UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

EVALUATION OF BOTANICALS FOR THE CONTROL OF ANTHRACNOSE (*Colletotrichum gloeosporioides* Penz) OF YAM (*Dioscorea rotundata* POIR)

BY

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DECLARATION

Student's

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

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Supervisor's

I 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

Anthracnose is the most serious leaf and vine epiphytotic disease of yam that causes yield loss. Severe infection results in vine dieback, defoliation, and tuber rot. Aqueous extracts of *Azadirachta indica* A Jus seed (Neem tree seed), *Jatropha curcas* (L) seed (Jatropha plant seed) and *Nicotiana tabacum* (L) leaf (Tobacco plant leaf) extracts were evaluated for the control of anthracnose disease of yam (*Dioscorea rotundata* Poir). The pathogen was isolated, identified and its growth was determined *in vitro*. The Potato Dextose Agar (PDA) was amended with the mother extract to make 35, 45, 55, 65 and 75% concentrations in the Petri dishes. Each Petri dish contained 2 ml of an extract and 20 ml of sterilized PDA. The solidified PDA plates were inoculated at the centre with 5 mm diameter mycelial disc of *C. gloeosporioides* Penz and incubated at 27 ^{0}C for 7 days (Nene and Thapliyal, 1979). PDA plates without extract served as negative controls whiles those with the thiophanate methyl (2 ml) served as positive controls. It was observed that 75 % of each plant extract recorded the least mycelial growth compared to the negative control. Percentage mycelial growth in PDA plates treated with *A. indica* seed extract, *N. tabacum* (L) leaf extract and *J. curcas* (L) seed extract were 15, 19.33 and 26.33 % respectively compared to the untreated control (100 %). Generally, the higher the concentration of each plant extract, the higher the inhibitory action against the fungus. Pathogenicity tests carried out showed that *C. gloeosporioides* Penz was the cause of the anthracnose symptoms observed. Each plant extract at 75 % concentration applied *in vivo* reduced the incidence and severity of the anthracnose disease. The plants extracts also promoted vegetative growth. Uninfected plants produced higher chlorophyll content than infected plants. The plant extracts at 75 % concentration also produced higher yield. Farmers may use 75 %

concentration of *A. indica* A Jus seed, *J. curcas* (L) seed and *N. tabacum* (L) leaf extracts as an alternative to synthetic fungicides for the control of yam anthracnose diseases.

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DEDICATION

To my dear family and in memory of my parents.

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CHAPTER ONE

1.0. INTRODUCTION

The use of plants to meet the world's needs is vital to our survival on a global basis. Over 65% of food proteins and more than 80% of food energy is supplied by plants. Yam is second to cassava among the root crops cultivated in Ghana (Coursey, 1976a) and second to cassava as the most important tropical root crop (Opara, 2003). Nigeria alone produces about 75% of the world's total, which is estimated at over 39 million tons (FAO, 2003). Domfeh (2013) reported that Ghana is the world's second largest yam producer after Nigeria but only 30,000 tons of Ghana's total productions of seven million tons are currently exported. Yam accounts for over 20, 8.1, and 4.6% of the total dietary calorie intake in the Kingdom of Tonga, Solomon Islands, and Papua New Guinea respectively (Coursey, 1976b).

Yam has contributed immensely to Ghana's agriculture. It is available throughout the year and plays a very vital role in the area of food security. Yam is consumed by the majority of Ghanaians in both rural and urban areas. Yam consumption turns to be higher in the urban areas (Aidoo, 2009). Most Ghanaians prefer the taste of the white yam to the yellow yam (Aidoo, 2009).

Ghana is the leading exporter of yam, despite the fact that it is the second largest yam producer in the world (Domfeh, 2013). Per capita consumption of yam increased by twelve percent (12%) between 1997 and 2007. Average daily consumption of yam is about 300kcal per capita (FAO STAT, 2012). Yam is the third important source of energy in the Ghanaian diet, accounting for 20% of total caloric intake (FAO STAT, 2012). Yam alone contributes about 16% of Ghana's Agricultural Gross Domestic

Product (GDP) (Anaadumba, 2013). In addition, 6.3% of Ghana's arable land is used for yam cultivation (Otto, 2005).

In Ghana, the yam growing belt is very narrowly delimited within the derived savannah area. The most important areas where yams are grown commercially are Berekum-Wenchi districts, covering Banda, Techiman, Kintampo, Nkoranza and Atebubu districts in Brong-Ahafo region; Northern Ashanti (Mampong and Ejura districts), Gonja and Dagomba districts, Bimbila in the Northern region, Mankessim and Bawjiase in the Central region, Asesewa in the Eastern region and Karachi, Kpando in the Volta region (Owusu and Ofori, 1969).

Anthracnose is one of the most serious leaf and vine epiphytotic diseases of yam, especially *D. rotundata* Poir and *D. alata* L (Ayodele *et al.* (www.iita.org. accessed on 25/10/14). The disease affects leaves, vines, and tubers. Severe infection causes defoliation and vine dieback resulting in yield loss (Bailey and Jeger, 1992). Abang *et al.* (2002) reported that foliar anthracnose caused by the fungus *Colletotrichum gloeosporioides* Penz. (teleomorph *Glomerella cingulata* (Stonem.) Spauld and Schrenk) is the most severe and damaging disease on yam with water yam (*D. alata* L) being particularly highly susceptible. It is particularly prevalent in the humid forest agro-ecological zone (Green, 1998).

Anthracnoses meaning blackening are diseases of the foliage, stems, tubers and fruits that typically appear as dark coloured spots or sunken lesions with slightly raised rim (Agrios, 2004). Anthracnoses (from anthrax = carbon = black) are caused by fungi that produce conidia within black acervuli (Agrios, 2004a). Severe yield losses in water yam caused by anthracnose infection have been reported from West Africa, the

Caribbean, India and the South Pacific (Winch *et al.*, 1984; McDonald *et al.*, 1998). Leaf necrosis and stem dieback seriously reduce photosynthetic surface area of the crop. Epidemics prior to or during tuber formation result in yield losses up to 85% (Green, 1994).

Mignouna *et al.* (2001) reported that resistant yam cultivars and chemical fungicides could potentially form the basis of sustainable management strategies for anthracnose disease. However, there is growing concern on the possible risks of the use of synthetic fungicides on food commodities to human health (Wilson and Wisniewski, 1994). Frequent use of fungicides could lead to development of resistance within the population of postharvest pathogens (Conway *et al.*, 2004). An alternative candidate to chemical fungicides for the management of anthracnose disease in a wide range of crops is plant extracts. In some instances, the mycelial growth of *Colletotrichum musae* P. was inhibited by *J. curcas* (L) leaves extracts in some banana varieties ('Robusta' 'Rasthali' and 'Ney Poovan') (Thangavelu *et al.*, 2004). Suleiman (2011) reported that *in vitro* application of plant extracts (*Nicotiana tabacum* (L) and *Azadirachta indica* A Jus) for the control of fungi showed that *N. tabacum* (L) at 60% concentration had fungitoxic effect on the mycelial growth of *Aspergillus viridae* and *Penicillium digitatum* Sacc. The inhibitory action of the extracts on the *in vitro* mycelial growth increased with increased concentrations.

1.1. Problem statement

Yam significantly plays a role in rural food security. This security arises from its diversity at farm level, which ensures a diversity of uses, and prolongs seasonal

availability of food and income. Yam has low Glycemic Index which provides a sustain form of energy and gives better protection against diabetes and obesity. It provides Vitmin B6 which breaks down homocysteine. A substance in the body when accumulated leads to heart attack, high blood pressure and stroke (Undie and Akubue, 1986).

Despite the numerous importance of yam, its production is hampered due to yield losses caused by anthracnose in excess of 85 to 90 % (Green, 1994). Several attempts have been made to protect crops with synthetic chemicals but only 0.1% of the synthetic chemicals do actually protect the crop. The 99.9% of it remains in the environment to cause hazardous effects (Pimental, 1995). Even with the 0.1% crop protection by the synthetic chemicals, approximately 10 % yield losses always occur (Wilm, 1998 - 2013). Ghana loses about 40% of its yam production to diseases and pests (Peters, 1999). Yam farmers at a workshop in the Northern Region 2012 reported 10% loss by anthracnose diseases and 50% loss by other diseases such as Nematodes.

1.2. Justification

Considering the negative effects usually posed on the environment and on non-target organisms, there is a public outcry for a paradigm shift from the use of synthetic fungicides to the use of better alternative methods such as plant extracts which are safe, cost effective, and feasible for crop protection (Amadioha, 2000).

The *phorbol ester* fraction from seed oil of *J. curcas* L is a candidate as a plant-derived protectant (Heller, 1996). *Azadirachtin* in *A.indica* A Jus, especially in the

seed kernel and *nicotine* in *N. tabacum* (L) leaf are potent against many fungal diseases. The bitter taste of *A. indica* A Jus is due to the presence of complex compounds called limnoids (triter penoids) (Heller, 1996). Ten limnoids have been isloated and identified in *A. indica* viz., Salannol, Salannol acetate, Diacetyl salanin, 14-Expoxy Azaradion, Gedunin, Nimbine, D-Acetyl nimbenin, Azadirachin and Azadirachtin.

Yam production could increase tremendously if anthracnose of yam is managed to the barest minimum (Nweke, 1996). Yam is a major source of income for small scale farmers. Export of yam produce would increase the foreign exchange earning of yam producing countries.

1.4. RESEARCH OBJECTIVE

1.4.1. Main objective

The study was focused on exploring phytochemical extracts of some selected botanicals (*Jatropha curcas* (L) seed, *Azadirachta indica* A Jus seed and Nicotiana tabacum (L) leaf extracts) to control anthracnose disease of yam.

1.4.2. Specific objective

The following were the specific objectives

-To isolate and identify anthracnose disease causing organism(s).

- To evaluate the efficacy of botanicals *in vitro* and *in vivo*.

- To determine incidence and severity of anthracnose on yam.

- To determine the effect of plant extracts on the vegetative growth and yield of yam.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Domestication of yam

2.1.1. Africa yam

Yams are members of the genus *Dioscorea*, which produce edible tubers. They are monocots, despite occasional evidence of a second cotyledon (Onwueme, 1978). Various species of food yams are cultivated in the tropics and sub-tropics (IITA, 1993). The six most economically important species grown as staple foods in Africa are *D. rotundata* Poir (white guinea yam), *D. cayenensis* Lam (yellow yam), *D. alata* L (water yam), D. *dumentorum* (Kunth) Pax (bitter yam) *D. bulbifera* L (aerial yam) and *D. esculenta* L (Chinese yam) (Onwueme, 1978). These six species constitute over 90% of the food yams produced in the tropics (Hahn *et al.* 1987).

2.1.2. West Africa yam

West Africa is believed to be the home of yams due to the fact that more yams are produced and consumed in this sub-region especially Nigeria. Clearly yams are produced and eaten in three continents: Africa, Asia and South America especially North Eastern parts of Brazil and the Caribbean Islands and South Pacific (Hahn *et al*, 1987). In West Africa, man began to gather yams for domestic use as early as 5000 BC, i.e. during the Paleolithic era (Davies, 1967). Agricultural archaeologists estimate that true yam-based agriculture started in West Africa approximately 3000 BC, the same time it started in South East Asia (Coursey, 1967; Davies, 1967). The earliest domesticated yams in West and Central Africa included *D. rotundata* Poir *D.*

cayenensis Lam and *D. dumentorum* (Kunth) Pax whilst in South East Asia, *D. alata* L was the first species cultivated (Coursey 1967, Onwueme 1978).

Domestication has been a traditional farmers practice in West Africa (Scarcelli *et al.*, 2006). Guinea yams (*Dioscorea cayenensis-rotundata* complex; *D. rotundata* Poir. and *D. cayenensis* Lam.) have been described as resulting from a process of domestication of wild yams of the section *Enantiophyllum* by African farmers (Mignouna and Dansi, 2003). Guinea yams were domesticated about 7000 years ago. White yam (*Dioscorea rotundata* Poir) originated in Africa and is the most widely grown and preferred yam species. Yellow yam (*Dioscorea cayenensis* Lam.) is a native to West Africa and very similar to the white yam in appearance (Agbaje *et al*, 2005). Water yam is believed to be a true cultigen that might have been domesticated in Indo-China from *Dioscorea hamiltoni* and *D. persimilis* (M) (Agbaje *et al.*, 2005). Water yam originated from South East Asia and it is the species most widely distributed throughout the world. In Africa, water yam is second to white yam in popularity (Lebot *et al*., 2005). Bitter yam (*Dioscorea dumetorum* (Kunth) Pax) also called trifoliate, originated in Africa (Lebot *et al.*, 2005).

2.1.3. Yam from other continent/country

The aerial yam *D. bulbifera* L originated both in Africa and Asia and spread to the other parts of the world. It is an important food in South East Asia, Indonesia, the Philippines and in the South Pacific Islands down to New Guinea. The spread of yam, particularly *D. alata* L from South-East Asia to Africa (Hahn *et al.*, 1987) is believed

to have occurred by the intervention of early agriculturalists and more recently by Portuguese and Spanish seafarers (east-west movement).

Although there is an extensive cultivation and use of *D. alata* L in the West Indies, these cultivars appear to have arrived in the West Indies from Africa. The African species, *D. rotundata* Poir and *D. cayenensis* Lam were taken westward to America and have now become important food crops in South and Central America and the Caribbean (Hahn *et al.*, 1987). The Niger and Benue river belts in Nigeria have the largest genetic base in cultivated *D. rotundata* Poir. The *D. rotundata* Poir is however cultivated in the West Africa zone/belt which stretches from west of the Cameroon mountains to the Bandama river in central Cote d'Ivoire (Coursey 1967) and (Hahn *et a.,L.*, 1987).

2.2. Yam and its production

2.2.1. Global production of yam

Yam is a major tuber crop produced and prepared in various forms for consumption (Onwueme, 1978; and Coursey, 1976). It is important to the livelihood of millions of people across the world especially, in West Africa, its major production zone (Onwueme, 1978; and Coursey, 1976). There are over 150 species of yam grown throughout the world. The global annual yam production was estimated to be 25 million Mt in 1974. This increased to 24 million Mt in 1992 and in 1993. It was estimated to be at 28.1 million tons. Out of the total of world's production, 96% came from West Africa with the main producers being; Nigeria, 75, Cote d'voire 8.1, Benin 4.3 and Ghana 3.5% (Opara, 1999). Between the years 1995 and 2000, total world

production increased from 32.7 million Mt in 1995 to 37.5 million Mt in 2000. Between 1990 and 2002 yam production increased from 18 million Mt to estimates of over 39 million (Opara, 1999). More than 95% (2.8 million ha) of the current global area under yam cultivation is in sub-Sahara Africa, where mean gross yields are 10t/h.

Table.2.1. World production and trade in yams (1995-2000).

	2000	**1999**	**1998**	**1997**	**1996**	**1995**
Production Mt	37,532138	37,552,383	35,753,519	34,705,657	33,587,195	32,765,435
Exports Mt	-	23,198	21,080	28,069	27,493	26,264
Exports 1000USS	-	20,077	19,212	20,873	20,810	21,108

Source: (FAO/STAT, 2000)

2.2.2. Production of yam in Africa

Yam is grown as an annual with tubers being planted between February (humid Forest zone) and April (Guinea savanna zone) in West Africa (Ile *et al.*, 2006). Thus, only one crop cycle is possible per annum, restricting supply. Harvested tubers remain dormant for 30 to 150 days depending on the date of harvest, species, and storage environmental conditions (Olayide *et al.*, 1972).

Yam is the most important tuber crop in Africa. It is also the primary agricultural commodity in West Africa (FAO, 2002). The production of yam is about one third of cassava. Over 90% of world yam production occurs in West and Central Africa with Nigeria alone accounting for about 68% of the world's total (FAO, 2002). More than 95% of the world's yam is currently grown in sub-Sahara Africa. In 2000, 96% of the global production of yam was from Africa where Nigeria alone accounts for 70%. More than 95% (2.8 million ha) of the current global are under yam cultivation in the sub-Sahara Africa, where mean gross yields are 10t/h. The domestic market is developed in the yam producing areas making it the main source of cash for a majority of small-scale farmers. Over 600 yam species are cultivated throughout the world but in West Africa, there three main species. They are; white yam, yellow yam and water yam (Nweke *et.al.*, 1991).

2.2.3. Production of yam in Ghana

Yam production has been on the decline despite the increasing demand for both local consumption and for export (Asiedu *et al.*, 1997). Farmers commercially cultivate mainly *Dioscorea rotundata* Poir (26 varieties) and to lesser extent 13 varieties of *Dioscorea alata* L (water yam). A few farmers grow *Dioscore cayenesis* Lam, *Dioscorea bulbifera* L, *Dioscorea dumetorum* (Kunth) Pax, and *Dioscorea esculenta* (L) for home use. The greater yam (*Dioscorea alata* L.) is the most widely distributed species of the genus in the humid and semi-humid tropics (Asiedu *et al.*, 1997). It is an important food in Africa, the Caribbean, and Melanesia. It has considerable social and cultural importance (Coursey, 1976). Table 2.2 presents a summary of percentage production statistics of yam in Africa for 2004. Out of the total percentage production

from Africa, Nigeria alone produced (66%), followed by Ghana (10%), Cote d'Ivoire (8%) and Benin (6%) in Table 2.2 below.

Table. 2.2. Production statistics for yam - 2004

Continent	Country	Percentage
Africa	Nigeria	66
	Ghana	10
	Cote d'Ivoire	8
	Benin	6
	Togo	1
Asia	-	1
South America	-	2
Other SSA	-	4
Rest of the world	-	2

Source: Adapted from FAO/STAT (2005).

Ghana, since the past decade (1999 - 2009) was ranked third in world's yam production (Ennin *et al.*, 2009). Yam ranks second to pineapple among the non-traditional export crops in Ghana and contributes about 16% of the national Agricultural Gross Domestic Product (AGDP) (Ennin *et al.*, 2009). In Ghana, it is a major food widely consumed (FAOSTAT, 2006). Its importance in the Ghanaian society and economy can therefore not be underestimated. However, yam has seen very little improvement in its husbandry practices over time because cultivation is

primarily carried out by small scale farmers using rudimentary hand tools. (MoFA, 2010). All phases of cultivation are labour-intensive, causing the youth to shy away from its cultivation, which limits the scale of production (Coursey, 1967). In Ghana, it is planted at random on mounds constructed with hoes, 1–2m apart. It may be planted on 'the flat', mounds, ridges, raised beds, in trenches or holes in other parts of Africa (Coursey, 1967). It requires well pulverized, loose soil consistent with high organic matter levels, for easy penetration and tuberisation. Acidic soils such as Oxisols, Ultisols and Histosols lead to low productivity (Ezumah, 1986).

Yam, *Dioscorea* spp is a twining herb grown from small tubers and setts. It is a short day (C4) plant that flower at 10 - 11h of daylight. Short daylight favours tuber formation while long daylight favours vine growth. Temperatures ranging from 25 to 30 ^{0}C are required (Visayas Consortium for Agriculture and Resources Program, 1987). Tuberisation starts 10 weeks after planting. Yam grows for 6 - 10 months and is dormant for two to four months depending on the species (Mignouna and Dansi, 2003). These two phases usually correspond to the wet and dry seasons. It requires an annual rainfall of over 1,500 mm throughout the growing season (Hiyane and Haley, 1977). Senescence of the main shoot is a sign of readiness for harvesting Hiyane and Haley, 1977). (White, yellow and water yams normally produce a single large tuber, often weighing 5-10kg annually (Hiyane and Haley, 1977).

2.3. Yam commonly grown in Ghana

There are over 600 yam species grown throughout the world, but in West Africa the most economically important species are the White yam (*Dioscorea rotundata*

Poir),Yellow yam (*Dioscorea cayenensis* Lam) and Water yam (*Dioscorea alata* L). Species predominantly grown throughout Ghana are the white yams (*D. rotundata* Poir), Bulbil bearing yams (*D. bulbifera L*), yellow yam (*D. cayenensis* Lam and *D. alata* L. These cultivars have significant differences that separate them into varieties.

White yam (*Dioscorea rotundata* Poir) originated in Africa and is the most widely grown and preferred yam species. The tuber is roughly cylindrical, with smooth and brown skin, and usually white and firm flesh. Yellow yam (*Dioscorea cayenensis* Lam.) derives its common name from its yellow flesh and is a native to West Africa and very similar to the white yam in appearance (Mignouna and Dansi, 2003). Its yellow flesh is caused by the presence of carotenoids. Mignouna and Dansi (2003) reported that it has a longer period of vegetation and a shorter dormancy than white yam. Water yam (*Dioscorea alata* L.) originated from South East Asia. It is the species most widely spread throughout the world. In Africa, it is second only to white yam in popularity (Lebot *et al.,* 2005). The tuber shape is generally cylindrical with white and "watery" flesh. Bitter yam (*Dioscorea dumetorum* Kunth Pax) originates in Africa where the wild cultivars also exist. It is characterized by the bitter flavour of its tubers (Mignouna and Dansi, 2003). Another marked undesired feature is its flesh hardens if not cooked and eating immediately after harvest.

The common (local) names of some of these varieties in northern Ghana are; kpagaa "laribako" "pono" "dundubanza" "chenchito" "sola" and "tantapurika" (Kumar *et al.*, 1979). In southern Ghana, these same varieties are called "Bayere fufuo" (Twi); "Etwo" (Fante); "Ete" (Ewe); and "Yele" (Ga).

2.4. Economic importance of yam

Besides the importance of yam as food source, yam also plays a significant role in the socio-cultural lives of some producing regions. In some parts of Southeastern Nigeria, the meals offered to the gods and the ancestors consist principally of mashed yam (Arinze, 1970). Yams store relatively longer in comparison with other tropical fresh produce and, therefore, stored yam represents stored wealth which can be sold all-year round by the farmers (Coursey, and Coursey, 1971). In parts of Igboland in Southeastern Nigeria, it is customary for the parents of a bride to offer her yams for planting as a resource to assist them in raising a family (Arinze, 1970).

Yam is a predominant source for the hemi synthesis of birth control pills in the medical field (Crabbe, 1979). *D. delatoidea* is used for producing shampoons in India. Also, due to its lower Glycemic Index than potato products, it provides a more sustained form of energy and gives better protection against obesity and diabetes (Undie and Akubue, 1986). The by-products such as the yam peels are fed to livestock preferably in the dried form which is said to have low nitrate compounds (Crabbe, 1979). In Nigeria, yam is part of their cultural heritage, and plays a key role in their religious ceremonies (Arinze, 1970). The new yam festival marking the onset of harvest period is an outstanding social event almost everywhere in the yam growing belt of West Africa (Coursey and Coursey, 1971).

In Ghana, agriculture is a tool for economic development, growth and poverty reduction. Over 60% of the population is engaged in agricultural production for food security, feed for livestock, export, income and wealth creation, and yam is among the top most crops cultivated (Yidana, *et al.*, 2006). Apart from carbohydrate, yam is also

a source of vitamin C, dietary fibre, vitamin B_6, potassium and manganese (Kochlar, 1981).

2.4.1. Contribution of yam to the Ghanaian Economy

Yam among other root and tuber crops contributes a significant role in the Ghanaian economy over the years. Agriculture contributes about 34% to Ghana's GDP (MoFA 2010). Roots and tubers alone contribute about 50% of agricultural GDP (MoFA, 2010). About 55% of farmers in Ghana grow root and tuber crop which is a major poverty reduction strategy for the country (The Project Team, 2013). Root and tuber crops are food security crops in Ghana and are consumed between 16% and 31% of per capita daily calorie consumption in the country (The Project Team, 2013). Yam production is a source of employment and poverty alleviation (The Project Team, 2013).

2.4.2. Nutritional and Medicinal importance of yam

Yams are nourishing plants in the diet of many inhabitants of inter-tropical region. Yams are major source of carbohydrates, minerals of iron, calcium, phosphorus, and vitamins such as thiamin, riboflavin and vitamin B and C. Yams are largely carbohydrate and are one of the cheapest sources of carbohydrate to mankind (Kochlar, 1981). Vitmin B6 is needed by the body to break down substance called homocysteine which can directly damage blood vessel walls. High levels of homocysteine can lead to heart attacks, high blood pressure and stroke. Some species of yam have been used medicinally to treat diseases like diabetes mellitus, coronary disorders and in preventing hypercholesterolemia (Undie and Akubue 1986). Yams have a sustained form of energy which makes it safer source of carbohydrate for

diabetes. It is a good source of manganese. Extracts from *D. detoidea* is used to produce anti-lice shampoo in India. Yam Contains a steroid sapogenin compound known as diosgenin, which is extracted and used as base for drugs such as cortisone and hormonal drugs. Some yam species contain alkaloids such as; dioscorine $C_{13}H_{19}O_2N$ and steroid derivatives. Table 2.3 below gives a summary ofnthen nutrient content of yam species per 100g edible tuber proteins.

Table.2.3. Nutrient content of yam species (*Dioscorea* spp.) per 100-g edible tuber proteins

	***D.* spp.**	***D. alata***	***D. bulbifera***	***D. cayenensis***	***D. esculenta***	***D. rotundata***
Water (ml)	69	65-76+	71-(79)	80	70-74+	80
Calories	119	135-87	112-(78)	71	112-102	71
Protein(g)	1.9	2.3-1.9	1.5-(1.4)	1.5	3.5-1.5	1.5
Fat(g)	0.2	0.1-0.2	0.1-(0.2)	0.1	0.1-0.2	0.1
Carbohydrate(g)	27.8	31-20	26-(18)	16	25-24	16
Fibre(g)	0.8	1.5-0.6	0.9-(1.2)	0.6	0.5-0.6	0.6
Calcium(mg)	52	28-38	69-(40)	36	62-12	36
Phosphorus(mg)	61	52-28	29-(58)	17	53-35	17
Iron(mg)	0.8	1.6-1.1	(2.0)	5.2	0.8	5.2
Vitamins	-	-	-	-	-	-
Carotene equiv.(ug)	10	10-5	-	-	-	-
Thiamine(mg)	0.11	0.05-10			0.10	
Riboflavin(mg)	0.02		0.03-0.04		0.01	

Source: (Opara, 1999).

2.4.3. Alimentary and Traditional uses of yam

The food value is based on the carbohydrate, protein, amino acids, vitamin and mineral content of the tuber (Kochlar, 1981). Based on this, different food preparations are prepared in combination with other foods. In Guyana, cultivars whose tubers contain anthocyanin are used to prepare beer (Grenand, 1980). In the Philippines, the most popular preparation is ice cream made with purple *D. alata* in addition to "guinatan" jellies and yam based candies (Brown, 1951). In their wild state, the *Dioscorea* are part of the diet of omnivorous and vegetarian burrowers of the humid tropics (Grenand, 1980). Yam peels are more commonly used today to feed livestock on family-run farms. Yam can also be processed into flour, crisp, chips or cubes and flakes in addition to common "ampesi" and "fufu", a traditional preparation of elastic, jellified, very dense paste that is obtained using pestle and mortar (Onyia *et al.,* 1987).

2.4.4. Non-Alimentary uses of yam

The non-alimentary uses of yam include the use of its toxic products for hunting. Wild species like *D. dragena*, *D. rupicola* and *D. piscatorum* are used for arrow poisoning and bait. The alkaloids, saponins that they contain have convulsive, paralysing or haemolytic effects. Certain yams are also used in human toxicology, such as an ordeal poison in criminal poisoning. Chevalier (1936) mentions *D. latifolia var contralatrones* as a type of repellent in plots of edible homomorphic cultivars. The toxicological properties of some yams used as insecticides for example are comparable to those of rotenone. For example *D. piscatorum* is used to protect rice in Malaysia and *D. deltoidea* is used for producing anti-lice shampoos in India. Yam contains

hormone diosgenin and has therefore become the predominant source for birth control pills (Crabbe, 1979).

2.4.5. Contribution of yam to employment in Ghana

Yam production is a major source of employment to Ghanaians nationwide. About 55% of farmers in Ghana grow root and tubers (The Project Team, 2013). About 90% of farmers who market and process root and tuber crops into several forms are women (The Project Team, 2015). The Government of Ghana has therefore recognized the major contributions of root and tuber crops in terms of employment generation and poverty alleviation and has formulated a policy targeted at root and tuber crop improvement as one of the key drivers of growth in the national economy through the value chain approach. Yam production is also a major source of income to farmers (The Project Team, 2015).

2.5. Propagation of yam

Yam sett is capable of sprouting from many points of the peel of the body starting from the head region, followed by the tail (Coursey, 1967). Sprouting from the immature tissue is usually delayed due to the time required for new bud formation (IITA, 1998). Sett from the middle section of the tuber is placed third in order of sprouting ability (Coursey, 1967).

According to Aighewi *et al.,* (2015). There four main ways of propagating yam and they are:

1. Tubers: The planting material (sett) should be between 400 and 500g. The setts can be pre-germinated in moist sawdust or coconut coir before planting. Three types of

setts can be obtained from the tuber; the head, middle and the tail. The head sett gives better sprout and emergence when planted because of the presence of the primary nodal complex which gives rise to the new plants.

2. **Seeds:** It is the use of true seeds found on vines of yam plants such as Cush-cush for propagation.
3. **Cuttings:** Cuttings are also used as propagative materials. Basal vine cuttings of 6– 8 cm pieces are best.
4. **Tissue culture:** Used to produce "clean" plantlets, that is free from diseases.

2.6. Sprouting process

Sprouting is a physiological process which demands a lot of energy. The sprouted tuber loses a lot of dry matter moisture and continues to deteriorate as the vine grows. According to Orkwor and Ekanayake (1998) long shelf life of healthy yam tubers could be achieved if sprouting process is delayed by prolonging dormancy. A yam tuber if cut into setts is capable of sprouting from many points of the peel of the tuber starting from the head region followed by the tail. Sprouting from fleshy tissue is usually delayed, due to the time required for new bud formation (IITA, 1998). Sprouting in most cultivated *D. rotundata* Poir starts through the growth of single bud at the apex due to apical dominance. The middle section comes third in order of sprouting ability (Coursey, 1967).

The point of sprout is normally whitish and delicate after which a purplish or whitish or cream colour, depending on the cultivar, appears on the young vine (Onwueme, 1973). The point of sprout grows a curved scale-leaf that eventually develops into a protective structure. The scale-leaf then stimulates the sequence of events involved in

the formation of subsequent leaves. Successful sprouting of yam takes place within one to two weeks.

2.7. Factors affecting the sprouting of yam tubers

2.7.1. Physiological age

The readiness with which a yam tuber sprouts depends on the physiological age of the tuber or how long the tuber has stayed in the soil or harvested (Onwueme, 1975). When yam tubers are harvested at the same time and planted at different times the duration each will take to sprout would vary. The tubers that would be planted earlier, would requiring a very long time to sprout, while progressively later plantings would require relatively shorter time to sprout due to the difference in their physiological age or dormancy period after harvest (Onwueme, 1975).

2.7.2. **Quality of tuber**

Any bruises or wounds on the tuber at harvest may render the tuber liable to infection by microorganisms such as fungi and bacteria which can cause tuber rot (Otoo, 1984). Transportation of yam from the farm after harvest or from place to place can render the yam tuber susceptible to disease infection. Nematodes (*Scutellonema* and *Melodogyne*) which infect tubers in the field before harvest can subsequently affect sprouting (Otoo, 1984).

2.7.3. Dormancy

Dormancy is a physiological resting period of yam during which sprouting is suppressed which is important in cultivation (Coursey, 1967). The yam tuber becomes resistant to infection when it is dormant (Coursey, 1967). After the period of

dormancy, tubers become more susceptible to infection (Passam and Noon, 1977). The ability to break yam dormancy early gives farmers the opportunity to grow two crops of early maturing varieties in a year where there is a relatively long period of the rains (Coursey, 1967). Dormancy in yams has duration from 28 to 180 days depending on the species and time of harvest. The average duration is 75 to 100 days where the dry season is very short (Coursey, 1967). Moisture loss occurs during dormancy, perhaps around 10% (Coursey, 1967). In 150 days, storage at 24-28 °C and at 70-90 % relative humidity, crude protein and starch levels fell as well as the vitamin C content (Coursey, 1967).

2.7.4. Part of the yam planted

Yam tuber, if cut into setts, is capable of sprouting from many points of the peel of the body starting from the head region, followed by the tail. Sprouting from the immature tissue is usually delayed, due to the time required for new bud formation (IITA, 1998). Setts from the middle section of the tuber come third in order of sprouting ability (Coursey, 1967).

2.7.5 Temperature

Temperature affects sprouting in yam (Onwueme, 1975). The optimum temperature for tuber sprouting is between 25 and 30 °C (Onwueme, 1975). Any appreciable change more than 5 °C below or above this range delays sprouting (Onwueme, 1975). This explains the significance of mulching the mounds after planting during hot season.

2.7.6. Soil moisture

Lack of soil moisture does not affect the rate of bud formation or sprout on the tuber rather the subsequent elongation of the bud is slowed by moisture stress (Onwueme, 1976). Tubers have long been known to sprout even in storage. Setts planted in dry sawdust, dry soil and on dry paper sprouted as readily as moistened setts (Onwueme, 1976). However, setts that sprouted under dry conditions tended to produce several more sprouting loci than those sprouted in moist media. However, buds produced by setts under dry conditions remain relatively un-elongated, unless moisture is supplied (Onwueme, 1976).

2.7.7. Oxygen

Sprouting of yam setts require sufficient oxygen (Onwueme, 1982). Sprouting was a process related to living cells and required an expenditure of energy by these cells by the process of oxidation (Mayer and Poljakoff, 1995). Lack of oxygen results in anaerobic respiration and metabolic processes will be depressed (Mayer and Poljakoff, 1995).

2.7.8. Soil drainage

Yams cannot tolerate water logging to any appreciable extent and it is therefore, important that soil be well drained (Onwueme, 1982). Tuber rot occurs as a result of poorly drained soils and the resultant water logging causing the roots to be poorly aerated. This could lead to high loss of the planting material and the whole plant if the poor drainage exist for a relatively long period of time.

2.8. Capping

It is the process of covering the top surface of the yam mounds just after planting with any biodegradable material to reduce the intense heat of the sun which could cause rot in the planting material. This also promotes percentage sprout, prevents soil erosion, and increases the moisture and the organic matter content of the soil (Igwilo, 1989).

2.9. Staking

In the cultivation of climbing crops, the use of suitable supports in the form of stakes or trellises, is very important. These supports help to expose the leaves of the crops to sunlight for optimum photosynthesis (Igwilo, 1989). According to Igwilo (1989) staking increases the tuber yield of yam. In Nigeria, staking increased tuber yield by 34-105% over unstaked yams (Ndegwe *et al.*, 1990).

2.10. Harvesting of yam

Yam harvesting is a labour intensive operation that involves standing, bending, squatting, and sometimes sitting on the ground depending on the size of the mound, size of tuber or depth of tuber penetration (Onwueme and Charles, 1994). Harvesting is done by hand using sticks, spades or diggers. Mechanical yam harvesting has been reported, especially for *D. composita* tubers for pharmaceutical uses (Nystrom *et al.*, 1987). It is still limited to research and demonstration purposes. The use of a potato spinner has been suggested for harvesting species which produce a number of small tubers (Onwueme, 1997).

Onwueme and Charles (1994) reported that yams can be harvested once or twice during the season to obtain early ('topping', 'beheading', and 'milking') and late harvest. Double harvesting is most applicable to short term varieties of *D. rotundata* Poir, *D. Cayenensis* Lam and *D. alata* L). According to Onwueme and Charles (1994) single harvested tubers had better eating quality than the double-harvested tubers. However, double harvesting has the advantage of making the new crop available for consumption earlier in the season and also produce excellent planting material at the second harvest.

2.11. Yield losses at harvest

Physical damage occurs to tubers during harvesting. Many also get deformed during growth as a result of the obstacles they encounter. These tubers are usually down-graded (Onwueme and Charles,1994).

2.12. Post harvest losses

These are losses incurred during storage caused by rot, tuber respiration and sprouting. There is also an appreciable loss of moisture from the tuber. During storage, the tubers are subject to losses of up to 50 % of the fresh matter (Morse *et al.*, 2000). Here, the losses due to microbial attack play a predominant role (Morse *et al.*, 2000). The fungal pathogens penetrate wounds caused by insects, nematodes and poor handling before, during and after harvest. Morse *et al.* (2000), revealed that most of the yam rot induced by fungi in specialized barns near Idah, Kogi State, Nigeria were predisposed by insect attack by mainly storage beetles (*Coleoptera*), mealy bug (*Planococcus citri*) and scale insect (*Aspidiella hartii*) during storage.

2.13. Yam diseases and their management

According to Amusa *et al.* (2003), different diseases are associated with yam both on the field and at storage (Wood *et al.*, 1980). Anthracnose is considered as the most widely spread of all the field diseases, while yam mosaic virus disease is considered to cause the most severe losses in yams (Amusa *et al.*, 2003). Dry, soft and wet rots cause both qualitative and quantitative tuber losses in storage. Dry rot is the most devastating of all the storage diseases of yam (Amusa *et al.*, 2003).. Dry rot of yams alone causes significant reduction in the quantity and quality of the edible portions of tubers in stored yams as high as 80 to 100% (Adesiyan *et al.*, 1975). Management strategies adopted for field diseases include the use of crop rotation, fallowing, planting of healthy material, use of resistant varieties and destruction of infected plants. Storage diseases could be managed the use of Tecto (Thiabendazole), wood ash before storage and processing of yam into chips or cubes (Amusah *et al.*, 2003).

2.14. Categories of yam rot

2.14.1. Soft rot

Yam tubers infected with soft rot usually have soft tissues and ramified by the fungal mycelium. The tissues then become brown, soft and at times wet due to a rapid collapse of the cell walls. The fungi associated with this type of rot are *Rhizopus* spp., *Mucor cinelloides* (F), *Scleratium .rolsii* (Curzi), *Rhizoctonia solani* (Kuhn) and *Armillarlia mellea* (Vahl: Fr) (Ikotuni, 1983; Green *et al.*, 1995; Amusa and Baiyewu, 1999).

2.14.2. Wet rot

It is characterized by the oozing of whitish fluid out of the tissue when pressed. This symptom is usually associated with bacterium, *Erwinia crotovor pv, carotovora* (IITA, 1993; Amusa and Baiyewu, 1999).

2.14.3. Dry rot

The symptoms of dry rot vary in colour depending on the invading pathogen. Infected tubers usually become hard and dry. Tubers infected with *Penicilim oxalicum* and *Penicilim cyclopium* (Var), become brown, hard and dry maintaining their integrity (IITA, 1993). Greenish mecylia covers the tissues if it is infected with *Scleratium marcescens* (Curzi). When tubers were infected by *Aspergilus niger* (A) and *A. tamari*, such tissues subsequently become brown with yellowish margin. *Rosellinia bunudes*, and *Lasiodidodia theobroma* (Pat), have been reported to cause dry black rot. They infected tubers first turn grey and then black. Such tubers become weak and break into smaller dry pieces (IITA, 1993).

The yam nematodes, *S.tellonema bradys* has been found to be the cause decay of yam tubers known as “dry rot disease” (Adesiyan *et al.*, 1975). The initial stage of the nematode dry rot consists of cream and light yellow lesions below the outer stem of the tuber (Adesiyan and Odihirin, 1975). No external symptoms are found at the stage. The disease then progresses and spreads into the tuber, normally to a maximum depth of 2 cm but sometimes deeper (Adesiyan *et al.*, 1975). In the later stages of dry rot, infected tissues first become light brown and then turn brown to black. External cracks appear on the skin of the infected tubers and parts can flake off exposing patches of

dark brown, dry rot tissues. The severe symptoms of dry rot are seen in matured tubers especially, during storage when it is often associated with generally decay of tubers (Adesiyan *et al.*, 1975).

2.15. Management of fungal diseases

Botanicals are extracts obtained from certain plants such as *Azadirachta indica* A Jus, *Jatropha curcas* L, *Carica papaya*, and *Nicotiana tabacum* and used as antipathogenic on a wide range of plants diseases (Amadioha, 2000). Plant extracts are non-phytotoxic, environmental friendly, biodegradable, relatively easily prepared, readily available and most importantly, cost effective (Amadioha, 2000). They have the ability to control plant pathogens and therefore the potential to be valued in plant disease management.

2.16. Epidemiology of anthracnose (incidence, distribution and control)

Environmental factors play major role in the development of disease epidemics. The relationships among rainfall intensity, duration and crop geometry and the dispersal of inoculum possibly lead to different levels of disease severity (Dodd *et al.*, 1992) and (Eni *et al.*, 2008, 2009). Temperature often interacts with other factors, such as leaf surface wetness humidity, light or competitive microbiota (Pallem *et al.*, 2012). Temperatures around 27°C and high humidity (a mean of 80%) are optimum for anthracnose disease development (Roberts *et al.*, 2001). *C. gloeosporioides* Penz infects leaves, petioles and blooms during periods of prolonged leaf moisture and high humidity (Pallem *et al.*, 2012).

Colletotrichum species utilize diverse strategies for invading host tissues, which vary from intracellular hemibiotrophy to subcuticular intramural necrotrophy (Bailey and

Jeger, 1992). *Colletotrichum* species produce a series of specialized infection structures such as germ tubes, appressoria, intracellular hyphae, and secondary necrotrophic hyphae (Perfect *et al*., 1999). Control measures for *C. gloeosporioides* Penz could be cultural, chemical (synthetic or botanicals), host resistance and treatment of planting material.

2.17. **Dispersal and life cycle of anthracnose of yam**

Colletotrichum gloeosporioides Penz colonizes dead twigs and injured plant tissues and forms an abundance of acervuli and conidia (Dodd *et al*., 1992). Conidia can spread over relatively short distances by rain splash or overhead irrigation (Dodd *et al*., 1992). Ascospores are airborne and important in long distance dispersal (Hong and Hwang, 1998). Conidia that come in contact with leaves, twigs, and fruit germinate to produce appressoria and quiescent infections which result in tissue necrosis. This necrotic tissue is subsequently colonized; acervuli are formed, thus completing the life cycle of *C. gloeosporioides* Penz (Dodd *et al*., 1992). Dead wood and plant debris are primary sources of inocula. It also survives on or in yam tubers (Dodd *et al*., 1992). Fruits with quiescent infections remain symptomatic up to harvesting. Injuries and tissues weakened by other factors cause further development of quiescent infections to form lesions during the post-harvest period (Brown, 2000).

2.18. Symptoms analysis and study of symptoms of yam anthracnose disease

Anthracnoses, meaning blackening, are diseases of the foliage, stems, or fruits that typically appear as dark coloured spots or sunken lesions with a slightly raised rim (Agrios, 2004). Some cause twig or branch dieback. In fruit infections, it often has a

prolonged latent stage. In some fruit crops, the spots are raised, with corky surfaces. It usually results in fruit drop and rot. Anthracnoses (from anthrax = carbon = black) are caused by fungi that produce conidia within black acervuli (Agrios, 2004).

Visual inspection to detect the presence of anthracnose of yam is made relatively easy when symptoms characteristic of the disease are clear (Agrios, 2004). However, many factors such as pathogen strain, host plant variety, time of infection and the environment can influence the symptoms exhibited by plants. It has been recorded on a wide range of hosts (Agrios, 2004). Symptoms vary with respect to the species of yam. It is reported to be most common in *D. alata* L. The symptoms are reported to be very prominent in leaves of susceptible varieties during the rainy season (Dodd *et al.*, 1992). Vine necrosis and superficial blackening also occur as symptoms.

Amusa (1991, 1997) reported that symptoms appeared at first as small dark brown or black lesion on the leaves, petioles and stems of yam. 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. The withered leaves and stem dieback gave the plant a scorched appearance hence the name 'scorch' disease. This immensely affects the photosynthetic activities of the plant and subsequently the yield (IITA, 1993).

According to Amusa (1997) that yam anthracnose is a disease complex, which has been associated with the activities of *Colletotrichum gloeosporioides* Penz, *Curvularia pallescens* (Boedijn), *Curvulariaer agrostides* (Hem), *Pestalotia* sps and *Rhizoctonia solani* (Kuhn). These associated fungi were also found to induce necrotic lesions of

varying sizes on the leaves of *D. alata* L. Simon (1993) reported that many of the symptoms attributed to yam anthracnose in Nigeria are probably caused by *Rhizoctonia solani* often in the absence of *C. gloeosporioides* Penz. However, Winch (1981) reported that interaction existed between *C. gloeosporioides* Penz and *Rhizoctonia solani (*Kuhn) in anthracnose disease complex of yam.

2.19. Other pathogens associated with Anthracnose disease complex

According to Kutama *et al.* (2013), the causal agent of anthracnose, *C. gloeosporioides* Penz, was isolated from yam leaves with foliar lesions in over 96% of all locations surved/sampled. The pathogen was also isolated from asymptomatic tissue. This suggests that the pathogen, as well as being the primary cause of anthracnose, might exist as an endophyte in yam tissue. Other pathogens such as *Colletotrichum capsici*, *Phoma* sp., *Curvularia* spp. *Cercospora apii* and *Fusarium* spp. were isolated in over 50% of yam lesions. These pathogens, along with *C. gloeosporioides* Penz, are generally regarded as pathogens associated with the anthracnose complex (IITA, 1993); *Curvularia* sp., *Phoma* sp. and *Cercospora apii* found associated with leaf symptoms were distinct from yam anthracnose. There was also non-anthracnose-type lesions found on yams throughout Ghana (IITA, 1993). Die-back was also associated with the anthracnose complex (Dodd *et al.*, 1992). Other forms of die-back were found to be associated with *Fusarium* spp. and *R. solani*. Infected plants become necrotic from the base upwards, in contrast to the anthracnose-type where plants become necrotic from the shoot tips downwards (IITA, 1993).

2.20. Economic importance of yam Anthracnose disease

Anthracnose disease causes serious yield loss on *D. alata* L throughout the world. In Nigeria, it is considered the single most important factor responsible for the decline of yam production. It might cause yield lost in production of up to 80% in Nigeria (Okigbo, 2005). Epidemics prior to or during tuber formation can result in yield losses up to 85 t0 90% (Green, 1994). Farmers in the Northern Region (Tamale) reported 10% loss at workshop. Farmers in most yam growing areas of the world no longer rely on the effectiveness of benzimidazole and related fungicides for the control of yam anthracnose due to the development of fungicide resistant *C. gloesporioides* Penz strains (McDonald and Linde, 2002).

2.21. Symptoms of infection

2.21.1. Above ground symptoms

Variations in symptoms are attributed to many factors, namely the age of the leaf, variety of yam and amount of rain (Milgroom, 2003). Mild infection produces very small brown spots on young leaves which become larger as the leaves grow to full size (Milgroom, 2003). Spots may coalesce to form large irregular blotches with a hole in the centre. Infected leaves finally fall off the plant. Long periods of rain favour epidemics of the disease because the fungal spores (conidia) are spread by rain splash (Milgroom, 2003). Young foliage is more susceptible to anthracnose. Therefore, if periods of high rainfall coincide with the stage of crop development, that is where a lot of young leaves are present, the disease can quickly spread throughout the crop (Milgroom, 2003).

2.21.2. Below ground symptoms

According to Milgroom (2003), dieback on young plants results in survival of few of the lower leaves which ends in producing no, or poor yield. New shoots sometimes grow from the planting piece of dead plants and become multi-stemmed in contrast to uninfected plants which usually have one or two stems. Each stem produces a tuber, therefore, affected plants may have several small tubers instead of the normal one or two (Milgroom, 2003). Anthracnose is systemic which usually has effect on the stem, root and tuber once the leaf is infected, though reported a most serious foliar disease of yam (Abang *et al.*, 2006).

2.22. Management of yam anthracnose disease

Extensive studies have been done on the control of anthracnose and other yam diseases and various management strategies have been recommended.

2.22.1. Chemical control methods

Chemical control is costly and difficult to apply. Weekly benomyl treatments alternating with applications of copper, dithiocarbonates or daconi have been tested. Resistance to benomyl is reported (Kutama *et al.*, 2011). Fungicides can delay the onset of epidemics, but cannot prevent them developing during the rainy season. Foliar sprays should be applied before symptoms of anthracnose appear in the crop, and weekly during the growing season (Kutama *et al.*, 2011).

Fungicides usually became ineffective in controlling the disease during the heavy rains. Explanations for the failure of chemical control include infrequent or poorly

timed applications (because of costs of chemicals, labour, and machinery), heavy rain washing fungicides off leaf surfaces, and the possible existence of fungicide-resistant strains of *C. gloeosporioides* Penz (Kutama *et al.*, 2011). This has consequently led to the growth of the disease, sometime reaching epiphytotic stage. Epidemiological studies have subsequently confirmed that high yields can be obtained if Weekly benomyl treatments alternating with applications of coper, dithiocarbonates or daconi spray programme is used to delay the onset of anthracnose until after root bulking (Sweetmore *et al.*, 2002).

2.22.2. Cultural control methods

Cultural control methods include; planting barrier crops, removal of weeds or alternative hosts, avoiding tuber damage at harvest, early staking, crop rotation and ploughing in plant residues immediately after harvest (Odu *et al.*, 2006). Early planting can also delay the development of anthracnose (Odu *et al.*, 2006). Early emerging yams have time to establish a canopy before the onset of weather conducive to disease development therefore; the impact of anthracnose would be mild. Mature leaves of 'White Lisbon' are known to be more resistant to anthracnose than intermediate or juvenile ones (Odu *et al.*, 2006).

According to International Society for Tropical Root Crops (1994), held in Salvador, Bahia, Brazil, October 23-29, 1994, no individual cropping practice or control measure was effective in eliminating yam anthracnose disease during the growing season, but particular combinations of cultural practices and environmental conditions

helped reduce disease development. Low rainfall and use of healthy planting material, for example, were factors of critical importance in controlling anthracnose.

2.22.3. Selection of planting setts

Tubers should be selected from uninfected plants and stored in a cool dry place during dormancy. Setts should be carefully inspected and any showing areas of rot rejected. The setts should be treated with a broad-spectrum fungicide to eliminate surface-borne fungi and cutting knives should be treated frequently with bleach (Amusa, 2001).

2.22.4. Use of resistant varieties

Some varieties of yam differ in their resistance to anthracnose disease. Some are highly resistant at all stages of growth. Others show good resistance only when leaves are mature and a full leaf canopy have formed. In some resistant varieties, infection does not occur until late in the life of the crop (McDonald and Linde, 2002). In this case, there may be dieback of young shoots, leaf curling with or without discolouration of vines on lower surfaces and infections of leaf stalks causing otherwise healthy mature leaves to fall off. Growers should be encouraged to select tubers from plants showing resistance and to use only these for propagation and increase of stock (McDonald and Linde, 2002).

2.22.5. Tracking pathogen populations

Yam is vegetatively propagated and planting materials, and yam tuber which are important source of *C. gloeosporioides* Poir inocula, are frequently exchanged within and across national borders in West and Central Africa (Mignouna *et al.*, 2002). Slow

growing grey (SGG), fast growing salmon (FGS), and fast growing grey (FGG) strains of *C. gloeosporioides* Poir have been described from yam in Nigeria. Only the SGG form has been observed to cause defoliation and premature death of inoculated plants (Mignouna *et al.*, 2002). The threat of the spread of the aggressive SGG strain in this region must be urgently tackled not only on account of the virulence of this strain, which appears to be linked to its ability to produce highly toxic metabolites (Abang *et al.*, 2004) but because of its epidemiological significance. Molecular differentiation of SGG and FGS populations using genetic markers will facilitate epidemiological studies (example, genotype tracking) as well as assist breeders develop improved strategies for resistance breeding against both pathogens populations (Mignouna *et al*, 2002).

Various viruses, bacterial and fungal diseases of yam have been reported in Nigeria since 1956. Pre- harvest and post -harvest tuber rot may result in storage loss of 25%. Pre-harvest rot is induced by the bacteria, *Corymebacterium* sp, *Erwinia* sp and the fungus *Botryodiplodia* sp. While the post harvest rot is caused by *Fusarium* sp, *Aspergillus sp*. amongst the foliar diseases described anthracnose can depress yield by 67% and 'Appollo' may result in total loss of planting seed. Storage losses have been controlled by carefully handling of the tubers and selections for resistant cultivars may help to control the other diseases (Mignouna *et al.*, 2002).

2.23. Isolation and identification of *C. gloeosporiodes*

Ayodele *et al.* (2004), cut pieces of sampled infected plant tissues and plated them on solidified Potato Dextrose Agar (PDA) using a pair of sterilized forceps. Incubation

was done at 28°C for four to seven days. Identification of isolates in pure cultures was done by observing cultural and microscopic features (photographs) as reported by Joseph *et al.* (2004), Sutton (1980) and Terna (2010). *Glomerella,* the teleomorph, of *C. gloeosporioides* Penz was isolated and identified on samples of yam leaves with typical anthracnose symptoms (Winch *et al.*, 1984). The leaves were collected and incubated under ambient laboratory conditions in large Petri dishes lined with moist sterile paper tissues.

According to Sutton (1992), *C. gloeosporioides* isolates were isolated from naturally infected fruits of papaya obtained from the field. Pieces of the infected fruits were cut from the advancing margin of the lesion, surface sterilized in 5% sodium hypochlorite solution and washed in three changes of sterilized distilled water. The cut tissues were blot dried on sterilized filter paper and then plated on PDA and incubated for seven days at 28^0C. The plates were then observed every 24h for seven days. Fungal colonies that emerged were transferred to fresh plates to obtain pure cultures. The fungal mycelium was then examined under a dissecting and compound microscope and identified by comparing their morphological and cultural distinctiveness with photographs (Sutton, 1992).

The tissue transplanting technique is another possibility of isolating a causal agent from infected plant parts (Nagel, 1934). The diseased plant parts were cut at the advanced margin of lesions into small pieces (5 × 5mm) and then surface disinfected with 10% Clorox for 1 minute followed by rinsing with sterilized distilled water two times and transferred onto isolating water agar (WA) medium. The mycelia growing

out of the plant tissue were sub-cultured onto PDA and incubated at room temperature 28 - 30°C for seven to ten days. Single spore isolation was performed to obtain pure cultures. The isolate was identified to species by morphological observation under a compound microscope.

Nagel (1934) isolated *Cercospora beticola* (Var) causing leaf spot disease of sugar beet by standard tissue isolation technique. Dange and Patel (1968) isolated *C. beticola* (Var*)* from naturally diseased spinach beet plants by standard tissue isolation method. Mallappa (2007) isolated *C. nicotianae* Ell.and Eve. from tobacco by tissue isolation technique and proofed pathogenicity.

Diseased portion of tomato fruits were scrapped with the aid of scalpel, and plated on PDA. The plates were incubated at room temperature and daily observations were made. The isolates were sub-cultured to obtain pure culture. The fungi isolates were identified under the microscope, using lactophenol in cotton blue as the staining agent based on morphological characteristics (Bernett and Hunter, 1992, and Altier and Thesis, 1995).

2.24. Cultural and morphological characteristics of *Colletotrichum gloeosporioides*

Stanley *et al.* (1998) reported that the optimal growth temperature of *C. gloeosporioides* Penz isolates from almond and avocado are 20 – 22 °C and 26 – 28 °C, respectively (Pallem *et al.*, 2012). Bem (2013) reported that cultures of *C. gloeosporioides* Poir exhibit white, grey, dark orange and pink-grey colours, while the

reverse sides of the colonies were white, dark grey and orange. The conidia are cylindrical with both apices rounded or with one apex rounded and the other end pointed (Swart, (1999). The growth patterns of *C. gloeosporioides* Poir also range from concentric with distinct rings to growth with less pronounced rings. Mycelia colour varied among isolates, namely grey, white, dark brown, orange, sometimes fluffy while some produced scanty mycelia. The fungus sporulated producing abundant and visible fruiting bodies with submerged acervuli. Some isolates sporulated superficially with scattered acervuli while in some isolates; the sporulation was both superficial and submerged. The growth rate also differed. Some isolates were fast growing while others were slow growing. Stanley *et al.* (1998) reported that the morphology of *C. gloeosporioides* Penz isolates from almond in culture to be white - grey and the isolates from avocado to be white, grey to black.

According to Kukdhrestha *et al.* (1976), *C. gloeosporioides* Penz from yam exhibits the following habit characters under stero-binocular microscope as acervuli single or in groups, sometimes appearing like pycnidial bodies emerging from ruptured, greyish black spots. Setae are absent or inconspicuous, and smaller than the conidial mass. Conidial mass is dull white to dull orange or bright orange. Mycelium mostly absent, when present white and shiny. At high moisture levels, conidia are formed in false heads (Moriwaki *et al.*, 2002). Conidia and setae under compound microscope exhibit characters such as: conidia hyaline, one-cell, and straight, oval, oblong or cylindrical, ends rounded. Moriwaki *et al.* (2002) observed that *C. gloeosporioides* Penz isolates produce conidia with obtuse ends while *C. capsici* isolates produce falcate conidia.

The fruiting bodies of *C. gloeosporioides* Penz from incubated yam leaves were collected on a microscope slide in a drop of cotton blue (0.5% in a 1: 1: 1 mixture of lactic acid, glycerol and water), crushed and identified based on the spore morphology (Abang *et al.*, 2006). Traditional morpho-physiological methods for differentiating *C. gloeosporioides* Penz isolates include conidia morphology, apressoria formation, presence or absence of setae, presence or absence of teleomorph, colour of colonies, mycelial growth rate and sensitivity to fungicides (Serra and Silva, 2004; Freeman, 2000).

2.25. Preparation of inoculants for inoculation

According to Kanchalika *et al.* (2010), inoculants were prepared by culturing isolates on PDA at room temperature (28 – 30 °C for ten days before used for inoculation. Isolations can be performed by culturing mycelia on potato dextrose agar and inoculated at room temperature (28 to 30 °C) for seven to ten days. Infected leaves can be incubated under ambient laboratory conditions in large Petri dishes lined with moist sterile paper tissues (Kanchalika *et al.*, 2010).

2.26. Pathogenicity test in fulfillment of Koch's Postulates

According to Abang *et al.*(2006), detached young healthy leaves of yam were surface sterilized, inoculated at 28 ^{0}C, incubated for seven days and assessed for percentage disease incidence. Fungal isolations were made onto PDA plates and isolates compared with inoculants (*C. gloeosporioides* isolates) based on colony and conidial morphology.

Sanders and Korsten (2003) used the plug inoculation method to carry out pathogenicity test on fruits of chilli pepper. Prior to inoculation, all chilli pepper fruits were swabbed with 70% (v/v) ethanol to reduce surface contamination and left for air-dry in the laboratory for 3 - 4 min. The fruits were then wounded by gentle pricking with a sterilized needle. Inoculants were prepared by culturing each isolate on PDA at room temperature for 10 days. Plugs of 5mm diameter were cut from actively-sporulating areas near to the colony periphery with a sterilized cork borer and placed over the wounded part of the sample. Fruits inoculated with only a PDA plug served as control. The inoculated fruits were kept in moist chamber at room temperature for 10 days.

2.27. Incidence of anthracnose disease of yam

Disease incidence according to Shatu *et al.* (2012) is the measure of proportion of plant population diseased at a time and place. Numbers of infected plants (whole plant) or plant parts (leaves, fruits, stems, roots etc) that are infected are counted and disease incidence is expressed as percent of infected plant in a sample or population. Chiarappa (1971); Kranz (1974) defined disease incidence as a qualitative measure of disease and can be calculated by the formula below:

$$\text{Percent diseased incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

2.28. Percentage severity of Anthracnose disease of yam

According to Simons and Green (1994), percentage anthracnose severity was measured using the individual leaf infected method. Inoculated leaves were rated for percentage disease severity on a 0–6 scale (1 ¼ = 1%, 2¼ = 2%, 3 ¼ = 5%, 4 ¼ =

10%, 5 ¼ = 25%, 6 > 50%). Percentage disease severity was measured by Cooke (2006) formula using the scale 0 – 3; where 0 = healthy (no leaf spot); 0.75 = mild infection (1 – 10 spots / leaf); 1.0 = moderate infection (11 – 20 spots / leaf); 2.0 = severe infection (21 – 40 spots/ leaf); and 3.0 = very severe infection (40 spots and above per leaf).

Disease severity is the measure of sickness of diseased plant. It is a quantitative trait, which measures the amount of disease on a plant at a particular place at a particular time in terms of intensity of symptoms or damage. It can be calculated by Cooke (2006) formula below.

$$\text{Percent diseased severity} = \frac{\text{Area of plants tissue affected}}{\text{Total area}} \times 100$$

Shatu *et al.* (2012), reported that the disease severity could be scored with a scale ranging from 1 - 5, Where; 1 = no obvious symptoms, 2 = symptoms on 0 - 24% of leaves, 3 = symptoms on 25 - 50% of leaves, 4 = symptoms on 51 - 74% of leaves and 5 = symptoms of 75 -100% of leaves.

2.29. Effect of plant extracts on plant vegetative growth

Datar (1999) reported that botanicals do not only control pathogens, but also stimulate the growth of host plants and this may be due to the presence of some growth hormones such as indole-3 acetic acid (IAA). According to Agrios (2004) plant growth is regulated by a small number of groups of natural occurring compounds in plants, which act as hormones and are generally called growth regulators. The most important ones are auxins (for example indole-3 acetic acid), gibberellins (example,

giberellic acid), cytokinin (for example, zeatin and isopentenyl adenosine), ethylene and some growth inhibitors.

2.30. Chlorophyll content

Amusa (1991, 1997) and IITA (1993) reported that anthracnose of yam immensely affects the photosynthetic activities of the plant and subsequently the yield. Hakan (2012) also asserted that leaves getting sun light contain higher chlorophyll than those receiving little or no sun light which usually has a correlation with the yield at the end.

The chlorophyll content is an important experimental parameter in agronomy and plant biology research (Lamb *et al.*, 2012). Amount of chlorophyll shows alteration depending on many edaphic and climatic factors such as salt stress (Yıldırım *et al.*, 2008; Avcıoğlu *et al.*, 2003) light (Johnston and Onwueme, 1998; Dai *et al.*, 2009; Khan *et al.*, 2000; Demircioğlu and Yılmaz, 2005; Güneş and İnal, 1995) water stress (Shubha and Tyagi, 2007; Karipçin, 2009; Demirel *et al.*, 2010; Kalefetoğlu and Ekmekçi, 2005), air pollution (Elkoca, 2003) fertilizing (Tunalı *et al.*, 2012) and also on time in vegetation period (Zavoruev and Zavorueva, 2002). In addition, plant species and position of leave affect the amount of chlorophyll (Gond *et al.*, 2012) and (Guerrero – Rodriguez *et al.*, 1995).

2. 31. Preparation of plant extracts

Plant extracts can be prepared by several methods depending on the type of plant; the active ingredient needed and type of solvent. They can either be extracted by water, ether or ethanol (Ajith *et al.*, 2012). Aqueous extraction was done from fresh leaves of

Cathranthus roseus, *Coleus aromaticus* (Benth), *Manilkara zapota* (L) and *Azadirachta indica* A Jus The aqueous extraction was done by washing 100g fresh leaf samples with sterile distilled water, blot dried and macerated with 100 ml distilled water (w/v) using blender (Warring International, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double layered muslin cloth and then centrifuged at 4000g for 30 minutes. The supernatant was filtered through Whatmann No.1 filter paper and sterilized at 60 °C for 30min. The filtrate served as the stock extract for *in vitro* application (Ajith *et al.*, 2012).

According to Sridhar and Vijayalakshine, (2002) and Singh and Singh, (2000) neem seed extract was prepared by pounding 2-5kg (2000 - 5000g) of shade dried seed with mortar and pestle into powder. The powder was then tied in a muslin cloth and soaked in a pot of 4-10 l of water for three days. The decoction was extracted by pressing the cloth pack for 10-15min. The mother extract was then amended with nine litres of water and 100ml of detergent (alata samina) (Sridhar and Vijayalakshine,2002).

2. 32. *In vitro* management of fungal diseases through plant extracts using the poisoned food technique

Methanolic extracts of *Jatropha curcas* L fruits, pulp and seeds have the potential of an antifungal compound phorbol ester to control *C. gloeosporioides* Penz which causes anthracnose disease in papaya, *in vitro* (Muklesur *et al.*, 2011). According to Thangavelu *et al.* (2004), the mycelial growth of *C. musae* was inhibited by *J. curcas* leaves extracts which were able to control the anthracnose disease in three banana varieties ('Robusta', 'Ney and Poovan'). Neem seed extract possesses active ingredients which exhibit fungitoxic action (Ajith *et al.*, 2012). Neem plant has been

shown to be a potential alternative for the control of plant fungal diseases, which have chemical compounds that are environmental friendly (Amadioha, 2000), and (Nwogbaga *et al.*, 2012).

Mishra and Dubey (1994) reported that plant extracts have ability to control plant pathogens and should be valued in diseases and pest management. Crude extract from *Allium sativum* (L) at 20% concentration indicated reduced mycelial growth of *R. solani* (Kuhn) *in vitro (*Dutta *et al.*, 2004). Growth inhibition of *C. gloeosporioides* Penz with methanol-chloroform extracts of *Flourensia cernua* (DC) was exhibited to be. 93 %.

2.33. *In vivo* management of fungal diseases by plant extracts

Some essential oils of 50 and 60% concentrations of different plant species showed inhibition of pathogen growth (Meazza *et al.*, 2003), and (Meepagala *et al.*, 2002). Ogbebor *et al.* (2007), reported that *J. curcas* leaves extract inhibited the mycelial growth of *C. gloeosporioides* Penz in Para rubber tree. It was also reported by Saetae and Suntornsuk (2010) that the seed cake extracts of *J. curcas* (L) caused complete inhibition of the mycelial growth of *C. gloeosporioides* Penz which is responsible for anthracnose, dieback, root rot, leaf spot, and blossom rot and seedling blight of tropical fruit.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Description of study area

The experiments were conducted on the experimental farms and in the Spanish laboratory at the Nyankpala campus of the University for Development Studies, Tamale during the 2013 and 2014 growing seasons. The site is located within the latitude 9^0 25'N, longitude $00^0$58'W with an altitude of 183 m above sea level. The soil is an Alfisol under USDA classification, and Savanna Ochrosol under the Ghanaian system of classification (Nyankpala Agricultural Experimental Station, 1984, 1986). The area has a mono modal rainfall, usually from April to November.

About 97% of the inhabitants derive their livelihood from agriculture as the main economic activity. Major crops cultivated are yam, maize, groundnuts, soya beans, pepper and cassava. Livestock reared include cattle, goats and sheep. Other economic activities are dress making, hair dressing and blacksmithing (source: field survey, 2014)

3.2. Experimental design

3.2.1. Laboratory studies

A Completely Randomized Design was used. There were five treatments and each was replicated four times. The main factor was the plant extracts (*A. indica* A Jus seed, *J. curcas* L seed and *N. tabaccum* L leaf extracts), thiophanate methyl (positive control) and Potato Dextrose Agar only (negative control). Each plant extract had five levels

but the controls had a level each. The second factor was the isolate (*C. gloeosporrioides* Penz). There were 68 experimental units.

3.2.2. Field studies

The experimental design was a 5 x 3 factorial laid in a Randomized Complete Block Design. There were 15 treatments each was replicated four times. Factor one was the plant extracts (*A. indica* A Jus seed, *J. curcas* L seed and *N. tabaccum* L leaf), thiophanate methyl (positive control) and water only (negative control). Each treatment had one level. The second factor was the yam varieties (Pona, Laribako, and Kpagaa). There were 120 experimental units.

3.3. Planting materials

Seed yams (setts) of *Dioscorea rotundata* Poir were obtained from local farmers at Tunayili. Some (setts) were provided by Dr. Elias N. K. Sowley of UDS, Tamale. The yam varieties used are presented in (Table. 3.1).

Table 3.1. Yam varieties used in the study

Variety	Source	Days to maturity
1.Pona	Farmers	180
2.Laribako	Dr. Sowley	180
3.Kpagaa	Dr. Sowley	180

3.4. Agronomic practices

3.4.1. Land preparation

The land was cleared of the existing vegetation using cutlass. Mounds were constructed using hoes after the site had been ploughed with a tractor. The mounds were 1m high and 1m apart.

3.4.2. Planting of the yam setts

The yam setts were planted in May, 2014. A sett was planted per mound. Yam setts were not treated before they were planted. After planting, each mound was capped with leaves to conserve soil moisture.

3.4.3. Staking yam plants

Staking was done with one stake per stand. It was done at the commencement of yam emergence. This was to help expose the leaves of yam plants to receive enough sunlight for photosynthetic activities.

3.5. Data collection

Data were collected on antifungal action of plant extracts *in vitro*, percentage yam emergence, number of leaves after emergence, percentage anthracnose incidence, percentage anthracnose severity, chlorophyll content (CC) and yield loss. Techniques used during the data collection were observations, isolation and identification of *C. gloeosporioides* Penz, pathogenicity tests and measurement of yield after harvest.

3.5.1. Number of yam tubers after harvest

Number of yam tubers for each treatment on each yam variety in the field was counted and recorded manually after harvest. The number of tubers for each treatment of each yam variety was, therefore, pulled together from the various blocks to find the means before data analysis

3.5.2. Yam tuber quality after harvest

Quality of yam tubers for each of the yam varieties was measured in the field by physical observation to detect defected and undefected yam tubers (for example rot, holes). The quality of yam tubers for each treatment on each yam variety in the field was observed, counted and recorded after harvest. The quality of yam tubers for each treatment of each yam variety was, therefore, pulled together from the various blocks to find the means before data analysis.

3.5.3. Yam tuber length after harvest

Length of yam tubers for each of the treatments for each of the yam varieties from each block was measured in the field with a surveyor's tape measure in centimeters after harvest. The length of yam tubers for each treatment of each yam variety was therefore pulled together from the various blocks to find the means before data analysis.

3.5.4. Yam tuber weight after harvest

Weight of yam tubers for each of of the treatments of the three yam varieties was measured with a top pan balance in kilograms in the field after harvest. The results on

the treatments for each of the three varieties of yam tubers were then pulled together from the various blocks to find the means before data analysis.

3.5.5. Number of emergence after planting

Number of yam emergence for each variety was counted and recorded at the first, fifth and ninth weeks after planting. The percent emergence for each yam variety was pulled together from the various blocks before data analysis.

3.5.6. Number of yam leaves after emergence

A yam plant was randomly sampled and tagged in each block for each treatment. The leaves of the sampled tagged plants were counted at the 1st, 2nd and 3rd month after emergence.

3.5.7. Determination of chlorophyll content

Chlorophyll content was determined with a chlorophyll meter based on the method described by Holden (1965). The determinations were done on sampled plants for each of the treatments. Chlorophyll content determinations were done on five leaves from each of the sampled plants. It was also determined on anthracnose infected and uninfected yam leaves on the field by the chlorophyll meter. The chlorophyll content was determined in four weeks after emergence. The determinations were done weekly for three conservative weeks.

3.6. Isolation and identification of anthracnose causing organism

3.6.1. Media preparation

Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instructions (Dickinson and Company Sparks, MD 21152 USA) by dissolving 39g of PDA in 1l of distilled water and autoclaved at 121^0 C for 15min. The autoclaved PDA was then allowed to cool to about 45^0C then dispensed into 9cm diameter sterile Petri dishes and allowed to solidify at room temperature in the laboratory.

3.6.2. Isolation of anthracnose causing organism

Based on the method described by Sutton (1992), *C. gloeosporioides* was isolated from two months old naturally infected leaves and vines and one week old tubers of yam from the field (Fig. 3.1). Pieces of tissues were cut from the advancing margin of the *C. gloeosporioides* lesion on the infected leaves, vines and tubers, surface sterilized separately in 5% sodium hypochlorite solution and rinsed separately in three changes of sterilized distilled water. The sterilized tissues were then blot dried separately on sterilized filter paper and plated five on a PDA plate. The plates were then incubated in the laboratory for seven days at 28^0C and observed every 24h according to Kanchalika *et al.* (2010). Each of the fungal colonies was subcultured to fresh PDA plates and incubated at room temperature separately for four days to obtain pure cultures of each *Colletotrichum* isolate.

a b c

Fig. 3.1. Lesions of infected yam leaves (plate a), vines (plate b), and tubers (plate c) used for fungi isolations

3.6.3. Identification of anthracnose causing organism

Identification of *C. gloeosporioides* Penz was done based on the method described by Sutton (1992). Fungal mycelium from fresh cultures were examined under the LCD Celestine microscope and identified by comparing their morphological and cultural characteristics with published photographs Sutton (1992). Morphological characteristics of *C. gloeosporioides* Penz on PDA plates of 10 days old observed for growth patterns, mycelial colour types, fruiting bodies, and the growth rate. These were compared with published photographs (Ulrike *et al.*, 2012).

3.7. Pathogenicity test

Pathoagenicity test was done according to Sanders and Korsten (2003). Susceptible yam leaves (two months old) and tubers (one week old) without anthracnose symptoms were randomly sampled for each yam variety at the field. Prior to inoculation, all detached susceptible yam leaves and tubers were thoroughly rinsed

under running water, swabbed with 70% (v/v) ethanol to reduce surface contamination. They were then oven dried for 4 mins in the laboratory. The leaves were then wounded by gentle pricking with a sterilized needle whiles the tubers were bored into with a 5mm sterilized cork borer. Inoculants were prepared by culturing each isolate of the organisms found in the yam infected sampled parts on PDA at room temperature for 10 days. Plugs (5mm diameter) were cut from actively sporulating areas near the colony periphery by using a sterilized cork borer and placed over the wounded part of each sample. Samples inoculated with only PDA plugs were controls. The inoculated leaves and tubers were kept in a moist chamber at room temperature for 10 days. After the ten day period, the inoculated yam tubers were cross-sectionally cut with a sterilized knife. Lesion development was then measured diagonally from the centre where the mycelial plug was placed in mm with a rule. Fungal pathogens were re-isolated on PDA plates and isolates compared with the inoculants based on colony and conidial morphology as described above.

3.8. Efficacy of plant extract against anthracnose disease of yam

3.8.1. Preparation of plant extracts

Plant extracts were prepared from matured dried seeds of *A indica* A Jus, *J. curcas* L and matured dried leaves of *N. tabacum* (L). The aqueous seed and leaf extractions were done according to Sridhar and Vijayalakshine, (2002) and Singh and Singh, (2000) with some modifications. One kilogramme (1kg) of seed or leaf sample was gently pounded separately into powder with a mortar and pestle. Each ground plant material was then soaked in 2l (w/v) sterilized distilled water in a sterilized flask and left to stand in the laboratory for 72h. The mouth of the flask in which it was soaked

was covered with aluminum foil to prevent volatility of the active ingredients (*Azadirachtin, Phorbol ester* and *Nicotine*). The mixture was then strained to obtain using a sterilized muslin cloth to obtain the filtrate. Each filtrate was centrifuged at 4000g for 30 min. to obtain a stock extract. The supernatant of the stock extract was filtered through Whatmann No. 1 filter paper and sterilized at 60 ^{0}C for 15min.

3.8.2. Effect of aqueous plant extracts on radial mycelial growth of *C. gloeosporioides in vitro*

Mycelial growth of *C. gloeosporioides* Penz was determined *in vitro*. The PDA was amended with the stock extract to make 35, 45, 55, 65 and 75% concentration in sterilized Petri dishes. Each Petri dish contained 2ml of an extract and 20ml of sterilized PDA. The solidified PDA amended plates were inoculated at the centre with 5 mm diameter mycelial disc of *C. gloeosporioides* Penz and incubated at 27^{0}C for seven days (Nene and Thapliyal, 1979) at PDA plates not amended with extract served as negative control whiles those amended with the thiophanate methyl (2ml) served as positive control. The colony diameter of C. *gloeosporioides* was measured and per cent inhibition of mycelial growth was calculated using Vincent (1927) formula below.

$$\text{Growth inhibition (\%)} = \frac{\text{Colony diameter of (Control} - \text{Treatment)}}{\text{Colony diameter of control}} \times 100$$

3.9. Incidence of anthracnose disease in the field

The plants in the field were assessed and scored for incidence of typical yam anthracnose symptoms expressed on the leaves and vines for each treatment. All experimental units were observed for each treatment and the number of experimental

units with symptoms was recorded. Observations were made immediately after emergence at three weeks interval for twelve consecutive weeks. The percentage incidence was calculated with Cooke (2006) formula.

$$\text{Percent diseased incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

3.10. Severity of anthracnose diseases in the field

All experimental units for each treatment in each block were observed immediately after emergence at three weeks interval for nine consecutive weeks. The disease severity was scored on a scale of 1 to 5, where; 1 = no obvious symptoms, 2 = symptoms on 0 - 24% of leaves, 3 = symptoms on 25 - 50% of leaves, 4 = symptoms on 51 - 74% of leaves and 5 = symptoms on 75 - 100% of leaves (Shatu *et al.*, 2012). Cooke (2006) formula was used in calculating percentage severity as follows:

$$\text{Percent diseased severity} = \frac{\text{Area of plant tissue infected}}{\text{total area}} \times 100$$

3.11. Data analysis

Data collected were subjected to analysis of variance using GenStat (Discovery Edition 4) statistical package software. Least significant difference was used to separate the means at 5 percent. Micro-soft excel was used to present results in graphs and tables Error bars were used. Analyzed data were presented in tables and graphs.

4.0. RESULTS

4.1. Isolation and identification of anthracnose causing agent(s)

Different fungal isolates, namely *Colletotrichum gloeosporioides* Penz, *Aspergilus niger* (A) and *Rhizopus* spp. were isolated from anthracnose infected leaf, tuber and vine samples of yam (Fig.4.1). *Colletotrichum gloeosporioides* Penz had the highest percentage occurrence in the leaf (80 %), followed by the tuber (30 %) and the vine (10 %) compared to *A. niger* and *Rhizopus* spp. Occurrence of *A. niger* (A) was higher than *Rhizopus* spp. both in the leaf and tuber samples. However, both *A. niger* (A) and *Rhizopus* spp. were not detected in the vine samples (Fig. 4.1). *Colletotrichum gloeosporioides* Penz was isolated and identified through LCD microscope, comparing to published photographs (Sutton, 1992) and through pathogenicity test to be the causal agent responsible for the anthracnose symptoms on the infected yam samples. *Colletotrichum gloeosporioides* Penz was significant in all the yam samples used for the isolation compared to *A. niger* (A) *and Rhizopus* spp.

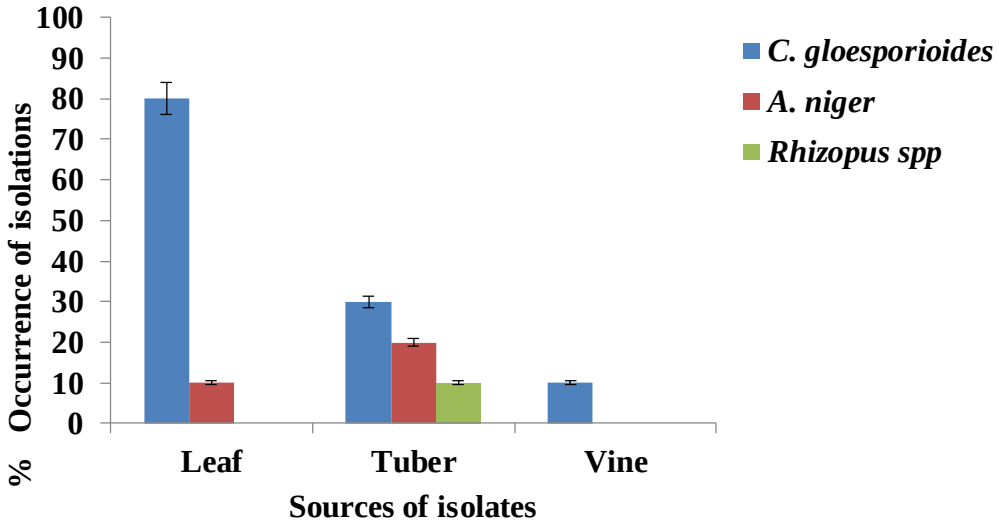
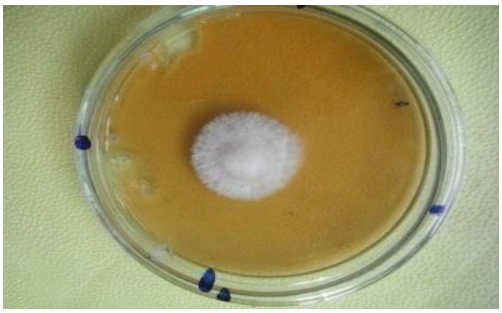


Fig. 4.1. Percentage occurrence of fungi in leaves tubers and vines

4.2. Cultural characteristics of *C. gloeosporioides* Penz

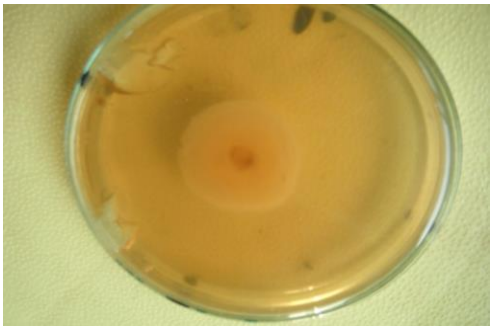
From Plate.4.1 below, it was observed that at a temperature of 28±2°C, *C. gloeosporioides* Penz grew rapidly on PDA, producing whitish-wooly or fluffy mycelia. It varied greatly in colony colour. The colour gradually changed from white to greenish (day 6) and finally to dark brown on the 10th day (Plate. 4.1). Pronounced concentric rings, abundant sporulation, and superficial production of distinct grey acervuli on the top side and orange on the reversed side 10 days of mycelial growth. The reverse side of the colonies were dark brown, orange or a mixture and with regular colony margins. The growth pattern was circular with the mycelia showing a uniform growth pattern and radial in a ring-like pattern.

On plate a, *C. gloeosporioides* Penz produced whitish-wooly mecylium on the top side of the plate but was yellow on the reverse side (plate b) of the plate (plate.4.1). Plate c produced whitish fluffy mycelim at the periphery but greenish at the center of the plate. Its reverse side (plate d) was yellow. The mecylium growth on plate e was completely light green and orange on the reverse side of the plate (plate f).



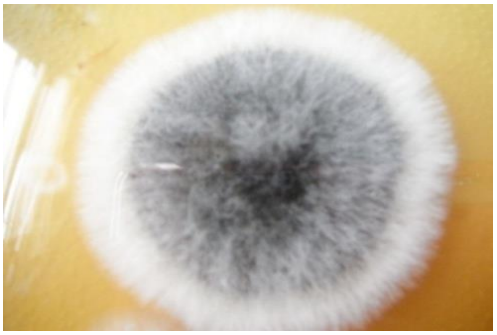
a

Top side (3 days old)



b

Reverse side (3 days old)

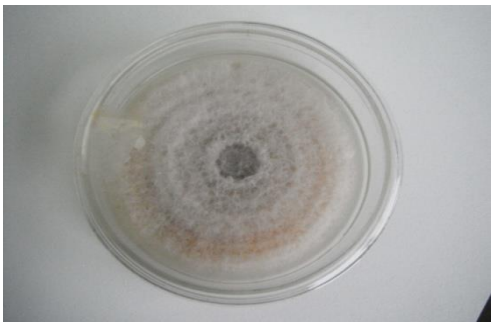


c

Top side (6 days old)

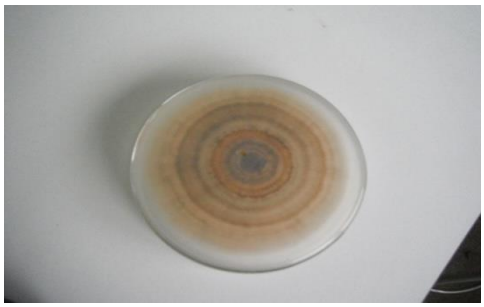
d

Reverse side (6 days old)



e

Top side (10 days old)



f

Reverse side (10 days old)

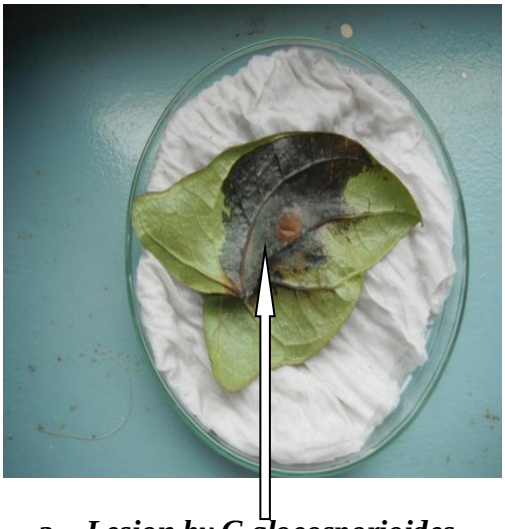
Plate.4.1. Characteristics of *C. gloeosporioides* Penz at 3(a,b), 6(c,d) and 10 (e,f) days growth stages after inoculation on PDA plates.

4.3. Pathogenicity test

Colletotrichum gloeosporioides Penz caused infection on the inoculated yam leaves compared to *A. niger* and *Rhizopus* spp (Table. 4.1). *Colletotrichum gloeosporioides* Penz (plate 4.2 a) produced a lesion size of 43.2cm. *A. niger* A (Plate 4.2.b) did not cause any infection and no lesion was developed on the inoculated yam leaves (Plate.4.2). *Rhizopus* spp. did not also cause any infection and therefore no lesion was developed. *C. gloeosporioides* Penz was re-isolated and identified, thus fulfilling Koch's postulates.

Table. 4.1. Lesion size of fungal isolates 10 days after inoculation in yam leaves.

Fungus/Isolate	Lesion size in leaf (cm)
C. gloeosporioides	43.2
A.*niger*	0.0
Rhizopus spp	0.0



a. *Lesion by C.gloeosporioides* **b. No lesion by *A. niger***

c. No lesion by *Rhizopus* spp **d. No lesion on Control**

Plate.4.2. Pathogenicity test on detached yam leaves showing lesions or not by the isolates

4.6.Effect of aqueous plant extract on growth of *C. gloeosporioides*

The various concentrations of the aqueous neem seed extract applied inhibited the mycelial growth of *C. gloeosporioides* (Fig.4.2)**.** The 75% concentration of the aqueous neem seed extract exhibited the best inhibitory effect considering the percentage mycelial growth it recorded (15%) followed by the 65, 55, 45 and 35% concentrations. The higher the concentration of the aqueous neem seed extract, the lower the mycelial growth and the lower the concentration of the neem seed extract the higher the mycelia growth. The 65 and 55% concentrations of the aqueous extract had similar growth pattern from day 1 to 7 but the 55% concentration increased in growth thereafter.

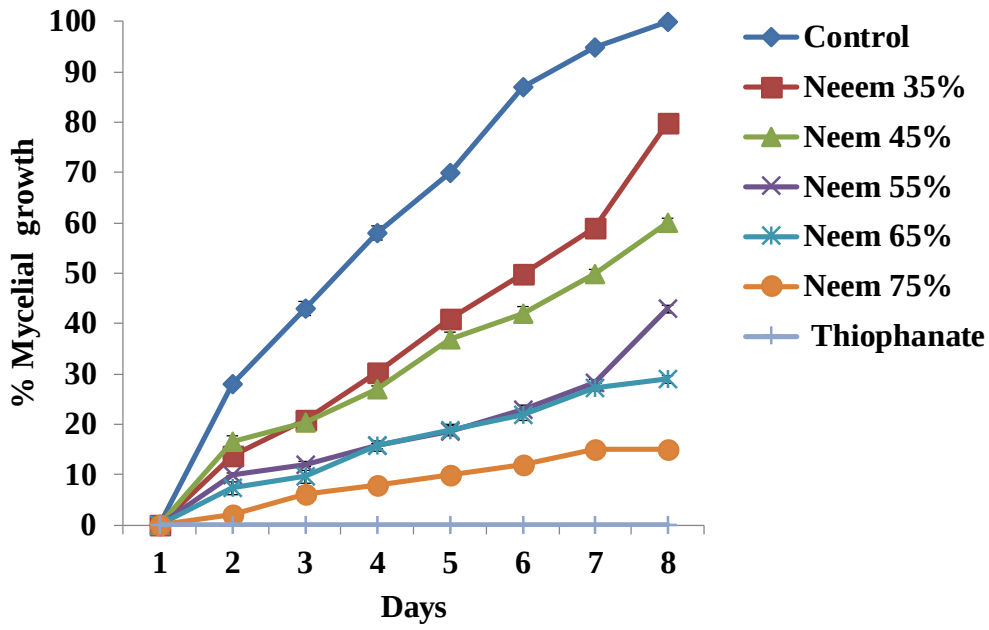


Fig. 4.2. Mycelial growth of *C. gloeosporioides* Penz during eight days of inoculation on PDA amended with 35, 45, 55, 65, and 75 % of aqueous neem seed extract, thiophanate methyl (positive control) and water (negative control)

The various concentrations, 35, 45, 55, 65, and 75 % of aqueous *J. curcas* L seed extract inhibited the mycelial growth of *C. gloeosporioides* Penz *in vitro* (Fig. 4.3). Higher concentrations of aqueous Jatropha seed extract gave higher inhibition of the mycelial growth than lower concentrations. The 75% concentration recorded the least mycelial growth (26.3%) compared to the negative control (100%).)

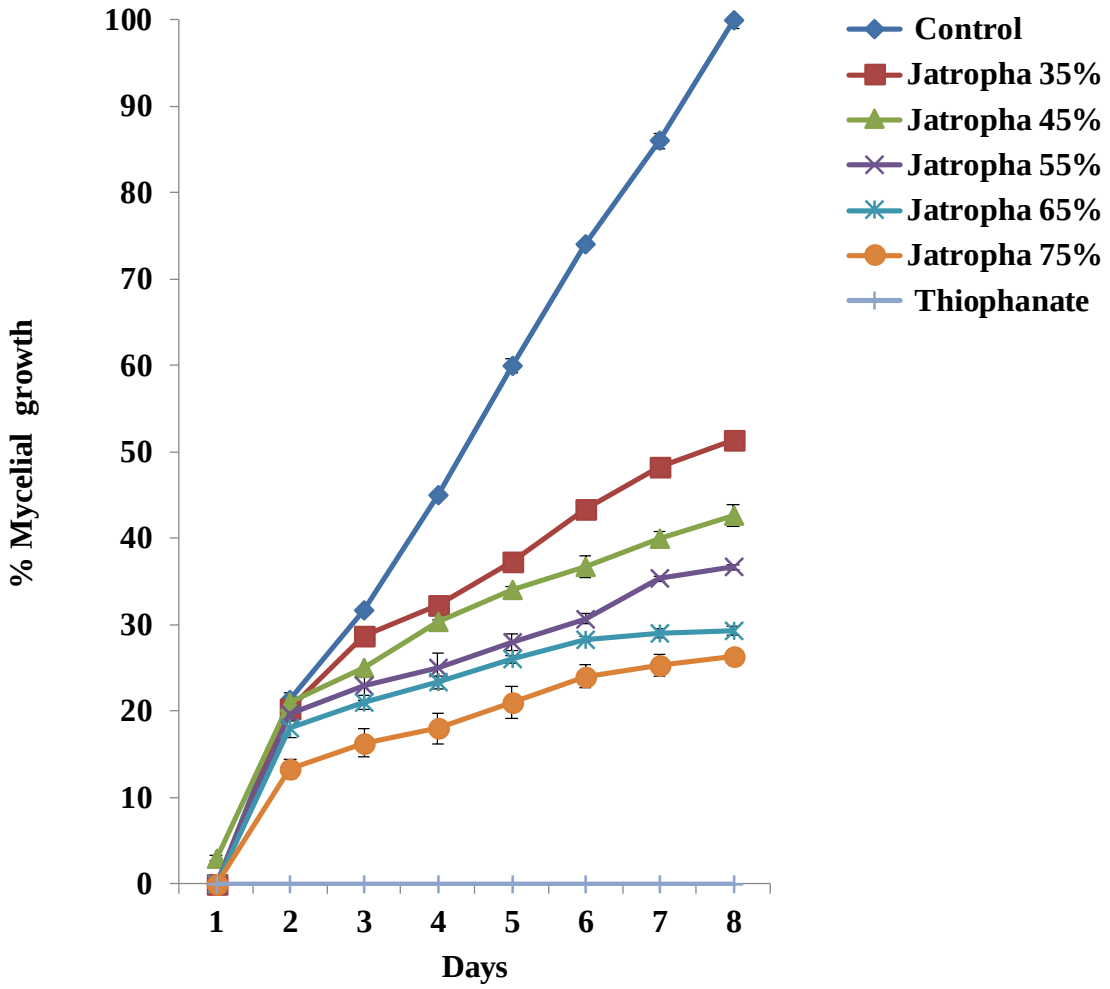


Fig. 4.3. Mycelial growth of *C. gloeosporioides* Penz during eight days of inoculation on PDA amended with 35, 45, 55, 65, and 75 % of *J. curcas* seed extract, thiophanate methyl (positive control) and water (negative control)

The aqueous *N. tabacum* (L) leaf extract was potent and inhibited the mycelial growth of *C. gloeosporioides* Penz *in vitro* at its various concentrations (Fig. 4.4). Aqueous *N. tabacum* (L) leaf extract at 75% concentration caused the lowest percentage mycelial growth (19.3%) followed by the 65, 55, 45 and 35 % concentrations. The higher the concentration of the aqueous *N. tabacum* leaf extract, the higher the suppressive effect on mycelial growth. The mycelial growth for each of the aqueous *N. tabcum* (L) leaf

extract differed from each other. Thiophanate methyl, the positive control completely inhibited mycelial growth.

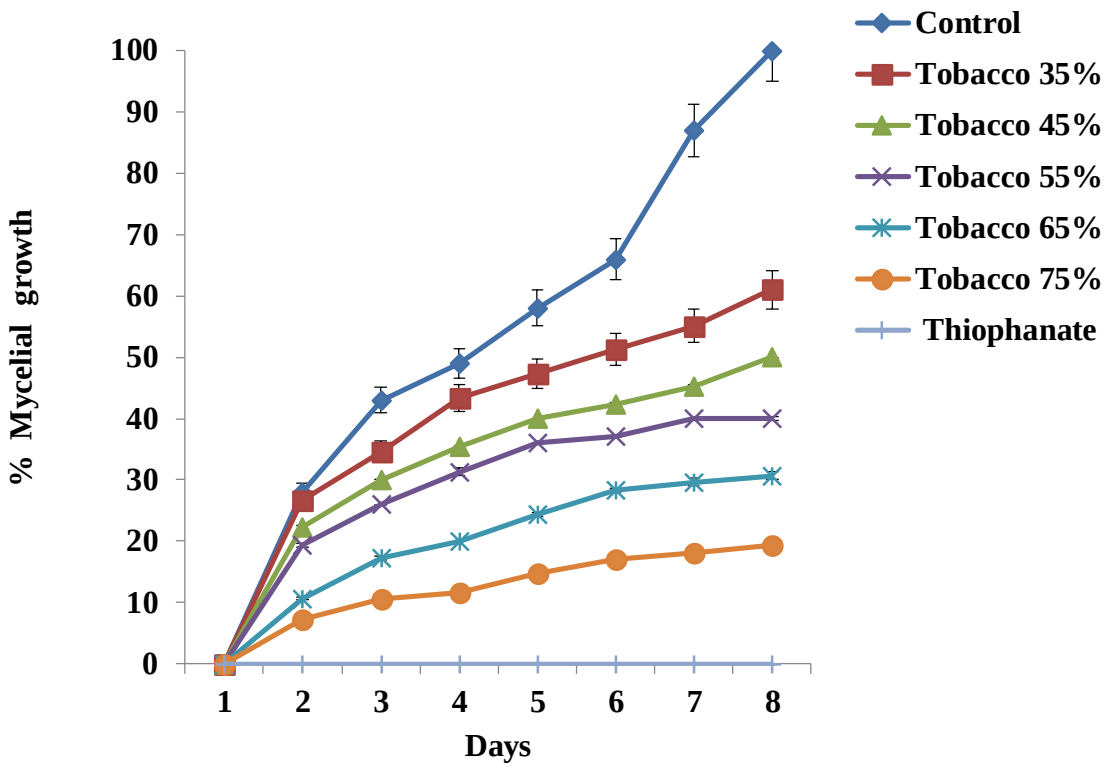


Fig. 4.4. Mycelia growth of *C. gloeosporioides* Penz during eight days of inoculation on PDA amended with 35, 45, 55, 65, and 75% of aqueous *N. tabacum* seed extracts, thiophanate methyl (positive control) and water (negative control).

4.7.Percentage emergence of yam after planting

There were some significant differences ($P < 0.05$) among the treatments (Fig.4.5). Pona and Kpagaa had higher percentage emergence than Laribako at nine weeks after plnting. However, there was no significant difference ($P > 0.05$) in percent emergence between Pona and Kpagaa. Kpagaa had the highest percentage emergence (40%) at week one followed by Pona (25%) and Laribako (15%). Pona and Kpagaa had higher

percentage emergence (60%) than Laribako (50%) at week five. By the ninth week, percentage emergence of Pona, Kpagaa and Laribako were 100, 100 and 80% respectively.

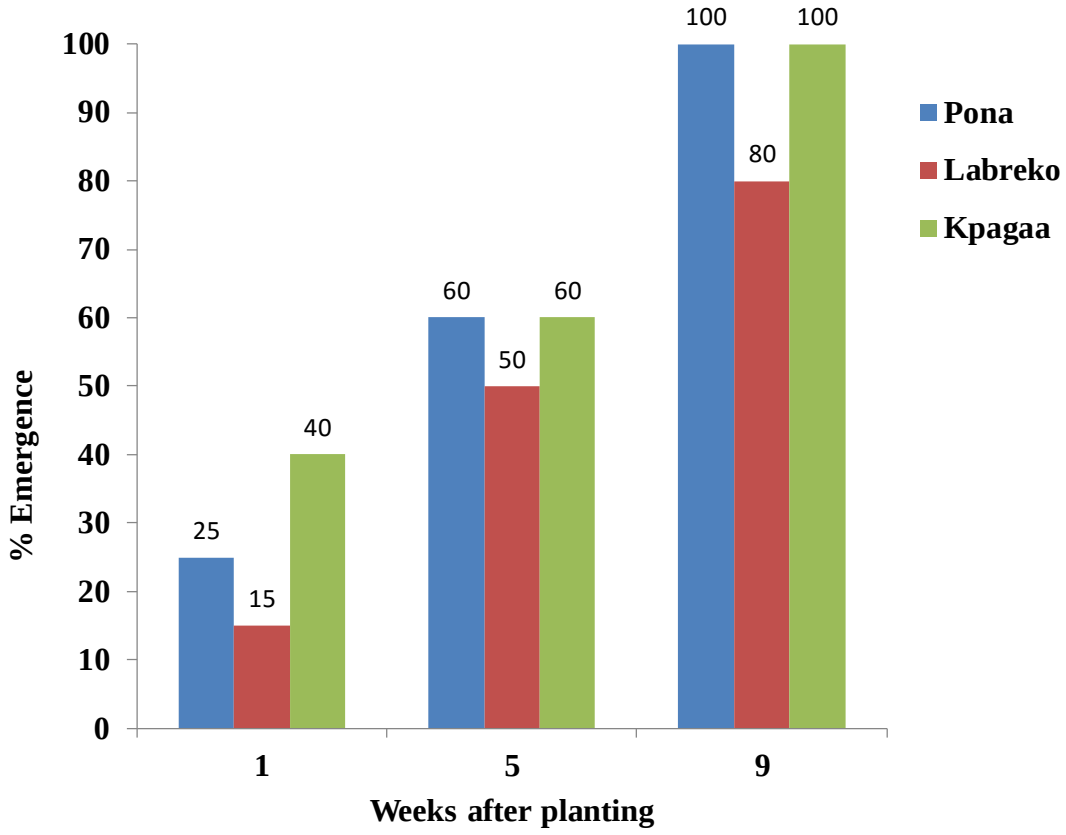


Fig.4.5. Emergence of three varieties at one, five and nine weeks after planting

4.6. The effect of aqueous plant extracts on the number of yam leaves in the field

The aqueous plant extracts had a positive influence on the number of yam leaves at the three and four months after emergence (Table. 4.2.). At four months, aqueous extracts of *A. indica* seed at 75% concentration produced *(*505) leaves for Pona followed by aqueous extracts of *J. curcas* L seed at 75% concentration (500) and aqueous extracts of *N. tabacum* (L) leaf at 75% (409) (Table.4.2). Laribako at four months (Table.4.2)

produced 500 leaves when aqueous extract of *A. indica* A Jus seed at 75% was applied to it in the field followed by aqueous extract of *J. curcas* L at 75% concentration (448) and aqueous extract of *N. tabacum* (L) at 75% concentration (380). On the other hand, Kpagaa at four months (Table.4.2) produced 498 yam leaves when aqueous extract of *J. curcas* L seed at 75% was applied to it in the field followed by aqueous extract of *A. indica* A Jus seed at 75% concentration (493) and aqueous extract of *N. tabacum* (L) at 75% concentration (422). However, there was no significant difference ($P > 0.05$) between thiophanate methyl (positive control) and the negative control for any of the yam varieties or aqueous plant extracts. Name of leaves for each treatment was rounded to the nearest whole number (Table. 4.2).

Table**.4.2. Effect of aqueous plant extracts on number of leaves of three varieties of yam leaves at 2, 3, and 4 months after emergence**

Extracts	Months								
	2			**3**			**4**		
	Pona	Laribako	Kpagaa	Pona	Laribako	Kpagaa	Pona	Laribako	Kpagaa
*J.curcas*75%	32	36	29	102	100	155	500	448	498
A.indica 75%	43	45	51	300	230	200	505	500	493
N. tabacum 75%	46	40	62	251	150	125	409	380	422
Thiophanate methyl	24	43	50	102	147	188	155	377	399
Control 75%	24	32	27	102	184	166	155	378	398

4.7. Effect of aqueous plant extracts on incidence of anthracnose of yam

In Pona, plants treated with 75% concentration of neem and *Jatropha* seed extracts recorded the least incidence (24%) at weeks three, six and nine but in week twelve, it recorded 27% incidence compared to the negative control (100%) at week twelve (Fig. 4.6). Pona plants treated with tobacco leaf extract had 29% disease incidence at week twelve. Pona plants treated with thiophanate methyl, recorded zero (0%) incidence throughout.

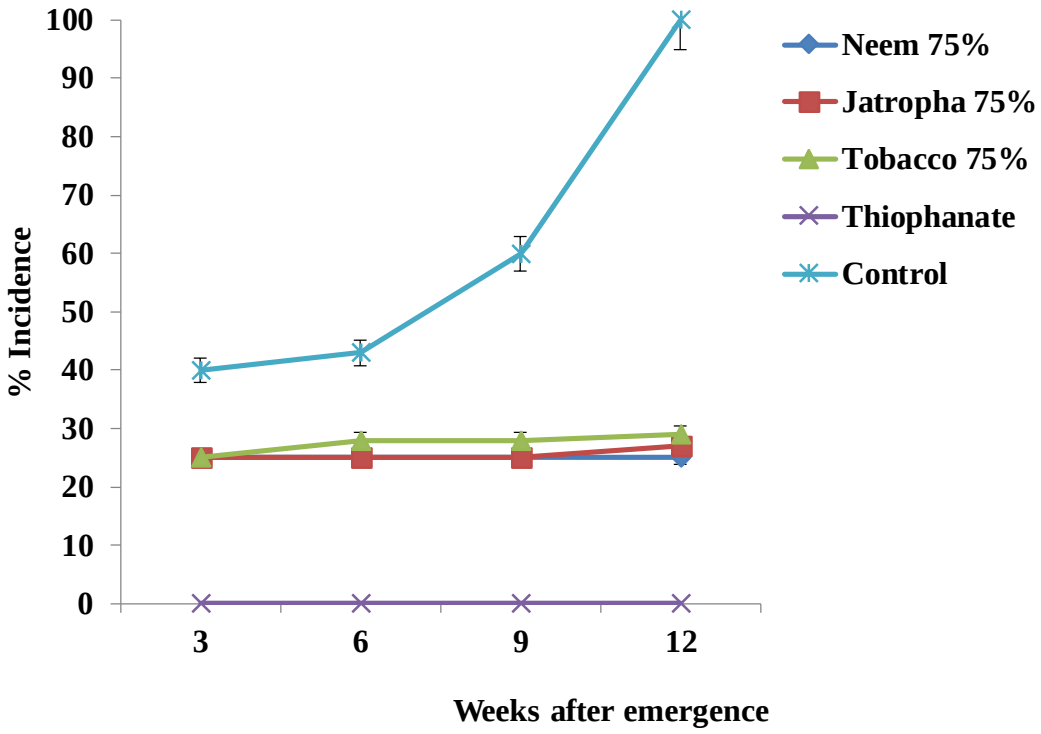


Fig. 4.6. Incidence of anthracnose disease on Pona after emergence

With Laribako, plants treated with aqueous tobacco leaf extract at 75% recorded the least incidence in laribako (29%) followed by aqueous neem seed extract (30%) and aqueous Jatropha seed extract (40%). The negative control had 100% incidence by the 12th week (Fig.4.7)

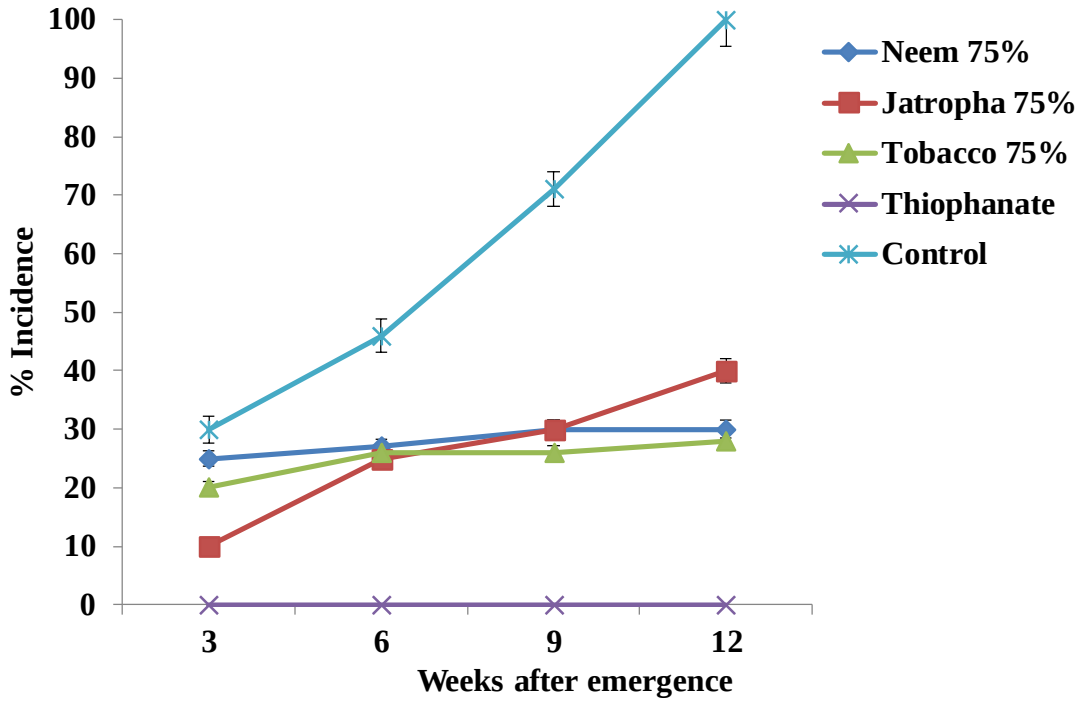


Fig. 4.7. Incidence of anthracnose disease on Laribako after emergence

Kpagaa plants treated with the 75 % concentration of aqueous neem seed plant extract had the least incidence (16%) followed by the aqueous tobacco leaf extract (26%) and the aqueous Jatropha seed extract (40%). There were significant differences ($P < 0.05$) among treatments (Fig. 4.8).

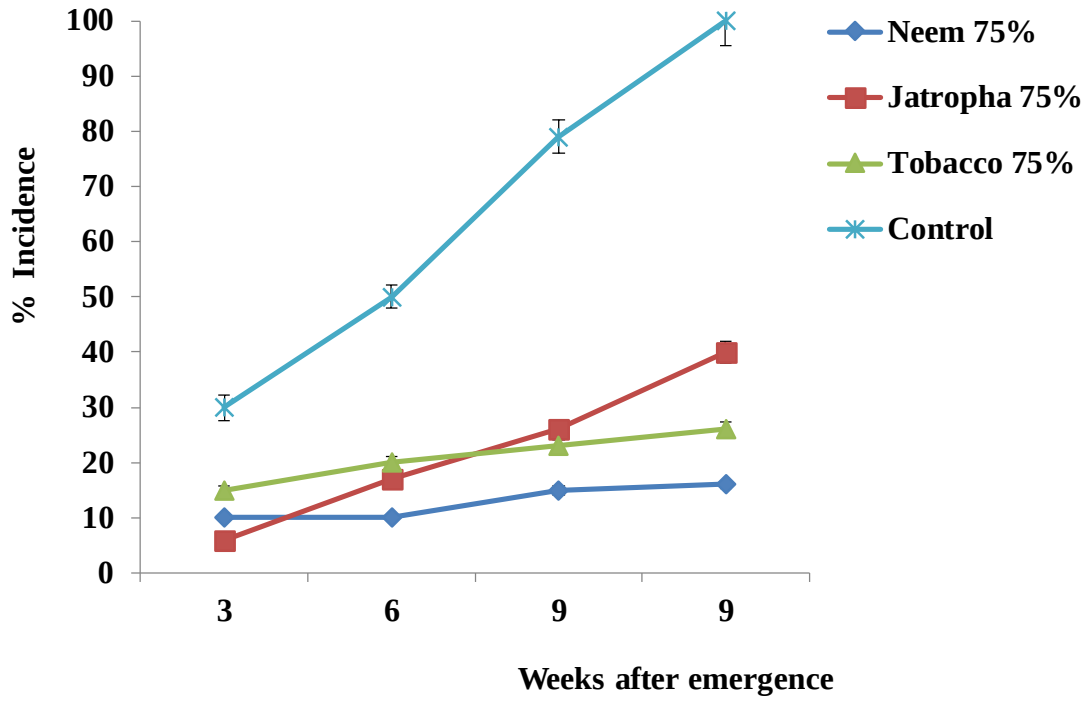


Fig. 4.8. Incidence of anthracnose disease on Kpagaa after emergence

4.8. Effect of aqueous plant extracts on the severity of anthracnose disease of three varieties of yam after emergence

Each of the aqueous plant extracts at 75% concentration was potent and, therefore, reduced the severity of anthracnose compared to the negative control. Pona plants treated with aqueous neem seed extract had the least severity (15%) followed by aqueous *J. curcas* L seed extract (35%) and aqueous tobacco leaf extract (47%) at week nine. The severity in the negative control reached 100% by the ninth week (Table. 4.3). The efficacy of the aqueous 75% neem seed extract was comparable to thiophanate methyl, the positive control at weeks one and three (0%) and then had a rise up to 15% in week nine.

Aqueous plant extracts at 75% concentration reduced anthracnose severity on Laribako yam plants in the field. Aqueous neem seed extracts recorded 12% aanthragnose disease incidence for Laribako yam plants in the field at the ninth week (Table. 4.13). Aqueous extracts of Jatropha seed and Tobacco leaf were comparable at week nine (30%). Thiophanate methyl (positive control) produced 0% anthracnose disease incidence while the negative control produced 85% anthracnose disease incidence (Table. 4.3).

At week one, each of the queous plant extracts and thiophanate methyl (positive) were comparable for Kpagaa yam plants (0%). Kpagaa Plants treated with aqueous Jatropha seed extract had the least severity (27%) followed by aqueous neem seed extract (29%) and aqueous tobacco leaf extract (31%) at week nine. The severity in the negative control reached 83% by the ninth week (Table. 4.3). The percentage severity for each treatment was rounded to the nearest whole number (Table. 4.3).

Table.4.3. Severity of anthracnose disease on yam after emergence

	Weeks after emergence														
	1			3			5			7			9		
Extracts	Pona	Lari-bako	Kpa-gaa	Pona	Lari-bako	Kpa-gaa	Pona	Lari-bako	Kpa-gaa	Pona	Lari-bako	Kpa-gaa	Pona	Lari-bako	Kpa-gaa
Jatropha 75%	0	0	0	10	5	10	25	20	17	32	20	23	35	30	27
Neem 75%	0	0	0	0	3	8	5	8	15	10	10	18	15	12	29
Tobacco 75%	0	0	0	15	13	6	22	18	13	45	23	22	47	30	31
Thiophanate nethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	4	45	20	21	64	33	45	85	35	60	100	85	83

4.9. The effect of aqueous plant extracts on the chlorophyll content of three varieties of yam after emergence

There was no significant (P > 0.05) effect of aqueous plant extracts on the chlorophyll content of Pona yam leaf at week one after emergence. However, at weeks two, three, and four after emergence, the chlorophyll content of the aqueous plant extracts treated plants differed significantly (P < 0.05). The chlorophyll content increased in each of the plant extracts but declined in the negative control over the weeks (Table. 4.4). By the fourth week after emergence plants treated with 75 % aqueous neem seed extract had 97.9 % chlorophyll followed by aqueous Jatropha seed extracts (80 %) and aqueous tobacco leaf extract (62 %) (Table 4.4).

There was significant (P < 0.05) difference of aqueous plant extracts on the chlorophyll content of Laribako yam leaf after emergence. At week one, aqueous neem seed extracts had 43% chlorophyll compared to the negative control (20%). However, aqueous Jatropha seed extracts and aqueous Tobacco leaf extracts were comparable (29%). The chlorophyll content for Laribako increased over the weeks. By the fourth week, aqueous neem extracts recorded 98% chlorophyll content followed by aqueous Jatropha seed extracts (74%) and Tobacco leaf extract (70%) (Table 4.4).

Aqueous plant extracts were each significant (P < 0.05) for Kpagaa yam plants in each of the weeks compared to the negative control. The highest chlorophyll content was recorded by Kpagaa yam plants treated with aqueous extract of Tobacco leaf (75.2%) followed by aqueous extract of neem seed (69%) and aqueous extract of Jatropha (56%) at the fourth week(Table 4.4)

Table. 4.4. Effect of aqueous plant extracts on chlorophyll content of yam

	Weeks after emergence														
	1			3			5			7			9		
Extracts	Pona	Lari-bako	Kpa-gaa	Pona	Lari-bako	Kpa-gaa	Pona	Lari-bako	Kpa-gaa	Pona	Lari-bako	Kpa-gaa	Pona	Lari-bako	Kpa-gaa
Jatropha 75%	0	0	0	10	5	10	25	20	17	32	20	23	35	30	27
Neem 75%	0	0	0	0	3	8	5	8	15	10	10	18	15	12	29
Tobacco 75%	0	0	0	15	13	6	22	18	13	45	23	22	47	30	31
Thiophanate nethyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	4	45	20	21	64	33	45	85	35	60	100	85	83

4.10. Effect of anthracnose disease infection on chlorophyll content of Pona

The anthracnose infection had a negative influence on the chlorophyll content of the yam leaves in each of the treatments (Table.4.5). The chlorophyll content in healthy (uninfected) Pona leaves was significantly higher ($P < 0.05$) than that in anthracnose disease infected leaves (Table.4.5). There was an increase in the chlorophyll content of Pona in each of the aqueous plant extracts over the weeks. At week three, aqueous extract of neem seed recorded 63.01% chlorophyll content for uninfected leaves and 39% for infected leaves followed by aqueous extract of Tobacco leaf (58%) for uninfected leaves and (40%) for infected leaves and aqueous extract of Jatropha seed (54.6%) for uninfected leaves and (40.45%) for the infected leaves.

Table.4.5. Chlorophyll content of anthracnose disease infected and uninfected Pona leaves

	Weeks afer emergence					
	1		2		3	
Extracts	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
Jatropha 75%	33.5	39.5	35.8	50.5	40.45	54.6
Neem 75%	25.1	40	24.3	46.2	39	63.01
Tobacco 75%	26.1	35.1	28	41	40	58
Thiophanate nethyl	30	31	32	33.1	43	47
Control	20	34	18	36	15	45

4.11. Effect of aqueous plants extracts on the yield of *D. rotundata* Poir

4.11.1. Effects of plant extracts on number of yam tubers at harvest

Pona plants treated with 75% concentration of each the plant extract produced a higher number of tubers per plant at harvest **(**Table. 4.6). There were significant differences ($P < 0.05$) among treatments. Plants treated with the 75% concentration of neem seed extract recorded the highest number of yam tubers per plant (5.5), followed by *Jatropha* seed extract (4) and tobacco leaf extract (2.6).

Laribako plants treated with the 75% concentration of aqueous neem seed extract produced the highest number of tubers per plant (4), followed by aqueous tobacco leaf extract (3.4). Aqueous *Jatropha* seed extract was comparable to the negative control and therefore not significantly different. Kpagaa plants treated with 75% concentration of neem seed extract produced the highest number of tubers per plant at harvest (8.7) followed by tobacco leaf extract at 75 % concentration (7) and Jatropha seed extract at 75% concentration (4.5) ($P < 0.05$).

Table. 4.6. Number of yam tubers at harvest

Plant Extract	Pona	Laribako	Kpagaa
Jatropha seed 75%	4	2.9	4.5
Neem seed 75%	5.5	4	8.7
Tobacco leaf 75%	2.6	3.4	7
Thiophanate methyl	3	4	3
Control	1	2	2.2

4.11.2. Effect of plant extracts on the quality of yam tubers at harvest

There were significant differences ($P < 0.05$) in the quality of yam tubers at harvest (Table. 4.7). Plants treated with aqueous plant extracts produced fewer defective tubers for each variety. Plants treated with aqueous neem seed extract at 75% concentration recorded the least percentage defect (11.3%) on Pona, followed by aqueous tobacco leaf extract at 75% concentration (30 %) and aqueous Jatropha seed extract at 75% concentration (40.2%). A similar trend was observed in both laribako and kpagaa. The pona variety had the least numbers of defective tubers for each of the treatments, followed by kpagaa and laribako.

Table.4.7. Quality percentage of yam tubers at harvest

Extract	Pona	Laribako	Kpagaa
Jatropha seed 75%	40.2	60	45.1
Neem seed 75%	11.3	20.1	15
Tobacco leaf 75%	30.4	70	30
Thiophanate methyl	00	10.5	00
Contro	90	100	60.2

4.11.3. Effect of aqueous plant extracts on the size of yam tubers at harvest

Each of the aqueous plant extracts applied at 75% concentration had a positive influence on the size of yam tubers at harvest (Table. 4.8). The highest number of yam tubers was produced by aqueous neem seed extract at 75% concentration which was comparable to thiophanate methyl (40) followed by aqueous Jatropha seed extract at 75% concentraction (35.3) and aqueous tobacco leaf extract at 75% concentration (35).

Pona yam plants produced the highest number of yam tubers when aqueous neem seed extract at 75% concentration was applied (36) followed by aqueous tobacco leaf extract at 75% concentration (30) and aqueous Jatropha seed extract at 75% concentration (23.4).

Kpagaa yam plants treated with aqueous neem seed extract produced the largest tubers (33.5cm), followed by aqueous Jatropha seed extract (32cm) and aqueous tobacco leaf extract (26cm).

Tab.4.8. Size of yam tubers at harvest (cm)

Plant extract	Pona	Laribako	Kpagaa
Jatropha seed 75%	23.4	35.3	32
Neem seed 75%	36	40	33.5
Tobacco leaf 75%	30	35	26
Thiophanate methyl	35	40	25.3
Control	19	30	24

4.11.4. Effect of aqueous plant extracts on the weight of yam tubers at harvest

Each of the plant extracts applied at 75% concentration was significant ($P < 0.05$). Kpagaa, plants treated with 75% aqueous Jatropha and aqueous neem seed extract produced the heaviest tubers (15kg) followed by thiophanate methyl (14kg) and aqueous tobacco leaf extract with 13kg (Table. 4.9) compared to the negative control. In the case of Laribako, thiophanate methyl, positive control produced the heaviest tubers (9kg) followed by aqueous tobacco leaf extract at 75% concentration (8kg) and aqueous Jatropha seed extract at 75% concentration (7.6kg). Pona plants, treated with aqueous neem seed extract at 75 % concentration produced the heaviest tubers (20kg) followed by aqueous Jatropha seed extract (15kg).

Table. 4.9. Weight of yam tubers at harvest (kg)

Plant extract	Pona	Laribako	Kpagaa
Jatropha seed 75%	15	7.6	15
Neem seed 75%	20	7	15
Tobacco leaf 75%	2.1	8	13.3
Thiophanate methyl	3.2	9	14
Control	5	4	7

4.11. 5. Effect of aqueous plant extracts on tuber length at harvest

The length of tubers of Pona was positively influenced by the plant extracts. (Table.4.10). Pona plants treated with aqueous neem seed extract at 75% concentration produced the longest tubers (27.5cm). The 75% concentration of aqueous tobacco leaf extract was higher (25cm) then the 75% concentration of the aqueous Jatropha seed extract (21cm). Laribako plants treated with plant extracts were significantly different in tuber length compared to the negative control. Aqueous Neem seed extract at 75% concentration produced the longest tubers (24cm) but was not significantly different from 75% concentration of aqueous Jatropha seed extract (23.9). Kpagaa plants treated with plant extracts were not significant. The negative control produced the longest tubers (37cm) than the plant extracts (Table. 4.10).

Table.4.10. Tuber length at harvest (cm)

Plant extract	Pona	Laribako	Kpagaa
Jatropha seed 75%	21	23.9	31
Neem seed 75%	27.5	24	24
Tobacco leaf 75%	25	19.6	27
Thiophanate methyl	11	25	30
Control	6	19	37

CHAPTER FIVE

5. 0. DISCUSSION

5.1. Isolation and identification of anthracnose causing agent(s)

Colletotrichum gloeosporioides Penz, *Aspergillus niger* (A) and *Rhizopus* spp. were isolated from anthracnose infected yam leaves, tubers and vines in the field (Alleyne, 2012). However, *C. gloeosporioides* Penz was identified as the causal agent responsible for all the symptom types observed in the field. Kutama *et al.* (2013) isolated *C. gloeosporioides* Penz from yam leaves with foliar lesions in over 96% of all locations. This confirms a similar observation reported by Ayodele *et al.*(2004), that in addition to *C. gloeosporioides* Penz, other fungi such as *C. truncatum* were isolated from the diseased yam leaves.

C. gloeosporioides Penz recorded the highest percentage occurrences (80 %). According to Abang *et al.* (2006), anthracnose disease of yam caused by *C. gloeosporioides* Penz is most common and severe in the leaves. According to Pallem *et al.* (2012), *Colletotrichum* is the most common fungal pathogen causing anthracnose in temperate, subtropical and tropical areas.

This fungus produced different growth patterns, and mycelial colour in culture. With this diversity, it is obvious that there are several other isolates of *C. gloeosporioides* either singly or in association involved in the yam anthracnose infections in the field. It was also observed during field inspections that the isolate causing the symptoms was very virulent, causing severe lesions, defoliation and dieback. These findings agree with Ayodele *et al.* (2004) who reported that colonies of *C. gloeosporioides*

Penz are easily identified by their dark grey mycelia, and superficial patches of white fluffy growth. According to Pallem et al.(2012), and Bem (2013), cultures of *C. gloeosporioides* Penz exhibit white, grey, dark orange and pink-grey colour, while the reverse side of the colonies are of white, dark grey and orange colour.

The presence of cylindrical conidia on the hyphae of *C. gloeosporioides* Penz also agrees with Joseph *et al.* (2004) and Bem *et al.* (2013) who reported that *C. gloeosporioides* Penz is distinct in its conidia (straight, cylindrical) and conidia with obtuse end (Moriwaki *et al.*, 2002), from those of any known pathogenic species. According to Moriwaki *et al.* (2002) *C. gloeosporioides* Penz isolates produce conidia with obtuse ends. The growth patterns of *C. gloeosporioides* Penz were also ranged from concentric with distinct rings to growth with pronounced rings by the 10th day in culture. This supports the report of Ayodele *et al.*(2004).

5.2. Pathogenicity test

Colletotrichum gloeosporioides Penz caused disease on the yam leaves resulting in development of lesions. *Aspergillus niger* (A) and *Rhizopus* spp. did not cause any infection. *Colletotrichum gloeosporioides* Penz was re-isolated and identified as the causal agent, thus fulfilling Koch's postulates. According to Alleyne (2012) isolates of *C. gloeosporioides* Penz were pathogenic only towards the host plants from which they were isolated. The findings of this study also supported Abang *et al.* (2006) who reported that foliar anthracnose caused by *C. gloeosporioides* Penz. (teleomorph *Glomerella cingulata* (Stonem.) Spauld and Schrenk), is the most common and damaging disease of yam. The inability of *A. niger* (A) and *Rhizopus* spp. to cause

infection could be due to the fact that they were opportunistic fungi and that they were not responsible for the anthracnose symptoms.

5.3. Effect of aqueous plant extracts on fungal diseases

The phytochemicals (*Jatropha curcas* L seed extract, *Azadirachta indica* A Jus seed extract and *Nicotiana tabacum* leaf extract) each at 75% concentration had positive effect on the fungal diseases (*C. gloeosporioides*). They are potent and have the ability to control plant pathogens and have potential to be valued in pest management (Mishra and Dubey, 1994). Botanicals contain a wide variety of secondary metabolites which are effective as plant protectants (Tripathi *et al*., 2004). Plant extracts are non-phytotoxic, easily biodegradable and cost effective.

5.4. Effect of aqueous plant extracts on growth of *C. gloeosporioides*

Three different aqueous plant extracts obtained from *A. indica* seed, *J. curcas* seed and *N. cotiana tabacum* (L) leaf were evaluated for the possible presence of fungitoxic activity against *C. gloeosporioides* Penz by using poison food technique. It was evident that each concentration of the plant extracts inhibited the radial mycelial growth of *C. gloeosporioides* Penz. The inhibitory effect of the aqueous plant extracts could be due to the presence of antifungal compound as reported by Thangavelu *et al.* (2004), Ajith *et al.* (2012), Amadioha (2000), and Nwogbaga (2012) that medicinal plants possess some compounds that are anti-pathogenic. The 75% concentrations of each the plant extracts showed maximum inhibition of radial mycelial growth of *C. gloeosporioides in vitro*. Percentage inhibition of the mycelial growth increased with increasing concentration of the plant extracts. Suleiman (2011) reported that inhibition

of mycelial growth of *A. viridae* and *P. digitatum* increased with increasing concentration of ethanolic extracts of *A. indica* A Jus and *N. tabacum* leaves.

5.5. Effect of aqueous plant extracts on incidence and severity of anthracnose disease of yam

Aqueous extracts of *A. indica* A Jus seed, *J. curcas* (L) seed and *N. tabacum* (L) leaf at 75% concentration reduced the percentage incidence of anthracnose *in vivo* (in the field). Hence the aqueous plant extracts at 75% concentration exhibited fungitoxic action that reduced the severity of anthracnose disease and prevented its further spread to uninfected plants of yam. This might be due to the fact that plant extracts possess active ingredients that are potent against plant pathogens (Nwogbaga, 2012).

Aqueous extracts of *J. curcas* (L) leaves inhibited the mycelial growth of *C. gloeosporioides* Penz in Para rubber tree (Ogbebor *et al.*, 2007). *Jatropha curcas* (L) extracts caused complete inhibition of the mycelial growth of *C. gloeosporioides* responsible for anthracnose; dieback, root rot, blossom rot and seedling blight of fruits such pwapwa (Saetae and Suntornsuk, 2010).

Singh *et al.*(1980), Locke, (1990), Locke and Lawson, (1990), Amadioha, (2000), Okigbo and Emogheme, (2003)) also reported that extracts of the leaves, roots and seeds of neem tree have the potency to inhibit the mycelial growth of fungi. There were significant differences among the plant extracts. The active components in the plant extracts could have also been influenced by the method of extraction, age of a plant, time of harvesting plant materials (Locke and Lawson, 1990).

Aqueous extracts from other plants (*C. aromaticus*) also reduced incidence of anthracnose by 26.09% Ajith (2012). Non-aqueous plant extracts **(**essential oils of neem seed) at 50% and 60% concentrations showed inhibition of *C. gloeosporioides* Penz growth in yam (Meazza *et al.*, 2003; Meepagala *et al.*, 2002). Neem seed oil is effective against anthracnose, *Rhizoctonia solani* (Kuhn) and *Scleratinia sclerotiorum* (S) Singh *et al.* (1980).

5.6. Effect of aqueous plant extracts on the number of leaves of yam

Aqueous extracts of *A. indica* A Jus seed, *J. curcas* (L) seed and *N. tabacum* (L) leaf were potent and promoted the number of leaves of yam. This could be attributed to the impact of reducing anthracnose (*C. gloeosporioides*) incidence and severity and presence of some growth hormones in the plant extracts as reported by Datar (1999), that plant extracts do not only control pathogens but as well stimulate the growth of plants which may be due to the presence of some growth hormones in them. Sridhar (2002), reported that "Limonoides" present in neem promote plant growth. According to Agrios (2004), plant growth are regulated by a small number of groups of naturally occurring compounds in plants, which act as hormones and were generally called growth regulators (auxins, giberellins, cytokinings, ethylene and some growth inhibitors).

5.7. Effect of aqueous plant extracts on chlorophyll content of yam leaves

The chlorophyll content was significantly higher ($P < 0.05$) in plants treated with 75% concentration of plant extracts than in the negative plants. The low chlorophyll content in the negative plants could be attributed to the effect of the anthracnose infection on

them. This was in agreement with Winch *et al.* (1984) and Green (1998) that the disease infects leaves, petioles and stems of yam plants resulting in extensive necrosis of the foliage and that severe infection ends up in reduction of chlorophyll content, increase in defoliation and vine dieback. It could also be attributed to the amount of sunlight received by the sampled leaves. Also, plant species and position of leaves affect the amount of chlorophyll content (Gond *et al.*, 2012). According to Amusa (1991, 1997) and International Institute of Tropical Agriculture (1993) anthracnose of yam immensely affects the photosynthetic activities of the plant and subsequently the yield.

5.8. Effect of aqueous plant extracts on yield of yam

Plants treated with the 75% concentrations of aqueous plant extracts produced a higher number of tubers compared to the negative control. In the case of yam tuber defectiveness, yam plants treated with 75 % plant extracts produced fewer defective tubers than the negative control. Longer and heavier tubers were also produced by plants treated with the plant extracts compared to the negative control.

This study has therefore revealed that application of plant extract to manage anthracnose of yam is a necessity for improving productivity. Significant differences ($P < 0.05$) in yield with respect to number, length, quality and weight of tubers of plants treated with plant extracts have been attributed to many factors. These factors include high vegetative growth and chlorophyll content (Hakan, 2012), low anthracnose incidence and severity. Application of 75% concentration of aqueous *A. indica* A Jus seed, *J. curcas* (L) seed and *N. tabacum* (L) leaf extracts on the field appeared adequate for maximizing tuber yield under the conditions of the experiment.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

The primary aim of the study was to; isolate and identify the causal agent of anthracnose of yam, evaluate the efficacy of botanicals *in vitro* and *in vivo*, determine incidence and severity of anthracnose on yam, determine the effect of plant extracts on the vegetative growth and yield of yam.

C. gloeosporioides Penz was isolated and identified as being responsible for the anthracnose symptoms. *C. gloeosporioides* Penz had the highest percentage occurrence in the leaf (80%), followed by the tuber (30%) the vine (10%) compared to *A. niger* and *Rhizopus* spp. It caused infection and produced lesions compared to *A. niger* and *Rhizopus* spp. with no lesions on yam leaves. The *in vitro* studies revealed that each concentration of the plant extracts were potent and, therefore inhibited the mycelial grow of *C. gloeosporioides* Penz. However, the 75% concentration of each plant extract was most effective. The higher the concentration of the plant extract, the higher its inhibitory action.

The *in vivo* (field) studies have revealed that incidence and severity of anthraces of yam were significantly ($P < 0.05$) reduced in plants treated with 75% concentration of plant extracts compared to the untreated control. The aqueous plant extracts had a positive influence on the number of yam leaves after emergence. Plants treated with aqueous *A. indica* A Jus and *J. curcas* (L) seed extracts were comparable with the highest number of yam leaves (500) followed by the leaf extract of *N. tabacum* (400).

However, there was no significant difference between thiophanate methyl (positive control) and the negative control. It was also revealed that uninfected (anthracnose free) leaves of yam plants had higher chlorophyll content by the 3rd week (50%) than anthracnose infected leaves (20%).

Generally, the plant extracts positively influenced the yield of yam in terms of number of tubers, length of tubers, weight and quality of tubers at harvest. For instance, pona plants treated with 75% concentration of aqueous *A. indica* A Jus seed extract recorded the highest number of yam tubers per plant (5), followed by *J. curcas* (L) seed extract (4) compared to the untreated control (1). In the case of tuber quality, plants treated with plant extracts produced fewer defective tubers for each variety**.** Plants treated with 75% aqueous neem seed extract recorded the least percentage defect (11%) on pona, followed by tobacco leaf extract (30%) and Jatropha seed extract (40%). A similar trend was observed in both laribako and kpagaa.

6.2. Recommendations

1. Extracts of *J. curcas* (L) seed, *A. indica* A Jus seed, and *N. tabacum* (L) leaf have the potential to control plant diseases. They are non-phytotoxic, environmental friendly and cost effective. Farmers, therefore, need to be educated, sensitized, and encouraged to promote their use rather than synthetic fungicides.

2. Use of 75% concentrations of aqueous extracts of *A. indica* A Jus seed, *J. curcas* (L) seed, and *N. tabacum* (L) leaf are generally recommended to farmers since aqueous extracts are relatively cheaper, and easier to prepare. They are equally effective in the control of plants pathogens. Neem should preferably be used by

farmers since it is common. The application needs to be done as soon as seedling emergence begins to inhibit infection and disease symptom development. The extracts are much more effective when applied as preventives than curatives

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APPENDICE

APENDIX.1.Field layout and labels

REP.1

T3 **PTL**	T13 KTL	T15 **KC**	T4 **PTH**	T11 KNS
T2 **PJS**	T12 **KJS**	T9 **LTH**	T1 **PNS**	T14 KTH
T8 **LTL**	T10 LC	T6 LNS	T5 PC	T7 LJS

REP.2

T10 **LC**	T4 **PTH**	T2 **PJS**	T15 KC	T5 **PC**
T1 **PNS**	T9 **LTH**	T3 **PTL**	T7 **LJS**	T11KNS
T6 **LNS**	T12KJS	T13KTL	T8 **LTL**	T14KTH

REP.3

T13KTL	T5 **PC**	T2 **PJS**	T7**LJS**	T4 **PTH**
T10 **LC**	T6 **LNS**	T11**KNS**	T9 **LTH**	T12**KJS**
T1 **PNS**	T14**KTH**	T3 **PTL**	T15 **KC**	T8 **LTL**

REP. 4

T9 LTH	T5 **PC**	T2 **PJS**	T7 **LJS**	T8 **LTL**
T11**KNS**	T6 **LNS**	T10 LC	T13**KTL**	T12**KJS**
T1 **PNS**	T14**KTH**	T3 **PTL**	T15 **KC**	T4 **PTH**

BOTANICALS (FACTOR 1)	YAM VARIETY (FACTOR 2)
1.Neem seed extract 75 %	**1**.Pona
2.*Jatropha* seed extract 75 %	**2**.Labreko
3.Tobacco leaf extract 75 %	**3K**pagaa
4.Thiophanate methyl	
5.Control.	

KEY

T1=Pona variety with 75% neem seed extract **T9**=Laribako variety with 75% Thiophanate m

T2=Pona variety with 75% *J curcas* seed extract **T10**=Laribako variety with control

T3=Pona variety with 75% tobacco leaf extract **T11**=Kpagaa variety with 75% neem seed

T4=Pona variety with 75% Thiophanate methyl **T12**=Kpagaa variety with 75% *J curcas* seed

T5= Pona variety with control **T13**= Kpagaa variety with 75% tobacco leaf extract

T6= Laribako variety with75% neem seed extract **T14**=Kpagaa with 75% Thiophanate m

T7=Laribako variety with 75% *J curcas* seed extract **T15**=Kpagaa with control.

T8=Laribako variety with 75% tobacco leaf extract

APPENDIX .2. Percentage emergence of yam after planting

VARIABLES	TREATMENTS			G.MEAN	LSD	CV%	Fpr
	Pona	Labreko	Kpagaa				
WEEK 1	2.75[a]	1.50[a]	3.5[a]	2.58	3.143	76.1	0.387
WEEK 2	6.25[b]	4.50[b]	6.50[b]	5.75	2.957	32.1	0.298
WEEK 3	9.75[c]	9.25[c]	9.75[c]	9.58	1.099	7.2	0.519

APPENDIX. 3. The effect of Plant extracts on chlorophyll content of *D. rotundata*

	Neem	Jatropha	Tobacco	Thiophanate	Control	Grand mean	LSD	CV%	Fpr
Pona wk 1	42.17^{e}	39.90^{e}	38.42^{e}	35.77^{e}	30.35^{e}	36.95	7.634	14.5	<.001
Pona wk 2	52.67^{m}	53.67^{m}	51.33^{m}	46.70^{m}	26.52^{n}	45.48	9.478	14.6	<.001
Pona wk 3	55.90^{v}	49.75^{v}	49.50^{v}	47.50^{v}	30.25^{w}	47.24	9.041	13.4	<.001
Labreko wk 1	40.30^{g}	44.28^{g}	40.90^{g}	40.17^{g}	29.62^{h}	36.95	7.634	14.5	<.001
Labreko wk 2	43.02^{p}	48.75^{p}	53.00^{p}	51.77^{p}	29.02^{q}	45.48	9.478	14.6	<.001
Labreko wk 3	45.98^{y}	49.98^{y}	48.98^{y}	51.62^{y}	39.75^{z}	47.24	9.041	13.4	<.001
Kpagaa	37.27^{j} 40	37.73^{j}	40.95^{j}	37.00^{j}	19.38^{k}	36.95	7.634	14.5	<.001
Kpagaa wk 2	49.00^{s}	49.70^{s}	53.92^{s}	47.60^{s}	25.50^{t}	45.48	9.478	14.6	<.001
Kpagaa week 3	50.00^{b}	50.70$_{b}$	54.12^{b}	52.33^{b}	32.25^{c}	47.24	9.041	13.4	<.001

APPENDIX. 4. Percentage incidence of anthracnose on Pona

VARIABLES	TREATMENTS					GRAND MEAN	LSD	CV%	Fpr
PONA	NS	JS	TL	TH	C				
Week 3	0.25[a]	0.25[a]	0.25[a]	0[a]	1[b]	0.700	1.0615	106.5	0.286
Week 6	0.25[b]	0.25[b]	0.5[b]	0[b]	1[c]	0.783	1.0126	90.8	0.053
Week 9	0.5[c]	0.25[c]	0.5[c]	0[c]	1.5[d]	0.917	1.0346	79.3	0.009
Week 12	0.5[d]	0.25[d]	0.5[d]	0[d]	1.5[e]	0.917	1.0346	79.3	0.009

APPENDIX. 5. Percentage incidence of anthracnose on Laribako

VARIALES	TREATMENTS					GRAND MEAN	LSD	CV%	Fpr
LABREKO	NS	JS	TL	M.TH	C				
Week 3	0.5[a]	1[a]	0.75[a]	1.25[a]	1.25[a]	0.700	1.0615	106.5	0.286
Week 6	0.5[b]	1[b]	1[b]	1.25[b]	1.75[b]	0.783	1.0126	90.8	0.053
Week 9	0.5[c]	1.25[c]	1.5[c]	1.5[c]	1.75[c]	0.917	1.0346	79.3	0.009
Week 12	0.5[d]	1.25[d]	1.5[d]	1.5[d]	1.75[d]	0.917	1.0346	79.3	0.009

APPENDIX. 6. Percentage incidence of anthracnose of Kpaga

VARIABLES	TREATMENTS					GRAND MEAN	LSD	CV%	Fpr
KPAGAA	NS	JS	TL	M.TH	C				
Week 3	0.5[a]	0.75[a]	1[a]	0.5[a]	1.25[a]	0.700	1.0615	106.5	0.286
Week 6	0.5[b]	0.75[b]	1[b]	0.5[b]	1.5[c]	0.783	1.0126	90.8	0.053
Week 9	0.5[c]	0.75[c]	1[c]	0.5[c]	1.75[d]	0.917	1.0346	79.3	0.009
Week 12	0.5[d]	0.75[d]	1[d]	0.5[d]	1.75[e]	0.917	1.0346	79.3	0.009

APPENDIX. 7. Percentage severity of anthracnose on *Discorea rotundata* Poir

VARIABLES	TREATMENTS					GRAND MEAN	LSD	CV%	Fpr
	NS	JS	TL	M.TH	C				
Pona wk 3	1.25^{c}	1.25^{c}	1.5^{d}	1^{e}	2.25^{f}	1.817	0.9554	36.9	<.001
Pona wk 6	1.5^{h}	1.25^{h}	1.5_{h}	1^{h}	2.75^{i}	1.950	1.0455	37.6	<.001
Pona wk 9	1.5^{n}	1.25^{p}	1.5^{q}	1^{r}	3.25^{x}	2.100	0.9495	31.7	<.001
Pona wk 12	1.5^{e}	1.25^{f}	1.75^{g}	1^{h}	4.5^{j}	2.333	0.9844	29.6	<.001
Labreko wk 3	1.25^{a}	1.75^{b}	2.25^{c}	1.5^{d}	4^{e}	1.817	0.9554	36.9	<.001
Labreko wk 6	1.25^{i}	2^{i}	2.5^{i}	1.5^{j}	4.25^{k}	1.950	1.0455	37.6	<.001
Labreko wk 9	1.5^{x}	2^{y}	2.75^{z}	1.75^{a}	4.75^{b}	2.100	0.9495	31.7	<.001
Labreko wk 12	1.5^{k}	2.25^{l}	3^{m}	2^{n}	4.75^{s}	2.333	0.9844	29.6	<.001
Kpagaa wk 3	1.25^{d}	1.25^{d}	1.75^{e}	1.25^{f}	3.75^{g}	1.817	0.9554	36.9	<.001
Kpagaa wk 6	1.25^{l}	1.5^{l}	2^{l}	1.25^{l}	3.75^{m}	1.950	1.0455	37.6	<.001
Kpagaa wk 9	1.25^{b}	1.5^{c}	2^{d}	1.25^{e}	4.25^{c}	2.100	0.9495	31.7	<.001
Kpagaa wk 12	1.5^{v}	1.5^{v}	2.25^{t}	1.25^{a}	5^{w}	2.333	0.9844	29.6	<.001

APPENDIX. 8. The inhibitory effect of *A. indica* A. Jus seed extract on the mycelial growth of *C. gloeosporioides* Penz *in vitro*

TREATMENTS	VARIABLES							
	Day1	Day2	Day3	Daay4	Day5	Daay6	Day7	Day8
Control	10.67^{a}	18.33^{b}	27.33^{c}	36^{d}	45.33^{e}	50.33^{f}	56^{g}	59.33^{h}
Neem 35%	8.67^{a}	13.67^{b}	21^{c}	30.33^{d}	39.33^{e}	47.33^{f}	52.33^{g}	56.67^{h}
Neem 45%	11^{a}	16.67^{b}	20.33^{c}	27^{d}	29.67^{f}	32^{g}	34^{h}	35^{i}
Neem 55%	6.33^{a}	9.67^{b}	12^{c}	15.67^{e}	18.67^{g}	23^{h}	25.33^{i}	26.67^{j}
Neem 65%	4.33^{a}	7.33^{b}	9.67^{c}	12^{e}	14^{g}	16.33^{h}	18.33^{j}	19.33^{k}
Neem 75%	2.33^{a}	4^{b}	6.67^{c}	8.67^{e}	10^{g}	11.67^{h}	13.67^{j}	14^{l}
Thiophanate	0.00^{a}	0.00^{b}	0.00^{c}	0.00^{e}	0.00^{h}	0.00^{i}	0.00^{k}	0.00^{m}
Grand mean	6.19	9.95	13.86	18.52	22.43	25.81	28.52	30.14
LSD (5%)	2.445	3.205	3.29	3.256	3.56	3.611	2.361	2.25
CV%	22.2	18.1	13.3	9.9	8.9	7.9	4.7	4.2
Fpr	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

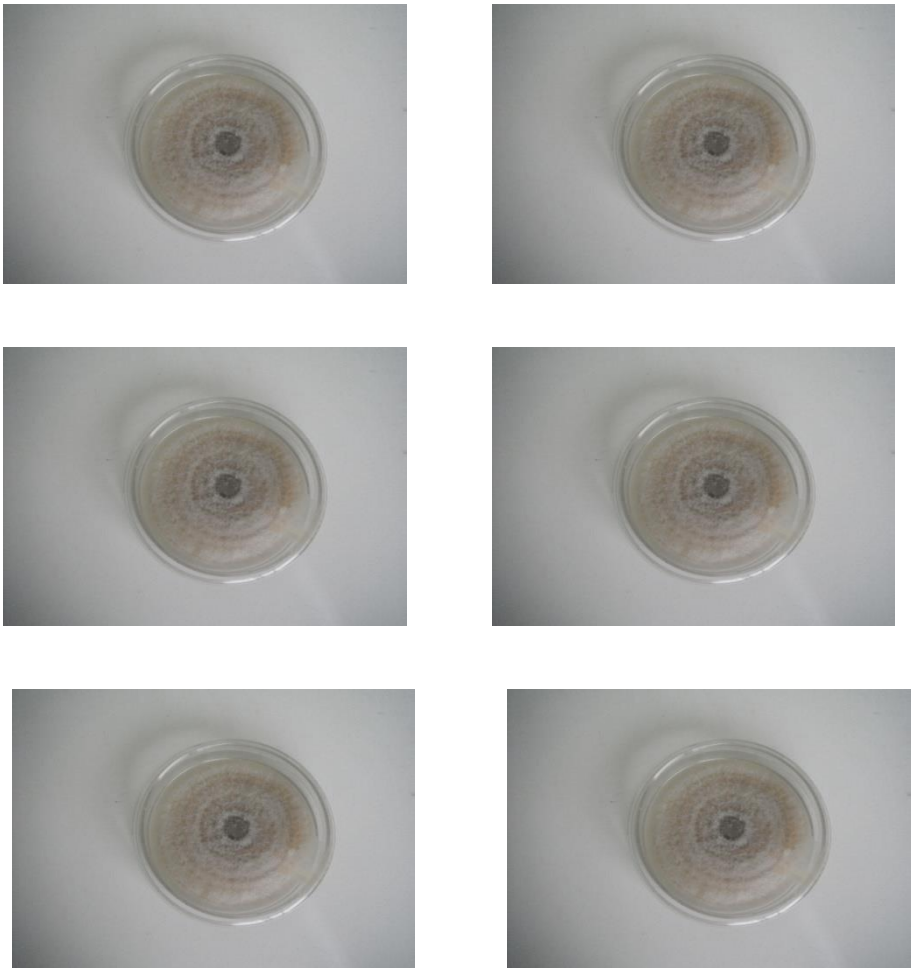
APPENDIX. 9. Yam plants under experiment in the field

APPENDIX.10. Experimenter carrying out experiment on isolation of anthracnose causing organisms from anthracnose infected yam leaves, vines and tubers in the fume chamber

APPENDIX. 11.Yam tubers at harvest

APPENDIX. 12. Symptoms of anthracnose infected yam leaves

APPENDIX. 13. Culture of *Colletotrichum gloeosporiodes* on PDA plates



APPENDIX. 14. Bull locks ploughing the experimental field.

APPENDIX. 15. Seeds of *J. curcas* and *A. indica* used during the study

APPENDIX.16. Determination of chlorophyll content using chlorophyll meter

APPENDIX.17. World production and trade in yams (1995-2000).

	2000	1999	1998	1997	1996	1995
Production Mt	37,532138	37,552,383	35,753,519	34,705,657	33,587,195	32,765,435
Exports Mt	-	23,198	21,080	28,069	27,493	26,264
Exports 1000USS	-	20,077	19,212	20,873	20,810	21,108

Source: (FAO/STAT, 2000).