cheaper compared to synthetic fungicides (Khan and Nasreen, 2010). It is, therefore, not surprising that, the eco-friendly nature of bio-pesticides has brought about an evolution which seeks to promote the development of plant materials with antifungal activity into natural fungicide products (Zaker, 2016). The importance of using plant extract as fungicides cannot be overlooked, and as such, natural fungicide products obtained from plant extracts are now commercially available. For instance, Milsana®, which is a commercially available natural fungicide extracted from the giant knotweed (*Reynoutria sacchalinensis*) is used to control powdery mildew in cucumber production, caused by *Sphaerotheca fuliginea* and other diseases such as gray mold in begonia, cucumbers, ornamentals, pepper and tomato caused by *Botrytis* spp. (Quarles, 2009; Zaker, 2016). TimorexGold® prepared from extract of *Melaleuca alternifolia* is available on a commercial scale for the management of powdery mildews, downy mildews, rust and early and late blight diseases in vegetables, grapevines and orchards and is harmless to beneficial insects such as bees (Zaker, 2016).

Yoon *et al*. (2004) reported that the management of anthracnose disease is still under extensive research. It is therefore not surprising that several researchers have reported the potential use of plant extracts as fungicides to control the disease (Naruzawa and Papa, 2011; Ademe *et al*., 2013; Rashid *et al*., 2015). The antimicrobial potential of several plants materials on various pathogenic microbes have been documented. For instance, authors such as El-Ghany *et al*. (2015); Sales *et al*. (2016) and Muthomi *et al*. (2017) have documented the antifungal potential of *Azadirachta indica*. Phytopathogenic fungi such as *Alternaria solani*, causal agent of early blight in potato and *Fusarium oxysporum* f. sp

lycopersici causing wilt disease in tomato crop have been reported to be susceptible to *A. indica* extracts (Hanif *et al*., 2013; Ramaiah and Garampalli, 2015).

The antimicrobial properties of *Balanites aegyptiaca* have been reported by researchers such as Khatoon *et al*. (2013) and Anywar *et al.* (2014). Abdulhamid and Sani (2016) revealed the antimicrobial properties of aqueous and methanolic leave extracts *B. aegyptiaca* against the microbes *Escherichia coli, Pheudomonas aeruginosa, Samonella typhi, Staphylococcus aureus, Aspergillus flavus* and *A. niger.* Mutwali and Abdelgadir (2016) also observed that both aqueous and ethanol extracts of *B. aegyptiaca* bark had antifungal activity against the fungi *Aspergillus niger* and *Candida albicans*, however, neither methanol nor ethyl acetate extracts could inhibit the fungi. A test conducted by Bonzi *et al*. (2012) to assess the antifungal potential of *B. aegyptiaca* leave extract against sorghum seed mould pathogens *Colletotrichum graminicola* and *Phoma sorghina* reavealed that *B. aegyptiaca* had antifungal activity against *C. graminicola* and not *P. sorghina.*

The potential use of *Jatropha curcas* as a botanical fungicide against phytopathogenic fungi of pineapple was documented by Sales *et al*. (2016). *Fusarium oxysporum, Alternaria alternata* and *Aspergillus flavus* are a few examples of phytopathogenic fungi that are susceptible to botanical fungicides obtained from *J. curcas* (El-Ghany *et al*., 2015). The *in vitro* potential of *J. curcas* seed and pulp extracts against phytopathogenic fungi of pawpaw such as *C. gloeosporioides* has been reported (Rahman, 2011).

Idu *et al*. (2014) reported the antimicrobial activity of *Khaya senegalensis* seed extracts against *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcous aureus, Candida albicans, Penicillum notatum* and *Aspergillus niger.* The fungicidal potential of *K. senegalensis* seed extract for the control of pearl millet midrib spot disease caused by *Curvularia eragrostidis* has been reported (Zarafi and Moumoudou, 2010).

Several researchers have reported the antimicrobial activity of *Capcicum* spp. (Omolo *et al*., 2014; Gayathri *et al*., 2016; Anikwe *et al*., 2017). Pepper extracts exhibited *in vitro* antifungal activities against some plant pathogenic fungi of tomato such as *Alternaria solani*, *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici* (Muthomi *et al*., 2017).

The antifungal activities of plant extract results from their secondary metabolites which act as protectors against pathogens (Khan and Nasreen, 2010). Secondary metabolites such as phenols, flavonoids, quinones, terpenens, tannins, alkaloids, lectins, polypeptides, saponins and sterols may possess some antifungal activities enabling them to create unfavourable environment for the establishment and multiplication of pathogenic microorganisms on host plants (Scheuerell and Mahaffee, 2002; Halama and Van Haluwin, 2004).

2.11 Phytochemicals and their role in plant disease management

Plants in their natural environment are prone to diseases resulting from infection by pathogens. As a result, plants have developed several natural means of protecting themselves against phytopathogenic infections. One of such strategies is the production of secondary metabolites (phytochemicals), which either are toxic to or create conditions that inhibit growth of pathogens (Pandey and Trivedi, 2006). The production of secondary metabolites by plants interferes with activities of pathogen and pathogenicity through toxic or lytic effect, thereby preventing or reducing infection (Chaube and Pundhir, 2009). It is therefore not surprising that, resistant plant varieties tend to have secondary metabolites in abundance while in susceptible varieties; these substances may be less or completely absent (Chaube and Pundhir, 2009).

Several classes of secondary metabolites have strong antimicrobial activity when tested *in vitro*; thus they have been proposed to function as defences against pathogens in the intact plant. Studies conducted by Okogbenin *et al*. (2014) established that; the presence of natural products (alkaloids, terpenoids, anthraquinones, flavonoids, tannins, and saponins) in *Aframomum sceptrum* extracts gave it a highly significant antifungal effect than the broad spectrum fungicide Mancozeb against *Fusarium oxysporum f.sp elaeidis* and *Hypocrea lixii* (IMI 501885*).* Tomatine in tomato, and avenacin in oats, have antifungal membranolytic activity (Chaube and Pundhir, 2009). This is a clear evidence of the importance of exploiting plant extracts in the treatment of plant diseases. Several secondary metabolites are found in the plant kingdom performing different functions in plants, some of which includes defending plants against phytopathogenic infections. These natural products include: alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatinnins, saponins, steroids, tannins and terpenoids.

Phenolic compounds have been reported to alter microbial cell permeability and interact with membrane proteins causing deformation in the structure and functionality of proteins (Pusztahelyi *et al.,* 2015). Tannins, a phenolic compound and some fatty acid-like compounds such as dienes which are potent inhibitors of many hydrolytic enzymes and pre-exist in higher concentrations in cells have been implicated for the resistance of young tissues to parasitic fungi such as *Botrytis* (Chaube and Pundhir, 2009). The phenolic compounds avenacins, saponins and chlorogenic acid present in some plants have been reported to inhibit the germination of the conidia of some fungal pathogens and hence contribute to disease resistance (Pandey and Trivedi, 2006). Saponins which have been documented to have strong antimicrobial activity against a wide range of pathogenic fungi have the ability to complex with sterols in fungal membranes and to cause loss of membrane integrity (keukens *et al*., 1995; Osbourn, 1996). Saponins also prevent fungal

pathogens which lack the enzymes (saponinases) that breakdown the saponins from infecting a plant host (Chaube and Pundhir, 2009). The phytochemical constituent cynogenic glycoside breaks down to produce hydrogen cyanide, a lethal chemical that halts cellular respiration in aerobic organisms (Freeman and Beattie, 2008). Anthraquinone derivatives show evidence of antifungal, anti-microbial, anti-parkinson, anti-proliferative, immune-enhancing, antiviral and antioxidant activities (Nazir *et al*., 2013). Anthraquinones have a broad spectrum antifungal activity and selective activity against some phytopathogenic bacteria species (Manojlovic *et al*., 2000). The antifungal properties of anthraquinones (aloin and aloe-emodin) against *Colletotrichum gloeosporioides* have been established (Nidiry *et al*., 2011). According to Gurjar *et al*. (2012) phytochemicals may exhibit different mode of action on a pathogenic microbe: for instance, terpernoids disrupts membrane; alkaloids intercalate into cell wall; flavonoids bind to adhesins, complex with cell wall, inactivate enzymes; and tannins bind to proteins causing enzyme inhibition. It can therefore be deduced that, the more diverse phytochemicals a plant extract contains, the higher its potency to serve as a natural fungicide; since the various phytochemicals would work in synergy to enhance its antifungal activity.

2.12 Effect of plant extracts on plant vegetative growth

Plant extracts have the capacity to modify physiological processes of plants in a way that provides potential benefits to growth, development and/or stress response (Du Jardin, 2012). Plant extracts functioning as biostimulants increase a crop's amino acid levels, enhancing protein synthesis and also heightens chlorophyll accumulation; improving net photosynthesis (Abbas and Akladious, 2013; Abdalla, 2013). Fuglie (1999) reported that plant extracts such as *M. oleifera* leaf extracts are capable of accelerating a plant's vegetative growth, improving resistance to pests and diseases, and enhancing productivity.

Moringa leaf extract has been reported to possess natural plant growth enhancing substances; hence its foliar spray on crops such as pepper, beans, melon, maize and sorghum enhanced their vegetative growth and yield (El–Hamied and El-Amary, 2015). Bulgari *et al* (2017) reported that both leaf and flower extracts of Borage (*Borago officinalis* L) increased the vegetative growth of lettuce plant (*Lactuca sativa 'Longifolia'*); resulting in yield improvement.

The effect of plant extracts used as foliar spray on crops is important, since these extracts have the capability of stimulating plant growth and development. It can therefore be conjectured that foliar application of plant extracts to manage anthracnose disease of yam may also enhance the crop's yield.

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Study site

The study was carried out in the Spanish Laboratory and on the experimental farm at the Nyankpala campus of the University for Development Studies, Tamale and also on yam farms in the Tolon District. The Nyankpala campus which is in the Tolon District is about 20 km South-West of Tamale, the capital of Northern Region of Ghana. The study area lies between latitudes 9° 15′` and 10°0 02` North and Longitudes 0° 53′and 1° 25′ West (Ghana Statistical Service, 2013). The area is characterised by a single rainy season, which starts in late April with little rainfall, rising to its peak in July - September and declining sharply and coming to a complete halt in October - November. The mean annual rainfall ranges between 950 to 1,200 mm. The dry season starts from November to March with day temperatures ranging from 33 to 39 °C, while mean night temperature ranges from 20 to 26 °C. Humidity between April and October can be as high as 95% in the night and falling to 70% in the day. Night humidity for the rest of the year range between 25 to 80%. The mean annual day sunshine is approximately 7.5 hours, with warm temperatures, dry and hazy in February to April. Harmattan is experienced from November to February. The soil is generally sandy loam except in the low lands where alluvial deposits are found and the vegetative cover is basically Guinea Savanna interspersed with short drought resistant trees and grassland.

3.2 Field assessment of yam anthracnose disease incidence and severity

3.2.1 Disease incidence

Yam plants were each scored for the presence or absence of anthracnose symptoms according to Abang *et al*., (2006) +/- scoring method, where (+) represented the presence of the disease and (-) absence of the disease on a plant. The disease incidence (%) was calculated using the formula.

$$\text{Disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100\%$$ (Chaube and Pundhir, 2009).

3.2.2 Disease severity

The extent of anthracnose damage on each plant was assessed using a disease assessment key with a score-scale of 1 – 5 (Table 3.1). The mean anthracnose severity for each farm/experimental plot was estimated by summing severity scores > 1 divided by the total number of symptomatic plants (Asfaw, 2016).

Table 3.1: Disease assessment key for severity of anthracnose disease of yam

Index	Qualitative rating	Pictorial rating
1	No visible symptoms of anthracnose disease.	
2	Few anthracnose spots or symptoms on 1 to ~25% of the plant.	
3	Anthracnose symptoms covering ~26 to ~50% of the plant.	
4	Symptom on > 51% of the plant.	
5	Severe necrosis and death of the plant.	

Source: Asfaw (2016)

3.3 Plant materials used for extract preparation

The plant materials farm which extracts were used for the study are presented in Table 3.2.

Table 3.2: Plant materials used for the study

Botanical name	Common name	Family	Part used
Azadiratcha indica A. Juss.	Neem	Meliaceae	seeds
Balanites aegyptiaca L. Delile	Desert date	Zygophyllaceae	seeds
Capsicum spp. L. (Legon 18 variety)	Hot pepper	Solanaceae	fruits
Icacina oliviformis Poir. J. Raynal	False yam	Icacinaceae	leaves
Jatropha curcas L.	Jatropha	Euphorbiaceae	seeds
Khaya senegalensis (Desr.) A. Juss.	Mahogany	Meliaceae	seeds

3.4 Sources of plant materials

Seeds of *A. indica, K. senegalensis* and leaves of *I. oliviformis* were obtained from the Nyankpala campus of the University for Development. Seeds of *B. aegyptiaca* and *J. curcas* were also collected from Dingoni in the Tolon district. Fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) were obtained from the Tamale market. These plant materials were carried to the laboratory in separate well labeled sterile polyethylene bags.

3.5 Preparation of plant materials for extraction

Fresh leaves of *I. oliviformis* were separated from the plant and washed thoroughly with tap water and subsequently with sterile distilled water to eliminate dust and other foreign materials. Seeds of *A. indica*, *B. aegyptiaca*, *J. curcas* and *K. senegalensis* were also removed from their shells. *Capsicum* spp. (hot pepper, Legon 18 variety) fruits were cut into fragments of 1 cm (to facilitate dryness). These plant materials were then air-dried separately at room temperature on laboratory trays under shady condition for 10 days. Each material was then pulverised using a hammer mail (Thomas Scientific Model 4). The powder obtained was then sieved with a 0.4 mm diameter mesh to obtain a fine powder which was then stored until required.

3.6 Preparation of plant extracts for *in vitro* and qualitative phytochemical studies

For both *in vitro* and qualitative phytochemical studies, a modified method of Janaidu *et al*. (2014) was used for extracts preparation. Aqueous and ethanol extracts were prepared by soaking 200 g of each plant material in 1000 ml of water and ethanol respectively in conical flasks which were covered and placed on a shaker for 8 hours. The extracts were filtered using a vacuum filtration system and concentrated to dryness using a rotary evaporator RE 300 (Barloworld Scientific, UK). Dry extracts were stored at 4 ^{0}C until required for analysis.

3.7 Synthetic fungicides used for the study

The synthetic fungicides Manlax and Rainmancoz were used for the study. They were each prepared according to the manufacturer's recommendation (Table 3.3).

Table 3.3: Active ingredients and rate of synthetic fungicides used in the study

Fungicide	Active ingredient	Concentration used
Manlax	mancozeb 64% + metalaxyl 8% WP	2.5g/1L of water
Rainmancoz	mancozeb 80% WP	2.0g/1L of water

3.8 Media preparation

The analytical grade Potato Dexrose Agar (PDA) media used in this study was prepared according to the manufacturer's (Sigma-Aldrich Company, Spain) directions. Thirty nine (39) g of powdered PDA was suspended in one litre (1 L) of distilled water in a 1 L Erlenmeyer flask and 250 mg of chloramphenicol added to suppress bacterial growth. The Erlenmeyer flask was covered with a non-absorbent cotton wool plug and then brought to the boil on an electric hot plate to dissolve the PDA powder completely. This was then sterilised by autoclaving at 121 ^{0}C and pressure of 1.03 Kg/cm^{2} for 15 minutes.

3.9 Isolation of *C. gloeosporioides*

Yam leaves with symptoms of anthracnose infection were obtained from 48 yam farms along a 'W' shaped sampling path; five plants per side at equal distance from each other (Toualy *et al*., 2014) in the Tolon District. These were taken to the laboratory in sterile polyethylene bags. The sampled leaves were washed separately with tap water to remove debris, cut into pieces (1 - cm fragment), surface sterilized with 0.1% sodium hypochlorite for three minutes and rinsed three times in changes of sterile distilled water. The pieces were then plated on PDA and incubated at ambient temperature (28 ± 2 °C) for 1 week. Mycelia that grew were sub-cultured onto fresh PDA and further sub-culturing carried out until pure cultures of *C. gloeosporioides* were obtained.

3.10 Identification of *C. gloeosporioides*

Slides of 7-day-old mycelia from pure cultures of *C. gloeosporioides* were examined under a compound microscope (Leica DME, Leica Microsystems, Shanghai, China). They were confirmed as *C. gloeosporioides* by comparing their morphological and cultural distinctiveness with images and descriptions documented by Barnett and Hunter (2006) and Gautam (2014). The isolate obtained was subjected to Koch's postulate to confirm the pathogenicity.

3.11 Maintenance of stock cultures

Stock cultures of the test fungus, *C. gloeosporioides* were grown on PDA contained in 9 cm Petri dishes. The cultures were stored at 4 ^{o}C and sub-cultured every two weeks.

3.12 Method of inoculation

A 5 mm disc of *C. gloeosporioides* mycelium, taken from advancing edge of the culture was used as inoculum in all *in vitro* investigations for mycelium growth.

3.13 Sterilization of glassware

Beakers, Erlenmeyer flasks, pipettes and Petri plates were washed with detergent and tap water and rinsed with distilled water. They were air-dried and then sterilized in an electric oven for two hours at 160 ^{o}C. Glass slides were washed with detergent, rinsed under running tap and stored in 70% ethanol.

3.14 Climatic data

Rainfall, relative humidity and temperature data were obtained from the Savanna Agriculture Research Institute (SARI) meteological.

3.15 Photography

The photographs in this thesis were taken with a Samsung ST72 camera, from Samson Electronics Co., Ltd.

3.16 Experimental precautions

a. All solutions were prepared with accurately measured volumes of solvents and weighed quantities of solutes.
b. It was ensured that there were no bubbles in the liquid in the pipette to ensure accurate volume was used.

c. Glassware were kept scrupulously clean, and those cleaned with water and detergent were thoroughly rinsed several times with tap water and three times with distilled water and allowed to drain dry before use.

d. The microflow laminar flow workstation was sterilized by using 70% rubbing alcohol to wipe the inside.

e. The microflow laminar flow workstation was switched on at least 20 minutes before use.

f. All plating and inoculation were done in the microflow laminar flow workstation.

g. Inoculations needles (pins), cork borer and forceps were kept in 70 % ethanol and sterilized over Bunsen flame until they appeared red hot and allowed to cool.

h. The opening edge of Erlenmeyer flasks containing molten medium (PDA) was flamed sterilized over a Bunsen flame before medium was poured into the Petri plates.

i. All working areas were wiped with 70 % rubbing alcohol before use.

j. In the *C. gloeosporioides* growth experiments, in the event of the incidence of bacterium or fungus contamination on any of the replicate cultures, the entire set of replicates was discarded and the experiment repeated.

3.17 Statistical analyses

Data obtained were subjected to Analysis of Variance (ANOVA) using GenStat (12th edition). The least significant difference (LSD) test was used to separate the treatment means at 5% significance level. The analysed outputs were either presented in tables or graphs. The tables and graphs were drawn using Microsoft word and excel respectively.

CHAPTER FOUR

4.0 INCIDENCE AND SEVERITY OF ANTHRACNOSE DISEASE OF YAM (*Dioscorea rotundata* POIR) IN THE TOLON DISTRICT

4.1 Introduction

Yam is a staple crop which is cultivated mainly in the tropical and subtropical regions for its tubers (Agrios, 2005; Achar *et al*., 2013). Unfortunately these regions also provide favourable conditions that support the growth and survival of *Colletotrichum gloeosporioides*, the causative agent of yam anthracnose disease; a major threat to yam production worldwide (Agrios, 2005; Chaube and Pundhir, 2009; Lebot, 2009; Reddy, 2015).

The intensification in the cultivation of yam to meet the increasing demand for yam tubers usually results in escalating incidence and severity of field diseases such as yam anthracnose (Asiedu and Sartie, 2010). Yam is mainly propagated using yam setts which are usually inadequate or unavailable. Traditionally, yam farmers often select from their harvest small tubers (usually ranging from 300 – 500 g), which unfortunately may come from anthracnose diseased plants and use them as planting setts (Aighewi *et al*., 2003). Other farmers also obtain their yam setts from family members, neighbours and other uncertified sources which may increase the chances of cultivating *C. gloeosporioides* infected yam sett seeds (Ayoola, 2012; Osei-Adu *et al*., 2016). Such a practice is a major source of inoculum spread, that initiate anthracnose disease cycle on the germinated yam; causing poor crop stand establishment (Asiedu and Sartie, 2010; Osei-Adu *et al*., 2016).

Colletotrichum gloeosporioides has the capability of surviving on yam crops and other alternate hosts, with the pathogen from yam crops capable of infecting a number of crops as well as weeds and vice versa (Lebot, 2009). The presence of potential *C. gloeosporioides* susceptible flora in or around yam farms and also mixed cropping yam with other

susceptible host crops could play an important role in accelerating the epidemiology of the pathogen in the farm (Lebot, 2009).

Colletotrichum gloeosporioides spores need moisture and other favourable conditions such as optimum temperature (20 – 30^{o}C) and relative humidity (above 95%) to germinate and cause infection before being dispersed by rain splash (Sharma and Kulshrestha, 2015). The most popular cultivars of yam, or those which are particularly adapted to commercial production, are also those which are the most susceptible to the anthracnose disease (Lebot, 2009). The status of *D. rotundata* as an important commercial yam in Ghana cannot be understated (CSIR/SARI, 2011; Otoo *et al*., 2015). Unfortunately, anthracnose is one of the most serious leaf and vine epiphytotic diseases of yam, especially *D. alata* and *D. rotundata*, in yam-growing countries of West Africa (Ayodele *et al*., 2000). **S**tudies on anthracnose disease of yam in Ghana have mainly been restricted to *D. alata* with minimal attention to *D. rotundata*, the major species cultivated in the country. The objective of the study was to determine the incidence and severity of anthracnose disease of *D. rotundata* in the Tolon District.

4.2 Materials and methods

4.2.1 Selection of yam farms for assessment of disease outbreak

A total of 48 yam farms in the Tolon District of the Northern Region of Ghana were selected for this study. The district was selected because yam is one of the major crops cultivated in the area (Tolon/Kumbumgu District Assembly, 2012). The Tolon District is also located within the same ecological zone as the Nyankpala campus of the University for Development Studies (Ghana Statistical Service, 2014) where the field experiment was conducted. This allowed for a better comparison of observations of yam anthracnose disease incidence and severity made on the experimental field and the farmers' fields since they were all cultivated under similar climatic conditions. The snowball sampling technique was used to select the top three yam farming zones in the district (Nyankpala, Wuribogu and Tolon) and two major yam farming communities within each zone. Eight yam farms were also selected in each yam farming community using the snowball technique (Table 4.1).

Table 4.1: Communities and number of farms surveyed for incidence and severity of yam anthracnose disease in the Tolon District

S/No	Yam farming zone	Yam farming community	Number of farms
1	Woribogu	Woribogu Kukuo	8
		Dingoli	8
2	Tolon	Fihini	8
		Sabegu	8
3	Nyankpalasogu	Kpalsogu	8
		Gawugu	8
Total	3	6	48

4.2.2 Field survey for disease incidence and severity

Data on the incidence and severity of yam anthracnose disease on the farmers' fields in the Tolon District were collected on monthly basis in July, August and September of the 2016 and 2017 yam cropping seasons.

4.2.2.1 Determination of disease incidence

Disease incidence which is a measure of proportion of plant population diseased at a time and a place (Chaube and Pundhir, 2009) was assessed. This was conducted by walking through a yam farm and inspecting individual sampled yam plants for symptoms of anthracnose infection. For each farm, disease incidence was estimated by assessing 20 plants along a 'W-shaped' sampling path and counting five plants per side spaced at an equal distance from each other (Toualy *et al*., 2014). These plants were each scored for the presence or absence of anthracnose symptoms. The method of Abang *et al*. (2006) described in section 3.2.1 was used for yam anthracnose incidence assessment. Percentage disease incidence was calculated using the formula of Chaube and Pundhir (2009) as described in section 3.2.1. Disease incidence for each community, zone and the district was also determined by the formulae below.

$$\text{Disease incidence (community)} = \frac{\Sigma \text{ Disease incidence in farm}}{\text{Total number of farms inspected}} \times 100\%$$

$$\text{Disease incidence (zone)} = \frac{\Sigma \text{ Disease incidence in Community}}{\text{Total number of Communities inspected in the zone}} \times 100\%$$

$$\text{Disease incidence (district)} = \frac{\Sigma \text{ Disease incidence in community}}{\text{Total number of zones inspected in the district}} \times 100\%$$

4.2.2.2 Determination of disease severity

Disease severity is a measure of sickness of diseased plant (Chaube and Pundhir, 2009). This was also evaluated by taking data on the same individual plants on which anthracnose

incidence was assessed along the 'W-shaped' sampling path (section 4.2.2.1). The disease severity was estimated according to Asfaw (2016) scoring key (section 3.2.2). The yam anthracnose disease severity index for the Tolon communities, zones and the district were determined using the formulae below:

$$\text{Disease severity index (community)} = \frac{\Sigma \text{ Disease severity index in farm}}{\text{Total number of farms inspected}}$$

$$\text{Disease severity index (zone)} = \frac{\Sigma \text{ Disease severity index in Community}}{\text{Total number of communities inspected in the zone}}$$

$$\text{Disease severity index (district)} = \frac{\Sigma \text{ Disease severity index in community}}{\text{Total number of zones inspected in district}}$$

4.3 Results

4.3.1 Incidence of yam anthracnose disease in the Tolon district

There were no significant differences ($P \leq 0.05$) in percentage anthracnose disease incidence among the communities (Kpalsogu, Gawugu, Fihini, Sabegu, Woribogu Kukuo and Dingoni) during the 2016 and 2017 cropping seasons (Table 4.2). During July and September, each of the communities recorded a higher disease incidence in the 2016 cropping season than that of the 2017 (Table 4.2). In August, the 2017 cropping season recorded a higher disease incidence than the 2016 (Table 4.2). Each of the communities with the exception of Kpalsogu recorded a higher percentage mean disease incidence in the 2016 cropping season than the 2017 (Table 4.2). The Tolon district recorded a higher disease incidence (46.36%) in the 2016 cropping season than that of the 2017 (41.95%) (Table 4.2). In July 2016, yam farms in Fihini recorded the lowest percentage disease incidence (6.9%) while Dingoni recorded the highest (17.5%) (Table 4.2). Percentage anthracnose disease incidence for July 2017 ranged from 4.4 % in Kpalsogu to 6.9% in Sabegu (Table 4.2). August 2016 had Kpalsogu recording the lowest (27.5%) and Dingoni recording the highest (40.0%) anthracnose disease incidence (Table 4.2). In August 2017, the lowest (43.1%) and highest (48.8%) anthracnose disease incidences were recorded for Fihini and Sabegu respectively (Table 4.2). The lowest anthracnose disease incidence (89.4%) and highest (94.4%) for September 2016 were observed for Kpalsogu and Woribogu Kukuo respectively, and that of 2017 ranged from 71.9% for Dingoni as the lowest and Sabegu recording the highest at 78.1% (Table 4.2).

Table 4.2: Anthracnose disease incidence (%) of *Dioscorea rotundata* in selected communities in the Tolon district for 2016 and 2017 cropping seasons over a period of three months

Zone	Community	Percentage disease incidence									
		July		August		September		Mean		Grand mean	
		2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Nyankpalasogu	Kpalsogu	8.13 ± 2.10[a]	4.38 ± 2.20[a]	27.50 ± 1.64[a]	46.25 ± 3.10[a]	89.38 ± 3.33[a]	74.38 ± 3.20[a]	41.67	41.67	46.36	41.95
	Gawugu	14.38 ± 4.38[a]	5.63 ± 2.20[a]	35.63 ± 5.46[a]	44.38 ± 4.38[a]	95.63 ± 2.40[a]	77.50 ± 3.13[a]	48.55	42.50		
Tolon	Fihini	6.88 ± 2.49[a]	5.00 ± 1.89[a]	28.13 ± 2.10[a]	43.13 ± 2.49[a]	93.13 ± 3.26[a]	73.75 ± 4.20[a]	42.71	40.63		
	Sabegu	10.63 ± 2.58[a]	6.88 ± 2.82[a]	34.38 ± 2.74[a]	48.75 ± 4.51[a]	93.75 ± 3.24[a]	78.13 ± 3.40[a]	46.25	44.59		
Woribogu	Woribogu Kukuo	15.00 ± 3.90[a]	6.25 ± 2.63[a]	37.50 ± 3.78[a]	46.88 ± 2.98[a]	94.38 ± 2.74[a]	72.50 ± 2.67[a]	48.96	41.88		
	Dingoni	17.50 ± 3.13[a]	5.63 ± 2.40[a]	40.00 ± 3.13[a]	43.75 ± 2.95[a]	92.50 ± 2.50[a]	71.88 ± 1.88[a]	50.00	40.42		
F (pr)		**0.151**	**0.982**	**0.069**	**0.860**	**0.756**	**0.649**				

Means ± standard errors in the same column followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's test.

Percentage yam anthracnose disease incidence varied among the various zones (Nyankpalasogu, Tolon and Woribogu), with the highest (93.4%) recorded in September 2016 for Tolon and Woribogu zones and the lowest (5.0%) observed in July 2017 for Nyankpalasogu (Figure 4.1). There were no significant differences ($P \leq 0.05$) in percentage disease incidence among the three zones during July 2016 and 2017 (Figure 4.1). With the exception of Woribogu, August of the 2017 cropping season recorded significantly higher ($P \leq 0.05$) percentage disease incidence for the Nyankpalasogu and Tolon zones than what was observed in the corresponding month for 2016 (Figure 4.1). However in September, each of the zones; Nyankpalasogu, Tolon and Woribogu recorded significantly higher ($P \leq 0.05$) percentage disease incidence in 2016 than 2017 (Figure 4.1).

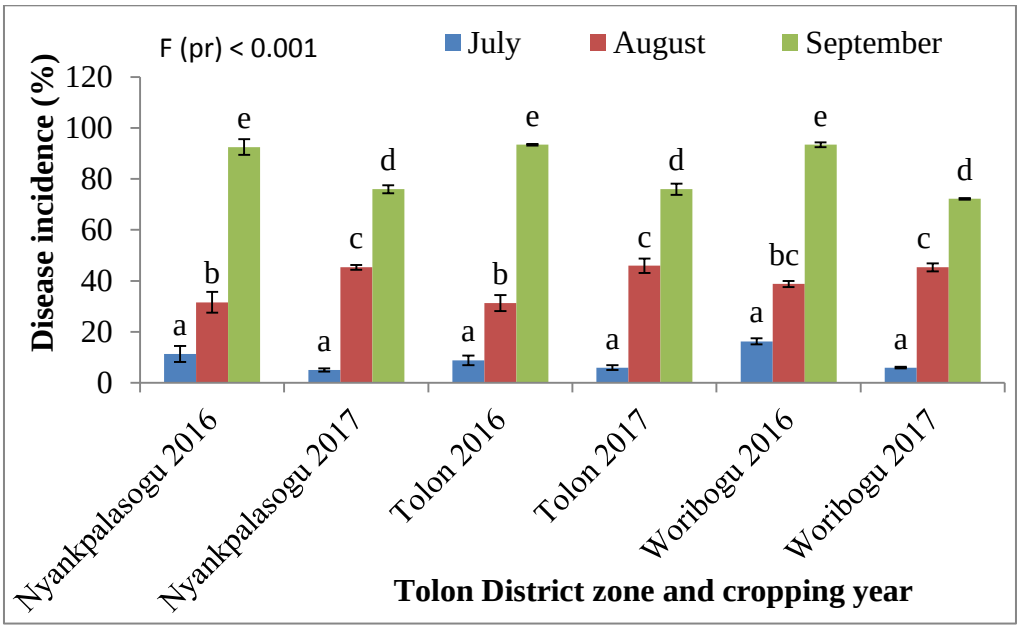

Figure 4.1: Percentage disease incidence in *D. rotundata* for different months of the 2016 and 2017 cropping seasons for various zones in the Tolon district. Error bars indicate standard errors of means.

For each of the 2016 and 2017 cropping seasons, there was a progressive increase in anthracnose disease incidence from July to September (Figure 4.2). The anthracnose disease incidence in the district varied significantly ($P \leq 0.05$) from July to September and also

from one cropping season to another (Figure 4.2). July and September of the 2016 cropping season each recorded a significantly higher ($P \leq 0.05$) yam anthracnose disease incidence in the district than their corresponding months in the 2017 cropping season (Figure 4.2). September 2016 recorded the highest mean anthracnose disease incidence of 93.1% followed by September 2017 (74.7%) and July 2017 recorded the least at 5.6% (Figure 4.2). The percentage disease incidence recorded during July (12.1%) and September (93.1%) of the 2016 cropping season, were significantly higher ($P \leq 0.05$) in comparison to their corresponding months in 2017 where July and September recorded 5.6% and 74.7% respectively (Figure 4.2). In August, the 2017 cropping season recorded a significantly higher ($P \leq 0.05$) percentage disease incidence (45.5%) than 2016 (33.9 %) (Figure 4.2).

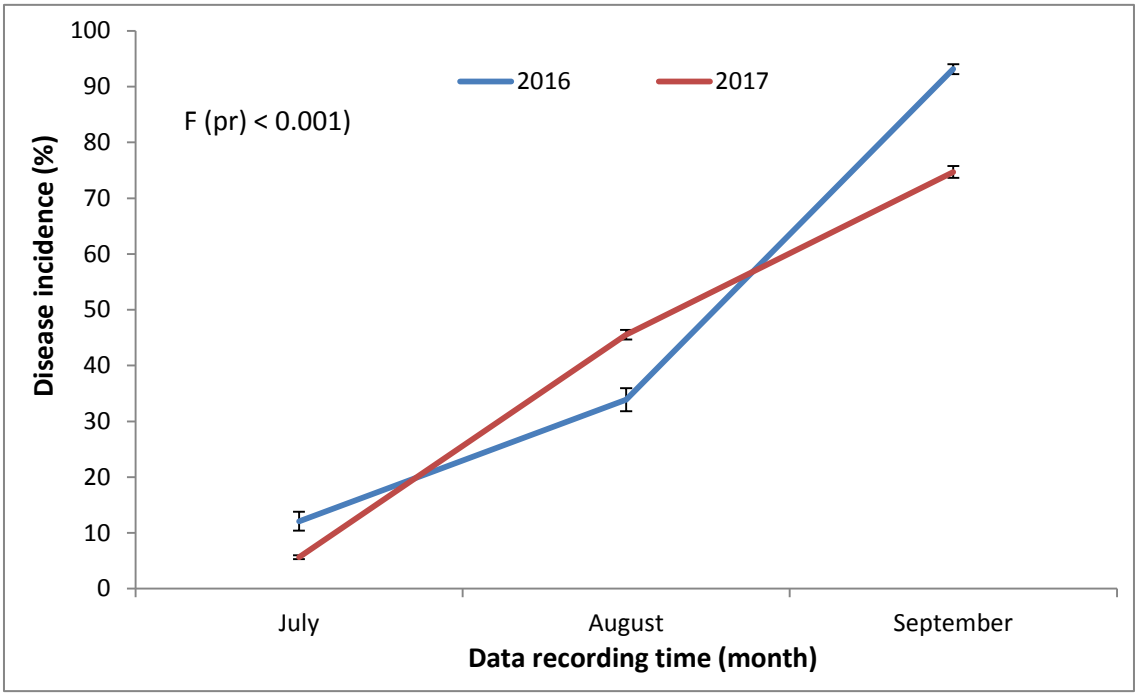

Figure 4.2: Percentage of *D. rotundata* anthracnose disease incidence for 2016 and 2017 cropping seasons in the Tolon district over a period of three months. Bars indicate standard errors of means.

4.3.2 Severity of yam anthracnose disease in the Tolon district

There were no significant differences ($P \leq 0.05$) in anthracnose disease severity index for July, August, September 2016 and 2017 cropping seasons for Kpalsogu, Gawugu, Fihini, Sabegu, Woribogu Kukuo and Dingoni communities (Table 4.3). For July and September, the 2016 cropping season recorded a higher disease severity for the the various communities than that of the 2017 (Table 4.3). During August, each of the communities recorded a higher disease severity in 2017 than 2016 (Table 4.3). Each of the communities recorded a higher mean disease severity in the 2016 cropping season than that of the 2017 (Table 4.3). The disease severity recorded for the 2016 cropping season was higher (2.25) than that of 2017 (2.02) (Table 4.3). In August, all the communities except Woribogu Kukuo and Dingoni had higher disease severity index in 2017 than 2016 (Table 4.3). In July of the 2016 cropping seson, Fihini and Dingoni recorded the lowest (1.63) and highest (1.94) disease severity index respectively (Table 4.3). During August of the 2016 cropping season, the lowest (2.07) and highest (2.19) disease severity index were recorded in Fihini and Dingoni respectively (Table 4.3). In September of the 2016 cropping season, the anthracnose disease severity index ranged from 2.66 to 2.99 in Fihini and Gawugu respectively (Table 4.3). For July 2017, Kpalsogu recorded the lowest disease severity index (1.38) while Dingoni recorded the highest (1.66) (Table 4.3). The least disease severity index (2.07) for August 2017 was recorded in Dingoni while Fihini recorded the highest (2.23) (Table 4.3). The anthracnose disease severity index for September 2017 ranged from 2.25 to 2.39 for Kpalsogu and Woribogu Kukuo respectively (Table 4.3).

Table 4.3: Anthracnose disease severity index of *Dioscorea rotundata* in selected communities in the Tolon district for 2016 and 2017 cropping seasons over a period of three months

Zone	Community	Percentage disease severity									
		July		August		September		Mean		Grand mean	
		2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Nyankpalasogu	Kpalsogu	1.81 ± 0.19[a]	1.38 ± 0.18[a]	2.12 ± 0.06[a]	2.18 ± 0.44[a]	2.87 ± 0.14[a]	2.25 ± 0.04[a]	2.27	1.94	2.25	2.02
	Gawugu	1.75 ± 0.16[a]	1.63 ± 0.18[a]	2.17 ± 0.04[a]	2.21 ± 0.08[a]	2.99 ± 0.10[a]	2.34 ± 0.06[a]	2.30	2.06		
Tolon	Fihini	1.63 ± 0.18[a]	1.50 ± 0.19[a]	2.07 ± 0.03[a]	2.23 ± 0.06[a]	2.66 ± 0.04[a]	2.33 ± 0.05[a]	2.12	2.02		
	Sabegu	1.88 ± 0.13[a]	1.56 ± 0.22[a]	2.09 ± 0.05[a]	2.13 ± 0.08[a]	2.90 ± 0.06[a]	2.36 ± 0.07[a]	2.29	2.02		
Woribogu	Woribogu Kukuo	1.69 ± 0.21[a]	1.63 ± 0.18[a]	2.13 ± 0.03[a]	2.08 ± 0.04[a]	2.76 ± 0.05[a]	2.39 ± 0.03[a]	2.19	2.03		
	Dingoni	1.94 ± 0.15[a]	1.66 ± 0.19[a]	2.19 ± 0.05[a]	2.07 ± 0.04[a]	2.91 ± 0.05[a]	2.37 ± 0.04[a]	2.35	2.03		
F (pr)		**0.801**	**0.910**	**0.444**	**0.277**	**0.075**	**0.416**				

Means ± standard errors in the same column followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's test.

D. rotundata disease severity index varied from zone to zone in the Tolon district, with the Nyankpalasogu zone recording the highest (2.93) and lowest (1.51) for September 2016 and July 2017 respectively (Figure 4.3). Generally, there were no significant differences ($P \leq 0.05$) in the recorded anthracnose disease severity index for July and August of the 2016 and 2017 cropping seasons for the Nyankpalasogu, Tolon and Woribogu zones (Figure 4.3). For September of the 2016 cropping season, each of the zones in the Tolon district recorded a significantly higher ($P \leq 0.05$) disease severity index than what was observed for the same month in 2017 (Figure 4.3).

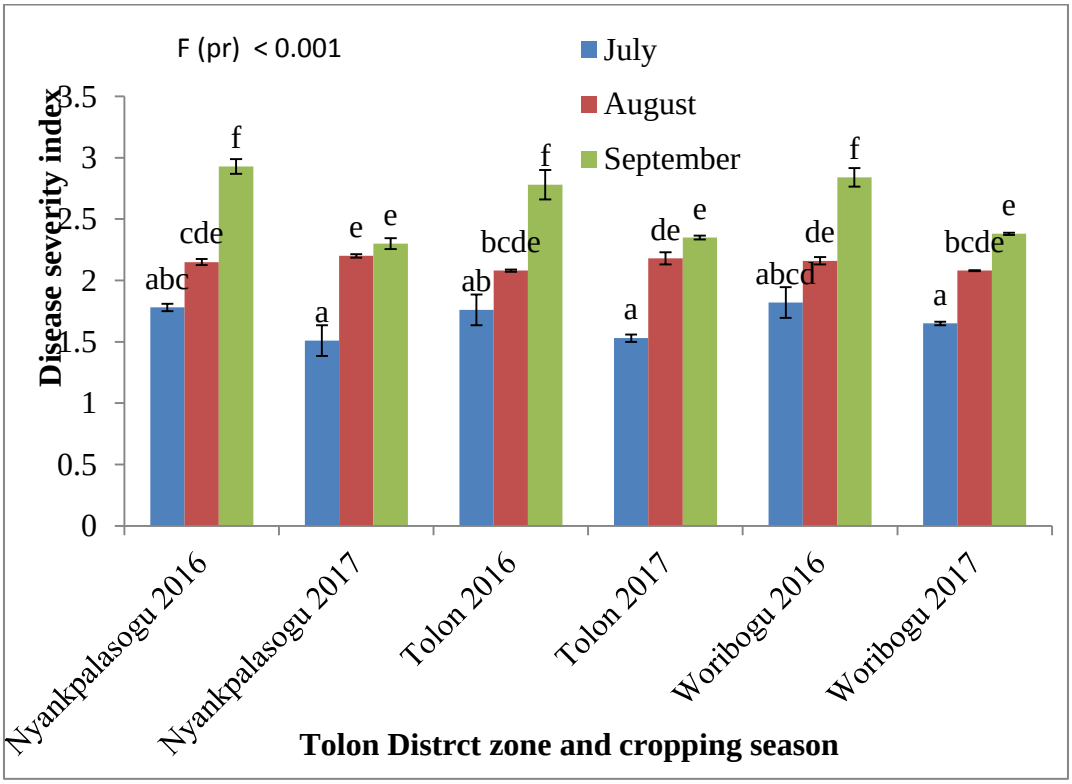

Figure 4.3: *Dioscorea rotundata* anthracnose disease severity for different zones during the 2016 and 2017 cropping seasons. Bars indicate standard errors of means.

The 2016 and 2017 cropping seasons each recorded progressive increases in yam anthracnose disease severity index from July to September (Figure 4.4). Disease severity

index varied significantly ($P \leq 0.05$) from month to month and from one cropping season to the other, with July 2017 and September 2016 recording the lowest and highest of 1.56 and 2.85 respectively (Figure 4.4). Although August 2017 recorded higher anthracnose disease severity index (2.15) than 2016 (2.13), there was no significant difference ($P \leq 0.05$) (Figure 4.4). However, the disease severity index of 1.78 for July and 2.85 for August 2016 were significantly higher ($P \leq 0.05$) than those of their corresponding months in the 2017 cropping season of 1.56 and 2.24 respectively (Figure 4.4).

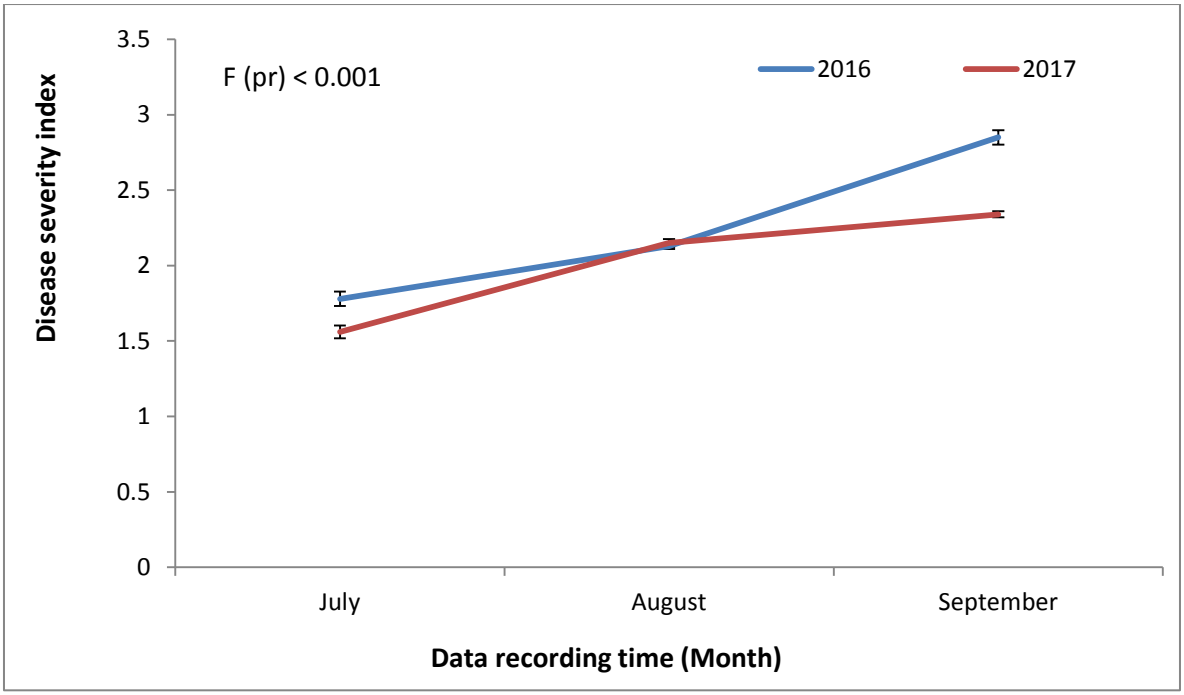

Figure 4.4: *D. rotundata* anthracnose disease severity index for 2016 and 2017 cropping seasons in the Tolon district over period of three months. Bars indicate standard errors of means.

4.4 Discussion

The high incidence of yam anthracnose disease recorded for the 2016 and 2017 cropping seasons across the yam farming communities in the Tolon District, could be attributed to many factors such as; intercropping with other *C. gloeosporioides* susceptible crops such as cassava, pepper and beans. Also highly infested weed farms served as reservoirs for *C. gloeosporioides* inoculum. These observations corroborate that of Lebot (2009) that *C. gloeosporioides* is a ubiquitous fungus capable of causing anthracnose disease in many weeds and several crops, and as such susceptible flora in or around yam fields harbouring the pathogen could serve as sources of inoculum which could initiate anthracnose infection in yam crops. It has also been established that infected yam tubers or setts used for cultivation could be a major source of anthracnose disease inoculum and spread (Green and Simons, 1994; Lebot, 2009; Asiedu and Sartie, 2010; Reddy, 2015). This implies that the wide spread of yam anthracnose disease in the district could also be attributed to the source of planting setts which may be harbouring *C. gloeosporioides* inoulum which include farmers' own setts, setts obtained from neighbours and/or local markets, some or all of which could be infected.

Generally the yam farmers in the Tolon District did not purposely employ control measures against anthracnose disease on their yam farms, which facilitated the spread of the disease on their farms. A similar observation was made by Moses and Lamptey (2001) and Appiah-Kubi *et al*. (2015) that majority of yam farmers in Ghana do not see the need to manage yam anthracnose diseases.

There were no significant differences ($P \leq 0.05$) in disease incidence and severity recorded among the various communities in the Tolon district; probably the yam plants were cultivated under similar climatic conditions of amount of rainfall, temperature and

humidity. The extent of anthracnose disease on a yam farm is dependent on the amount of rainfall, favourable conditions of temperature and humidity (Agrios, 2005; Chaube and Pundhir, 2009; Reddy, 2015). During the period of data collection, the Tolon district recorded 24 – 170 mm of rain, a temperature range of 26.4 – 27.5^{o}C and relative humidity of 82.9 – 84.3% (Appendix 1). These temperatures, relative humidities recorded and rainfall figures in the district fall within those specified by Sharma and Kulshrestha (2015); hence provided favourable environmental conditions for the establishment of anthracnose disease on yam fields. Even though July 2016 and 2017 each had higher rainfall than August and September of the same cropping years, the incidence and severity in July for the two seasons were significantly lower than what was recorded for August and September for the two seasons. This was because in July of the two cropping seasons, most of the yam plants were at the stage of sprouting and establishment; hence were yet developing leaves from which anthracnose infection will start. A similar observation was made by Achar *et al*. (2013) that, new yam plants that sprout following the onset of the rainy season are not immediately infected by anthracnose disease; however, upon leaf maturity disease incidence occurs at its lowest and intensifies during the post rainy months. Also in July, the young plants could have had a latent infection of *C. gloeosporioides* which began to show signs of anthracnose disease symptoms and becoming more aggressive in August and intensified in September. This is supported by Agrios (2005) who stated that anthracnose disease is usually marked with little or no visible symptoms initially, with the infection becoming aggressive with time revealing symptoms of the disease.

There were generally significant differences ($P \leq 0.05$) in the yam anthracnose disease incidence and severity that occurred in the district from August to September for the two cropping seasons (2016 and 2017). This could be due to the excessive rainfall from August to September (Appendix 1A), aiding the dispersal of *C. gloeosporioides* spores from

infected plants to healthy ones; facilitating secondary infection on a particular yam field. The dissemination of anthracnose inoculums by rain splash has been documented by Agrios (2005), Farr *et al*. (2006), Chaube and Pundhir (2009), Achar *et al*. (2013), Gautam (2014) and Reddy, 2015. Also Abang *et al*. (2003) observed that, spatial distribution of yam anthracnose disease was random initially due to localized infection focus on the host and later became aggravated as a result of secondary spread of inoculum from infected crops to other ones.

The general significant difference ($P \leq 0.05$) observed for anthracnose disease incidence and severity in the Tolon district between the 2016 and 2017 cropping seasons could be attributed to variations in seasonal weather conditions; that is, higher rainfalls recorded in 2016 than 2017, alongside the favourable conditions of temperature and relative humidity (Appendix 1). These observations attested the finding of Akem and Asiedu (1994) who stated that, because anthracnose disease is affected by environmental factors, it extent of occurrence on a particular field may vary from year to year. Also under optimal temperature and humidity conditions, yam anthracnose disease intensification is directly proportional to the amount of rainfall (Lebot, 2009). Interestingly, August 2017 recorded a significantly higher ($P \leq 0.05$) anthracnose disease incidence and severity than 2016. This could be attributed to the higher rainfall recorded in August 2017 cropping season (39 mm) than what was observed in 2016 of the same month (24 mm). The generally low disease severity index in the district could be due to the fact that, yam farmers in the district generally cultivate varieties of *D. rotundata* which have some level of inherent resistance to the anthracnose disease compared to *D. alata* which has been documented to be more prone to the disease. This is supported by Lebot (2009) that, *D. rotundata* and *D. cayenensis* are less susceptible to the anthracnose disease compared to *D. alata*. The recorded anthracnose disease on cultivars of *D. rotundata* crops in the district could have a dwindling effect on

yam production. As such there is the need to sensitize yam farmers in the studied district to adapt appropriate measures in controlling the disease.

CHAPTER FIVE

5.0 CHARACTERISATION OF *Colletotrichum gloeosporioides* ISOLATES CAUSING ANTHRACNOSE DISEASE ON *Dioscorea rotundata* IN THE TOLON DISTRICT, GHANA

5.1 Introduction

Anthracnose disease caused by *Colletotrichum gloeosporioides* has been reported as having a dwindling effect on yam yield; hence limiting its productivity (Palaniyandi *et al*., 2016). There is, therefore, the need to manage the disease in order to enhance yam production. A major factor to consider in effective plant disease management is proper identification of the causative pathogen; since it aids in adapting to a better strategy in managing diseases.

Even though *C. gloeosporioides* has been documented as the main causative agent of yam anthracnose disease, its identification based on culture characteristics and conidial morphology, can be mistaken with other species within the genus, more especially *C. acutatum* (Chowdappa *et al*., 2012; Reddy, 2015). Serra *et al*. (2011) stated that different species of *Colletotrichum* are capable of infecting a single host. It has also been reported that the foliage infection of *C. acutatum* and *C. gloeosporioides* are difficult to differentiate in terms of their symptoms and cultural morphology (Shi *et al*., 2008).

The difficulty involved in using features such as cultural and morphological characteristics in distinguishing among various species of *Colletotrichum*, more especially *C. acutatum* and *C. gloeosporioides* has necessitated the use of molecular methods in differentiating among species within the genera (Serra *et al*., 2011). Polymerase Chain Reaction **(**PCR) analysis has been documented as one of the effective methods that can be employed for proper identification of *C. gloeosporioides* (Shi *et al*., 2008; Serra *et al*., 2011; Raj *et al*., 2013; Chagas *et al*., 2017)

The combined method of cultural, morphological characteristics as well as molecular method will aid in the accurate identification of *Colletotrichum* species. The use of molecular methods such as PCR will help ascertain the true identity of the pathogen; facilitating the correct diagnosis of the plant disease and an appropriate control measure. *Colletotrichum gloeosporioides* has reported as having the capability to infect yam leaves, vines and tubers/setts (Ayodele *et al*., 2000; Reddy, 2015). This study was therefore conducted to confirm the identification of *C. gloeosporioides* isolates obtained from yam leaves, vines and setts in the Tolon distrct of Ghana using PCR analysis. The specific objectives were to:

- describe cultural characteristics of isolates of *C. gloeosporioides* obtained from *D. rotundata* leaves, vines and setts.
- determine the mycelia growth rate of isolates of *C. gloeosporioides* obtained from *D. rotundata* leaves, vines and setts.
- confirm the identity of isolates of *C. gloeosporioides* obtained from *D. rotundata* leaves, vines and setts using PCR.

5.2 Materials and methods

5.2.1 Sampling of yam leaves, vines and setts

Dioscorea rotundata leaves, vines and planting setts that exhibited typical anthracnose symptoms were obtained from the Tolon District in the Northern Region of Ghana. Forty eight yam farms were surveyed and yam leaves with typical anthracnose disease symptoms obtained as described in section 3.9. During the survey, yam vines showing anthracnose disease die-back symptoms were also obtained. For the 2016 cropping season, 2 samples of *D. rotundata* vine die-back were each obtained from Woribogu Kukuo and Dingoni communities and 1 from Fihini in 2017. A total of 5 symptomatic *D. rotundata* die-back vines were obtained. *Dioscorea rotundata* setts suspected to be diseased were obtained from 30 farmers whose yam farms were surveyed (5 farmers randomly selected from each community). A total of 150 (5 setts per farmer) were obtained. In the laboratory, these yam setts were carefully examined and a total of 21 setts showing symptoms of "dead skin" were selected for the study.

5.2.2 Isolation of *C. gloeosporioides* from *D. rotundata* leaves, vines and setts

The method employed in the isolation of *C. gloeosporioides* from the yam leaves has been described in section 3.9. The die-back yam vines were cut into 1 cm long pieces and about 0.5 cm^3 fragments also obtained from portions of the yam setts with the "dead skin" symptoms. These were washed, surface sterilized and placed on PDA plates as per the method described in section 3.9 to obtain pure cultures of *C. gloeosporioides*. The pure cultures obtained were then grouped into six isolates based on their distinctive cultural appearance on PDA in Petri dishes.

5.2.3 Determination of mycelia growth rate

This was determined according to the method of Than *et al*. (2008) with some modifications. The radial growth of the mycelia of each of the *C. gloeosporioides* isolates on PDA in Petri dish (9 cm diameter) was measured daily for five days. The mycelia growth rate (mm per day) of each isolate was then averaged to obtain the mean daily growth. Each isolate had four replicates.

5.2.4 Primers used for the study

The primers used for the study were obtained from Inqaba Biotec West Africa Ltd., Nigeria (Table 5.1).

Table 5.1: Primers used for the study

Name	Sequence (5′→3′)	Bases
ITS1	TCC GTA GGT GAA CCT GCG G	19
ITS4	TCC TCC GCT TAT TGA TAT GC	20
NS1	GTA GTC ATA TGC TTG TCT C	19
NS2	GGC TGC TGG CAC CAG ACT TG	20
CgInt	GGC CTC CCG CCT CCG GGC GG	20
Ca-f1	TGA ACA TAC CTA ACC GTT GC	20
Ca-r1	AGG GTC CGC CAC TAC CTT TA	20
CaGlu-f2	CGT TCA CGA CAA ACA CCT TG	20
CaGlu-r2	ATC GAG TCG TGA TCG AAT CC	20
CgLac-f	GAA GAT CTC GGC ACC ATC AT	20
CgLac-r	AAC AAC AGG GAC CAG GTC AG	20

5.2.5 DNA extraction using the CTAB protocol

DNA was extracted from fungal mycelia using the CTAB protocol as described by Lodhi *et al.* (1994). The mycelia were grinded to a fine paste in 400 µl of extraction buffer in microfuge tubes using a pestle. It was then incubated in a recirculating water bath at 65°C for 15 minutes, followed by centrifugation at 12000 revolutions per minutes (rpm) for 5 minutes. Four hundred microlitres of supernatant was transferred into new eppendorf tubes and 250 µl of Chloroform: Iso Amyl Alcohol (24:1) was added to each tube, mixed with the solution by inversion and centrifuged at 13,000 rpm for 1 minute. The upper aqueous phase

was transferred into a clean microfuge tube and 50 µl of 7.5 M Ammonium Acetate, followed by the addition of 400 µl of ice cold ethanol to each tube to precipitate the DNA. This was then mixed by slow inverted movements that caused the DNA to precipitate at the bottom of the tubes. The tubes containing the DNA were then centrifuged at 13,000 rpm for 5 minutes after which the propanol was decanted. This was washed twice with 0.5 ml of 70% ethanol and centrifuged at 15,000 rpm for 5 minutes. The DNA was then dried and 50 µl of TE buffer was added to dissolve it. It was then stored at -20 °C.

5.2.6 PCR amplification of *C. gloeosporioides* isolates

The reaction volume was 20 µl containing 2 µL of genomic DNA, 2X MasterMix with standard buffer (New England Biolab, UK) and 1µL of each primer. PCR amplification was started at an initial denaturation step at 94ºC for 5 min followed by 35 cycles of denaturation at 93ºC for 1 minute, for each primer pair, DNA annealing was done at a specific temperature (Table 5.2) for 1 minute, extension at 72 ºC for 2 minute, and a final extension at 72 ºC for 5 min. The formation and size of the bands were checked by electrophoresis on a 1.0 % agarose gel stained with ethidium bromide.

Table 5.2: Annealing temperatures of various primer pairs

Primer name	Annealing temperature (^{o}C)
ITS1/ITS4	56.0
NS1/NS2	53.1
CgInt/ITS4	65.0
Ca-f1/Ca-f1	55.0
CaGlu-f2/CaGlu-r2	55.0
CgLac-f/CgLac-r	56.0

5.2.7 Pathogenicity test

Pathogenicity of isolates was determined by the leaf detached method (Shivanna and Mallikarjunaswamy, 2009) with some modifications. Fully expanded apparently healthy yam leaves (Pona and Labreko) were detached from their plants, washed with tap water to remove any attached dust particles. The leaves were then surface sterilized with 70% alcohol for 3 minutes and rinsed in three changes of sterile distilled water contained in 500 ml beakers and left to air-dry in a microflow laminar flow workstation. A leaf was then placed on moistened blotter discs (filter paper) in a Petri plate. The leaf was then wounded by gentle pricking with a sterilized needle and inoculated with the spore suspension (2×10^6 spore/ml) of *C. gloeosporioides* and incubated under light-dark cycle of 12/12 hours at 23 ± 2 ^{0}C for 7 days. The leaves that served as control were prick and sterile distilled water applied. Fungal pathogens were re-isolated on PDA plates and isolates compared with the inoculants based on colony and conidial morphology as described above (3.9).

5.3 Results

5.3.1 Cultural characteristics of isolates

The cultures of the *C. gloeosporioides* isolates obtained during this study had varied characteristics (Table 5.3 and Plate 5.1).

Table 5.3: *Colletotrichum gloeosporioides* isolates obtained from *D. rotundata* crops, their cultural characteristics and mycelia growth rate

Isolate	Crop part for isolation			Cultural characteristics	Mycelia growth rate per day (mm)
	leaves	vines	setts		
CDr1	+	-	-	Mycelia growth with a pinkish centre and whitish towards the margins.	5.30 ± 0.208[a]
CDr2	+	+	+	Whitish mycelia growth with concentric rings and having abundant orange conidia masses at the centre.	5.20 ± 0.141[a]
CDr3	+	+	-	White cottony sparse mycelia growth.	5.35 ± 0.263[a]
CDr4	+	-	-	White cottony dense mycelia growth.	4.55 ± 0.287[a]
CDr5	+	-	+	Whitish cottony mycelia with dark conidia masses occurring in concentric masses.	5.05 ± 0.171[a]
CDr6	+	+	-	White mycelia growth with concentric rings.	4.95 ± 0.377[a]
F (pr)					**0.291**

Means ± standard errors in the same column followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's test. Key: (+) = isolated, (-) = not isolated.

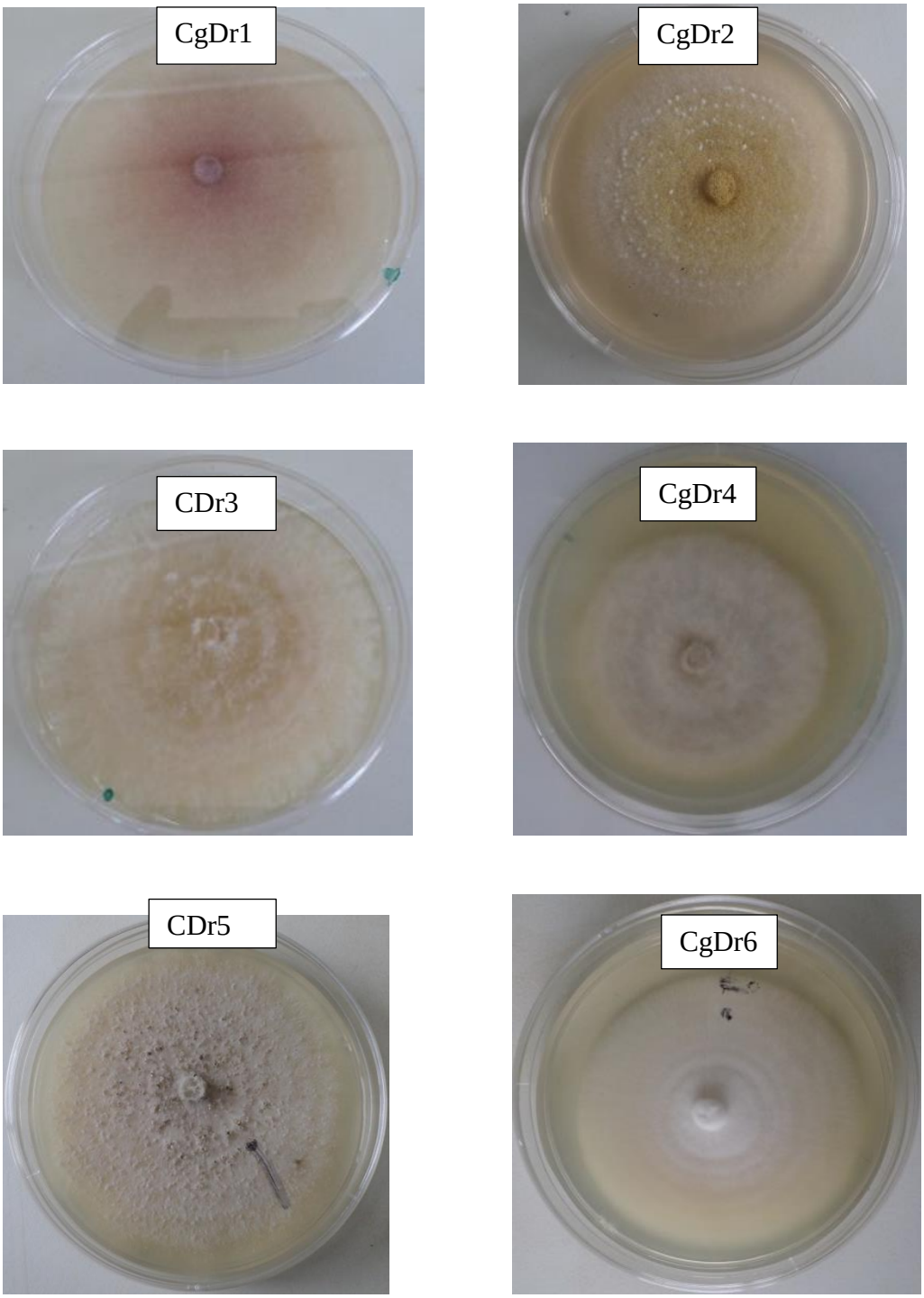

Plate 5.1: Culture characteristics of *C. gloeosprioides* isolates on PDA

Each of the isolates (CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6) had cylindrical conidia with both ends rounded (Plate 5.2 A). Setae (Plate 5.2 B) were not recorded for the isolates CDr1, CDr2, CDr3, CDr4, and CDr6 except CDr5. There were no significant differences ($P \leq 0.05$) among the mycelia growth rate of the various *C. gloeosporioides* isolates (Table 5.3). The mycelia growth rate of the *C. gloeosporioides* isolates ranged from 4.55 ± 0.287 to

5.35 ± 0.263 mm per day with CDr4 and CDr3 recording the lowest and highest respectively (Table 5.3).

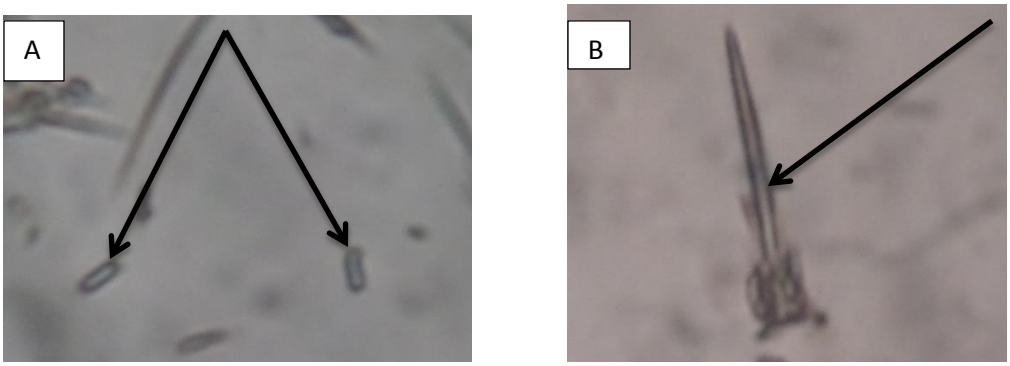

Plate 5.2: Micrograph of *C. gloeosporioides* (A) cylindrical conidia with both ends rounded (B) Setae

5.3.2 Polymerase chain reaction

The PCR analysis carried out for the characterization of the ITS region of the *C. gloeosporioides* isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 produced characteristic band sizes of approximately 580 bp when run on the universal primer pair ITS1/ITS4 (Plate 5.3). A band size of 560 bp was amplified when the DNA of each of the isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 were run with the 18S rRNA universal primer pair NS1/NS2 (Plate 5.4). The species specific primer pairs to *C. gloeosprioides* CgInt/ITS4 produced DNA fragment at 463 bp for each of the isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 (Plate 5.5). Also CgLac-f/CgLac-r primer pair which is specific for *C. gloeosporioides* produced bands which ranged from 1300bp to 200bp for the isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 (Plate 5.6). The *C. acutatum* species specific primer pairs CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r2 each did not produce DNA fragments for the various isolates of *C. gloeosporioides* (CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6) (Plates 5.7 and 5.8). There was no PCR amplification for the negative control (nuclease-free PCR

water) on the various primer pairs ITS1/ITS4, NS1/NS2, CgInt/ITS4, CgLac-f/CgLac-r, CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r2 (Plates 5.3, 5.4, 5.5, 5.6, 5.7 and 5.8).

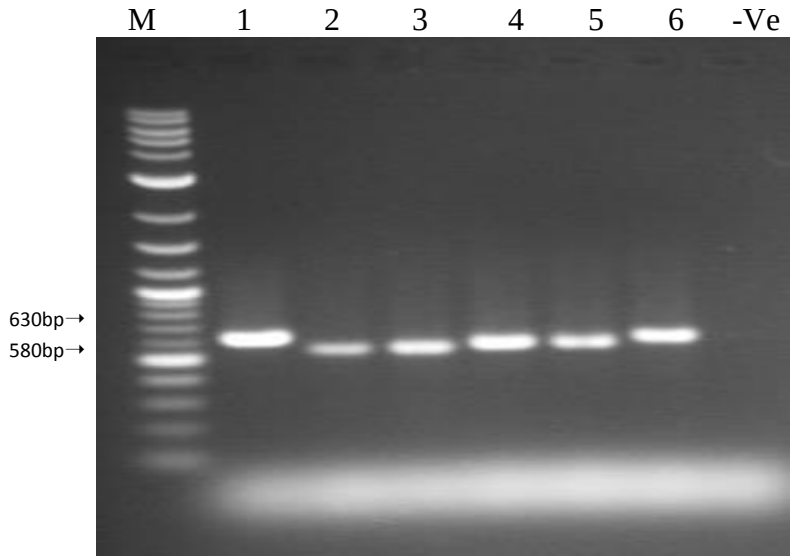

Plate 5.3: Amplified DNA fragments of *Collectotrichum* isolates (lanes – 1, 2, 3, 4, 5, 6) using ITS1/ITS4 universal primer pair. M = Molecular size marker, Quick-Load Purple 2-Log DNA Ladder (0.1-10.0 kb); 1 = CDr1, 2 = CDr2, 3 = CDr3, 4 = CDr4, 5 = CDr5, 6 = CDr6, -Ve = Negative controls (nuclease-free PCR water).

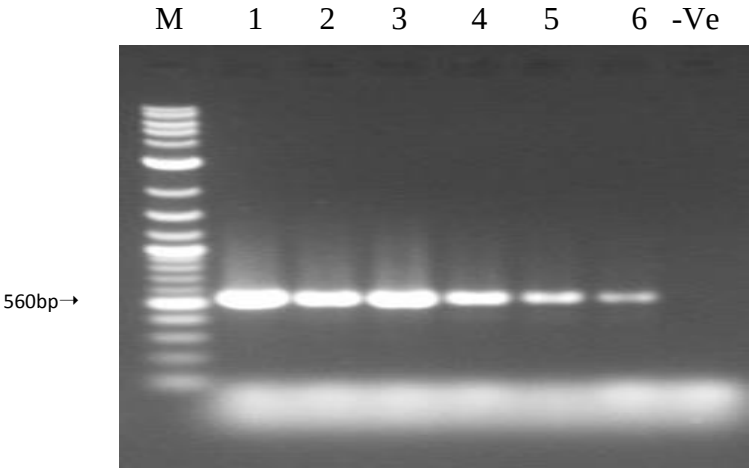

Plate 5.4: Amplified DNA fragments of *Collectotrichum* isolates (lanes – 1, 2, 3, 4, 5, 6) using NS1/NS2 universal primer pair. M = Molecular size marker, Quick-Load Purple 2-

Log DNA Ladder (0.1-10.0 kb); 1 = CDr1, 2 = CDr2, 3 = CDr3, 4 = CDr4, 5 = CDr5, 6 = CDr6, -Ve = Negative controls (nuclease-free PCR water).

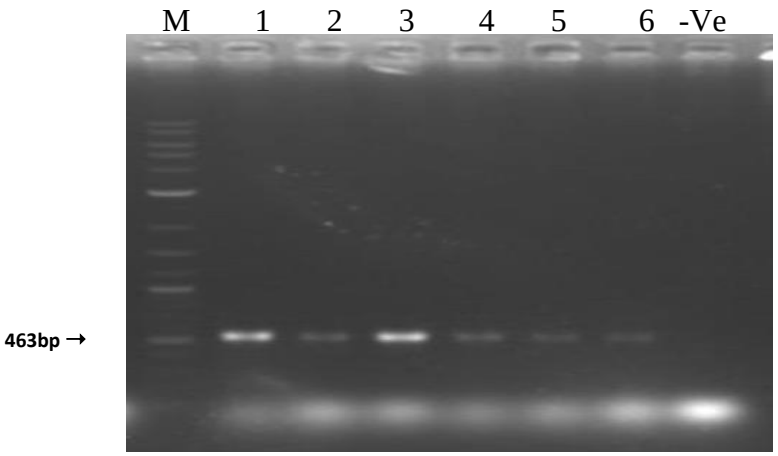

Plate 5.5: Amplified DNA fragments of *Collectotrichum* isolates (lanes – 1, 2, 3, 4, 5, 6) using CgInt/ITS4 species-specific primer pair. M = Molecular size marker, Quick-Load Purple 2-Log DNA Ladder (0.1-10.0 kb); 1 = CDr1, 2 = CDr2, 3 = CDr3, 4 = CDr4, 5 = CDr5, 6 = CDr6, -Ve = Negative controls (nuclease-free PCR water).

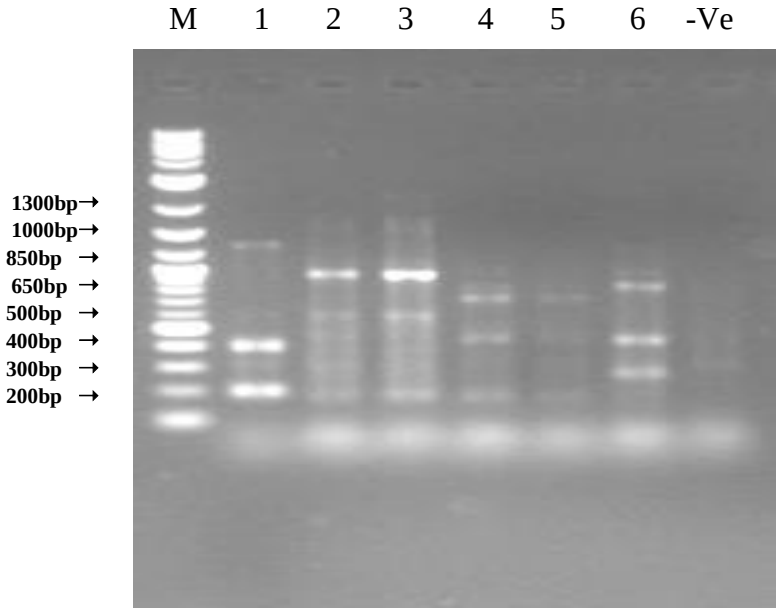

Plate 5.6: Amplified DNA fragments of *Collectotrichum* isolates (lanes – 1, 2, 3, 4, 5, 6) using CgLac-f/CgLac-r species-specific primer pair. M = Molecular size marker, Quick-

Load Purple 2-Log DNA Ladder (0.1-10.0 kb); 1 = CDr1, 2 = CDr2, 3 = CDr3, 4 = CDr4, 5 = CDr5, 6 = CDr6, -Ve = Negative controls (nuclease-free PCR water).

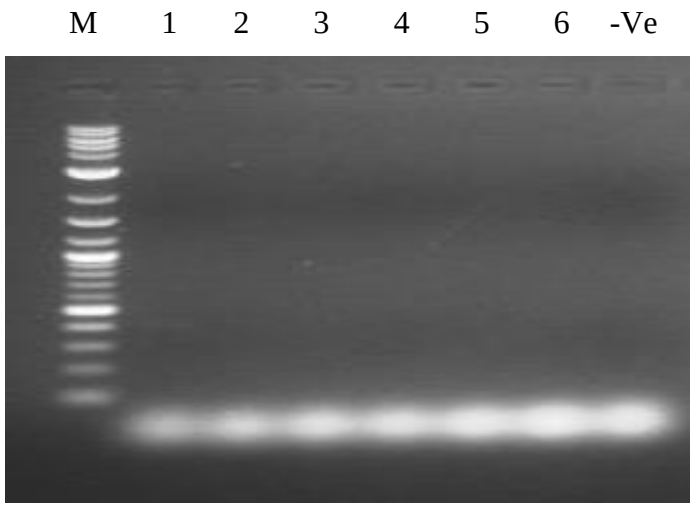

Plate 5.7: Amplified DNA fragments of *Collectotrichum* isolates (lanes – 1, 2, 3, 4, 5, 6) using CaGlu-f2/CaGlu-r2 species-specific primer pair. M = Molecular size marker, Quick-Load Purple 2-Log DNA Ladder (0.1-10.0 kb); 1 = CDr1, 2 = CDr2, 3 = CDr3, 4 = CDr4, 5 = CDr5, 6 = CDr6, -Ve = Negative controls (nuclease-free PCR water).

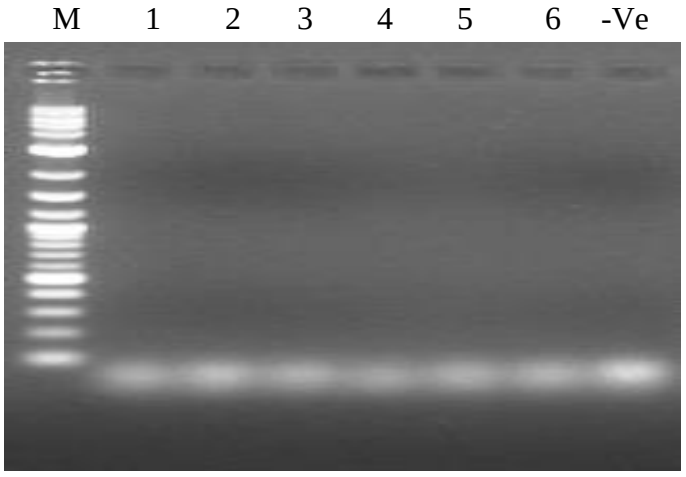

Plate 5.8: Amplified DNA fragments of *Collectotrichum* isolates (lanes – 1, 2, 3, 4, 5, 6) using Ca-f1/Ca-r1 species-specific primer pair. M = Molecular size marker, Quick-Load

Purple 2-Log DNA Ladder (0.1-10.0 kb); 1 = CDr1, 2 = CDr2, 3 = CDr3, 4 = CDr4, 5 = CDr5, 6 = CDr6, -Ve = Negative controls (nuclease-free PCR water).

5.3.3 Pathogenicity test using detached leaves

The yam leaves that were inoculated with the conidia suspension of *C. gloeosporioides* developed anthracnose symptoms (Plate 5.8). The re-isolation of the pathogen from these symptomatic leaves on PDA produced similar cultural characteristics as their mother culture. The negative control leaves which were inoculated with sterile distilled water did not develop any disease symptom. These observations fulfilled Koch's postulate.

Plate 5.9: Anthracnose disease symptom development on yam leaf 7 days after it was prick inoculated with *C. gloeosporioides* conidia suspension (A) no disease symptom development on the negative control leaf 7 day after it was prick inoculated with sterile distilled water (B).

5.4 Discussion

The cylindrical shaped conidia with rounded ends recorded for each of the isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 were similar to the observations made by Abera *et al*. (2015) who also worked on *C. gloeosporioides*. The mycelia growth rate per day for the various isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 which ranged from 4.55 ± 0.287 to 5.35 ± 0.263 mm were within the growth rate range of 3.6 to 11.2 mm recorded for *C. gloeosporioides* (Than *et al*., 2008; Abera *et al*., 2015). The lack of significant differences ($P \leq 0.05$) among the growth rate of the *C. gloeosporioides* isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 confirmed the report of Than *et al*. (2008). However, this also contradicted the findings of Abera *et al*. (2015) who also recorded significant differences in mycelia growth among isolates of *C. gloeosporioides* isolates. These contradictory reports on the significant differences in mycelia growth among *C. gloeosporioides* isolates showed that, it is unreliable to distinguish among *Colletotrichum* spp. using mycelia growth rate as a measure.

The observed variation in colour and pattern of growth among the pure cultures of CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 agreed with the findings of Than *et al*. (2008), Gautam (2014), Abera *et al*. (2015) and Appiah-Kubi *et al*. (2016) who also documented differences in cultural characteristics among isolates of *C. gloeosporioides*. The differences in cultural characteristics among the *C. gloeosporioides* isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 could be attributed to environmental rather than genetic factors. This is because according to Than *et al*. (2008) the use of morphological and phenotypic characteristics in distinguishing among C*olletotrichum* species can be deceptive since different environmental conditions can cause variation among these traits. It is therefore unreliable to depend solely on the cultural and morphological characteristics of *Colletotrichum* species

for their identification. According to Cannon *et al*. (2000) the DNA traits of an organism are not directly influenced by environmental conditions; hence the most reliable method of distinguishing among *Colletotrichum* species will be the use of molecular techniques such as PCR.

When the DNA extracts of the C. *gloeosporioides* isolates were subjected to PCR run with the universal primer pairs ITS1/ITS4, the band size amplification of approximately 580 bp was yielded for the isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6. This agrees with the findings of Raj *et al*. (2013) who also observed band amplifications of 580 bp when C. *gloeosporioides* isolates were subjected to PCR using the universal ITS1/ITS4 primer pair. The various isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 were also not separated when run on NS1/NS2 primer pair because each of them produced DNA fragment of 560 bp. Shi *et al*. (2008) who observed a 560 bp DNA fragments for *C. gloeosporioides* isolates after subjecting them to PCR using the universal primer pair NS1/NS2. The 463 bp recorded for the PCR running of the DNA extracts of the isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 with the *C. gloeosporioides* specific primer pair CgInt/ITS4 also confirmed all the isolates as the same strain of *C. gloeosporioides.* A similar observation was made by Shi *et al*. (2008). Serra *et al*. (2011) also stated that *Colletotrichum* isolates that produced DNA fragments on the primer pair CgInt/ITS4 was a confirmation of their identity as *C. gloeosporioides.*

The PCR analysis of the Isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 with the *C. gloeosporioides* species specific primer pair CgLac-f/CgLac-r produced similar bands for the various isolates; also confirming them as *C. gloeosporioides.* This agreed with the detection of similar DNA bands by *C. gloeosporioides* with the primer pair CgLac-f/CgLac-r reported by Shi *et al*. (2008) and Chagas *et al*. (2017).

The difficulty in distinguishing between *C. gloeosporioides* and *C. acutatum* using cultural and morphological characteristics necessitated the PCR run of the isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 on the *C. acutatum* species specific primers CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r1 to further clarify their identity as *C. gloeosporioides*. The absence of bands when various *C. gloeosporioides* isolates were subjected to PCR with *C. acutatum* species specific primer pairs CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r1further confirmed their identification as *C. gloeosprioides*. A similar observation was made by Shi *et al*. (2008) who also recorded no DNA amplification for the PCR analysis of *C. gloeosporioides* on each of the *C. acutatum* species specific primer pairs CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r1. The pathogenicity test which showed that the *C. gloeosporioides* isolates produced anthracnose disease symptoms on the yam leaves proved that the isolates were the causative agents of the disease on the crops.

The characteristic bands amplified in the *C. gloeosprioides* isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 with the universal primer pairs ITS1/ITS4 and NS1/NS2, *C. gloeosprioides* species specific primer pairs CgInt/ITS4 and CgLac-f/CgLac-r but not *C. acutatum* specific primer pairs CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r2 confirmed all the isolates as *C. gloeosporioides*. The proper identification of the pathogen causing the anthracnose symptoms on the *D. rotundata* crops as *C. gloeosporioides* is important for the management of the disease.

CHAPTER SIX

6.0 QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS USED AS FUNGICIDES

6.1 Introduction

For several decades, synthetic fungicides have been used for the management of plant diseases. However, environmental and health concerns have also been raised about synthetic fungicide usage in crop disease management (Kuberan *et al*., 2012). In order to promote sustainable crop production, there is the need to patronise natural based substances such as plant extracts as alternatives to synthetic fungicides. This is because unlike synthetic fungicides which residues persist in the environment and cause harm, plant extracts (used as fungicides) are natural products; hence, are easily biodegraded to make them eco-friendly (Kuberan *et al*., 2012).

The antifungal activities of plant materials results from their phytochemicals (Wink, 2003; Pusztahelyi *et al*., 2015; Daniel, 2016). These phytochemicals are produced as bi- products of the plant's primary metabolism (Bako and Aguh, 2007). According to Sherief *et al*. (2014), phytochemicals are deposited in various parts of plants and are responsible for many bioactive properties. Also the types and quantities of phytochemicals a plant extract contains, is a determinant of the level of its biological activity (Fokunang *et al*., 2000). Harinder *et al.* (2007) and Tabasum *et al.* (2016) documented alkaloids, cyanogenic glycosides, flavonoids, saponins, steroids, tannins and terpenes as major phytochemicals which can be developed into therapeutic products.

Most plant resources, especially those in the tropical regions are rich in phytochemicals; and as such there is the need to explore them in order to derive their benefits (Harinder *et al*., 2007). Yadav *et al.* (2014) also stated that, there is the need for qualitative screening of plant materials for the presence of phytochemicals; in order to detect bioactive agents which

can be developed into products to combat microbial infection. The exploitation of plants for their antimicrobial properties is one of the most intensive areas of natural product research today, yet the field is far from being exhausted (Sofowora, 2006). This implies that the phytochemical analysis of indigenous plant materials will help reveal their active ingredients, which will aid in the exploitation of their extracts as fungicides or their formulation into eco-friendly fungicide products. This study seeks to carry out phytochemical analysis of some plant materials in order to reveal their antifungal potential. The specific objectives were to:

- detect qualitatively the presence of various phytochemical constituents in extracts of seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, leaves of *I. oliviformis*, and fruits of *Capsicum* spp. (hot pepper, Legon 18 variety).
- Assess the quantities of total phenolics, flavonoids, tannins and alkaloids in extracts of seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, leaves of *I. oliviformis*, and fruits of *Capsicum* spp. (hot pepper, Legon 18 variety).

6.2 Materials and methods

6.2.1 Sources of plant materials

The locations from which the plant materials (seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, *I. oliviformis* leaves and *Capsicum* spp. - hot pepper, Legon 18 variety fruits) were obtained have been stated in section 3.4. The parts of the various plant materials used for the study have been stated in section 3.3, Table 3.2.

6.2.2 Preparation of plant materials

The seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, *I. oliviformis* leaves and *Capsicum* sp. (hot pepper, Legon 18 variety) fruits were pulverised into powdered forms as described in section 3.5. For each powdered dried plant material, 10 g was weighed into a dry conical flask and 300 ml of ethanol added. This was then placed on a shaker (LAB-LINE Instrument, Inc. Melrose Park, ILL Orbit Shaker) for 24 hours. The extract was then filtered through Whiteman No. 1 filter paper and the filtrate evaporated to dryness using the rotary evaporator. For each sample, 1 mg/ml of stock solution was prepared. Each sample was then analysed in triplicates.

6.2.3 Preparation of standard solution

Gallic acid, rutin and tannic acid standard solutions were prepared with distilled water (1 mg/ml). Caffeine standard solution was also prepared by dissolving 1 mg pure Caffeine in 10 ml distilled water. These solutions were serially diluted to 1 µg/ml.

6.2.4 Qualitative test for phytochemicals

6.2.4.1 Alkaloids

This was determined with the method of Trease and Evans (1989). One millilitre of plant extract was placed in a test tube. Two drops of Mayer's reagent were added along the sides of the test tube. A cream coloured precipitate was taken as evidence of alkaloids.

6.2.4.2 Saponins

This was done according to the method of Adegoke *et al*. (2010). About 0.5 g of each plant extract was shaken with 10 ml of distilled water in a test tube. Frothing which persisted on warming was taken as evidence for the presence of saponins.

6.2.4.3 Tannins

Two drops of Ferric chloride were added to 0.5 ml of plant extract in a test tube. The appearance of a blue green colour confirmed the presence of tannins in the sample (Iyengar, 1995).

6.2.4.4 Phlobatinnins

About 0.5 g of each plant extract was boiled with one percent aqueous Hydrochloric acid. Deposition of a red precipitate was taken as evidence for the presence of phlobatannins (Trease and Evans, 1989).

6.2.4.5 Anthraquinones

The determination was done according to the method of Trease and Evans (1989). The plant extract (0.5 g) was added to 5 ml benzene. The mixture was filtered and to the filtrate, 5 ml

of 10 percent ammonia solution added. The appearance of a pink, red, or violet colour after the mixture was shaken was taken as evidence of anthraquinones.

6.2.4.6 Cardiac glycosides

To 2 ml of each extract, 1 ml of glacial acetic acid containing 1 ml Ferric chloride was added, followed by 2 ml of concentrated Sulphuric acid. The appearance of a greenish-blue colouration confirmed the presence of cardiac glycosides (Ayoola *et al.*, 2008).

6.2.4.7 Steroid

One millilitre of each extract was added to 2 ml of chloroform and then mixed with 2 ml of Acetic acid and 2 ml of concentrated Sulphuric acid. Appearance of greenish colour indicated the presence of steroids (Bargah, 2015).

6.2.4.8 Terpenoids

Two millilitres of chloroform was mixed with 5 ml of the extract before adding 3 ml of concentrated sulphuric acid. Appearance of a reddish brown colour confirmed the presence of terpenoids (Wadood *et al.*, 2013).

6.2.4.9 Flavanoids

To two millilitres of the test solution, a small piece of magnesium ribbon and 1 ml concentrated Hydrochloric acid were added. Appearance of a crimson red colour confirmed the presence of flavonoids in the sample (Kumar *et al.*, 2007).

6.2.5 Estimation of quantities of phytochemicals in plant extracts

6.2.5.1 Total phenols

The method of Kaur and Kapoor (2002) with some midifications was used for the estimation of total phenolic content. To every 0.1 ml of extract sample, 3.9 ml of distilled water and 0.5 ml of Folin-Ciocalteau reagent were added and the mixture incubated at room temperature for 3 min before adding 2 ml of 20% sodium carbonate. The mixture was heated in boiling water for 1 min and its absorbance at 650 nm was measured. Gallic acid was used as a standard for constructing a calibration curve.

6.2.5.2 Total flavonoids

Total flavonoid content was determined by a modified method of Zhishen *et al.* (1999). Distilled water was added to 0.1 ml of the extract to make it up to the 5 ml. After 5 min, 0.3 ml of 5% $NaNO_2$ and 3 ml of 10% $AlCl_3$ were added and allowed to stand. Six (6) min later, 2 ml of 1 M NaOH was added. The absorbance of the resulting solution was measured at 510 nm. Rutin was used as a standard for constructing a calibration curve.

6.2.5.3 Total tannins

The method of Graham (1992) was used in determining total tannins content. To 0.1 ml of the plant extract, 6.9 ml of distilled water, 1ml of 0.008M potassium Ferric cyanide, 1 ml of 0.2M ferric chloride in 0.1M HCl were added and mixed. The absorbance of the resulting solution at 700 nm was measured. A calibration curve was constructed using tannic acid as standard.

6.2.5.4 Total alkaloids

The method of Ajanal *et al*. (2012) was employed for the determination of total alkaloids. The plant extract (1 mg/ml) was added to 2 N HCl. This was then filtered and one millilitre of the filtrate washed with 10 ml chloroform in a separating funnel, and the procedure repeated thrice. About 0.1 N NaOH was then added to neutralize the pH of the solution. To this solution, 5 ml each of bromocresol green solution and phosphate buffer were added and agitated. The complex formed was extracted with 4 ml chloroform by vigorous shaking. The filtrate was then transferred into a volumetric flask (10 ml) and diluted to volume with chloroform. The absorbance of the solution at 470 nm was measured. A calibration curve was constructed using Caffeine as standard.

6.3 Results

6.3.1 Qualitative test for phytochemicals

Alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatinnins, saponins, steroids, tannins and terpenoids were detected in the extracts (Table 6.1). Alkaloids were detected in aqueous extracts of *A. indica*, *J. curcas*, *K. senegalensis* and ethanol extract of *B. aegyptiaca* seeds, *I. oliviformis* leaves and both aqueous and ethanol fruits extracts of *Capsicum* spp. (Legon 18 variety). Anthraquinones were also detected in ethanol extracts of *Capsicum* spp. (Legon 18 variety) fruits, *I. oliviformis* leaves, *J. curcas* seeds and aqueous extract of *I. oliviformis* leaves. Ethanol extracts of *A. indica* and *B. aegyptiaca* seeds, *I. oliviformis* leaves as well as both aqueous and ethanol extracts of *Capsicum* spp. (Legon 18 variety) fruits and *K. senegalensis* seeds contained cardiac glycosides.

Flavonoids were detected in ethanol extracts of *A. indica* seeds, *B. aegyptiaca* seeds, *Capsicum* spp. (Legon 18 variety) fruits, *I. oliviformis* leaves and aqueous extract of *Capsicum* spp. (Legon 18 variety) fruits. Aqueous extract of *Capsicum* spp. (Legon 18 variety) fruits, and both aqueous and ethanol extracts of *I. oliviformis* leaves contained phlobatinnins. Ethanol extracts of *A. indica*, *B. aegyptiaca*, *K. senegalensis* seeds and aqueous extract of *Capsicum* spp. (Legon 18 variety) fruits contained saponins.

Steroids were detected in aqueous extracts of *A. indica* seeds, *Capsicum* spp. (Legon 18 variety) fruits, ethanol extracts of *I. oliviformis* leaves as well as in both aqueous and ethanol *J. curcas* seeds extracts. With the exception of ethanol extract of *A. indica* and aqueous extract of *B. aegyptiaca* seeds, tannins were detected in all the other extracts used in this study. Terpenoids was detected in aqueous *A. indica* seeds, aqueous *Capsicum* spp. (Legon 18 variety) fruits and ethanol *I. oliviformis* leaves extracts.

Table 6.1: Phytochemical constituents of aqueous and ethanol extracts of the plant extracts

Phytochemical constituent	Plant extracts											
	***A. indica* seeds**		***B. aegyptiaca* seeds**		***Capsicum* spp. (Legon 18) fruits**		***I. olivoformis* leaves**		***J. curcas* seeds**		***K. senegalensis* seeds**	
	Aqueous	**Ethanol**	**aqueous**	**ethanol**	**Aqueous**	**Ethanol**	**aqueous**	**ethanol**	**aqueous**	**ethanol**	**aqueous**	**ethanol**
Alkaloids	+	-	-	+	+	+	+	-	+	-	+	-
Anthraquinones	-	-	-	-	-	+	+	+	-	+	-	-
Cardiac glycosides	-	+	-	+	+	+	-	+	-	-	+	+
Flavanoids	-	+	+	+	+	+	-	+	-	-	-	-
Phlobatinnins	-	-	-	-	+	-	+	+	-	-	-	-
Saponins	-	+	+	+	+	-	-	-	-	-	-	+
Steroids	+	-	-	-	+	-	-	+	+	+	-	-
Tannins	+	-	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	-	-	-	+	-	-	+	-	-	-	-

Key: +=present; -=absent

The highest number of phytochemical constituents was recorded for aqueous extract of *Capsicum* spp. (Legon 18 variety) fruits. With the exception of anthraquinones, all other phytochemicals (alkaloids, cardiac glycosides, flavonoids, phlobatinnins, saponins, steroids, tannins and terpenoids) screened for in this study were detected in aqueous *Capsicum* spp. (Legon 18 variety) fruits extract (Table 6.1).

6.3.2 Estimation of amount of phytochemicals in plant extracts

6.3.2.1 Total phenols

The curve of absorbance versus concentration of the gallic acid used as standard was described by the equation y = 1.2667x – 0.6 with R^2 = 0.9576, where y = absorbance at 650 nm and x = total phenol in the extracts (Figure 6.1).

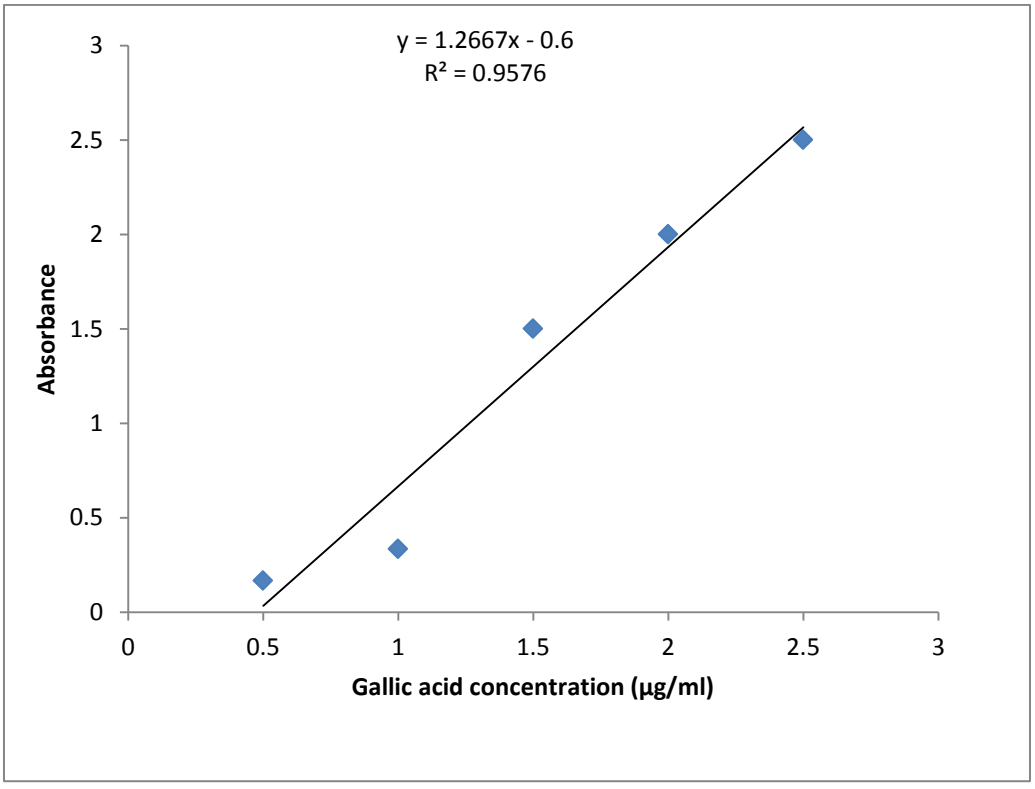

Figure 6.1: Standard calibration curve for total phenolic content for standard gallic acid

Total phenolic content of the samples obtained were expressed in terms of gallic acid equivalent in µg/ml. The total phenolic content of the plant extracts ranged from 0.494 ± 0.002 to 1.800 ± 0.006. *A. indica* and *K. senegalensis* seeds extracts recorded the lowest (0.494 ± 0.002) and highest (1.800 ± 0.006) values respectively (Figure 6.2).

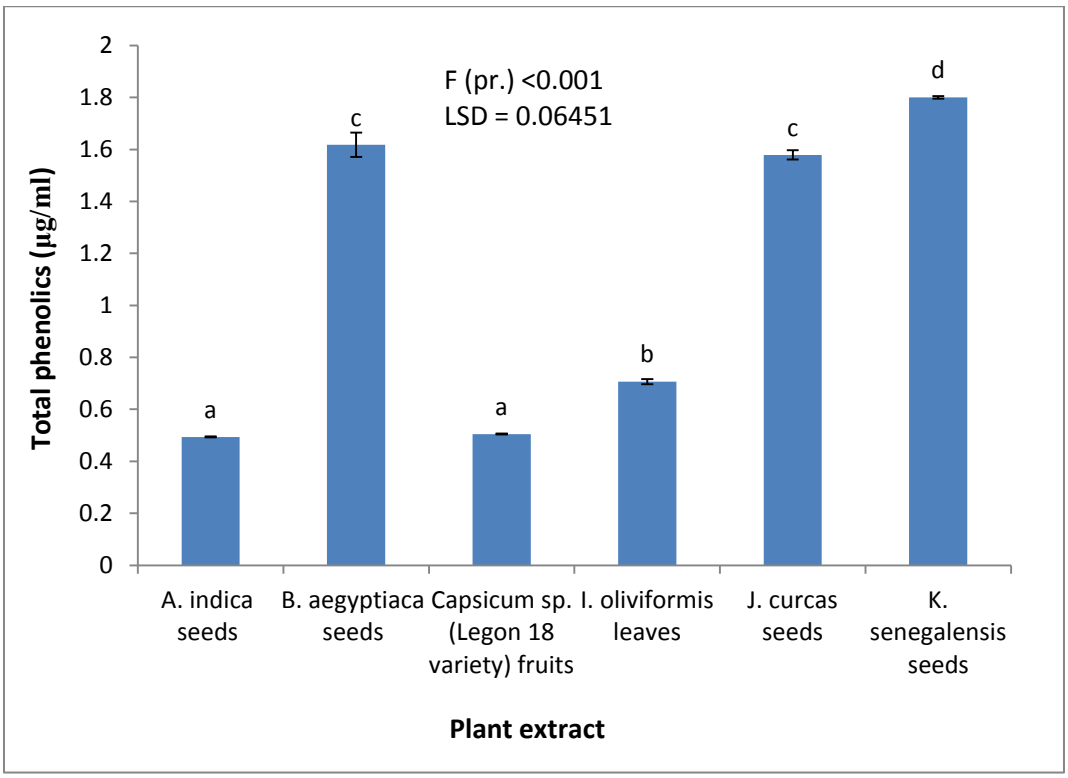

Figure 6.2: Total phenolic content of some plant extracts

Generally there were significant differences ($P \leq 0.05$) in total phenolic content among the various extracts (Figure 6.2). No significant difference ($P \leq 0.05$) was observed between total phenolic content for *A. indica* seeds (0.494 ± 0.002) and *Capsicum* spp. (Legon 18 variety) fruits (0.505 ± 0.002) extracts (Figure 6.2), which each had significantly ($P \leq 0.05$) lower total phenolic content in comparison to extracts of seeds of *B. aegyptiaca*, *J. curcas*, *K. senegalensis* and *I. oliviformis* leaves (Figure 6.2). *K. senegalensis* seeds extract had a significantly higher ($P \leq 0.05$) total phenolic content than those of *B. aegyptiaca*, *J. curcas* and *I. oliviformis* leaves (Figure 6.2). Even though no significant difference was observed

between total phenolic content for extracts of seeds of *B. aegyptiaca* and *J. curcas*, each of them had a significantly higher ($P \leq 0.05$) total phenolic content than that of *I. oliviformis* leaves (Figure 6.2).

6.3.2.2 Total flavonoids

The total flavonoid content was expressed as µg/ml rutin equivalent using the standard curve equation: y = 0.0594x + 0.0323, R^2 = 0.09551, where y = Absorbance at 510 nm and x = Total flavonoid content in the extracts (Figure 6.3).

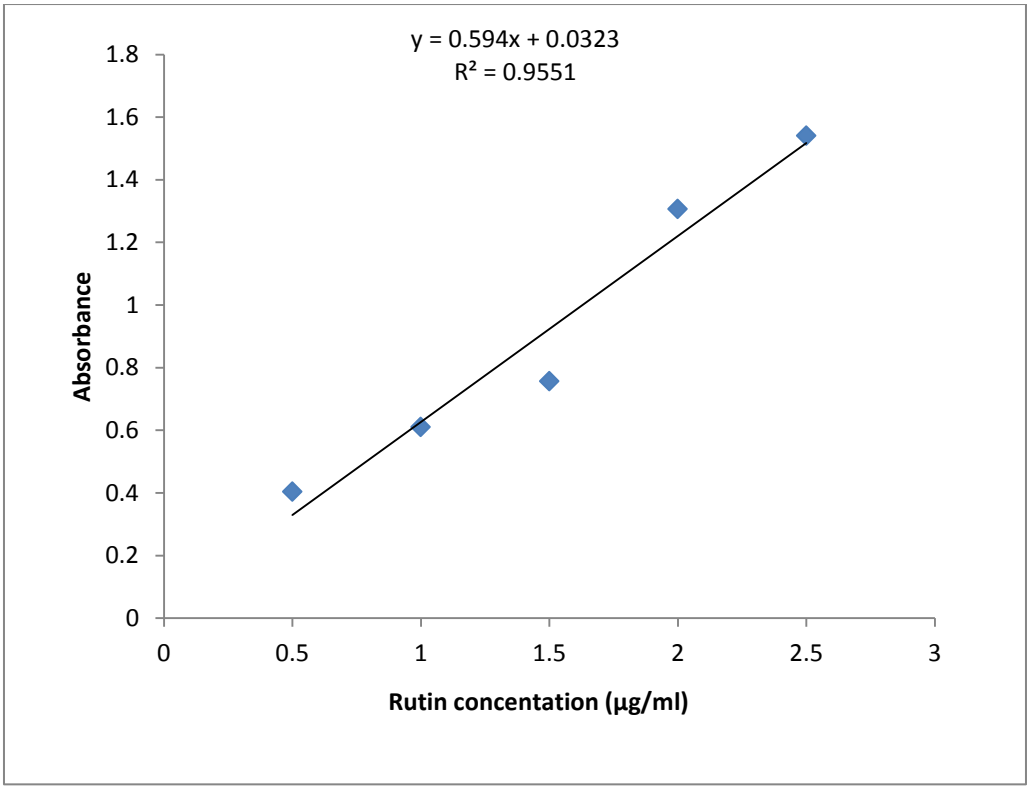

Figure 6.3: Standard calibration curve for total flavonoid content for standard rutin

The lowest (1.109 ± 0.001) and highest (2.663 ± 0.007) total flavonoid contents were recorded for *B. aegyptiaca* seeds and *Capsicum* sp. (Legon 18 variety) fruits extract respectively (Figure 6.4). Total flavonoid contents of extracts of *Capsicum* sp. (Legon 18 variety) fruits, *I. oliviformis* leaves and *J. curcas* seeds were significantly higher ($P \leq 0.05$)

than those of *B. aegyptiaca* (1.109 ± 0.001) and *K. senegalensis* (1.355 ± 0.007) seeds (Figure 6.4). There were also no significant differences ($P \leq 0.05$) in total flavonoid contents among extracts of *Capsicum* spp. (Legon 18 variety) fruits (2.663 ±0.007), *I. oliviformis* leaves (2.503 ± 0.008) and *J. curcas* seeds (2.501 ± 0.016) (Figure 6.4). Generally there were significant differences ($P \leq 0.05$) in total flavonoid content between extracts of *A. indica* seeds in comparison to that of the other plant materials (Figure 6.4).

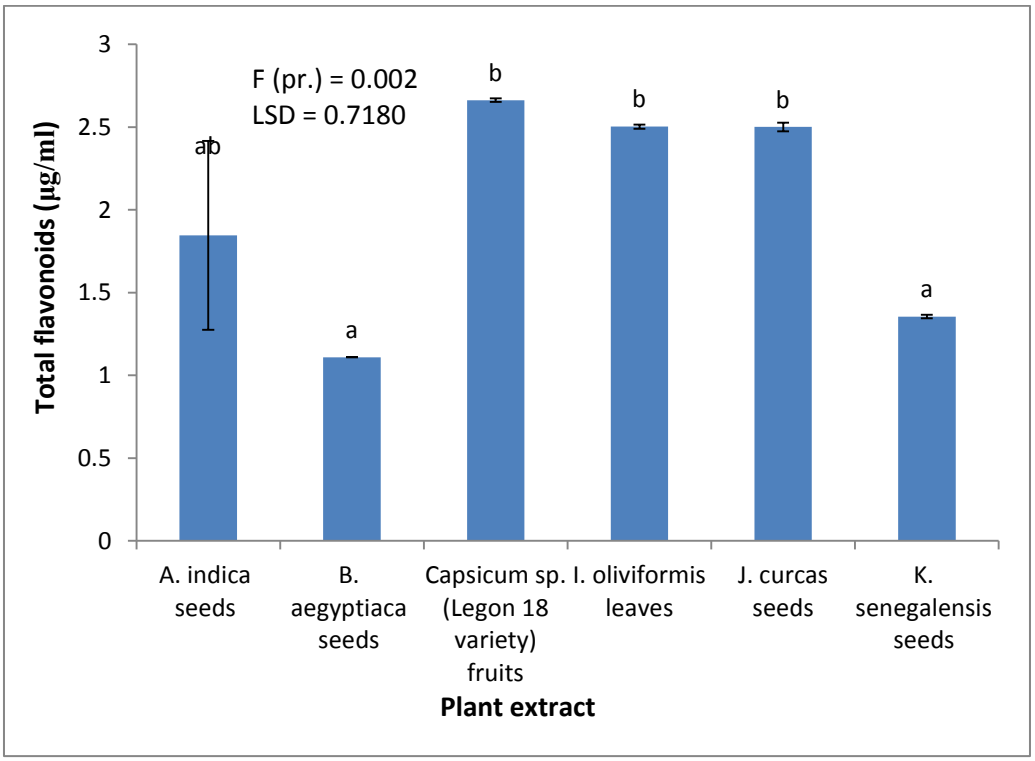

Figure 6.4: Total flavonoid content of some plant extracts

6.3.2.3 Total tannins

For total tannins content, the curve of absorbance versus concentration of the tannic acid used as standard was described by the equation y = 0.5025x + 0.0007, R^2 = 0.9998, where y = absorbance at 700 nm and x = total tannins concentration in the extracts (Figure 6.5).

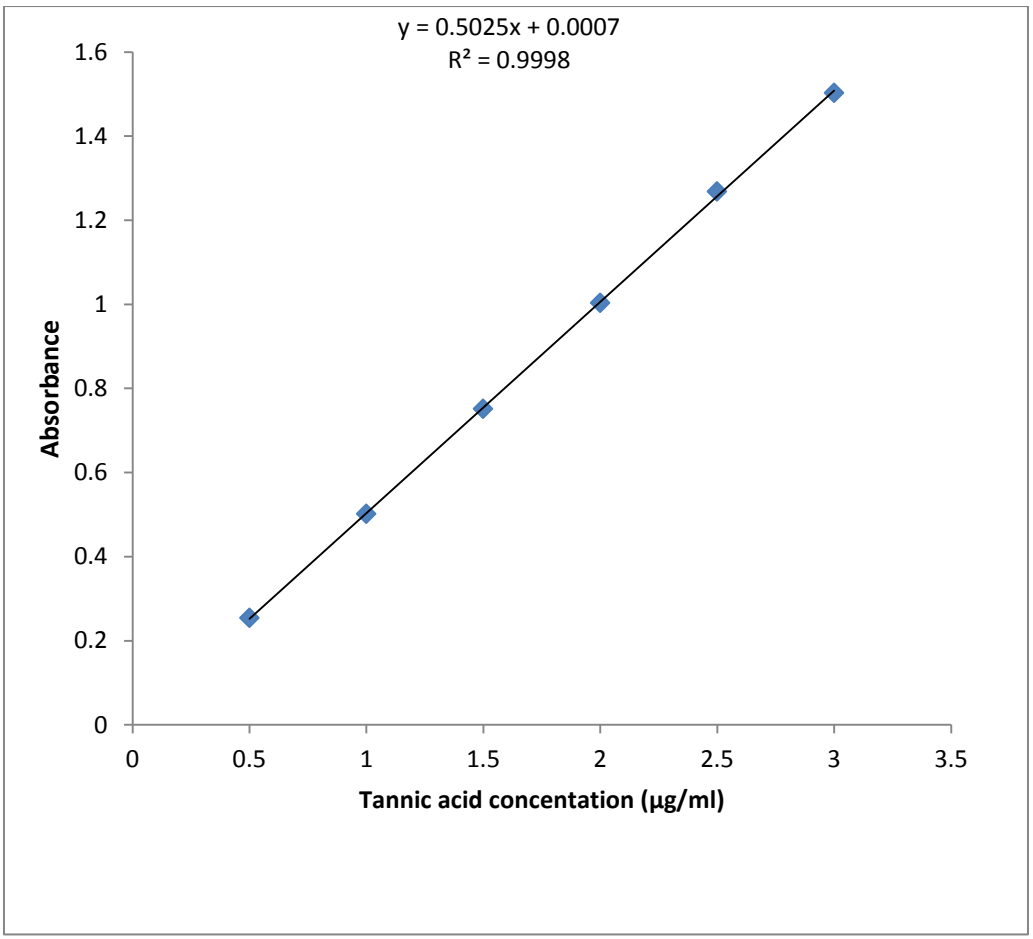

Figure 6.5: Standard calibration curve for total tannin content for standard tannic acid

Total tannins content of the samples obtained were expressed in terms of tannic acid equivalent in µg/ml. *I. oliviformis* leaves extract had a significantly higher ($P \leq 0.05$) total tannins content (0.07 ± 0.001) than extracts of seeds of *A. indica* (0.011 ± 0.001), *B. aegyptiaca* (0.003 ± 0.001), *J. curcas* (0.005 ± 0.001), *K. senegalensis* (0.015 ± 0.000), and *Capsicum* sp. (Legon 18 variety) fruits (0.009 ± 0.002) (Figure 6.6). Although no significant difference ($P \leq 0.05$) was observed for total tannins content between A. *indica* seeds and *Capsicum* sp. (Legon 18 variety) fruits extracts, there was a general significant difference ($P \leq 0.05$) between these two and extracts of seeds of *B. aegyptiaca*, *J. curcas*, *K. senegalensis* and *I. oliviformis* leaves (Figure 6.6). There was also no significant difference

($P \leq 0.05$) in total tannin contents between *B. aegyptiaca* and *J. curcas* seed extracts (Figure 6.6).

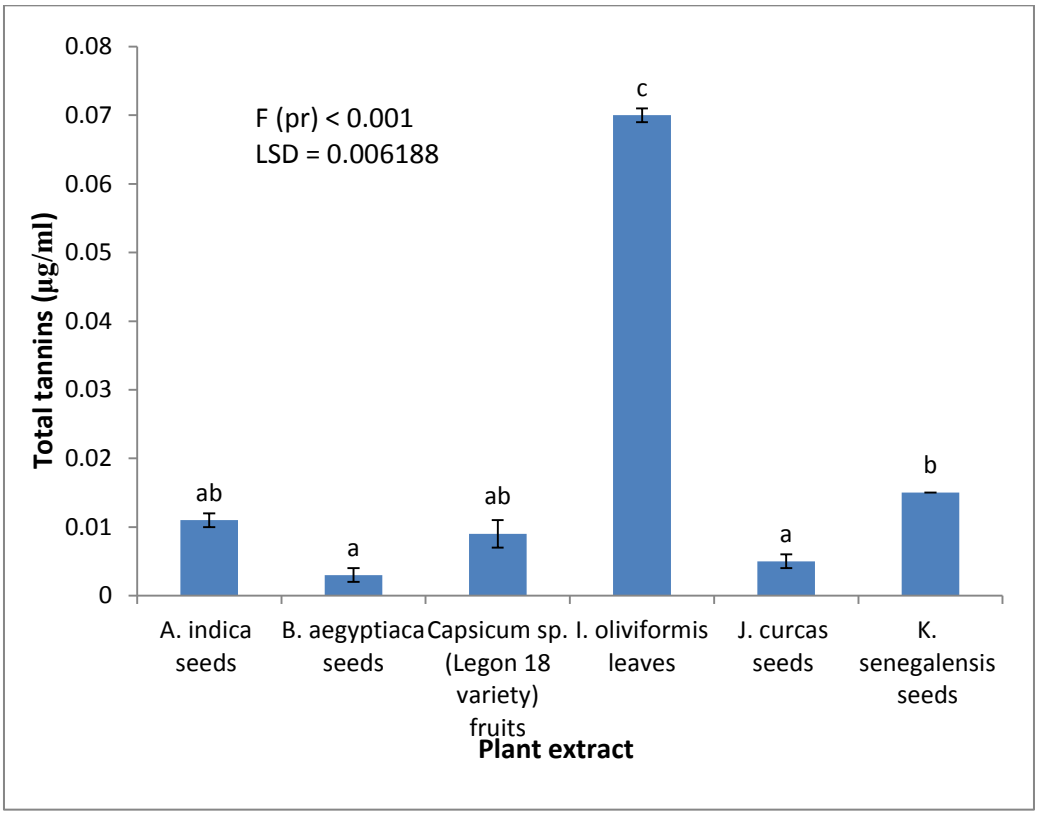

Figure 6.6: Total tannins content of some plant extracts

6.3.2.4 Total alkaloids

For total alkaloid content, the curve of absorbance versus concentration of the caffeine used as standard was described by the equation y = 0.6336x - 0.1445, R^2 = 0.9507, where y = absorbance at 470 nm and x = total tannin concentration in the extracts (Figure 6.7).

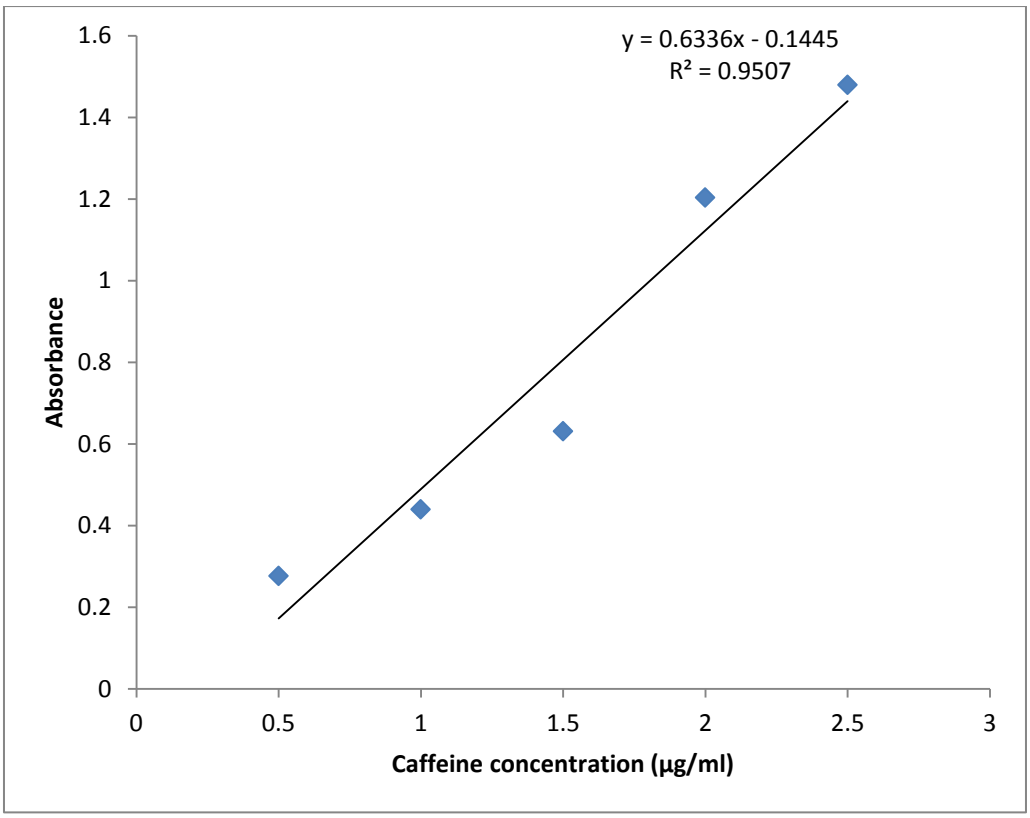

Fig 6.7: Standard calibration curve for total alkaloid content for standard caffeine

Total alkaloid content of the samples obtained was expressed in terms of caffeine equivalent in µg/ml. The total alkaloid content of extracts of seeds of *B. aegyptiaca* (0.232 ± 0.000), *J. curcas* (0.238 ± 0.002), leaves of *I. oliviformis* leaves (0.230 ± 0.001) as well as *Capsicum* sp. (Legon 18 variety) fruits (0.230 ± 0.001) were significantly ($P \leq 0.05$) lower than those of seeds extracts of *A. indica* (1.406 ± 0.002) and *K. senegalensis* (0.747 ± 0.007) (Figure 6.8). Seeds extracts of *A. indica* also recorded a significantly higher ($P \leq 0.05$) total alkaloid content than that of *K. senegalensis* (Figure 6.8).

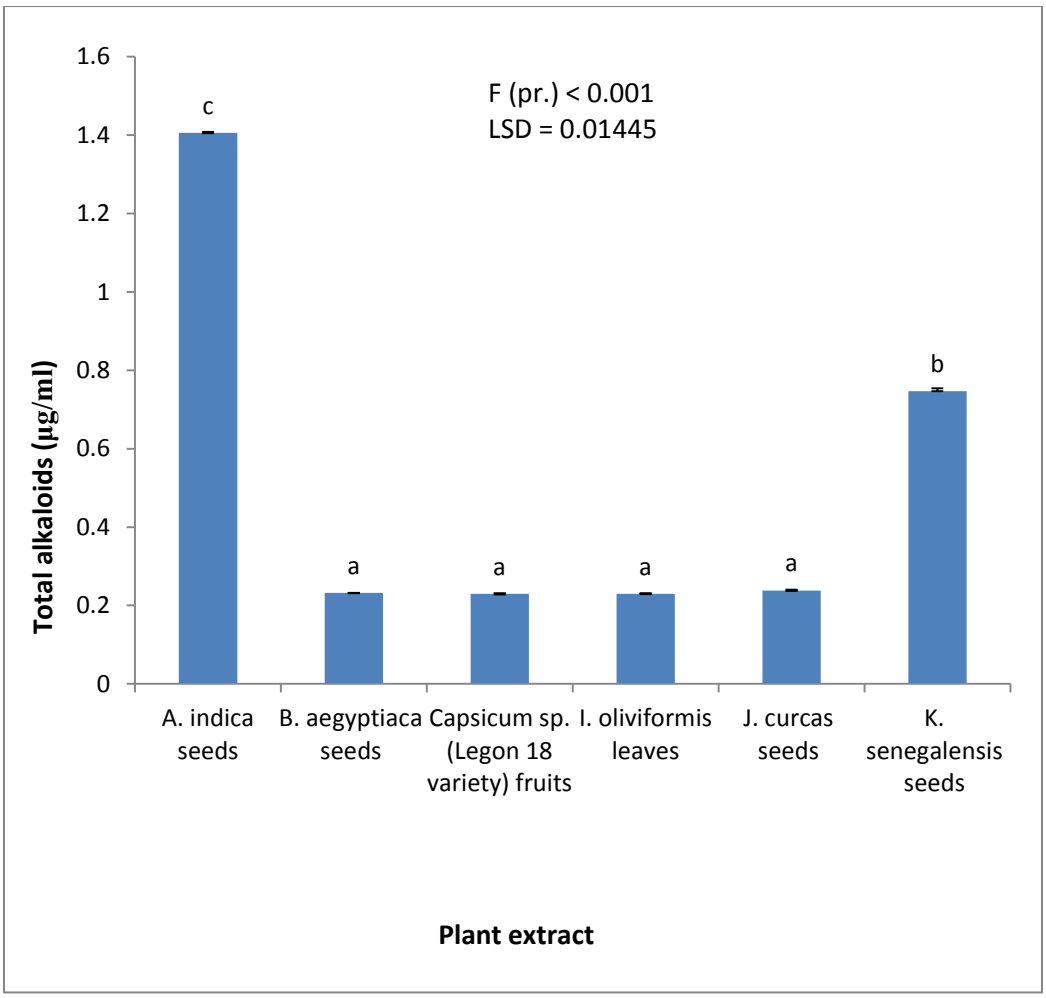

Figure 6.8: Total alkaloid content of some plant extracts

6.4 Discussion

The presence of alkaloids, cardiac glycosides, flavanoids, saponins, steroids, tannins and terpenoids in *A. indica* seeds extracts confirms the findings of Kosma *et al.* (2011) who also detected alkaloids, phenols, saponins, steroids, tannins and triterpenoids in seeds extracts of *A indica.* Deteection of alkaloids, cardiac glycosides, flavanoids, saponins and tannins in the seeds extracts of the *B. aegyptiaca* in this study confirms the findings of Ogori (2017) who reported the presence of alkaloid, saponin, flavonoid, Tannin and phenol for the same extract type. The presence of alkaloids, anthraquinone, saponins, steroids and tannins in the *J. curcas* seeds extracts confirms the findings of Rampadarath *et al*. (2016) who detected alkaloid, coumarins, flavonoids, phenol, steroids and tannins in extracts of *J. curcas* seeds. The *K. senegalensis* seeds also contained alkaloids, cardiac glycosides, saponins, tannins as reported by Idu *et al*. (2014) who also detected alkaloids, tannins, saponins, phytates and oxalates in seeds extract of *K. senegalensis.*

The phytochemicals alkaloids, anthraquinone, cardiac glycosides, flavanoids, phlobatinnins, saponins, steroids, tannins and terpenoids recorded for *Capsicum* sp. (Legon 18 variety) fruits confirms the findings of Koffi-Nevry *et al.* (2012) and Emmanuel-Ikpeme *et al.* (2014) who also detected alkaloids, anthraquinones, carotenoids, flavonoïds, glycosides, limonoids, phenolic, saponin, steroïds, tannins and terpenoids in extracts of *Capsicum annuum* fruits. The alkaloids, anthraquinone, cardiac glycosides, flavanoids, phlobatinnins, saponins, steroids, tannins and terpenoids detected in *I. olivoformis* leaves confirmed the findings of David-Oku *et al*. (2017) who also detected alkaloids, cardiac glycosides, oxalate, phenols, phytate, saponin and steroids in tuber extracts of *Icacina senegalensis (*synonym of *I. olivoformis).*

The presence of various phytochemical constituents in seeds extracts of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis* as well as extracts of *Capsicum* sp. (Legon 18 variety) fruits and *I. oliviformis* leaves (Table 6.1) indicated their potential use as fungicides. This is supported by Wink (2003) and Pusztahelyi *et al*. (2015) reports that secondary metabolites of plants have a defensive action against microbes, viruses or other competing plants and as such, are important for plants' survival and fitness. Phytochemicals usually have a toxic or lytic effect on pathogens; hence reducing or preventing infection (Chaube and Pundhir, 2009). Studies conducted by Okogbenin *et al*. (2014) established that the presence of natural products (alkaloids, terpenoids, anthraquinones, flavonoids, tannins, and saponins) in *Aframomum sceptrum* extracts gave it a highly significant antifungal effect than the broad spectrum fungicide, Mancozeb against *Fusarium oxysporum f.sp elaeidis* and *Hypocrea lixii* (IMI 501885*).*

Secondary metabolites of plants usually affect phytopathogenic fungi through interference with molecular targets in the pathogens' organs, tissues and cells; with major targets including biomembranes, proteins and nucleic acid (Engelmeier and Hadacek, 2006**;** Ribera and Zuñiga, 2012**).** Other secondary metabolites also affect plant pathogenic fungi by inhibiting cutinase and laccase production (Goetz *et al*., 1999; Bostock *et al*., 1999). Antifungal compounds of plant extracts may also have an effect on spore germination leading to its inhibition or on microbial cell wall altering its permeability (William, 2008). Pandey and Trivedi (2006) reported the inhibition of fungal spore germination by certain phytochemicals.

The different levels in total phenolics, flavonoids, tannins and alkaloids observed among the various extracts of seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis,* fruits of *Capsicum* sp. (Legon 18 variety) and *I. oliviformis* leaves was expected. This confirms the

findings of Khan *et al*. (2010) that, the type and quantity of phytochemical vary among plant species and also in various organs of the same plant.

The detection of the phytochemicals alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatinnins, saponins, steroids, tannins and terpenoids in extracts of seeds of *A. indica, B. aegyptiaca, J. curcas, K. senegalensis,* leaves of *I. oliviformis,* and fruits of *Capsicum* sp. (Legon 18 variety) and at varying concentrations of total phenolics, flavonoids, tannins and alkaloids confirmed their antifungal potential. This shows that potentially these extracts can be used to produce botanical fungicides for the management of anthracnose disease of yam.

CHAPTER SEVEN

7.0 *IN VITRO* STUDIES OF ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) THE CAUSATIVE AGENT OF YAM ANTHRACNOSE DISEASE

7.1 Introduction

Colletotrichum gloeosporioides is a pathogen that causes anthracnose disease in a wide range of plant host including yam (Agrios, 2005). The anthracnose disease is a major threat to yam production resulting in greater yield losses (Agrios, 2005; Egesi *et al*., 2007). Symptoms of the disease appear first on yam leaves as black or brown spots usually with yellow halo, and can enlarge and merge; resulting in leaf necrosis and vine die-back (Amusa *et al*. 2003). The leaf necrosis and vine dieback reduces the available photosynthetic area on crops thereby limiting productivity (Egesi *et al*. 2007). Even though several strategies have been adapted in the management of yam anthracnose disease, it is prudent to use ecologically friendly control measures.

Synthetic fungicides have been produced mainly for the control of plant diseases in order to enhance productivity. However, inappropriate and continuous synthetic fungicide usage have been associated with problems such as development of resistance to chemicals by pathogens, harmful effect on non-target organisms and environmental pollution resulting from fungicide residue (Kuberan *et al*., 2012). In order to encourage sustainable agriculture, there is the need to promote natural based products such as plant extracts as fungicides since they are eco-friendly, indigenously available and relatively cost effective (Kuberan *et al*., 2012).

The exploitation of local plant materials as fungicides is one major means of reducing crop diseases; increasing crop yield and also sustaining farm lands for future generations. The

antimicrobial activities of extracts of *A. indica* (Ramaiah and Garampalli, 2015), *B. aegyptiaca* (Bonzi *et al*. (2012), *Capcicum* spp. (Anikwe *et al*., 2017), *J. curcas* (Sales *et al*., 2016) and *K. senegalensis* (Zarafi and Moumoudou, 2010) against certain pathogens that cause crop diseases have been documented. The antimicrobial properties exhibited by plant extracts have necessitated their exploration as botanical fungicides in the management of yam anthracnose disease. The phytochemical analysis conducted on the plant extracts used in this study revealed various phytochemical constituents (Table 6.1). This showed the antifungal potential of these plant extracts. This study was conducted *in vitro* to test seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) and leaves of *I. oliviformis* for their potential antifungal activity against mycelia growth and spore germination of *C. gloeosporioides*.

7.2 Materials and methods

7.2.1 Plant materials

The sources and parts of plant materials (*A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, *Capsicum* sp. - hot pepper, Legon 18 variety and leaves of *I. oliviformis*) which extracts were used for the study have been stated in sections 3.3, 3.4 and Table 3.2.

7.2.2 Preparation of plant materials for extraction

The processes involved in the preparation of seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) and leaves of *I. oliviformis* before their extraction have been described in section 3.5.

7.2.3 Preparation of plant extracts for *in vitro* studies

The method used for the extraction of seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) and leaves of *I. oliviformis* has been described under section 3.6.

7.3 Synthetic fungicides preparation

The preparations of the concentrations of the synthetic fungicides manlax and rainmancoz used for the study have been described in Section 3.7, Table 3.3.

7.4 Media preparation

The preparation of the Potato Dextrose Agar (PDA) medium used for the study has been described in section 3.8.

7.5 Isolation and identification of *C. gloeosporioides*

The methods employed for the isolation and identification of *C. gloeosporioides* have been described under sections 3.9 and 3.10 respectively.

7.6 Inhibitory effect of plant extracts on mycelia growth and spore germination of *C. gloeosporioides*

7.6.1 Effect of plant extracts on *C. gloeosporioides* mycelia growth

The effect of 10, 25, 50, 75 and 100% concentrations of each plant extract on *C. gloeosporioides* mycelia growth was determined with the food poison method. Five (5) ml of each extract concentration was delivered into a 9 cm diameter Petri dish after which 20 ml of melted Potato Dextrose Agar (PDA) was added to obtain plant extract amended PDA. The mixture was then swirled before the PDA solidified. A mycelia disc (5 mm) of *C. gloeosporioides* pure culture was placed at the centre of the plate and incubated at 28±2 ^{o}C for 7 days. Mycelia growth was determined by measuring the colony radius daily with a transparent rule along two perpendicular lines drawn at the bottom of the plate and the average recorded (Singh and Tripatti, 1999). Each treatment was replicated 5 times. PDA to which sterilized distilled water was added served as negative control. Two positive controls which consisted of PDA amended with the synthetic fungicides Manlax (mancozeb 64% + metalaxyl 8% WP) and Rainmancoz (mancozeb 80% WP) each prepared according to the manufacturer's recommendations were used. Percentage mycelia growth inhibition was determined using the formula of Hokkanen and Kotiluoto (1992).

$$\text{Mycelia growth inhibition} = \frac{C - T}{C} \times 100\%$$

Where; C = radial growth of fungus in control, T = radial growth of fungus in treatment.

7.6.2 Inhibitory effect of plant extracts on spore germination of *C. gloeosporioides*

C. gloeosporioides spore suspension was prepared with a modified method of Slawecki *et al*. (2002). Twenty (20) ml of 10, 25, 50, 75 and 100% concentrations of each plant extract were added separately to pure cultures of *C. gloeosporioides*, and the surface scraped gently with a sterile loop to dislodge spores from hyphae. Manlax and rainmancoz were the positive control and distilled water was the negative control. The resulting spore suspension was filtered through four folds of cotton cloth to remove any hyphal fragment present and left standing for 30 min. The suspension was adjusted approximately to 10^3 spores/ml with a haemocytometer. A drop of the suspension was placed on a glass slide and incubated at 28 ^{o}C ± 2 for 24 hours in a humid chamber. The control contained sterile distilled water. After incubation a drop of lactophenol in cotton blue was added to each suspension and observed microscopically for spore germination. One hundred (100) spores were randomly selected, counted and the number germinated noted. Each treatment was replicated five times. Percentage spore inhibition for each treatment was calculated using Gemeda *et al*. (2014) formula:

$$\text{Spore germination inhibition (\%)} = \frac{\text{GC} - \text{GT}}{\text{GC}} \times 100$$

Where: GC = number of spores germinated in control, GT = number of spores germination in treatment

7.7 Results

7.7.1 Effect of plant extracts on mycelia growth of *C. gloeosporioides*

7.7.1.1 Aqueous plant extracts

There was total inhibition of mycelia growth of *C. gloeosporioides* by manlax and rainmancoz (Table 7.1). Generally there were significant differences ($P \leq 0.05$) in mycelia growth among the various aqueous plant extracts treatments. (Table 7.1). The mycelia growth for the negative control treatment (26.80 ± 0.37 mm) was significantly higher ($P \leq 0.05$) than each of the aqueous plant extracts treatments (Table 7.1). Mycelia growth inhibition ranged from 76.12 to 11.19 % with the highest and lowest recorded for *Capsicum* sp. (Legon 18 variety) fruits at 100% and *K. senegalensis* seeds at 10% respectively (Table 7.1). There were no significant differences ($P \leq 0.05$) in mycelia growth for aqueous *Capsicum* sp. (Legon 18 variety) fruits extracts at 50 (7.60 ± 0.25 mm), 75 (6.80 ± 0.20 mm) and 100% (6.40 ± 0.25 mm) concentrations (Table 7.1).

7.7.1.2 Ethanol plant extracts

There were significant differences ($P \leq 0.05$) in mycelia growth among the various ethanol plant extracts treatments (Table 7.1). Manlax and rainmancoz treatments completely inhibited mycelia growth of *C. gloeosporioides* (Table 7.1). With the exception of ethanol extract of *K. senegalensis* seeds at 10% which recorded a mycelia growth of 25.40 ± 0.25 mm, the negative control treatment recorded a significantly higher (26.80 ± 0.37 mm) mycelia growth in comparison to the other ethanol plant extract treatments (Table 7.1). Among the various ethanol plant extract treatments, the least mycelia growth of 7.00 ± 0.32 mm (representing 73.88% mycelia growth inhibition) and the highest of 25.40 ± 0.25 mm (5.22% mycelia growth inhibition) were recorded for ethanol extracts of *Capsicum* sp. (Legon 18 variety) fruits at 100% and *K. senegalensis* seeds at 10% respectively (Table

7.1). It was also observed that for the aqueous and ethanol extracts, the efficacy of each plant extract on *C. gloeosporioides* mycelia growth increased with increasing concentration of the extract (Table 7.1, Plate 7.1 A, B, C, D and E).

Table 7.1: Effect of plant extracts on mycelial growth of *C. gloeosporioides*

Treatment	Mycelia growth (mm) ± S.E.		% growth inhibition	
	Aqueous	**Ethanol**	**Aqueous**	**Ethanol**
Manlax	0.00±0.00^{a}	0.00±0.00^{a}	100.00	100.00
Rainmancoz	0.00±0.00^{a}	0.00±0.00^{a}	100.00	100.00
AIS@10%	21.20±0.37^{rst}	20.60±0.40^{op}	20.90	23.13
AIS@25%	20.00±0.32^{opqrs}	16.60±0.40^{jk}	25.37	38.06
AIS@50%	18.00±0.32^{imno}	14.00±0.32^{gh}	32.84	47.76
AIS@75%	15.20±0.37^{hij}	12.80±0.37^{fgh}	43.28	52.24
AIS@100%	10.40±0.25^{cd}	7.60±0.25^{bc}	61.19	71.64
BAS@10%	19.00±0.00^{nopq}	17.60±0.25^{jklm}	29.10	34.33
BAS@25%	17.40±0.25^{klmn}	16.80±0.20^{jkl}	35.07	37.31
BAS@50%	15.60±0.25^{hijk}	14.60±0.25^{hi}	41.79	45.52
BAS@75%	14.00±0.32^{fgh}	14.00±0.00^{gh}	47.76	47.76
BAS@100%	13.00±0.55^{efg}	12.40±0.25^{efg}	51.49	53.73
CF@10%	11.80±0.21^{cde}	13.80±0.67^{gh}	55.97	48.52
CF@25%	9.80±0.20^{c}	12.20±0.45^{efg}	63.43	54.48
CF@50%	7.60±0.25^{b}	11.00±0.00^{def}	71.64	58.96
CF@75%	6.80±0.20^{b}	8.80±0.37^{bc}	74.63	67.16
CF@100%	6.40±0.25^{b}	7.00±0.32^{b}	76.12	73.88
IOL@10%	20.40±0.51^{pqrst}	18.60±0.51^{imn}	23.88	30.60
IOL@25%	18.40±4.00^{mnop}	17.40±0.25^{jkl}	31.34	35.07
IOL@50%	16.40±3.16^{ijklm}	12.80±0.20^{fgh}	38.81	52.24
IOL@75%	16.00±0.32^{hijkl}	10.8±0.58^{de}	40.30	59.70
IOL@100%	12.40±0.25^{def}	9.40±0.25^{cd}	53.73	64.93
JCS@10%	22.00±0.32^{stu}	20.80±0.49^{op}	17.91	22.39
JCS@25%	20.60±0.25^{qrst}	20.40±0.40^{nop}	23.13	23.88
JCS@50%	19.60±0.81^{opqr}	19.40±0.25^{mno}	26.87	27.61
JCS@75%	17.00±0.32^{jklmn}	18.20±0.58^{klm}	36.57	32.09
JCS@100%	14.80±0.49^{ghi}	16.20±0.20^{ij}	44.78	39.55
KSS@10%	23.80±0.37^{u}	25.40±0.25^{qr}	11.19	5.22
KSS@25%	22.40±0.51^{tu}	23.60±0.25^{q}	16.42	11.94
KSS@50%	21.00±0.55^{qrst}	21.60±0.68^{p}	21.64	19.40
KSS@75%	19.80±0.20^{opqr}	18.60±0.51^{lmn}	26.12	30.60
KSS@100%	18.40±0.25^{mnop}	16.60±0.40^{jk}	31.34	38.06
Negative control	26.80 ±0.37^{v}	26.80±0.37^{r}	0.00	0.00
F (pr)	**<0.001**	**<0.001**		

Means ± standard errors in the same column followed by different letter(s) are significantly different as determined by Tukey's test. Key: AIS = *A. indica* seeds extract, BAS= *B.*

aegyptiaca seeds extract, CF = *Capsicum* sp. (Legon 18 variety) fruits extract, IOL= *I. oliviformis* leaves extract, JCS= *J. curcas* seeds extract, KSS = *K. senegalensis* seeds extract

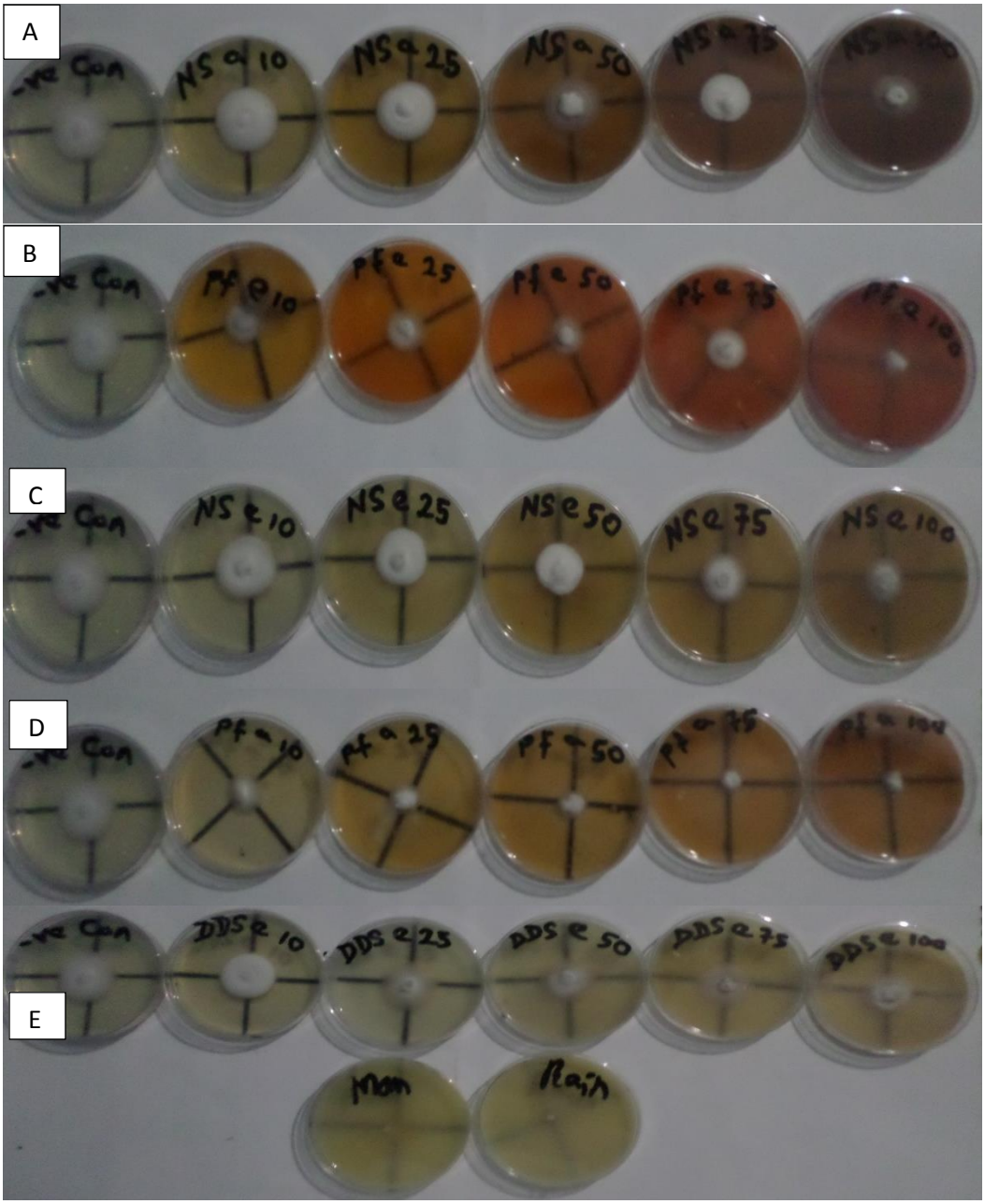

Plate 7.1: Effect of different concentration levels (10, 25, 50, 75 and 100 %) of extracts of aqueous *Capsicum* sp. (Legon 18 variety) fruits (A) aqueous *A. indica* seeds (B) ethanol *Capsicum* sp. (Legon 18 variety) fruits (C) ethanol *A. indica* seeds (D) ethanol *B. aegyptiaca* seeds (E) on *C. gloeosporioides* mycelia growth, -ve con = negative control, Man = manlax, Rain = rainmancoz

Key: – ve = negative control, pf a = aqueous extract of *Capsicum* sp. (Legon 18 variety) fruits, Ns a = aqueous extract of *A. indica* seeds. pf e = ethanol extract of *Capsicum* sp.

(Legon 18 variety) fruits, Ns e = ethanol extract of *A. indica* seeds, DDS e = ethanol extract of *B. aegyptiaca* seeds, Man = manlax, Rain = rainmancoz.

7.7.2 Effect of plant extracts on spore germination

7.7.2.1 Aqueous plant extracts

Total inhibition of *C. gloeosporioides* spore germination was observed for each of manlax and rainmancoz treatments (Table 7.2). Generally there were significant inhibition ($P \leq 0.05$) of spore germination among the various aqueous plant extract treatments, with *K. senegalensis* seeds at 100% recording the highest spore germination of 77.20 ± 1.36 (13.84% spore germination inhibition) and *Capsicum* sp. (Legon 18 variety) fruits at 100% recording the least of 7.40 ± 1.96 (91.74% spore germination inhibition) (Table 7.2). The negative control also recorded significantly higher ($P \leq 0.05$) mean germinated spores (89.60 ± 2.42) than what was recorded for each of the aqueous plant extract treatments (Table 7.2).

7.7.2.2 Ethanol plant extracts

Generally significant differences ($P \leq 0.05$) were also observed in spore germination among the ethanol plant extracts treatments (Table 7.2). These ranged from 86.60 ± 2.32 (3.35% spore germination inhibition) to 8.20 ± 1.53 (90.85% spore germination inhibition) for *K. senegalensis* seeds at 10% and *Capsicum* sp. (Legon 18 variety) fruits at 100% respectively (Table 7.2).

Table 7.2: Effect of plant extracts on *C. gloeosporioides* spores germination

Treatment	Mean germinated spores ± S.E.		% spore inhibition	
	Aqueous	Ethanol	Aqueous	Ethanol
Manlax	0.00±0.00^{a}	0.00±0.00^{a}	100.00	100.00
Rainmancoz	0.00±0.00^{a}	0.00±0.00^{a}	100.00	100.00
AIS@10%	64.60±1.57^{jklmn}	58.80±2.82^{lmno}	27.90	34.38
AIS@25%	51.00±1.87^{ghi}	44.80±2.46^{ijk}	43.08	50.00
AIS@50%	43.80±2.08^{fg}	39.80±2.42^{ghi}	51.12	55.58
AIS@75%	38.20±1.53^{def}	29.80±1.43^{defg}	57.37	66.74
AIS@100%	18.60±1.60^{c}	16.60±1.63^{bc}	79.24	81.47
BAS@10%	59.60±3.40^{ijkl}	56.60±1.36^{lmn}	33.48	36.83
BAS@25%	56.20±2.13^{hij}	51.40±2.52^{jkl}	37.28	42.63
BAS@50%	45.60±2.89^{fg}	42.20±3.62^{hij}	49.11	52.90
BAS@75%	41.80±1.59^{efg}	29.80±3.02^{defg}	53.35	66.74
BAS@100%	29.20±2.13^{d}	25.60±1.36^{cde}	67.41	71.14
CF@10%	31.80±2.03^{d}	37.80±2.42^{fghi}	64.51	57.81
CF@25%	19.20±1.28^{c}	32.40±2.04^{efgh}	78.57	63.84
CF@50%	17.80±0.97^{c}	24.00±1.84^{cde}	80.13	73.21
CF@75%	14.60±1.29^{bc}	18.40±2.04^{bcd}	83.71	79.46
CF@100%	7.40±1.96^{ab}	8.20±1.53^{ab}	91.74	90.85
IOL@10%	66.20±1.88^{klmn}	65.60±2.38^{mnop}	26.12	26.79
IOL@25%	56.40±1.91^{hij}	54.20±2.06^{klm}	37.05	39.51
IOL@50%	48.40±1.03^{gh}	38.20±2.92^{fghi}	45.98	57.37
IOL@75%	42.20±1.85^{efg}	31.80±2.13^{efgh}	52.90	64.51
IOL@100%	29.40±0.93^{d}	26.80±1.02^{cdef}	67.19	70.09
JCS@10%	70.60±1.57^{mno}	68.20±1.93^{op}	21.21	23.88
JCS@25%	67.40±1.44^{lmn}	67.60±0.87^{nop}	24.78	24.55
JCS@50%	64.60±1.50^{jklmn}	61.40±0.93^{lmno}	27.90	31.47
JCS@75%	51.40±2.48^{ghi}	55.80±3.02^{klm}	42.63	37.72
JCS@100%	33.20±1.20^{de}	38.00±0.71^{fghi}	62.95	57.59
KSS@10%	77.20±1.36^{o}	86.60±2.32^{q}	13.84	3.35
KSS@25%	73.20±0.92^{no}	74.40±1.66^{p}	18.30	16.96
KSS@50%	67.40±0.81^{lmn}	68.40±0.60^{op}	24.78	23.66
KSS@75%	63.20±0.66^{jklm}	55.40±2.32^{klm}	29.46	38.17
KSS@100%	56.60±2.94^{hijk}	52.60±2.84^{jkl}	36.83	41.29
Negative control	89.60±2.42^{p}	89.60±2.42^{q}	0.00	0.00
F (pr)	**<0.001**	**<0.001**		

Means ± standard errors in the same column followed by different letter(s) are significantly different as determined by Tukey's test.

Key: AIS= *A. indica* seeds extract, BAS= *B. aegyptiaca* seeds extract, CF = *Capsicum* sp. (Legon 18 variety) fruits extract, IOL= *I. oliviformis* leaves extract, JCS= *J. curcas* seeds extract, KSS = *K. senegalensis* seeds extract

The negative control recorded significantly higher spore germination (89.60 ± 2.32) than those of the other ethanol plant extract treatments except that of *K. senegalensis* seeds at 10% (86.60 ± 2.32) (Table 7.2). Manlax and rainmancoz recorded significantly lower (0.0 ± 00) *C. gloeosporioides* spore germination in comparison to each of the ethanol plant extract treatments (Table 7.2).

7.8 Relative efficacy of aqueous and ethanol plant extracts (100%) in inhibiting mycelia growth of *C. gloeosporioides*

The aqueous and ethanol extracts at 100% had varying effect on the mycelia growth of *C. gloeosporioides* (Table 7.3, Plate 7.2). Aqueous extracts of seeds of *A. indica, K. senegalensis* and leaves of *I. oliviformis* had a significantly higher ($P \leq 0.05$) inhibitory effect on mycelia growth of *C. gloeosporioides* than the ethanol extracts (Table 7.3). There were no significant differences ($P \leq 0.05$) between the aqueous and ethanol extracts of seeds of *B. aegyptiaca*, *J. curcas* and fruits of *Capsicum* sp. (Legon 18 variety) (Table 7.3).

Table 7.3: Effect of plant extract (100% concentration) on mycelia growth of *C. gloeosporioides*

Plant extract	Mycelia growth (mm) ± S.E.	
	Aqueous	Ethanol
A. indica seeds	10.40±0.25^{a}	7.60±0.25^{b}
B. aegyptiaca seeds	13.00±0.55^{a}	12.40±0.25^{a}
Capsicum sp. fruits	6.40±0.25^{a}	7.00±0.32^{a}
I. oliviformis leaves	12.40±0.25^{a}	9.40±0.25^{b}
J. curcas seeds	14.80±0.49^{a}	16.20±0.20^{a}
K. senegalensis seeds	18.40±0.25^{a}	16.60±0.40^{b}

Means with different letter within the same row are significantly different ($P \leq 0.05$) as determined by Tukey's test.

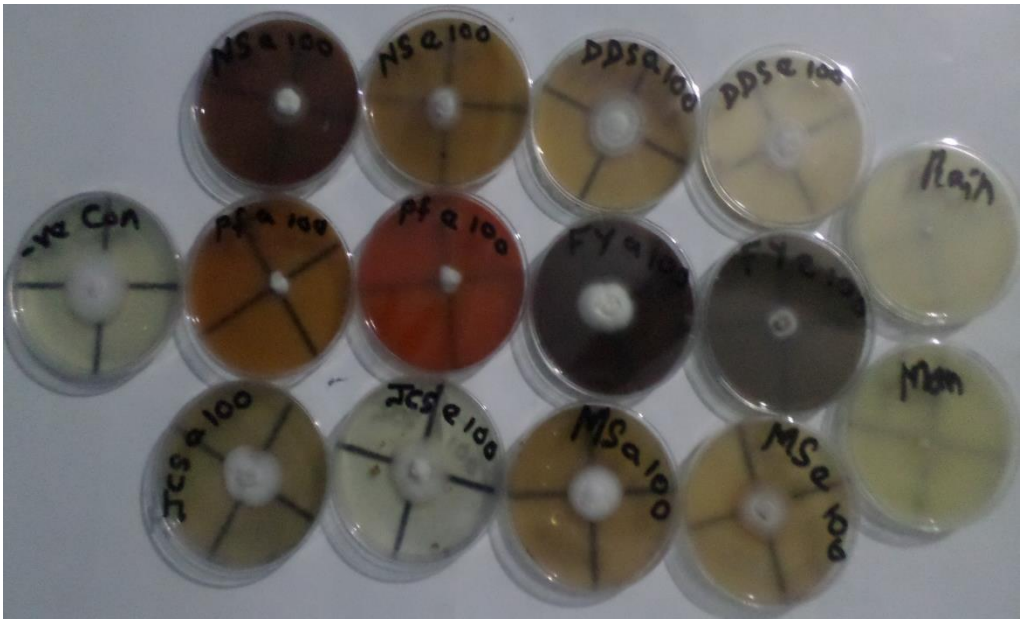

Plate 7.2: Effect of various plant extracts at 100 % concentration on mycelial growth of *C. gloeosporioides*

Key: Key: – ve = negative control, NS a = aqueous extract of *A. indica* seeds, NS e = ethanol extract of *A. indica* seeds. , DDS a = aqueous extract of *B. aegyptiaca* seeds, DDs e = ethanol extract of *B. aegyptiaca* seeds, pf a = aqueous extract of *Capsicum* sp. (Legon 18 variety) fruits, pf e = ethanol extract of *Capsicum* sp. (Legon 18 variety) fruits, Fy a = aqueous extract of *I. oliviformis* leaves, FY e = ethanol extract of *I. oliviformis* leaves, JCS a = aqueous extract of *J. curcas* seeds, JCS e = ethanol extract of *J. curcas* seeds, MS a = aqueous extract of *K. senegalensis* seeds, MS e = ethanol extract of *K. senegalensis* seeds, Rain = rainmancoz, Man = manlax

7.9 Relative efficacy of aqueous and ethanol plant extracts (100 %) in inhibiting spore germination of *C. gloeosporioides*

Aqueous extracts of seeds of *A. indica, B. aegyptiaca, K. senegalensis* and leaves of *I. oliviformis,* with the exception of *Capsicum* sp. (Legon 18 variety) fruits and *J. curcas* seeds each had a higher inhibitory effect on spore germination of *C. gloeosporioides* in comparison to their respective ethanol extracts (Table 7.4). There were however no significant differences ($P \leq 0.05$) between the aqueous and their corresponding ethanol extracts for seeds of *A. indica*, *B. aegyptiaca, J. curcas, K. senegalensis; Capsicum* sp.

(Legon 18 variety) fruits and *I. oliviformis* leaves on *C. gloeosporioides* spore germination (Table 7.4).

Table 7.4: Effect of plant extract (100% concentration) on spore germination of *C. gloeosporioides*

Plant extract	Mean germinated spores ± S.E.	
	Aqueous	Ethanol
A. indica seeds	18.60±1.60[a]	16.60±1.63[a]
B. aegyptiaca seeds	29.20±2.13[a]	25.60±1.36[a]
Capsicum sp. fruits	7.40±1.96[a]	8.20±1.53[a]
I. oliviformis leaves	29.40±0.93[a]	26.80±1.02[a]
J. curcas seeds	33.20±1.20[a]	38.00±0.71[a]
K. senegalensis seeds	56.60±2.94[a]	52.60±2.84[a]

Means with different letter within the same row are significantly different ($P \leq 0.05$) as determined by Tukey's test.

7.10 Discussion

All the plant extracts (seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, fruits of *Capsicum* sp. - Legon 18 variety and leaves of *I. oliviformis*) used in this study had fungitoxic effect on *C. gloeosporioides*. This could be due to the secondary metabolites detected in these extracts (Section 6.3.1, Table 6.1). This observation was supported by the report of Okogbenin *et al*. (2014) and Pusztahelyi *et al*. (2015) that secondary metabolites are responsible for the antifungal properties of plant extracts. The plant extracts also exhibited variations in their efficacy levels in inhibiting spore germination and mycelia growth of *C. gloeosporioides*. This may be an indication that, the type and quantity of secondary metabolites contained in each plant extract had varying action mechanisms on *C. gloeosporioides*. For example, phenolic compounds alter microbial cell permeability and interact with membrane proteins causing deformation in the structure and functionality of the proteins (Pusztahelyi *et al.*, 2015). Saponins are reported to have strong antimicrobial activity against a wide range of pathogenic fungi through formation of complex with sterols in fungal membranes to cause loss of membrane integrity (Keukens *et al*., 1995; Osbourn, 1996). Cynogenic glycoside breaks down to produce hydrogen cyanide, a lethal chemical that halts cellular respiration in aerobic organisms (Freeman and Beattie, 2008). Since the phytochemical constituents of the various plant extracts used in this study varied, it was not surprising that the level of their antifungal activity on *C. gloeosporioides* differed.

Comparatively, the aqueous *Capsicum* sp. (Legon 18 variety) fruits extract at various concentrations generally inhibited spore germination and mycelia growth of *C. gloeosporioides* to the highest extent. This is not surprising because with the exception of anthraquinones, this extract contained all other phytochemical constituents; thus making it more potent than the other extracts. It can therefore be deduced that, the more diverse phytochemicals a plant extract contains, the higher its potency as a natural fungicide; since

the various phytochemicals work in synergy to enhance antifungal activity. The variations in the quantity of phytochemical (total phenolics, flavonoids, tannins and alkaloids) observed in the different plant extracts might have also contributed to the differences in their antifungal activity. This agreed with the findings of Fokunang *et al*. (2000) who reported that the antifungal activity of any plant extract is dependent on the nature and amount of active phytochemicals it contains. Antifungal activity of each plant extract against *C. gloeosporioides* increased with increasing concentration. This may be an indication that, the quantity of secondary metabolites in a plant extract is directly proportional to the extract concentration. Therefore the antifungal activity of any extract may be enhanced by increasing it concentration.

Generally, the plant extracts suppressed spore germination compared to mycelia growth; which indicated that the spores were more sensitive to the extracts than the mycelia. Similar trends were observed by Naruzawa and Papa (2011) who attributed it to the fact that the spores were directly immersed in the extract suspension, while the mycelium grew on the medium containing the extract, thus having a more restricted contact with the plant extract.

The significant differences ($P \leq 0.05$) observed between the effects of aqueous extracts of seeds of *A. indica, K. senegalensis* and *I. oliviformis* leaves and their corresponding ethanol extracts on *C. gloeosporioides* mycelial growth, could be attributed to the extraction method. This confirmed Tiwari *et al*. (2011) report that the type of solvent used for botanical extraction has an influence on the antimicrobial activity of the extract. The plant extracts used in this study inhibited spore germination and mycelia growth of *C. gloeosporioides*. This showed that potentially these extracts have antifungal properties that can be used to produce formulations for the control of anthracnose disease of yam.

CHAPTER EIGHT

8.0 FIELD STUDIES OF THE ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) THE CAUSATIVE AGENT OF YAM ANTHRACNOSE DISEASE

8.1 Introduction

The hazards associated with synthetic fungicides have heightened the advocacy to minimise it usage in the agriculture sector. Several researches have been carried out to promote the use of natural based substances such as plant extracts as fungicides; since they are easily biodegradable, locally available and may be obtained at no cost (Quarles, 2009; Kuberan *et al*., 2012). There is no doubt that in order to promote sustainable agriculture, the usage of natural based products such as plant extracts as fungicides is the way to go.

However, the use of plant extract in plant disease management is usually restricted to *in vitro* studies or green house pot trials; limiting its usage on crop fields. In order to explore the full antifungal potential of plant extracts, there is the need to buttress *in vitro* studies with experimental field studies. This would help ascertain the ability of the plant extract in controlling the disease under field conditions and also establish its effect on the host plant.

The usage of plant extracts in plant disease management unlike synthetic fungicides which are harmful to the environment, have the advantage of being eco-friendly, enhancing plant growth and productivity (Kuberan *et al*., 2012; El-Hamied and El-Amary, 2015). For instance, El-Hamied and El-Amary (2015) reported about the enhanced vegetative growth and productivity of pear plants when sprayed with extracts of *Allium sativum*, *Glycyrrhiza glabra* and *Moringa oleifera*. Also extracts of tea seeds have been reported to contain

substances that promote the vegetative growth and yield of various crops (Andresen and Cedergreen, 2010).

At higher concentration certain plant extracts have however, been reported to exhibit allelopathic effect on plant growth; which reduced productivity (Andresen and Cedergreen, 2010; Iqbal *et al*., 2015; Sarbeng *et al*., 2016; Shah *et al.,* 2017). Interestingly, the phytochemicals contained in plant extracts responsible for their antifungal properties have also been documented as the main allelochemical constituents responsible for the extract phytotoxic activity. For instance, Aliyu and Mustapha (2014) documented tannins, flavonoids, glycosides, steroids, saponins and cardiac glycosides as the allelochemical constituents of milkweed plant extracts. The allelopathic effects of *A. indica* (El–Hamid *et al*., 2017), *Capsicum annuum* (Iqbal *et al*., 2015) and *J. curcas* extracts (Rejila and Vijayakumar, 2011) on certain crops have been established. It is therefore necessary to perform field trials to ascertain the efficacy of the various plant extracts (seeds of *A. indica*, *B. aegyptiaca*, *J. curcas* and *K. senegalensis, I. oliviformis* leaves and *Capsicum* sp. - Legon 18 variety fruits) adapted in this study on yam anthracnose disease control and also determine the effect of their concentration levels on the vegetative growth of the *D. rotundata* (laribako and pona) crops. The study was therefore conducted to:

- assess the potential of extracts of seeds of *A. indica, B. aegyptiaca*, *J. curcas*, *K. senegalensis*, leaves of *I. oliviformis* and fruits of *Capsicum* sp. (Legon 18 variety) for the field management of yam (pona and laribako) anthracnose disease.
- evaluate the effect of extracts of seeds of *A. indica, B. aegyptiaca*, *J. curcas*, *K. senegalensis*, *I. oliviformis* leaves and fruits of *Capsicum* sp. (Legon 18 variety) on yield of pona and laribako yam crops.

8.2 Materials and methods

8.2.1 Preparation of aqueous plant extracts for *in vivo* (field) experiment

Plant extracts were prepared according to the method of Kuberan *et al*. (2012) with some modifications. Hundred (100) g of powdered plant material (prepared as described in section 3.5) was soaked in 1 L tap water (w/v), stirred vigorously with a glass rod and allowed to stand for 24 h at room temperature. After this period, the supernatant was then filtered through a doubled layered cotton cloth. The extract obtained served as the stock solution (100%). To the stock solution, 1 g key soap was added to enhance the adhesiveness of the active ingredient and to reduce its volatility in the field. For each extract, a concentration of 75% was also prepared from the stock solution by diluting it with appropriate quantity of tap water. The stock solution and the 75% level extract concentration were used for the field studies.

8.2.2 Experimental field design

This study was conducted under rain fed conditions in the 2016 and 2017 yam cropping seasons. The experimental design used for the study was Randomised Complete Block Design (RCBD). The factors were the yam cultivars Pona and Laribako of *D. rotundata* and aqueous plant extracts prepared from seeds of *A. indica*, *B. aegyptiaca*, *J. curcas* and *K. senegalensis*, I. *oliviformis* leaves and *Capsicum* sp. (Legon 18 variety) fruits. There were 30 treatments, made up of 75 and 100% (100g/1L of water) extract concentrations each of seeds of *A. indica*, *B. aegyptiaca*, *J. curcas* and *K. senegalensis*, *I. oliviformis* leaves and *Capsicum* sp. (Legon 18 variety) fruits. Tap water served as the negative control. Manlax (mancozeb 64% + metalaxyl 8% WP) and Rainmancoz (mancozeb 80% WP) prepared at the manufacturer's recommendation rate of 2.5 g/1L and 2.0 g/1L of water respectively

were the positive controls. There were 4 replications per treatment. Each replication consisted of 15 plots and each measured 3 m x 2 m. The distances between plots in a replication and between replications were 1 m and 2 m respectively. The plots were ploughed and yam mounds prepared using a hand hoe.

Table 8.1: Detailed treatment descriptions

Treatment code	Treatment descriptions
AIS@75%L	*A. indica* seeds extract-75% + Labreko
AIS@75%P	*A. indica* seeds extract-75% + Pona
AIS@100%L	*A. indica* seeds extract -100% + Labreko
AIS@100%P	*A. indica* seeds extract -100% + Pona
BAS@75%L	*B. aegyptiaca* seeds extract -75% + Labreko
BAS@75%P	*B. aegyptiaca* seeds extract -75% + Pona
BAS@100%L	*B. aegyptiaca* seeds extract -100% + Labreko
BAS@100%P	*B. aegyptiaca* seeds extract -100% + Pona
IOL@75%L	*I. oliviformis* leaves extract -75% + Labreko
IOL@75%P	*I. oliviformis* leaves extract -75% + Pona
IOL@100%L	*I. oliviformis* leaves extract -100% + Labreko
IOL@100%P	*I. oliviformis* leaves extract -100% + Pona
JCS@75%L	*J. curcas* seeds extract -75% + Labreko
JCS@75%P	*J. curcas* seeds extract -75% + Pona
JCS@100%L	*J. curcas* seeds extract -100% + Labreko
JCS@100%P	*J. curcas* seeds extract -100% + Pona
KSS@75%L	*K. senegalensis* seeds extract -75% + Labreko
KSS@75%P	*K. senegalensis* seeds extract -75% + Pona
KSS@100%L	*K. senegalensis* seeds extract -100% + Labreko
KSS@100%P	*K. senegalensis* seeds extract -100% + Pona
CF@75%L	*Capsicum* sp. (Legon 18) fruits extract -75% + Labreko
CF@75%P	*Capsicum* sp. (Legon 18) fruits extract -75% + Pona
CF@100%L	*Capsicum* sp. (Legon 18) fruits extract -100% + Labreko
CF@100%P	*Capsicum* sp. (Legon 18) fruits extract -100% + Pona
ManlaxL	Manlax + Labreko
ManlaxP	Manlax + Pona
RainmancozL	Rainmancoz + Labreko
RainmancozP	Rainmancoz + Pona
Neg conL	Negative control + Labreko
Neg conP	Negative control + Pona

Six mounds (3 x 2) were raised per plot. The inter and intra row distance were 1 m each. Six yam setts comprising of 3 each of puna and labreko cultivars of *D. roundata* were randomly

planted one per yam mound in each of the plots at a depth of 10 cm. The application of treatments started from 5 Weeks after Planting (WAP) to 15 WAP using a 15 L knapsack sprayer. This was done at 2 weeks interval. The spraying was targeted at the leaves and vine of the yam plant. The spray volume was 150 L/ha. The treatments (T) were combined as follows (Table: 8.1)

8.2.3 Effect of plant extracts on the incidence and severity of anthracnose disease

The data for determining the anthracnose disease incidence and severity of pona and laribako crops were collected every two weeks from 5 and to 15 WAP.

8.2.3.1 Determination of disease incidence

This was determined by inspecting individual yam plants on the experimental field for symptoms of anthracnose infection. For a particular replicate, plants were examined one plot at a time starting from plot 1 in each replicate and ending up with plot 15 (last plot). This was done until all 4 replicates and their individual plots were examined. Individual yam plants (pona and labreko) for each treatment were scored for presence or absence of anthracnose infection according to the method employed by Abang *et al*., (2006) as described in section 3.2.1. For each treatment, data were collected on the total number of plants examined and the number of anthracnose infected plants. The disease incidence for each treatment was calculated with the formula of Chaube and Pundhir (2009) stated at section 3.2.1.

8.2.3.2 Determination of disease severity

Individual yam plants on the experimental field were examined for the presence and extent of anthracnose infection. For each replicate, plants were examined and data taken and

recorded according to treatment plots. Disease severity was determined using Asfaw (2016) score-scale of 1-5 described in section 3.2.2, Table 3.1.

8.2.4 Effect of plant extracts on growth and yield

8.2.4.1 Measurement of vine length

The height of each yam vine was obtained with a tape measure. The tape measure was stretched along the vine starting from the soil mark level to the apex and the height recorded. Measurements were taken for each plot and the average recorded. This was done after harvesting the yam crops.

8.2.4.2 Determination of effect of plant extracts on growth

After harvesting and obtaining the yam tuber, the yam plant was divided into shoot and root components by cutting through a transverse section at the soil level mark of the plant with a scalpel. The roots were washed with tap water to remove the soil. Three replicates were used for each study.

Each plant shoot and root systems obtained above were kept in separate labeled envelops and dried in an oven at 80 ^{o}C for 24 h. They were removed from the oven and allowed to cool in a desiccator and their mass determined using an electronic balance (Model number CP224S, Sartorious, Germanay). Each sample was reheated and reweighed until a constant mass was obtained.

8.2.4.3 Determination of yam tuber yield after harvest

The weight of harvested yam tubers from each treatment were determined with an electronic balance (Model number ACS-15-JE21, Zhongshan Camry Manufacturer and Trading Co., Ltd., China) and the average recorded. This was then converted to tuber yield in t/ha using Kyle's converter (http://www.kylesconverter.com).

8.3 Results

8.3.1 Effect of plant extracts on anthracnose disease incidence of yam plants

8.3.1.1 Laribako yam plants

For the 2016 and 2017 cropping seasons, no anthracnose disease incidence was recorded for the various laribako treatments at 5 and 7 WAP (Table 8.2). At 9 WAP of the 2016 cropping season, disease incidences were only recoreded for laribako plants treated with *K. senegalensis* seeds extract at 75% concentration (8.33%) and the negative control (24.98%) (Table 8.2). The disease incidence recorded for the negative control (24.98%) at 9 WAP of the 2016 cropping season was significantly higher ($P \leq 0.05$) than that of the *K. senegalensis* seeds extract at 75% concentration treatment (8.33%). Interestingly at 9 WAP of the 2017 cropping season, none of the treatments recorded anthracnose incidence (Table 8.2). At 11 WAP of the 2016 cropping season, the negative control recorded a significantly higher ($P \leq 0.05$) disease incidence (58.35%) than manlax, rainmancoz and 100% extract concentration of *Capsicum* sp. (Legon 18 variety) fruits treatments. Also at 11 WAP of the 2017 cropping season the negative control recorded a significantly higher ($P \leq 0.05$) disease incidence (33.33%) than the other treatments with the exception of 75% extract concentrations of seeds of *B. aegyptiaca, K. senegalensis* and leaves of *I. oliviformis*. For the 2016 cropping season, no significant differences ($P \leq 0.05$) in disease incidence were observed among the various treatments at 13 WAP. At 11 WAP during the 2017 cropping season, the negative control recorded a significantly higher ($P \leq 0.05$) anthracnose disease incidence (66.70%) than the 100% extract concentrations of seeds of *A. indica, B. aegyptiaca, J. curcas, K. senegalensis,* fruits of *Capsicum* sp. (Legon 18 variety) and leaves of *I. oliviformis*. At 15 WAP of 2016 there were no significant differences ($P \leq 0.05$) among the treatments with the lowest (66.65%) and highest (91.68%) disease incidences recorded

Table 8.2: Effects of plant extracts on percentage anthracnose disease incidence of laribako yam plants for 2016 and 2017 cropping seasons for the experimental farm

Treatment	WAP											
	5		7		9		11		13		15	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Manlax	0.00	0.00	0.00	0.00	0.00[a]	0.00	0.00[a]	0.00[a]	33.30[a]	33.33[ab]	66.65[a]	33.30[a]
Rainmancoz	0.00	0.00	0.00	0.00	0.00[a]	0.00	0.00[a]	0.00[a]	33.30[a]	33.30[ab]	66.70[a]	41.65[a]
AIS@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	24.97[ab]	0.00[a]	58.35[a]	33.30[ab]	66.70[a]	50.03[a]
AIS@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	16.65[ab]	0.00[a]	41.65[a]	8.33[a]	66.68[a]	50.00[a]
BAS@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	33.30[ab]	8.33[ab]	50.00[a]	33.33[ab]	75.03[a]	58.35[a]
BAS@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	25.00[ab]	0.00[a]	50.00[a]	16.65[a]	75.00[a]	50.03[a]
CF@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	16.68[ab]	0.00[a]	50.00[a]	8.33[a]	75.03[a]	41.68[a]
CF@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	0.00[a]	0.00[a]	41.65[a]	8.33[a]	66.68[a]	33.30[a]
IOL@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	33.30[ab]	8.33[ab]	58.35[a]	33.33[ab]	75.03[a]	58.35[a]
IOL@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	24.97[ab]	0.00[a]	50.03[a]	16.65[a]	75.03[a]	50.00[a]
JCS@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	41.67[ab]	0.00[a]	58.35[a]	33.30[ab]	75.03[a]	58.35[a]
JCS@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	16.65[ab]	0.00[a]	50.03[a]	16.65[a]	75.03[a]	50.03[a]
KSS@75%	0.00	0.00	0.00	0.00	8.33[a]	0.00	41.65[ab]	16.65[ab]	58.35[a]	25.00[ab]	75.03[a]	58.33[a]
KSS@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	24.97[ab]	0.00[a]	50.00[a]	16.65[a]	75.00[a]	58.35[a]
Negative control	0.00	0.00	0.00	0.00	24.98[b]	0.00	58.35[b]	33.33[b]	75.03[a]	66.70[b]	91.68[a]	75.03[a]
P - value	**NAS**	**NAS**	**NAS**	**NAS**	**<0.001**	**NAS**	**0.005**	**0.002**	**0.596**	**<0.001**	**0.993**	**0.801**

Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test; NAS, not analysed statistically. Key: AIS = *A. indica* seeds, BAS = *B. aegyptiaca* seeds, *Capsicum* sp. (Legon 18 variety) fruits, IOL = *I. oliviformis* leaves, JCS = *J. curcas* seeds and KSS = *K. senegalensis* seeds

for manlax and negative control respectively. There were also no significant differences ($P \leq 0.05$) among the treatments at 15 WAP of 2017 cropping season with the negative control recording highest (75.03%) and manlax the lowest (33.30%) disease incidence for the laribako crops (Table 8.2). Generally, for the laribako treated crops there was a higher anthracnose disease incidence in the 2016 cropping season in comparison to that of 2017.

8.3.1.2 Pona yam crops

For the 2016 and 2017 cropping seasons, 5 and 7 WAP recorded no anthracnose disease incidence for the pona crops (Table 8.3). At 9 WAP in the 2016 cropping season only the negative control treatment recorded a significantly higher ($P \leq 0.05$) disease incidence (16.65%). No anthracnose disease incidence was recorded at 9 WAP in the 2017 cropping season for the pona treated crops. At 11 WAP in 2016, all treatments recorded disease incidence without any significant differences ($P \leq 0.05$) among them. In the 2017 cropping season at 11 WAP, disease incidence was recorded for *K. senegalensis* seeds extract concentrations at 75% (16.65%), 100 % (8.33%) and the negative control (25.0%) which were all not significantly different ($P \leq 0.05$) from the other treatments. At 13 WAP in the 2016, no significant differences ($P \leq 0.05$) in disease incidence were observed among the various treatments which ranged from 41.65 to 66.68%. The disease incidence (8.33%) recorded for each of *Capsicum* sp. (Legon 18 variety) fruits extracts concentration at 75% and 100% were significantly lower ($P \leq 0.05$) than that of the negative control (58.35%) at 13 WAP of the 2017 cropping season. At 15 WAP of the 2016 cropping season, the lowest disease incidence (66.68%) was recorded for each of manlax, 100% extract concentrations of *A. indica* seeds and *Capsicum* sp. (Legon 18 variety) fruits and the highest (83.33%) recorded for the negative control treatment (Table 8.3). There were no significant

Table 8.3: Effects of plant extracts on percentage anthracnose disease incidence of pona yam plants for 2016 and 2017 cropping seasons for the experimental farm

Treatment	WAP											
	5		7		9		11		13		15	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Manlax	0.00	0.00	0.00	0.00	0.00[a]	0.00	16.65[a]	0.00[a]	41.65[a]	16.65[ab]	66.68[a]	41.68[a]
Rainmancoz	0.00	0.00	0.00	0.00	0.00[a]	0.00	24.98[a]	0.00[a]	50.00[a]	33.30[ab]	66.70[a]	41.65[a]
AIS@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	24.98[a]	0.00[a]	50.00[a]	24.98[ab]	66.70[a]	50.00[a]
AIS@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	16.65[a]	0.00[a]	50.00[a]	16.65[ab]	66.68[a]	41.68[a]
BAS@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	24.98[a]	0.00[a]	50.00[a]	33.30[ab]	75.03[a]	50.03[a]
BAS@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	24.98[a]	0.00[a]	50,00[a]	33.30[ab]	75.03[a]	50.03[a]
CF@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	16.65[a]	0.00[a]	50.03[a]	8.33[a]	75.03[a]	50.00[a]
CF@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	16.68[a]	0.00[a]	50.00[a]	8.33[a]	66.68[a]	41.65[a]
IOL@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	24.98[a]	0.00[a]	58.35[a]	41.68[ab]	75.03[a]	58.35[a]
IOL@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	16.65[a]	0.00[a]	50.00[a]	16.65[ab]	75.03[a]	41.68[a]
JCS@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	41.65[a]	0.00[a]	58.35[a]	33.30[ab]	75.03[a]	58.35[a]
JCS@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	33.33[a]	0.00[a]	50.00[a]	16.65[ab]	75.03[a]	50.00[a]
KSS@75%	0.00	0.00	0.00	0.00	0.00[a]	0.00	33.33[a]	16.65[a]	58.35[a]	33.30[ab]	75.03[a]	58.35[a]
KSS@100%	0.00	0.00	0.00	0.00	0.00[a]	0.00	33.30[a]	8.33[a]	58.35[a]	24.98[ab]	75.03[a]	50.03[a]
Negative control	0.00	0.00	0.00	0.00	16.65[b]	0.00	50.00[a]	25.00[a]	66.68[a]	58.35[b]	83.33[a]	66.70[a]
P - value	**NAS**	**NAS**	**NAS**	**NAS**	**0.003**	**NAS**	**0.475**	**0.033**	**0.993**	**0.038**	**0.999**	**0.967**

Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test; NAS, not analysed statistically. Key: AIS = *A. indica* seeds, BAS = *B. aegyptiaca* seeds, *Capsicum* sp. (Legon 18 variety) fruits, IOL = *I. oliviformis* leaves, JCS = *J. curcas* seeds and KSS = *K. senegalensis* seeds

differences ($P \leq 0.05$) in the disease incidence among the various treatments at 15 WAP of the 2016 cropping season (Table 8.3). Generally the 2016 cropping season recorded higher anthracnose disease incidence for the various pona treatments than that of the 2017 (Table 8.3).

8.3.2 Effect of plant extracts on anthracnose disease severity of yam plants

8.3.2.1 Laribako

No disease severity were recorded for 5 and 7 WAP of the 2016 and also 5,7 and 9 WAP of the 2017 cropping seasons (Table 8.4). At 9 WAP of 2016 the negative control recorded a significantly higher ($P \leq 0.05$) disease severity than the other treatments. In 2016 at 11WAP, the negative control recorded a significantly higher ($P \leq 0.05$) disease severity (2.25) than those of manlax, rainmancoz and *Capsicum* sp. (Legon 18 variety) fruits extract concentration at 100%. At 11 WAP of the 2017 cropping season, with the exception of 75% extract concentrations of seeds of *B. aegyptiaca, K. senegalensis* and leaves of *I. oliviformis*, the negative control recorded a significantly higher ($P \leq 0.05$) disease severity than the other treatments (Table 8.4). In the 2016 cropping season, disease severity was recorded for all the treatments at 13 WAP with no significant differences ($P \leq 0.05$) among them (Table 8.4). However at 13 WAP of the 2017 cropping season, the negative control treatment recorded a significantly higher ($P \leq 0.05$) disease severity (2.13) in comparison to the other treatments. There were no significant differences ($P \leq 0.05$) in disease severity index recorded for each of the 2016 and 2017 cropping seasons at 15 WAP (Table 8.4). Generally the 2016 cropping season recorded higher laribako anthracnose disease severity in comparison to 2017 for the various WAP (Table 8.4).

Table 8.4: Effects of plant extracts on anthracnose disease severity index of laribako yam plants for 2016 and 2017 cropping seasons for the experimental farm

Treatment	WAP											
	5		7		9		11		13		15	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Manlax	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.00^{a}	1.00^{a}	1.25^{a}	1.17^{a}	1.25^{a}	1.33^{a}
Rainmancoz	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.00^{a}	1.00^{a}	1.25^{a}	1.33^{a}	1.50^{a}	1.42^{a}
AIS@75%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.50^{ab}	1.00^{a}	1.50^{a}	1.33^{a}	1.50^{a}	1.50^{a}
AIS@100%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.25^{ab}	1.00^{a}	1.50^{a}	1.08^{a}	1.50^{a}	1.50^{a}
BAS@75%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.75^{ab}	1.25^{ab}	1.75^{a}	1.33^{a}	1.75^{a}	1.58^{a}
BAS@100%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.50^{ab}	1.00^{a}	1.50^{a}	1.17^{a}	1.50^{a}	1.50^{a}
CF@75%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.25^{ab}	1.00^{a}	1.50^{a}	1.33^{a}	1.50^{a}	1.42^{a}
CF@100%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.00^{a}	1.00^{a}	1.25^{a}	1.33^{a}	1.25^{a}	1.33^{a}
IOL@75%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.50^{ab}	1.25^{ab}	1.50^{a}	1.33^{a}	1.50^{a}	1.50^{a}
IOL@100%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.50^{ab}	1.00^{a}	1.50^{a}	1.17^{a}	1.50^{a}	1.50^{a}
JCS@75%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.50^{ab}	1.00^{a}	1.50^{a}	1.33^{a}	1.50^{a}	1.59^{a}
JCS@100%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.50^{ab}	1.00^{a}	1.50^{a}	1.17^{a}	1.50^{a}	1.50^{a}
KSS@75%	1.00	1.00	1.00	1.00	1.25^{a}	1.00	1.70^{ab}	1.50^{ab}	1.75^{a}	1.25^{a}	2.25^{a}	1.67^{a}
KSS@100%	1.00	1.00	1.00	1.00	1.00^{a}	1.00	1.70^{ab}	1.00^{a}	1.75^{a}	1.17^{a}	1.75^{a}	1.50^{a}
Negative control	1.00	1.00	1.00	1.00	1.75^{b}	1.00	2.25^{b}	1.75^{b}	2.50^{a}	2.13^{b}	2.75^{a}	2.00^{a}
P - value	**NAS**	**NAS**	**NAS**	**NAS**	**<0.001**	**NAS**	**0.044**	**0.003**	**0.278**	**<0.001**	**0.133**	**0.349**

Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test; NAS, not analysed statistically. Key: AIS = *A. indica* seeds, BAS = *B. aegyptiaca* seeds, *Capsicum* sp. (Legon 18 variety) fruits, IOL = *I. oliviformis* leaves, JCS = *J. curcas* seeds and KSS = *K. senegalensis* seeds

8.3.2.2 Pona

Anthracnose disease severity were not recorded for pona treated yam crops at 5 and 7 WAP of the 2016 cropping season (Table 8.5). Also at 5, 7 and 9 WAP in the 2017 cropping season anthracnose disease severity for the various treatments of the pona yam crop (Table 8.5). At 9 WAP in the 2016 cropping season, the negative control recorded a significantly higher ($P \leq 0.05$) disease severity (1.5) than the other treatments (Table 8.5). For each of the 2016 and 2017 cropping seasons at 11 WAP, there were no significant differences ($P \leq 0.05$) in the disease severity among the various treatments (Table 8.5). At 13 WAP of the 2016 cropping season, no significant differences ($P \leq 0.05$) in disease severity were observed among the various treatments. However at 13 WAP of the 2017 cropping season, the negative control recorded a significantly higher ($P \leq 0.05$) disease severity (2.25) than the other treatments. In 2016 at 15 WAP, the negative control recorded a significantly higher ($P \leq 0.05$) disease severity (2.75) than manlax (1.75). For the 2017 cropping season at 15 WAP, the negative control recorded a significantly higher ($P \leq 0.05$) disease severity (2.34) than the other treatments except that of 75% *K. senegalensis* seeds extract concentration. For the pona crop treatments, 2016 generally recorded a higher anthracnose disease severity for the various WAP (9 to 15) than that of 2017 (Table 8.5).

Table 8.5: Effects of plant extracts on anthracnose disease severity index of pona yam plants for 2016 and 2017 cropping seasons for the experimental farm

Treatment	WAP											
	5		7		9		11		13		15	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Manlax	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.50[a]	1.00[a]	1.50[a]	1.17[a]	1.75[a]	1.42[a]
Rainmancoz	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.50[a]	1.00[a]	1.75[a]	1.33[a]	2.00[ab]	1.42[a]
AIS@75%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.50[a]	1.00[a]	2.00[a]	1.25[a]	2.25[ab]	1.42[a]
AIS@100%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.50[a]	1.00[a]	1.75[a]	1.17[a]	2.00[ab]	1.42[a]
BAS@75%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.75[a]	1.00[a]	2.00[a]	1.33[a]	2.25[ab]	1.50[a]
BAS@100%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.75[a]	1.00[a]	1.75[a]	1.42[a]	2.00[ab]	1.42[a]
CF@75%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.20[a]	1.00[a]	1.75[a]	1.33[a]	2.00[ab]	1.50[a]
CF@100%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.20[a]	1.00[a]	1.75[a]	1.08[a]	2.00[ab]	1.42[a]
IOL@75%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.75[a]	1.00[a]	2.00[a]	1.42[a]	2.25[ab]	1.42[a]
IOL@100%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.50[a]	1.00[a]	1.75[a]	1.17[a]	2.00[ab]	1.42[a]
JCS@75%	1.00	1.00	1.00	1.00	1.00[a]	1.00	2.00[a]	1.00[a]	2.00[a]	1.33[a]	2.25[ab]	1.59[a]
JCS@100%	1.00	1.00	1.00	1.00	1.00[a]	1.00	1.75[a]	1.00[a]	1.75[a]	1.17[a]	2.00[ab]	1.42[a]
KSS@75%	1.00	1.00	1.00	1.00	1.00[a]	1.00	2.00[a]	1.50[a]	2.25[a]	1.42[a]	2.25[ab]	1.92[ab]
KSS@100%	1.00	1.00	1.00	1.00	1.00[a]	1.00	2.00[a]	1.25[a]	2.00[a]	1.25[a]	2.25[ab]	1.50[a]
Negative control	1.00	1.00	1.00	1.00	1.50[b]	1.00	2.00[a]	1.50[a]	2.25[a]	2.25[b]	2.75[b]	2.34[b]
P - value	**NAS**	**NAS**	**NAS**	**NAS**	**0.003**	**NAS**	**0.247**	**0.027**	**0.461**	**<0.001**	**0.125**	**0.002**

Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test; NAS, not analysed statistically. Key: AIS = *A. indica* seeds, BAS = *B. aegyptiaca* seeds, *Capsicum* sp. (Legon 18 variety) fruits, IOL = *I. oliviformis* leaves, JCS = *J. curcas* seeds and KSS = *K. senegalensis* seeds

8.3.3 Growth and yield parameters

8.3.3.1 Vine length

8.3.3.1.1 Laribako

The vine lengths of the laribako crops in the 2016 cropping season ranged from 109.2 ± 7.771 to 137.8 ± 7.653 cm, with no significant differences ($P \leq 0.05$) among the various treatments (Table 8.5). The lowest laribako vine length (109.2 ± 7.771 cm) was recorded for the negative control and the highest (137.8 ± 7.653 cm) for *B. aegyptiaca* seed extract concentration at 100% (Table 8.5).

In the 2017 cropping season, the lowest laribako vine length (113.5 ± 12.926 cm) and the highest (140.8 ± 17.684 cm) were recorded for the negative control and *A. indica* seed extract at 100% concentration respectively (Table 8.5). There were no significant differences ($P \leq 0.05$) in the vine lengths recorded for the various treatments in the 2017 cropping season (Table 8.5).

8.3.3.1.2 Pona

The vine lengths of the pona crops ranged from 102.5 ± 5.331 (negative control) to 131.0 ± 8.406 cm (100% extract concentration of *Capsicum* sp. fruits) and 106.8 ± 7.443 (negative control) to 136.8 ± 15.802 cm (100% extract concentration of *Capsicum* sp. fruits) for the 2016 and 2017 cropping seasons respectively (Table 8.7). There were no significant differences ($P \leq 0.05$) among each of the treatments (Table 8.7).

8.3.3.2 Shoot dry weight/crop

8.3.3.2.1 Laribako

The lowest and highest laribako plant shoot dry weights were 0.081 ± 0.008 kg for the negative control and 0.322 ± 0.057 kg for *Capsicum* sp. (Legon 18 variety) fruits extract at 100% concentration treatments in the 2016 cropping season (Table 8.6). There were no significant differences ($P \leq 0.05$) in shoot dry weight among the various treatments in the 2016 cropping season (Table 8.6).

There were significant differences ($P \leq 0.05$) in shoot dry weight among the various treatments during the 2017 cropping season (Table 8.6). The 2017 laribako shoot dry weights ranged from 0.133 ± 0.012 kg for the negative control to 0.352 ± 0.056 kg for the *Capsicum* sp. (Legon 18 variety) fruits extract at 100% concentration (Table 8.6). In the 2017 cropping season, the recorded laribako shoot dry weight for the negative control (0.133 ± 0.012 kg) was significantly lower ($P \leq 0.05$) than those of manlax (0.342 ± 0.025 kg), *Capsicum* sp. (Legon 18 variety) fruits extracts at 75 (0.331 ± 0.044 kg) and 100 % (0.352 ± 0.056 kg).

8.3.3.2.2 Pona

In the 2016 cropping season, 100% extract concentrations of seeds of *B. aegyptiaca* (0.338 ± 0.061 kg) and fruits of *Capsicum* sp. (Legon 18 variety) (0.348 ± 0.151 kg) had significantly higher ($P \leq 0.05$) shoot dry weight than the negative control treatment (0.111 ± 0.005 kg) (Table 8.8). There was also a significant difference ($P \leq 0.05$) between the shoot dry weight of plants treated with extracts of *Capsicum* sp. (Legon 18 variety) fruits at 100% (0.348 ± 0.151 kg) and those treated with *K. senegalensis* at 75% concentrations (1.187 ± 0.092 kg) in the 2016 cropping season (Table 8.8)

In the 2017 cropping season, shoot dry weight of plants treated with manlax (0.353 ± 0.043 kg), rainmancoz (0.299 ± 0.021 kg), 100% extract concentrations of seeds of *B. aegyptiaca* (0.042 ± 0.003 kg), fruits of *Capsicum* sp. (Legon 18 variety) (0.346 ± 0.031 kg) and leaves of *I. oliviformis* (0.298 ± 0.014 kg) recorded significantly higher ($P \leq 0.05$) shoot dry weight than those of the negative control (0.136 ± 0.021 kg), 75% extract concentrations of seeds of *B. aegyptiaca* (0.158 ± 0.021 kg), *K. senegalensis* (0.137 ± 0.025 kg), leaves of *I. oliviformis* (0.159 ± 0.015 kg) as well as 100% concentration of *K. senegalensis* seeds (0.154 ± 0.025 kg) (Table 8.8). Also in the 2017 cropping season, the shoot dry weight recorded for plants treated with manlax (0.353 ± 0.043 kg) and 100% extract concentration of *Capsicum* sp. (Legon 18 variety) fruits (0.346 ± 0.031 kg) were significantly higher ($P \leq 0.05$) than those of 75% concentrations of seeds of *A. indica* (0.200 ± 0.028 kg), *J. curcas* (0.185 ± 0.016 kg), *Capsicum* sp. (Legon 18 variety) fruits (0.218 ± 0.014 kg) and 100% *J. curcas* seeds (0.207 ± 0.040 kg) (Table 8.8).

8.3.3.3 Root dry weight/crop

8.3.3.3.1 Laribako

Generally there were significant differences ($P \leq 0.05$) in root dry weight among the various treatments in the 2016 cropping season (Table 8.6). In the 2016 cropping season, treatments of manlax, extracts seeds of *B. aegyptiaca* at 100%, fruits of *Capsicum* sp. at 75 and 100% concentrations which recorded root dry weights of 0.031 ± 0.001, 0.032 ± 0.001, 0.032 ± 0.004 and 0.036 ± 0.001 kg respectively were each highly significant ($P \leq 0.05$) in comparison to what was observed for the negative control (0.017 ± 0.002 kg) (Table 8.6).

In the 2017 cropping season, there were no significant differences ($P \leq 0.05$) among the various treatments (Table 8.6). The lowest (0.019 ± 0.003 kg) and highest (0.041 ± 0.007

kg) root dry weights were recorded for the negative control and *Capsicum* sp. (Legon 18 variety) at 100% extract concentration in the 2017 cropping seasons (Table 8.6).

8.3.3.3.2 Pona

The highest root dry weight (0.046 ± 0.003 kg) recorded for the 2016 cropping season was observed for plants treated with 100% extract concentration of *Capsicum* sp. (Legon 18 variety) fruits while the negative control recorded the lowest (0.023 ± 0.001 kg) (Table 8.8). In the 2016 cropping season, the root dry weight for treatments of manlax (0.041 ± 0.002 kg), 100% extract concentration seeds of *B. aegyptiaca* (0.042 ± 0.003 kg), 75 (0.040 ± 0.001 kg) and 100% (0.046 ± 0.003 kg) concentrations of *Capsicum* sp. (Legon 18 variety) fruits were significantly higher ($P \leq 0.05$) than those of the negative control (0.023 ± 0.001 kg), extracts concentrations of 75% of seeds of *B. aegyptiaca* (0.028 ± 0.003 kg), *K. senegalensis* (0.024 ± 0.003 kg) and 100% of *K. senegalensis* (0.024 ± 0.001 kg) (Table 8.8). Also for the 2016 cropping season, the root dry weight recorded for *Capsicum* sp. (Legon 18 variety) fruits at 100% extract concentration (0.046 ± 0.003 kg) with the exception of that of manlax (0.041 ± 0.002 kg), rainmancoz (0.034 ± 0.005 kg), *B. aegyptiaca* seeds at 100% (0.042 ± 0.003 kg) and *Capsicum* sp fruit at 75% (0.040 ± 0.001 kg) extract concentrations was significantly higher ($P \leq 0.05$) than what was observed for the other treatments.

For the 2017 cropping season, *Capsicum* sp. (Legon 18 variety) fruits extract at 100% concentration treatment recorded the highest (0.049 ± 0.008 kg) root dry weight and the lowest was recorded for the negative control (0.025 ± 0.004 kg) (Table 8.8). Root dry weight recorded for 100% extract concentrations of *B. aegyptiaca* seeds (0.048 ± 0.007 kg)

and *Capsicum* sp. (Legon 18 variety) fruits (0.049 ± 0.008 kg) were significantly higher ($P \leq 0.05$) than that of the negative control (0.025 ± 0.004 kg).

8.3.3.4 Tuber weight/crop and yield

8.3.3.4.1 Laribako

Among the various treated plots, laribako tuber weight/crop for the 2016 cropping season ranged from 1.785 ± 0.132 to 0.796 ± 0.019; representing a yield range of 17.85 to 7.96 t/ha (Table 8.7). In the same cropping season, the negative control recorded a significantly lower ($P \leq 0.05$) laribako tuber weight/crop (0.796 ± 0.019 kg) than those obtained for the other treated plots (Table 8.7). It was also observed in the 2016 cropping season that there were no significant differences ($P \leq 0.05$) in tuber weight/crop among manlax, 100% seeds extracts concentrations of *A. indica*, *B. aegyptiaca*, *J. curcas,* and 75 and 100% *Capsicum* sp. (Legon 18 variety) fruits. In the 2016 cropping season, with the exception of the 75% *K. senegalensis* seeds treatment tuber weight/crop (0.877 ± 0.046 kg), there were no significant differences ($P \leq 0.05$) between rainmancoz and the other plant extract.

In the 2017 cropping season, the highest (2.186 ± 0.037 kg) and the lowest (1.061 ± 0.103 kg) tuber weight/crop were recorded for *Capsicum* sp. (Legon 18 variety) fruits at 100% extract concentration and the negative control treatments respectively (Table 8.7). This represented tuber yield range of 10.61 to 21.86 t/ha (Table 8.7). *Capsicum* sp. (Legon 18 variety) fruits extract at 100% concentration had a significantly higher ($P \leq 0.05$) tuber weight/crop than 75 (1.087 ± 0118 kg) and 100% (1.120 ± 0.093 kg) concentrations of *K. senegalensis* seeds and the negative control treatments (1.061 ± 0.103 kg) for the 2017 cropping season (Table 8.7).

8.3.3.4.2 Pona

The tuber weight/crop for the 2016 cropping season ranged from 0.905 ± 0.037 kg for the negative control to 1.841 ± 0.089 kg for manlax, representing tuber yield range of 9.05 to 18.41 t/ha (Table 8.9). There were significant differences ($P \leq 0.05$) among the tuber weight/crop recorded for the various treatments in the 2016 cropping season (Table 8.9). The tuber weight/crop recorded for manlax (1.841 ± 0.089 kg), rainmancoz (1.764 ± 0.094 kg), fruit extracts of *Capsicum* sp. (Legon 18 variety) at 75 (1.628 ± 0.129 kg) and 100% (1.686 ± 0.158 kg) treatments in the 2016 cropping season were significantly higher ($P \leq 0.05$) than what was recorded for the negative control (0.813 ± 0.037 kg) (Table 8.9).

For the 2017 cropping season, the lowest (1.185 ± 0.123 kg) and highest (2.360 ± 0.197 kg) tuber weight/crop were recorded for the negative control and manlax treatments respectively (Table 8.9). This represented tuber yield range of 11.85 to 23.60 t/ha (Table 8.9). In the 2017 cropping season it was also observed that tuber weight/crop recorded for manlax (2.360 ± 0.197 kg), rainmancoz (2.299 ± 0.175 kg), fruits extracts of *Capsicum* sp. (Legon 18 variety) at 75 (2.265 ± 0.166 kg) and 100% (2.322 ± 0.258 kg) treatments were significantly higher ($P \leq 0.05$) than those of 75% extracts concentrations of seeds of *A. indica* (1.309 ± 0.204 kg), *B. aegyptiaca* (1.257 ± 0.134 kg), *J. curcas* (1.232 ± 0.132 kg), *K. senegalensis* (1.187 ± 0.092 kg), leaves of *I. oliviformis* (1.238 ± 0.118 kg), 100% extracts concentrations of *J. curcas* (1.370 ± 0.183 kg) and *K. senegalensis* (1.193 ± 0.097 kg), and the negative control (1.185 ± 0.123 kg).

Table 8.6: Effect of plant extracts on the vegetative growth of laribako

Treatment	Vine length (cm) ± S.E		Shoot dry weight/crop (kg) ± S.E		Root dry weight/crop (kg) ± SE	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**
Manlax	123.8 ± 11.995^{a}	132.8 ± 13.930^{a}	0.292 ± 0.196^{a}	0.342 ± 0.025^{c}	0.031 ± 0.001^{bcd}	0.037 ± 0.007^{a}
Rainmancoz	121.5 ± 17.876^{a}	127.8 ± 17.764^{a}	0.194 ± 0.008^{a}	0.200 ± 0.041^{abc}	0.024 ± 0.001^{abc}	0.029 ± 0.006^{a}
AIS@75%	111.5 ± 8.302^{a}	116.0 ± 5.196^{a}	0.148 ± 0.011^{a}	0.172 ± 0.029^{a}	0.022 ± 0.002^{abc}	0.024 ± 0.005^{a}
AIS@100%	129.5 ± 16.357^{a}	140.8 ± 17.684^{a}	0.224 ± 0.036^{a}	0.213 ± 0.023^{abc}	0.026 ± 0.001^{abcd}	0.031 ± 0.006^{a}
BAS@75%	121.0 ± 8.766^{a}	127.5 ± 5.852^{a}	0.168 ± 0.009^{a}	0.149 ± 0.013^{a}	0.022 ± 0.002^{abc}	0.026 ± 0.003^{a}
BAS@100%	137.8 ± 7.653^{a}	141.0 ± 10.222^{a}	0.345 ± 0.218^{a}	0.201 ± 0.030^{abc}	0.032 ± 0.001^{cd}	0.037 ± 0.004^{a}
CF@75%	126.5 ± 8.057^{a}	130.2 ± 10.857^{a}	0.157 ± 0.005^{a}	0.331 ± 0.044^{bc}	0.032 ± 0.004^{cd}	0.034 ± 0.006^{a}
CF@100%	127.0 ± 10.230^{a}	137.8 ± 6.799^{a}	0.322 ± 0.057^{a}	0.352 ± 0.056^{c}	0.036 ± 0.001^{d}	0.041 ± 0.007^{a}
IOL@75%	119.2 ± 6.511^{a}	118.2 ± 4.230^{a}	0.138 ± 0.006^{a}	0.163 ± 0.026^{a}	0.022 ± 0.001^{abc}	0.027 ± 0.004^{a}
IOL@100%	122.2 ± 12.351^{a}	123.5 ± 9.717^{a}	0.207 ± 0.005^{a}	0.253 ± 0.044^{abc}	0.025 ± 0.003^{abc}	0.035 ± 0.005^{a}
JCS@75%	119.0 ± 7.071^{a}	117.2 ± 9.022^{a}	0.135 ± 0.004^{a}	0.142 ± 0.015^{a}	0.020 ± 0.002^{ab}	0.027 ± 0.005^{a}
JCS@100%	121.8 ± 2.428^{a}	122.8 ± 10.086^{a}	0.189 ± 0.011^{a}	0.185 ± 0.028^{ab}	0.023 ± 0.004^{abc}	0.029 ± 0.005^{a}
KSS@75%	117.8 ± 4.347^{a}	121.8 ± 13.047^{a}	0.124 ± 0.009^{a}	0.135 ± 0.014^{a}	0.020 ± 0.003^{ab}	0.021 ± 0.004^{a}
KSS@100%	124.8 ± 14.755^{a}	122.5 ± 10.275^{a}	0.127 ± 0.005^{a}	0.138 ± 0.021^{a}	0.021 ± 0.002^{abc}	0.022 ± 0.004^{a}
Negativ econtrol	109.2 ± 7.771^{a}	113.5 ± 12.926^{a}	0.081 ± 0.008^{a}	0.133 ± 0.012^{a}	0.017 ± 0.002^{a}	0.019 ± 0.003^{a}
F (pr)	**0.955**	**0.835**	**<0.001**	**<0.001**	**<0.001**	**0.121**

Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test; NAS, not analysed statistically. Key: AIS = *A. indica* seeds, BAS = *B. aegyptiaca* seeds, *Capsicum* sp. (Legon 18 variety) fruits, IOL = *I. oliviformis* leaves, JCS = *J. curcas* seeds and KSS = *K. senegalensis* seeds

Table 8.7: Effect of plant extracts on laribako tuber weight (kg/stand) and yield (t/ha) for the 2016 and 2017 cropping seasons

Treatment	2016		2017	
	Tuber weight (kg/stand) ± S.E	**Tuber yield (t/ha)**	**Tuber weight (kg/stand) ± S.E**	**Tuber yield (t/ha)**
Manlax	1.785 ± 0.132^{d}	17.85	1.984 ± 0.378^{ab}	19.84
Rainmancoz	1.598 ± 0.055^{cd}	15.98	1.883 ± 0.357^{ab}	18.83
AIS@75%	1.120 ± 0.186^{abc}	11.20	1.293 ± 0.148^{ab}	12.93
AIS@100%	1.332 ± 0.136^{abcd}	13.32	1.761 ± 0.325^{ab}	17.61
BAS@75%	1.104 ± 0.048^{abc}	11.04	1.205 ± 0.117^{ab}	12.05
BAS@100%	1.224 ± 0.091^{abcd}	12.24	1.592 ± 0.287^{ab}	15.92
CF@75%	1.469 ± 0.307^{bcd}	14.69	2.053 ± 0.125^{ab}	20.53
CF@100%	1.534 ± 0.044^{cd}	15.34	2.186 ± 0.037^{b}	21.86
IOL@75%	1.058 ± 0.135^{abc}	10.58	1.177 ± 0.136^{ab}	11.77
IOL@100%	1.170 ± 0.077^{abc}	11.70	1.497 ± 0.193^{ab}	14.97
JCS@75%	1.046 ± 0.026^{abc}	10.46	1.158 ± 0.115^{ab}	11.58
JCS@100%	1.186 ± 0.082^{abcd}	11.86	1.334 ± 0.205^{ab}	13.34
KSS@75%	0.877 ± 0.046^{ab}	8.77	1.087 ± 0118^{a}	10.87
KSS@100%	0.998 ± 0.029^{abc}	9.98	1.120 ± 0.093^{a}	11.20
Negative control	0.796 ± 0.019^{a}	7.96	1.061 ± 0.103^{a}	10.61
F (pr)	**<0.001**		**< 0.001**	

Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test. Key: AIS = *A. indica* seeds, BAS = *B. aegyptiaca* seeds, *Capsicum* sp. (Legon 18 variety) fruits, IOL = *I. oliviformis* leaves, JCS = *J. curcas* seeds and KSS = *K. senegalensis* seeds

Table 8.8: Effect of plant extracts on the vegetative growth of pona

Treatment	Vine length (cm) ± S.E		Shoot dry weight (kg) ± S.E		Root dry weight (kg) ± SE	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**
Manlax	109.5 ± 4.500^{a}	129.8 ± 4.498^{a}	0.226 ± 0.013^{abc}	0.353 ± 0.043^{d}	0.041 ± 0.002^{bc}	0.038 ± 0.003^{ab}
Rainmancoz	126.8 ± 7.375^{a}	126.8 ± 10.522^{a}	0.209 ± 0.009^{abc}	0.299 ± 0.021^{bcd}	0.034 ± 0.005^{abc}	0.046 ± 0.006^{ab}
AIS@75%	105.8 ± 5.588^{a}	109.2 ± 4.661^{a}	0.165 ± 0.003^{abc}	0.200 ± 0.028^{ab}	0.030 ± 0.001^{ab}	0.030 ± 0.003^{ab}
AIS@100%	108.2 ± 7.432^{a}	110.5 ± 6.701^{a}	0.171 ± 0.004^{abc}	0.262 ± 0.021^{abcd}	0.032 ± 0.002^{ab}	0.036 ± 0.003^{ab}
BAS@75%	107.0 ± 5.715^{a}	112.8 ± 7.454^{a}	0.148 ± 0.008^{abc}	0.158 ± 0.021^{a}	0.028 ± 0.003^{a}	0.031 ± 0.003^{ab}
BAS@100%	118.2 ± 4.571^{a}	115.2 ± 4.171^{a}	0.338 ± 0.061^{bc}	0.333 ± 0.019^{cd}	0.042 ± 0.003^{bc}	0.048 ± 0.007^{b}
CF@75%	117.8 ± 3.637^{a}	126.2 ± 8.596^{a}	0.216 ± 0.013^{abc}	0.218 ± 0.014^{abc}	0.040 ± 0.001^{bc}	0.037 ± 0.003^{ab}
CF@100%	131.0 ± 8.406^{a}	136.8 ± 15.802^{a}	0.348 ± 0.151^{c}	0.346 ± 0.031^{d}	0.046 ± 0.003^{c}	0.049 ± 0.008^{b}
IOL@75%	107.5 ± 8.139^{a}	123.5 ± 9.500^{a}	0.161 ± 0.005^{abc}	0.159 ± 0.015^{a}	0.031 ± 0.002^{ab}	0.033 ± 0.003^{ab}
IOL@100%	122.5 ± 9.535^{a}	127.8 ± 5.963^{a}	0.170 ± 0.006^{abc}	0.298 ± 0.014^{bcd}	0.031 ± 0.001^{ab}	0.042 ± 0.007 ^{ab}
JCS@75%	116.2 ± 2.496^{a}	114.8 ± 3.705^{a}	0.163 ± 0.007^{abc}	0.185 ± 0.016^{ab}	0.030 ± 0.002^{ab}	0.029 ± 0.002^{ab}
JCS@100%	116.5 ± 2.901^{a}	120.8 ± 2.175^{a}	0.171 ± 0.004^{abc}	0.207 ± 0.040^{abc}	0.031 ± 0.002^{ab}	0.035 ± 0.006^{ab}
KSS@75%	107.0 ± 3.559^{a}	110.5 ± 4.518^{a}	0.130 ± 0.003^{ab}	0.137 ± 0.025^{a}	0.024 ± 0.003^{a}	0.027 ± 0.002^{ab}
KSS@100%	119.5 ± 5.377^{a}	117.2 ± 3.326^{a}	0.137 ± 0.001^{abc}	0.154 ± 0.025^{a}	0.024 ± 0.001^{a}	0.029 ± 0.003^{ab}
Negative control	102.5 ± 5.331^{a}	106.8 ± 7.443^{a}	0.111 ± 0.005^{a}	0.136 ± 0.021^{a}	0.023 ± 0.001^{a}	0.025 ± 0.004^{a}
F (pr)	**0.045**	**0.176**	**<0.001**	**<0.001**	**<0.001**	**0.003**

Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test. Key: AIS = *A. indica* seeds, BAS = *B. aegyptiaca* seeds, *Capsicum* sp. (Legon 18 variety) fruits, IOL = *I. oliviformis* leaves, JCS = *J. curcas* seeds and KSS = *K. senegalensis* seeds

Table 8.9: Effect of plant extracts on pona tuber weight (kg/stand) and yield (t/ha) for the 2016 and 2017 cropping seasons

Treatment	2016		2017	
	Tuber weight (kg/stand) ± S.E	Tuber yield (t/ha)	Tuber weight (kg/stand) ± S.E	Tuber yield (t/ha)
Manlax	1.841 ± 0.089^{d}	18.41	2.360 ± 0.197^{b}	23.60
Rainmancoz	1.764 ± 0.094^{cd}	17.64	2.299 ± 0.175^{b}	22.99
AIS@75%	1.262 ± 0.199^{abcd}	12.79	1.309 ± 0.204^{a}	13.09
AIS@100%	1.525 ± 0.233^{abcd}	15.25	1.873 ± 0.174^{ab}	18.73
BAS@75%	1.186 ± 0.125^{abc}	11.86	1.257 ± 0.134^{a}	12.57
BAS@100%	1.344 ± 0.158^{abcd}	13.44	1.573 ± 0.186^{ab}	15.73
CF@75%	1.628 ± 0.129^{bcd}	16.28	2.265 ± 0.166^{b}	22.65
CF@100%	1.686 ± 0.158^{cd}	16.86	2.322 ± 0.258^{b}	23.22
IOL@75%	1.248 ± 0.047^{abcd}	12.48	1.238 ± 0.118^{a}	12.38
IOL@100%	1.272 ± 0.713^{abcd}	12.72	1.549 ± 0.227^{ab}	15.49
JCS@75%	1.224 ± 0.131^{abcd}	12.24	1.232 ± 0.132^{a}	12.32
JCS@100%	1.279 ± 0.111^{abcd}	12.79	1.370 ± 0.183^{a}	13.70
KSS@75%	0.977 ± 0.052^{a}	9.77	1.187 ± 0.092^{a}	11.87
KSS@100%	1.029 ± 0.015^{ab}	10.29	1.193 ± 0.097^{a}	11.93
Negative control	0.905 ± 0.037^{a}	9.05	1.185 ± 0.123^{a}	11.85
F (pr)	**<0.001**		**< 0.001**	

Means in the same column followed by different letter(s) are significantly different as determined by Tukey's test. Key: AIS = *A. indica* seeds, BAS = *B. aegyptiaca* seeds, *Capsicum* sp. (Legon 18 variety) fruits, IOL = *I. oliviformis* leaves, JCS = *J. curcas* seeds and KSS = *K. senegalensis* seeds

8.4 Discussion

Generally, treatment with the plant extracts (seeds of *A. indica*, *B. aegyptiaca*, *J. curcas* and *K. senegalensis, I. oliviformis* leaves and *Capsicum* sp. - Legon 18 variety fruits) reduced the incidence and severity of anthracnose disease in laribako and pona cultivars of *D. rotundata* compared to the negative control. This indicated that these extracts have the potential to be used as botanical fungicides in the management of yam anthracnose disease under field conditions. The observation can be attributed to the presence of the phytochemical constituents contained in the plant extracts (Section 6.3.1, Table 6.1) which might have exhibited a fungitoxic activity on the spore germination and growth of *C. gloeosporioides* or enhanced the crop's resistance to infection. This was supported by Amadioha (2000) and Shabana (2017) who stated that secondary metabolites contained in plant extracts are capable of reducing the growth of phytopathogenic fungi and their multiplication or induce the defence responses of plants to diseases. Generally the improved yam tuber yield, shoot and root dry weights for the various plant extract treatments as obtained for manlax and rainmancoz; confirmed the antifungal activity of the plant extracts against the anthracnose disease of yam.

The progressive increase in the anthracnose disease incidence and severity observed among the various treatments of the laribako and pona cultivars of *D roundata* for the 2016 and 2017 cropping seasons as the seasons progressed from 5 WAP to 15 WAP was expected. This is because it has been established that yam anthracnose disease will always intensify with seasonal progress and increasing rainfall (Agrios, 2005; Achar *et al*., 2013; Gautam, 2014). The rainfall data (Appendix 1A) recorded during this study generally showed increasing rainfall along seasonal progress.

In the 2016 and 2017 cropping seasons at the 13 and 15 WAP for the pona crops, there were no significant differences ($P \leq 0.05$) in disease severity among the treatments; probably the plant extracts and synthetic fungicides applications only delayed the onset of the anthracnose disease but could not prevent its establishment. A similar observation was made by Kutama *et al.* (2013) who stated that foliar fungicide application can only delay the start of yam anthracnose disease but cannot prevent it during the rainy season. Also during the 13 and 15 WAP the rainy season was at its peak and therefore rain splash might have facilitated the dissemination of *C. gloeosporioides* spores on the yam field as documented by Reddy (2015).

For the various treatments, the 2016 cropping season recorded higher yam anthracnose disease incidence and severity than that of 2017, which could be attributed to the higher rainfall recorded in the former season than the latter. This confirms the findings of Akem and Asiedu (1994) and Lebot (2009) who reported that the extent of anthracnose disease on the same farm for a particular crop may vary from one year to another due to seasonal variations in weather conditions; with higher disease incidence occurring in seasons with higher rainfall.

Also the various plant extract treatments recorded higher tuber yield, shoot and root dry weights than their corresponding negative controls in the 2016 and 2017 cropping seasons. This indicated that the plants extracts might have enhanced the vegetative growth and yield of the laribako and pona crops. This observation agreed with the report that plant extracts contain growth promoting substances capable of enhancing the vegetative growth and yield of crops (Fuglie, 1999; Bulgari *et al.*, 2017).

The enhanced vegetative growth in laribako and pona plants treated with the plant extracts during the 2016 and 2017 cropping seasons could also be an indication that, the extracts

were applied at concentrations below the levels at which they would have exhibited allelopathic effect on the yam crops. This agreed with the report of Shah *et al.* (2017) that the allelopathic effect of plant extracts on crop growth is concentration dependent; where growth retardation increases with increasing extract concentration and vice versa.

It was also observed that, the tuber weight recorded for a particular treatment tended to be directly proportional to shoot and root dry weights of the treatment. These findings corresponded to that of Oyetunji and Afolayan (2014) who stated that a positive correlation existed between yam tuber yields, shoot and root dry weights.

This study has revealed that extracts of seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) and leaves of *I. oliviformis* possess antifungal properties that can be exploited in the management of yam anthracnose disease under field conditions.

CHAPTER NINE

9.0 GENERAL CONCLUSION AND RECOMMENDATIONS

9.1 Conclusion

This study has revealed the prevalence of yam anthracnose disease infection on *D. rotundata* crops (laribako and pona) in the Tolon District of Ghana. The disease incidence and severity recorded for each of the 2016 and 2017 cropping seasons for the Tolon communities (Kpalsogu, Gawugu, Fihini, Sabegu, Woribogu Kukuo and Dingoni), zones (Nyankpalasogu, Tolon and Woribogu) and district generally increased as the cropping season progressed from July through August and to September. Generally the 2016 cropping season recorded higher anthracnose disease incidence and severity than what was observed for 2017. For the 2016 and 2017 cropping seasons there were no significant differences among each of the anthracnose disease incidence and severity recorded for Kpalsogu, Gawugu, Fihini, Sabegu, Woribogu Kukuo and Dingoni communities for each of the months of July, August and September. A similar observation was made for Nyankpalasogu, Tolon and Woribogu zones of the Tolon district.

The *Colletotrichum* spp. isolates obtained from the leaves, vines and setts of *D. rotundata* in the Tolon District were grouped into six (CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6) based on their cultural characteristics on PDA. They were all identified as *C. gloeosporioides* isolates based on their cultural and morphological characteristics. The identification of the isolates CDr1, CDr2, CDr3, CDr4, CDr5 and CDr6 were further confirmed using PCR techniques. All the *C. gloeosporioides* isolates obtained produced DNA fragments on each of the universal primer pairs ITS1/ITS4 and NS1/NS2, *C. gloeosporioides* species primer pairs ITS4/CgInt and CgLac-f/CgLac-r. However none of the isolates CDr1, CDr2, CDr3,

CDr4, CDr5 and CDr6 yielded DNA fragments on each of the *C. acutatum* species specific primer pairs CaGlu-f2/CaGlu-r2 and Ca-f1/ Ca-r1.

The aqueous and ethanol extracts of seeds of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, leaves of *I. oliviformis*, and fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) were screened qualitatively and quantitatively for certain phytochemicals. The qualitative analysis revealed the presence of the phytochemicals alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatinnins, saponins, steroids, tannins and terpenoids. The number of phytochemicals detected varied from one plant extract to the other. Aqueous extract of *Capsicum* sp. (hot pepper, Legon 18 variety) recorded the highest number (8) of phytochemicals (alkaloids, cardiac glycosides, flavonoids, phlobatinnins, saponins, steroids, tannins and terpenoids). The quantitative phytochemicals analysis carried out on the ethanol extracts of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, leaves of *I. oliviformis*, and fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) also showed that contents of total phenolics, flavonoids, tannins and alkaloids varied among the plant extracts.

The presence of the phytochemicals in the various plant extracts, showed their antimicrobial potential. Each of the aqueous and ethanol extracts of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, leaves of *I. oliviformis*, and fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) at various concentrations (10, 25, 50, 75 and 100%) significantly reduced ($P \leq 0.05$) the mycelia growth and spore germination of *C. gloeosporioides* in comparison to the negative control treatment (distilled water). There were variations among the antifungal activities of the various concentrations of the plant extracts on the mycelia growth and spore germination of *C. gloeosporioides. Capsicum* sp. (hot pepper, Legon 18 variety) had the most promising results of inhibiting the mycelia growth (76.1%) and spore germination (91.7%) of *C. gloeosporioides* in the *in vitro* studies.

The foliar applications of 75 and 100% aqueous extracts concentrations of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, leaves of *I. oliviformis*, and fruits of *Capsicum* sp. (hot pepper, Legon 18 variety) as botanical fungicides against anthracnose disease of *D. roundata* (laribako and pona) under field conditions showed some promising results in managing the disease. The various concentrations of each of the plant extracts were able to reduce the incidence and severity of the anthracnose disease on the laribako and pona crops in comparison to that of the negative control treatment (water). Each of the plant extracts also improved the crop's (laribako and pona) growth trait such as shoot and root dry weight over that of the negative control. Generally the foliar application of the plant extracts also enhanced the tuber yield of the laribako and pona crops. Among the plant extract treatments the best growth traits and tuber yield results were obtained for aqueous extract of *Capsicum* sp. (hot pepper, Legon 18 variety) at 100%.

9.2 Recommendations

From the findings of this study, the following recommendations were made;

- Yam farmers should be encouraged to practice phytosanitary measures such as the removal and burning of *C. gloeosporioides* infected crop debris on their farms, weeding their farms regularly to minimise the presence of other potentially alternate host of *C. gloeosporioides* and avoid planting infected yam setts. These measures would help reduce *C. gloeosporioides* inoculum and their spread on the yam farms.
- Yam farmers should adapt to the usage of plant extracts which have antifungal activities against *C. gloeosporioides* as botanical fungicides in managing the anthracnose disease. The potential antifungal activity of *A. indica*, *B. aegyptiaca*, *J. curcas*, *K. senegalensis*, leaves of *I. oliviformis*, and fruits of *Capsicum* sp. (hot pepper, Legon 18 variety)

against yam anthracnose disease under field conditions implies that yam farmer can employ them as botanical fungicides for foliage spray of their yam crops in managing the disease.

- Further research should be carried out to determine the concentrations at which the various plants extracts used for this study can totally inhibit the mycelia growth and spore germination of *C. gloeosporioides.* Also field experiments should be carried out to establish the highest concentration at which each of the extracts, especially *Capsicum* sp. (hot pepper, Legon 18 variety) can control the yam anthracnose disease without having allelopathy effect on the host crop.
- There is the need to also research into the effect of various mixture combinations of extracts of the plant materials employed for this study in the management of the yam anthracnose disease. This is because a combination of various plant extracts would likely enhance its antifungal activity than a single one.
- Researches should be conducted leading to the development of plant extracts such as *Capsicum* sp. (hot pepper, Legon 18 variety) which has exhibited a high antifungal activity against *C. gloeosporioides* into commercial botanical fungicide products for the management of yam anthracnose disease.

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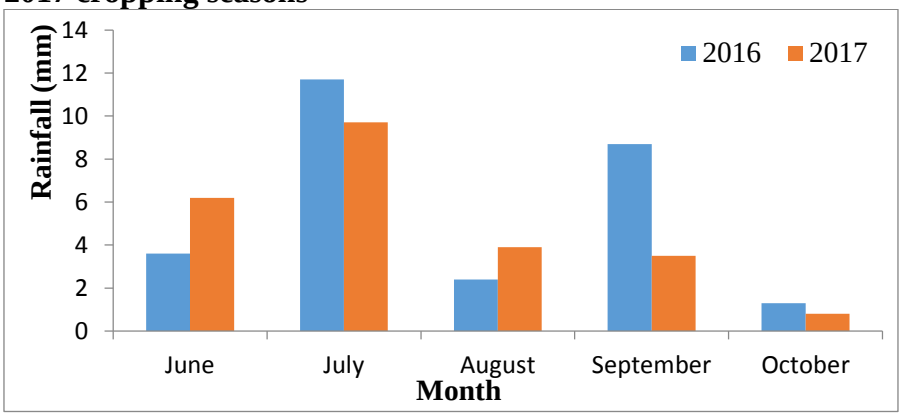
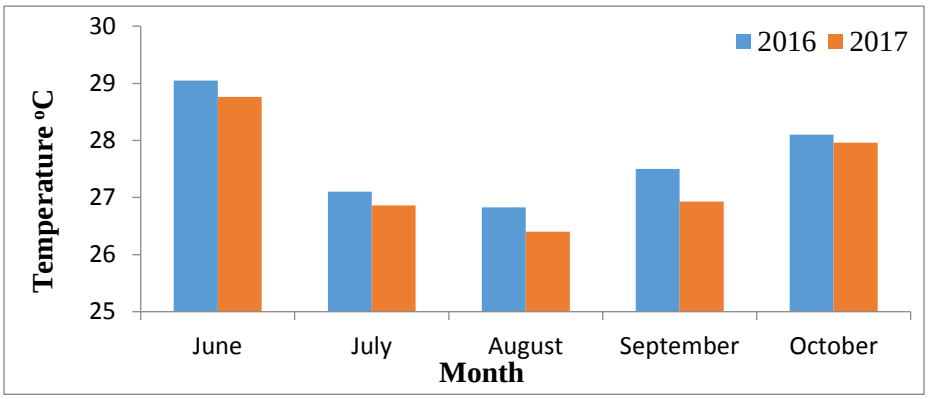
APPENDICES

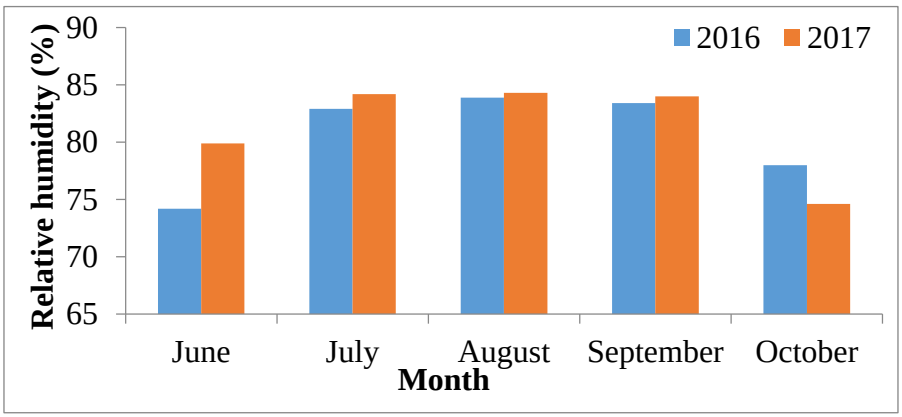
Appendix 1: Weather data of the study area from June to October for the 2016 and 2017 cropping seasons



Monthly mean rainfall



Monthly mean temperature



Monthly mean relative humidity

Source: SARI weather data

Appendix 2: A photograph of the experimental yam field

Appendix 3: Some anthracnose infection on yam observed during the study

Anthracnose lesion on yam leaves (A) Die back of yam vine due to anthracnose infection (B) "Dead skin" of yam sett resulting from anthracnose infection (C)

Appendix 4: Analysis of *D. rotundata* disease incidence for different months of the 2016 and 2017 cropping seasons for various zones in the Tolon District

Analysis of variance

Variate: Disease incidence (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Communities_month	17	35860.084	2109.417	258.80	<.001
Residual	18	146.714	8.151		
Total	35	36006.798			

Tables of means

Variate: Disease incidence (%)

Grand mean 44.15

Communities and month	Nyankpalasogu(August2016)	Nyankpalasogu(August2017)
	31.57	45.31
Communities and month	Nyankpalasogu(July2016)	Nyankpalasogu(July2017)
	11.26	5.01
Communities and month	Nyankpalasogu(September2016)	Nyankpalasogu(September2017)
	92.50	75.94
Communities and month	Tolon(August2016)	Tolon(August2017)
	31.26	45.94
Communities and month	Tolon(July2016)	Tolon(July2017)
	8.76	5.94
Communities and month	Tolon(September2016)	Tolon(September2017)
	93.44	75.94
Communities and month	Woribogu(August2016)	Woribogu(August2017)
	38.75	45.31
Communities and month	Woribogu(July2016)	Woribogu(July2017)
	16.25	5.94
Communities and month	Woribogu(September2016)	Woribogu(September2017)
	93.44	72.19

Standard errors of means

Table	Communities and month
rep.	2
d.f.	18
e.s.e.	2.019

Standard errors of differences of means

Table	Communities and month
rep.	2
d.f.	18
s.e.d.	2.855

Least significant differences of means (5% level)

Table	Communities and month
rep.	2
d.f.	18
l.s.d.	5.998

Stratum standard errors and coefficients of variation

Variate: Disease and incidence (%)

d.f.	s.e.	cv%
18	2.855	6.5

Tukey's 95% confidence intervals

Communities and month

	Mean	
Nyankpalasogu(July2017)	5.01	a
Tolon(July2017)	5.94	a
Woribogu(July2017)	5.94	a
Tolon(July2016)	8.76	a
Nyankpalasogu(July2016)	11.26	a
Woribogu(July2016)	16.25	a
Tolon(August2016)	31.26	b
Nyankpalasogu(August2016)	31.57	b
Woribogu(August2016)	38.75	bc
Nyankpalasogu(August2017)	45.31	c
Woribogu(August2017)	45.31	c
Tolon(August2017)	45.94	c
Woribogu(September2017)	72.19	d
Nyankpalasogu(September2017)	75.94	d
Tolon(September2017)	75.94	d
Nyankpalasogu(September2016)	92.50	e
Tolon(September2016)	93.44	e
Woribogu(September2016)	93.44	e

43 ENDIF
44 SET [IN=*]

Appendix 5: Analysis of *D. rotundata* anthracnose disease severity index for different months of the 2016 and 2017 cropping seasons for various zones in the Tolon district.

Analysis of variance

Variate: Disease severity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Communities_month	17	6.113200	0.359600	40.84	<.001
Residual	18	0.158500	0.008806		
Total	35	6.271700			

Tables of means

Variate: Disease severity

Grand mean 2.135

Communities and month		
	Nyankpalasogu(August2016)	Nyankpalasogu(August2017)
	2.145	2.195
Communities and month	Nyankpalasogu(July2016)	Nyankpalasogu(July2017)
	1.780	1.505
Communities and month	Nyankpalasogu(September2016)	Nyankpalasogu(September2017)
	2.930	2.295
Communities and month	Tolon(August2016)	Tolon(August2017)
	2.080	2.180
Communities and month	Tolon(July2016)	Tolon(July2017)
	1.755	1.530
Communities and month	Tolon(September2016)	Tolon(September2017)
	2.780	2.345
Communities and month	Woribogu(August2016)	Woribogu(August2017)
	2.160	2.075
Communities and month	Woribogu(July2016)	Woribogu(July2017)
	1.815	1.645
Communities and month	Woribogu(September2016)	Woribogu(September2017)
	2.835	2.380

Standard errors of means

Table	Communities and month
rep.	2
d.f.	18
e.s.e.	0.0664

Standard errors of differences of means

Table	Communities and month
rep.	2
d.f.	18
s.e.d.	0.0938

Least significant differences of means (5% level)

Table	Communities and month
rep.	2
d.f.	18
l.s.d.	0.1971

Stratum standard errors and coefficients of variation

Variate: Disease severity

d.f.	s.e.	cv%
18	0.0938	4.4

Tukey's 95% confidence intervals

Communities_month

	Mean	
Nyankpalasogu(July2017)	1.505	a
Tolon(July2017)	1.530	a
Woribogu(July2017)	1.645	a
Tolon(July2016)	1.755	ab
Nyankpalasogu(July2016)	1.780	abc
Woribogu(July2016)	1.815	abcd
Woribogu(August2017)	2.075	bcde
Tolon(August2016)	2.080	bcde
Nyankpalasogu(August2016)	2.145	cde
Woribogu(August2016)	2.160	de
Tolon(August2017)	2.180	de
Nyankpalasogu(August2017)	2.195	e
Nyankpalasogu(September2017)	2.295	e
Tolon(September2017)	2.345	e
Woribogu(September2017)	2.380	e
Tolon(September2016)	2.780	f
Woribogu(September2016)	2.835	f
Nyankpalasogu(September2016)	2.930	f

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43  ENDIF
44  SET [IN=*]
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Appendix 6: Analysis of effect of plant extracts on mycelial growth of *C. gloeosporioides*

Analysis of variance

Variate: aqueous extract mycelia growth (mm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	32	6443.3818	201.3557	299.31	<.001
Residual	132	88.8000	0.6727		
Total	164	6532.1818			

Tables of means

Variate: aqueous extract mycelia growth (mm)

Grand mean 15.636

Treatment	AIS@10%	AIS@100%	AIS@25%	AIS@50%
	21.200	10.400	20.000	18.000
Treatment	AIS@75%	BAS@10%	BAS@100%	BAS@25%
	15.200	19.000	13.000	17.400
Treatment	BAS@50%	BAS@75%	CF@10%	CF@100%
	15.600	14.000	11.800	6.400
Treatment	CF@25%	CF@50%	CF@75%	IOL@10%
	9.800	7.600	6.800	20.400
Treatment	IOL@100%	IOL@25%	IOL@50%	IOL@75%
	12.400	18.400	16.400	16.000
Treatment	JCS@10%	JCS@100%	JCS@25%	JCS@50%
	22.000	14.800	20.600	19.600
Treatment	JCS@75%	KSS@10%	KSS@100%	KSS@25%
	17.000	23.800	18.400	22.400
Treatment	KSS@50%	KSS@75%	manlax	negative control
	21.000	19.800	0.000	26.800
Treatment	rainmancoz			
	0.000			

Standard errors of means

Table	Treatment
rep.	5
d.f.	132
e.s.e.	0.3668

Standard errors of differences of means

Table	Treatment
rep.	5
d.f.	132
s.e.d.	0.5187

Least significant differences of means (5% level)

Table	Treatment
rep.	5
d.f.	132
l.s.d.	1.0261

Stratum standard errors and coefficients of variation

Variate: aqueous extract mycelia growth (mm)

d.f.	s.e.	cv%
132	0.8202	5.2

Tukey's 95% confidence intervals

Treatment

	Mean	
manlax	0.00	a
rainmancoz	0.00	a
CF@100%	6.40	b
CF@75%	6.80	b
CF@50%	7.60	b
CF@25%	9.80	c
AIS@100%	10.40	cd
CF@10%	11.80	cde
IOL@100%	12.40	def
BAS@100%	13.00	efg
BAS@75%	14.00	fgh
JCS@100%	14.80	ghi
AIS@75%	15.20	hij
BAS@50%	15.60	hijk
IOL@75%	16.00	hijkl
IOL@50%	16.40	ijklm
JCS@75%	17.00	jklmn
BAS@25%	17.40	klmn
AIS@50%	18.00	lmno
IOL@25%	18.40	mnop
KSS@100%	18.40	mnop
BAS@10%	19.00	nopq
JCS@50%	19.60	opqr
KSS@75%	19.80	opqr
AIS@25%	20.00	opqrs
IOL@10%	20.40	pqrst
JCS@25%	20.60	qrst
KSS@50%	21.00	qrst
AIS@10%	21.20	rst
JCS@10%	22.00	stu
KSS@25%	22.40	tu
KSS@10%	23.80	u
negative control	26.80	v

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51  ENDIF
52  SET [IN=*]
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Appendix 7: Analysis of effect of plant extracts on Pona shoot dry weight (kg/stand) for the 2017 cropping season

Analysis of variance

Variate: pona shoot dry weight (kg/stand) 2017 cropping season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	14	0.346603	0.024757	9.76	<.001
Residual	45	0.114205	0.002538		
Total	59	0.460808			

Tables of means

Variate: pona shoot dry weight (kg/stand) 2017 cropping season

Grand mean 0.2296

Treatment	DDS@100%	DDS@75%	FYL@100%	FYL@75%	JS@100%	JS@75%
	0.3330	0.1580	0.2980	0.1590	0.2070	0.1850

Treatment	KSS@100%	KSS@75%	Manlax	Neg contol	NS@100%	NS@75%
	0.1540	0.1370	0.3530	0.1360	0.2620	0.2000

Treatment	PF@100%	PF@75%	Rainmancoz
	0.3460	0.2180	0.2987

Standard errors of means

Table	Treatment
rep.	4
d.f.	45
e.s.e.	0.02519

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	45
s.e.d.	0.03562

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	45
l.s.d.	0.07175

Stratum standard errors and coefficients of variation

Variate: pona shoot dry weight (kg/stand) 2017 cropping season

d.f.	s.e.	cv%
45	0.05038	21.9

Tukey's 95% confidence intervals

Treatment

	Mean	
Neg contol	0.1360	a
KSS@75%	0.1370	a
KSS@100%	0.1540	a
DDS@75%	0.1580	a
FYL@75%	0.1590	a
JS@75%	0.1850	ab
NS@75%	0.2000	ab
JS@100%	0.2070	abc
PF@75%	0.2180	abc
NS@100%	0.2620	abcd
FYL@100%	0.2980	bcd
Rainmancoz	0.2987	bcd
DDS@100%	0.3330	cd
PF@100%	0.3460	d
Manlax	0.3530	d

40 ENDIF
41 SET [IN=*]

Appendix 8: Analysis of effect of plant extracts on Laribako root dry weight (kg/stand) for the 2016 cropping season

Analysis of variance

Variate: Laribako root dry weight (Kg/stand) 2016 cropping season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	14	0.00155833	0.00011131	6.00	<.001
Residual	45	0.00083500	0.00001856		
Total	59	0.00239333			

Tables of means

Variate: Laribako root dry weight (Kg/stand) 2016 cropping season

Grand mean 0.02467

Treatment	DDS@100%	DDS@75%	FYL@100%	FYL@75%	JS@100%	JS@75%
	0.03150	0.02200	0.02450	0.02150	0.02275	0.02025
Treatment	KSS@100%	KSS@75%	Manlax	Neg contol	NS@100%	NS@75%
	0.02125	0.01975	0.03050	0.01725	0.02575	0.02175
Treatment	PF@100%	PF@75%	Rainmancoz			
	0.03550	0.03175	0.02400			

Standard errors of means

Table	Treatment
rep.	4
d.f.	45
e.s.e.	0.002154

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	45
s.e.d.	0.003046

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	45
l.s.d.	0.006135

Stratum standard errors and coefficients of variation

Variate: Laribako root dry weight (Kg/stand) 2016 cropping season

d.f.	s.e.	cv%
45	0.004308	17.5

Tukey's 95% confidence intervals

Treatment

	Mean	
Neg contol	0.01725	a
KSS@75%	0.01975	ab
JS@75%	0.02025	ab
KSS@100%	0.02125	abc
FYL@75%	0.02150	abc
NS@75%	0.02175	abc
DDS@75%	0.02200	abc
JS@100%	0.02275	abc
Rainmancoz	0.02400	abc
FYL@100%	0.02450	abc
NS@100%	0.02575	abcd
Manlax	0.03050	bcd
DDS@100%	0.03150	cd
PF@75%	0.03175	cd
PF@100%	0.03550	d

40 ENDIF
41 SET [IN=*]

Appendix 9: Analysis of effect of plant extracts on pona tuber weight (kg/stand) for the 2016 cropping season

Analysis of variance

Variate: Pona tuber weight (kg/stand): 2016 cropping season

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	14	4.58053	0.32718	5.31	<.001
Residual	45	2.77015	0.06156		
Total	59	7.35068			

Tables of means

Variate: Pona tuber weight (kg/stand) 2016 cropping season

Grand mean 1.345

Treatment	DDS@100%	DDS@75%	FYL@100%	FYL@75%	JS@100%	JS@75%
	1.344	1.186	1.272	1.248	1.279	1.224

Treatment	KSS@100%	KSS@75%	Manlax	Neg contol	NS@100%	NS@75%
	1.029	0.977	1.841	0.905	1.525	1.262

Treatment	PF@100%	PF@75%	Rainmancoz
	1.686	1.628	1.764

Standard errors of means

Table	Treatment
rep.	4
d.f.	45
e.s.e.	0.1241

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	45
s.e.d.	0.1754

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	45
l.s.d.	0.3534

Stratum standard errors and coefficients of variation

Variate: Pona tuber weight (kg/stand) 2016 cropping season

d.f.	s.e.	cv%
45	0.2481	18.5

Tukey's 95% confidence intervals

Treatment

	Mean	
Neg contol	0.905	a
KSS@75%	0.977	a
KSS@100%	1.029	ab
DDS@75%	1.186	abc
JS@75%	1.224	abcd
FYL@75%	1.248	abcd
NS@75%	1.262	abcd
FYL@100%	1.272	abcd
JS@100%	1.279	abcd
DDS@100%	1.344	abcd
NS@100%	1.525	abcd
PF@75%	1.628	bcd
PF@100%	1.686	cd
Rainmancoz	1.764	cd
Manlax	1.841	d

```
 40  ENDIF
 41  SET [IN=*]
```