UNIVERSITY OF CAPE COAST

"THE STUDY OF PHYTOPHTHORA HEART ROT DISEASE OF PINEAPPLE IN SOME SELECTED DISTRICTS IN THE CENTRAL REGION"

AMMA KISSIWAA AMOATENG

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BY

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Thesis submitted to the Department of Crop Science, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirement for the award of Master of Philosophy (MPhil) degree in Crop Science

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DECLARATION

I, Amma Kissiwaa Amoateng do herewith state that this work titled "The study of Phytophthora heart rot in some selected districts in the central region" was undertaken by me and that it is the documentation of my own research work and has not been submitted anywhere else neither in full nor part for the award of a degree.

Amma Kissiwaa Amoateng	…….	……
(Student Name)	(Signature)	(Date)

The above statement / declaration is confirmed

Dr. Mrs. Grace van der Puije	……	……
(Supervisor's Name)	(Signature)	(Date)

ABSTRACT

Phytophthora heart rot disease (PHRD) of pineapple is one of the damaging diseases that attack pineapple plants of all ages and can cause 100% yield loss. Documented information on the disease in Ghana is quite limiting even though Ghana is among the top exporting countries in Africa. It is therefore essential to know the status of this disease, the particular species of *Phytophthora* causing the disease in the selected areas of study (Komenda-Edina-Eguafo-Abrem, Abura-Asebu-Kwamankese and Ekumfi districts) and how it could be managed using botanicals and bio-control organism. The study was conducted in three main phases: surveys (demographic and field), pathogen identification and characterisation and *in vitro* disease management. One hundred and twenty (120) farmers were interviewed using structured questionnaires. The disease was present in seven (7) out of the twelve (12) communities selected for the survey. The highest incidence was recorded at Abrenu-Akyinim (3.40%) and Asofa (3.40%) with the least at Nanaben (0.4%). Disease severity was highest at Abrenu-Akyinim (1.50) and lowest at Atabadze (0.2). *P. nicotiannae* and *P. cinnamomi* were both found to be the causative organism of PHRD in the study area. *In vitro* control studies using Neem, Prekɛsɛ, Mahogany extract and *Trichoderma asperellum* via Minimum Inhibitory Concentration test showed that all treatments with the exception of Mahogany were inhibitory to *P. cinnamomi* with Prekɛsɛ recording the highest mean inhibition index of 3.00. A dual culture technique test performed using the *Trichoderma* sp. and *Phytophthora* isolates on different application days, showed that, the application of the *Trichoderma* 24 hrs before plating the isolate gave the highest inhibition percentage.

KEYWORDS

Ananas comosus (Pineapple)

Bio-control

Botanicals

Central region

Phytophthora spp

Heart rot disease

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DEDICATION

I dedicate this work to all my lecturers especially Dr. Mrs. Grace C. van der Puije and Dr. Festus Annor - Frempong. I also dedicate this work to my colleagues on the Pineapple Value Chain research team.

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CHAPTER ONE

INTRODUCTION

Pineapple (*Ananas comosus*) is one of the most important fruits in the world. It belongs to the Family *Bromeliaceae,* Sub-family *Bromelioideae* and Order *Bromeliales* (Barthelomew *et al*., 2003). The origin of pineapple is not known but *Ananas comosus var ananassoides* is known to be of a wild ancestry. It has very tiny, seedy fruit and spiny leaves (Bartholomew *et al*., 2003).

Pineapple was cultivated first by the Tupi-Guarani Indians, before the discovery of pineapple by Christopher Columbus in November 1493 (Collins, 1960). It was largely distributed within tropical America and then extended to Africa, Asia, and the South Pacific (Ho-a-Shu, 1999). As at 2013, the major producing and exporting countries of pineapple included Philippines, Brazil, Costa Rica, Thailand and China with Ghana at the 26th and 8th positions with respect to production and export on international market (UNCTAD, 2016).

Pineapple is third to banana and citrus in world fruit production (Collins, 1960). There are 30 cultivars of *Ananas comosus* that are grown in subtropical and tropical countries in the world (d'Eeckenbrugge & Leal, 2003). Among these are the smooth cayenne, queen, red Spanish, Pernambuco, sugar loaf, and MD2. In Ghana, the cultivars grown are smooth cayenne, MD2 and sugar loaf. In Ghana, the crop is one of the most important for the local and foreign markets. Lucrative markets for pineapples have brought about the upsurge of fruit processing industries. Areas of pineapple cultivation in Ghana are Greater Accra, Central, Eastern and the Volta Regions.

Pineapple contains significant amounts of vitamins and other essential nutrients. Initially only the juice from pineapple fruit was consumed but in

recent times different products can be obtained from the fruit. Processing industries can now prepare jam, syrup, crushed, chunks and diced pineapples from the pineapple fruit. The by-product from processing the fruit can be used for wine, vinegar, animal feed and manure (Ho-a-Shu, 1999). Different parts of the pineapple crop like the leaves of certain varieties are used in making fibres (d'Eeckenbrugge & Leal, 2003), which are in turn used in manufacturing clothes, rope, fishing net and pulp for the paper industry (Ho-a-Shu, 1999).

In addition to its use the pineapple fruit has medicinal properties due to the presence of a protein digesting enzyme, bromelain, which provides relief for arthritis sufferers, helps in reducing blood clotting, act as a digestive aid and an analgesic agent (Bartholomew *et al.*, 2003). Bromelain is used to make meat tender, produce vegetable oils, dehydrate eggs and clarify beer. The juice from the leaf is used as a vermifuge for cleansing worms and also as a purgative (d'Eeckenbrugge & Leal, 1996).

Pineapple like any other plant is attacked by different diseases caused by different pathogens such as bacteria, fungi and viruses. The most damaging of the fungal diseases is the Phytophthora heart rot disease (PHRD). PHRD attacks plants of all ages, most especially three to four months old plants if it is not managed (Hegde, 2014). According to Fulcher and Bowers, (2013) extended periods of high soil moisture favour the disease condition but disease development can occur in relatively dry locations, with occasional heavy rainfall (Marçais *et al.*, 2004). The reason is that sporangia of the pathogen cause new infections by germinating directly and colonizing roots and basal leaf or releasing many zoospores. In some areas such as Hawaii in the United

States of America, high rainfall coupled with poor drainage promotes or increases disease severity (Green & Nelson, 2015).

The Phytophthora heart rot disease is characterised by rotting of the entire middle portion of the stem where the basal white leaf becomes water soaked, causing a foul odour and wilting of heart leaves which become brown and are easily pulled off (Shen *et al*., 2013; Green & Nelson, 2015). On fruit-bearing plants of susceptible varieties of pineapple, the infection can extend to the peduncle and rot the fruit (Green & Nelson, 2015). The disease manifestation of the PHRD varies depending on the variety of pineapple (Rodriguez *et al*., 2002). The manifestation is further complicated by the great diversity, high aggressiveness and the multicyclic nature of *Phytophthora* (Milenkovic *et al*., 2014; Ocwa *et al.*, 2018). Pineapple heart rot disease can cause yield losses up to 100% (Rohrbach & Schenke, 1985). Several researchers (Joy & Sindhu, 2012; Shen *et al*., 2013) have reported that pineapple heart rot disease is caused by a wide range of *Phytophthora* species.

Due to the severe damage caused by PHRD, certain management practices are undertaken to reduce severity of the disease. Management practices such as good sanitation and crop rotation which reduce level of inoculum of oospore and chlamydospore, planting of resistant variety, disease free planting materials and pesticides (Green & Nelson, 2015) are used by farmers to keep disease level below the threshold.

Research has shown that systemic fungicides, e.g. fosetyl Al and metalaxyl are used to manage the pathogen (Dalldorf, 1993; Hegde, 2014). Pesticides are central to the management of plant pathogenic organisms to protect crops from pest damage (Rani *et al*., 2017). The downside of pesticide use is its effect on

the environment as it is mostly harmful to beneficial soil microorganisms (Rani *et al*., 2017). The constant use of pesticides also results in resistance in the pathogen it is meant to control, and as such its effectiveness is reduced (Alkhail, 2005; Rani *et al*., 2017).

Also, in recent times, studies have shown that price premiums of about 15% - 30% are placed on produce that use these organic pesticides in place of the synthetic ones. Such produce termed organic is mainly aimed at the export markets where these premiums are common (CBI, 2008). Farmers are increasingly moving away from the use of synthetic chemicals on their crops in favour of botanicals and bio-control agent mainly to protect the environment, sustain their farm businesses, and increase their economic rewards from their farm enterprises.

Statement of the Problem

According to FAO, 2019 during the tropical pest and disease conference it was said that there has been an increase in demand for tropical fruit like pineapple and demand will continue to increase but like any other crop pest and disease incidence have an impact on sustainable production.

In 2014, there was a decline of about 10% - 30% in production and earnings reduced from US$ 24 million to US$ 19 million (Gatune *et al*., 2016; 'Pineapple yields unsettle farmers', 2016). The decline was attributed to many factors; one of such important factor was pests and diseases. One of the most devastating diseases of pineapple is the Pineapple heart rot disease (Rohrbach & Johnson, 2003) which is caused by *Phytophthora* spp. This disease can cause crop losses up to US$ 2.4 billion on the average globally (Drenth & Guest, 2004). Different species of *Phytophthora* are known to cause heart rot

in pineapple: *P. palmivora*, *P. cinnamomi* and *P. nicotianae* (Ratti *et al*., 2018). Not much work has been done with respect to the disease in Central region and Ghana as a whole, even though Central region is a major pineapple production area with government flagship programmes like 1D1F (One district, one factory) been undertaken and also Ghana being among the major producers and exporters in Africa. It is therefore important to determine the prevalence of PHRD, the species occurring in the production area and a possible control measure.

Significance of the study

To ensure sustainable production and increase yields, information on disease status is needed. It is also important to know the species responsible for causing the disease for holistic management. Also, the effectiveness of the bio-control agents and botanical extract treatment will be a valuable additional knowledge.

1.1 General Objectives

The general objective is to assess the prevalence of Phytophthora heart rot disease in pineapple, identify the causal organism and evaluate botanicals and a bio-control organism against the identified pathogen.

1.2 Specific Objectives of the study

Specifically this study aims to

1. determine the prevalence of PHR disease in three districts in Central Region through a field and household survey.
2. identify and characterize the causal agent by morphological and molecular methods.

3. evaluate the efficacy of botanicals (Neem, Mahogany, and Prekese extracts) and biological control organism (*Trichoderma* spp.), on the *Phytophthora* spp. identified.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany

Pineapples are perennial plants that are grown all year long. They are grown by using propagules such as crowns, slips and suckers (Bartholomew et al., 2003).

They are herbaceous plants that are about 2 metres tall. The leaves of pineapple plants are long, trough-shaped, wide at the base and tapered at the tip, and arranged spirally on the stump. They shade leaves when large and mature. The number of leaves varies from cultivar to cultivar, but a mature pineapple has about 82 leaves. The leaves are spirally arranged such that the thirteenth leaf partly covers and shades the lower leaf on the plant. The leaf arrangement differs between large fruited and small fruited pineapples (d'Eckenbrugge and Leal, 2003). A mature plant weighs 3.6 Kg with leaf area of about 2.2 m^2 (Collins, 1960) and few stomata located on the underside of the leaf. The stomata are protected by waxy trichomes and have low transpiration rate.

The stem is erect, cylindrical and club shaped. It is wider at the base, measuring 25 -50 cm and narrow at the top, measuring 5- 8 cm. It has nodes and anti-nodes (Purseglove, 1972). The stem is a reservoir for about 11% starch. The stem cannot be seen unless leaves are removed. There are lateral buds on the stem which is connected to each leaf and can form ratoon suckers to be used in replant fields. (Collins, 1960).

The pineapple plant has a depthless root system which does not grow again when attacked by pests and diseases. The roots are of two types, the axillary

roots and the main roots. All roots start from the growing point of the planting material. They grow through the cortex and emerge just below the leaf base of the growing tip. Axillary roots are formed when the growing tips move farther away from the soil and wraps around the stem. Main roots are formed when the growing tip emerges within a centimetre above or below the soil ground. The main root appear within the first 12 months and last till harvest. Root growth starts right after planting to flowering but can slow down depending on temperature and soil moisture conditions. The root shape is flat and reddish brown in colour. (Pineapple Best Practice Manual, 2009).

The flower of the pineapple develops from the apex of the meristem in an ascending succession with the youngest at the top. The inflorescence consists of between 50-200 individual flowers and arranged in a spiral form capped with a crown and 150 short leaves. The phase at which the flowers emerge is called the 'red heart' due to the reddish nature of the peduncle bracts produced at the stem (Bartholomew *et al.*, 2003). Flowering occurs when plants have reached sufficient maturity or are artificially induced (Collins, 1960). Natural flowering occurs when plants are a year old and weighs greater than 500 g (Australian Government, 2008). Artificial induction which is known as 'forcing' is performed to avoid uneven natural flowering. Flower induction is done using forcing agents or chemicals like ethylene, naphthalene acetic and acetylene (Gowing & Leeper, 1959; Kuan *et al.*, 2005).

Fruiting in pineapple occur at an irregular manner and needs many rounds of harvest. The fruit is formed as a result of many fruitlets combining to form a unit. Each fruitlet is a whole fruit. When fruitlet formation is complete, the growing point returns to a vegetative state and forms the crown. Ripening of

the fruitlets is from the bottom to the top with the bottom riper and sweeter than the top (Pineapple Best Practice Manual, 2009). The fruit weighs 2.3 kg or more (Bartholomew *et al*., 2003). The pulp colour varies from white to yellow/golden yellow. Seeds are rarely produced, but when produced are curved on one side and appear flat on the other side, with a hard seed coat (Australian Government, 2008).

2.2 Propagation

Pineapples are propagated using crowns, slips, suckers or butts (Bartholomew *et al*., 2003). Each plant is therefore true to type and therefore produces uniform crops. Planting depth is influenced by the kind of planting material; crowns are usually planted at a depth of 5-10 cm, whereas slips and suckers are planted at a depth of 10 - 15 cm (Australian Government, 2008). Crowns and slips are normally the propagules that are used for planting. Slips are normally larger than crowns and reach maturity earlier. Slip and crown planting have different times of flowering even when planted at the same time. Slips flower at about 12 months, whereas crowns flower at about 14 months. The plant crop is ready for harvest at 20 to 24 months after planting. Plants are grown on flat ground or raised bed with intra row distance of 60 – 80 cm and inter row distance of 35 - 40 cm (d'Eckenbrugge and Leal, 2003).

2.3 Ecology

Pineapple production is influenced by factors, such as cultivars, climatic conditions, altitude and cultural practices (Hassan *et al*., 2011). Pineapple grows best in warm climates with temperatures between 26˚C - 33˚C. Temperatures of about 40ºC affect plant growth and therefore not suitable

(Bartholomew *et al*., 2003). It is fairly drought resistant requiring low but regular rainfall, but for high yields a well distributed annual rainfall of at least 1000 mm is necessary. Pineapple requires a regular supply of soil moisture and medium altitudes of 1350 - 1750 m above sea level. Below 1350 m the fruit has little fibre, leading to a mushy fruit containing too much sugar resulting in a bland taste. At high altitudes above 1750 m, growth is slow and the fruit contains much acid (Rohrbach & Johnson, 2003).

2.3.1 *Effect of Temperature*

Temperature greatly affects flowering, growth and yield of plant. Pineapple is a plant of the tropics and growth is best at warm temperatures. It can survive in hot, dry as well as cool conditions (Bartholomew *et al*., 2003). The optimal climatic conditions for growing pineapple are between 21 – 27˚C combined with relative humidity of 70% - 80%. When pineapple is produced during winter or under colder conditions, according to Australian Government (2008), fruits produced have high acidity levels and experience a chilling related physiological disorder (Hassan *et al*., 2011). At high temperatures when lots of sunlight is received fruit development is affected, growth retarded (Malezieux *et al.*, 1994) and ascorbic acid content increases (Singleton and Gortner, 1965; Hassan *et al*., 2011). Extremely high temperatures above 35°C increases spoilage.

2.3.2 *Effect of Moisture*

The relative humidity required for the growth of pineapple is 75%-78% (Sarkar, 1994). A high relative humidity is said to improve growth in areas of low rainfall due to moisture condensation on the leaves, which runs down the

middle of the leafs to the base of the plant (Boucher, 1991). The pineapple plant has different ways of conserving moisture and is a very good water efficiency crop (Bartholomew *et al*., 2003). The leaf orientation is such that the large cup formed where the leaf is attached to the stem is effective reservoir for capturing water. Also, the leaves have fewer stomata and are insulated to reduce loss of water. Due to this, water is pulled up to maintain plant growth and fruit development when there is water stress. The pineapple plant can tolerate drought condition to a certain degree. At low moisture levels, growth and yield of plant is reduced significantly (Bartholomew *et al*., 2003). Fruit that mature under this condition is susceptible to cracking. In areas of high rainfall, plant growth is affected and becomes susceptible to diseases which could lead to total crop loss (Le Van, 1991; Bartholomew *et al*., 2003)

2.3.3 *Soils*

Pineapple plants require a rich loamy soil with a pH of 4.5 - 6.5 (Bartholomew *et al*., 2003). A pH greater than 6.5 adversely affects yields (Boucher, 1991) and creates a conducive environment for the multiplication of disease-causing organisms like *Phytophthora* spp., which parasitizes on the stem and root of pineapple (Bartholomew *et al*., 2003). Different soil types are used to cultivate pineapple as long as they are rich in nutrients and not waterlogged. Soil types that have been used are organic peat, volcanic ash and sandy soils (Hepton, 2003; Hassan *et al*., 2011). Pineapple plants require essential nutrients in high and low amounts for good growth. Nutrients such as Nitrogen, Potassium and Calcium are needed in high amounts by the plant. The presence of these nutrients can lead to increase in leaf number, size and green colouration,

crown production, fruit yield, quality and aroma, slip production and enhanced plant growth (Swete, 1993). Potassium, Magnesium, Zinc, Sulphur and Iron are required by the plant in low quantities. They are responsible for leaf colouration by increasing chlorophyll concentration for photosynthesis, strong stem, enhanced root growth and increased fruit sizes (Malézieux & Bartholomew, 2003). The absence of all these nutrients can lead to small leaf size, small number of leaves, yellowing of leaves, small fruit sizes, stunted growth and weak root system (Australian Government, 2008).

2.4 Pests of Pineapples

Pests and diseases are a major problem to pineapple production. Some insect pests that attack pineapple plants include mealy bugs, scales, mites, ants, thrips, and bud moths (Rohrbach & Johnson, 2003). The most important of these insect pests are scales. These pests cause some serious production and postharvest problems. They are usually found or hidden under the bracts of individual fruitlets, in the crown and the basal portions of the plant. They can be controlled by washing them away using the force of water in a water jet (Hassan *et al*., 2011).

The pineapple scale, *Diaspis bromeliad,* always attacks the leaves and fruit of pineapples plants (Waite, 1993). Its effect varies from pineapple to pineapple; in some instance it affects fruit appearance and not yield (Waite, 1993), while in others high scale numbers kill plants (Carter, 1967). They are found under the leaves and in some cases on the surface of the leaf. Plants become stunted, weak, produce a grey appearance and gradually, foliage dieback occurs. Fruits may have cracks between fruitlet. This pest can be controlled biologically using tiny wasp and ladybird beetles (Waite, 1993).

The mealybugs, *Dysmicoccus* spp. attack the plant throughout the growth period, from planting to harvesting (Rohrbach & Johnson, 2003). They tend to carry viruses which cause the mealy bug wilt disease of pineapple. The mealybug is usually found in the roots of pineapple plants few inches below the soil and in the leaf axils of developing fruit (Rohrbach & Johnson, 2003). When mealybugs are not controlled pineapple plantings are lost, due to the wilt disease (Rohrbach & Johnson, 2003).

The pineapple tarsonmide mite, *Steneotarsonemus ananas*, only infests pineapple plants (Jeppson *et al.*, 1975). These pests are most abundant during the period just before flowering and ingest the trichomes on the flower bracts, sepals and white basal leaf leading to the development of brown necrotic regions (Rohrbach & Johnson, 2003). This can be controlled using recommended pesticides three weeks before forcing (Rohrbach *et al*., 1981).

2.5 Diseases of Pineapple

Diseases in pineapple are mostly caused by Pathogens: bacteria, fungi, viruses and nematodes (Joy & Sindhu, 2012) and most of the diseases are economically significant. Disease occurrence and severity on pineapple can be as a result of different pineapple characteristics, mineral deficiency and commercial pineapple production systems (Rohrbach & Johnson, 2003). Different pathogens attack different parts of the plant and cause different diseases. Severity of disease is indicated by reduction in growth and yield of plants (Rohrbach & Johnson, 2003). Some fungal diseases of pineapple are Phytophthora Heart Rot Disease (PHRD), Phytophthora Root Rot, Butt Rot, Black Rot, Fruitlet core rot, Fusariosis, Green fruit rot, Inter fruitlet corking, Leathery pocket, Water blister, and White leaf spot. Nematode associated

diseases include, Root knot disease, Root lesion disease, and Reniform disease. Bacteria associated diseases include, Marbling and Pink disease and that of viruses are Mealy bug wilt and yellow spot.

2.5.1 *Bacterial diseases of pineapple*

Pink disease of pineapple is caused by *Gluconobacter oxydans* or *Acetobacter aceti*. This disease does not show any external symptoms on the fruit. The inside of the fruit becomes watery and gives off an odour. The bacterium is carried by nectar feeding insects and mites and infection starts when the insect invades the flower when it opens during cool weather (Joy & Sindhu, 2012). The disease is not usually managed because the bacteria are killed by high temperatures.

Marbling disease is caused by *Pantoea ananatis* and *Acetobacter* spp. This disease is generally a minor disease in pineapple (Rohrbach & Schmitt, 2008). It becomes severe when fruit matures under warm environmental conditions. The disease affects the fruit with the internal portion becoming reddish-brown and granular in appearance, and of a woody consistency. There is no known practical way of managing marbling. Smooth Cayenne variety is resistant to the disease to some extent (Rohrbach & Schmitt, 2008; Joy & Sindhu, 2012).

2.5.2 *Viral diseases of pineapples*

The mealy bug wilt disease is the most important viral disease of pineapple. It is caused by different strains of viruses. It affects the plant from planting time to harvest (Rohrbach & Johnson, 2003). The virus is spread by the mealy bug insect which is commonly found in pineapple planting (Rohrbach & Johnson, 2003). Initial symptoms are the red colouration of leaves, which later change

to pink. With time the leaves become loose and roll at the margins and the tips starts to die, root tissues collapse and plant appears wilted (Joy & Sindhu, 2012). The disease is managed by using disease-free planting materials and by applying recommended pesticides on mealy bugs and associated ants when plants show symptoms of wilt (Joy & Sindhu, 2012).

Yellow spot viral disease is caused by the virus that causes Tomato spotted wilt (Illingworth, 1931). Symptoms include the appearance of yellow spot on the surface of young leaves which joins to form a streak and later turns brown and eventually dies. Infection kills the plants, so that, the virus is not transmitted to subsequent plantings. Keeping plantation free from weeds, and avoiding the destruction of old weed patches near fields with developing fruits, are some of the management practices performed by farmers (Joy & Sindhu, 2012) to manage the disease.

2.5.3 *Nematodes- associated diseases of pineapples*

Nematodes like Root-knot nematode (*Meloidogyne javanica*), root lesion nematode (*Pratylenchus brachyurus*) and reniform nematodes (*Rotylenchus reniformis*) are known to cause disease symptoms on the pineapple plant.

Symptoms of Root-knot nematodes are apparent swellings at the root terminal which stops further root development leading to root stunting. There is also the yellowing and dieback which affect the leaves. The root lesion nematode causes the plant cell on the root surface to blacken. The reniform nematode also causes stunting in plant which may lead to total collapse and death of plant (Johnson & Rohrbach, 2003). This can be controlled by soil fumigation as pre-plant treatment and a nematicide application as a post-plant treatment (Rohrbach & Johnson, 2003). Attempts have also been made to use

Trichoderma sp. to control plant parasitic nematodes, where Bokhari, 2009 used *Trichoderma* sp. to control successfully Root-knot and reniform nematodes on eggplant in vitro.

2.5.4 *Fungal diseases of pineapple*

Butt rot disease, also known as Top rot or soft rot, caused by the fungus *Chalara paradoxa* occurs in areas where pineapple is grown (Rohrbach, 1983). This disease affects the crown, slip and sucker before or immediately after planting. Vegetative seed materials exhibit symptoms such as soft rot and blackening of the areas below the stem tissue (Rohrbach & Johnson, 2003). To manage the disease, planting material must be dipped in recommended fungicide. Also, seed material of mother plant is stored in a cool dry place where it is not exposed to infested soil (Rohrbach & Schmitt, 2008).

Fruitlet core rot (Green eye) is caused by *Fusarium guttiforme* and *Penicillin funiculosum*. It is a disease that occurs within the fruit. Some varieties of pineapple may produce fruitlets that fail to colour, and fruit that are badly affected become brownish and sink in as they ripen (Joy & Sindhu, 2012). Symptoms produced by the *Penicillin* sp. are dark to medium brown colouration of the core of the fruitlet, with a watery middle portion. The colour may extend into the non-capillary tissue. Symptoms produced by the *Fusarium* spp. ranges from light to dark brown and cover the entire or part of the fruitlet (Rohrbach & Johnson, 2003). This disease can be managed by the application of a fungicide directly into the opening of a terminal leaf (Joy & Sindhu, 2012).

Fusariosis is caused by the fungi *Fusarium guttiforme*. This disease affects all plant parts but very noticeable and damaging on the fruit (Joy & Sindhu,

2012). The symptoms are, discoloration of the fruitlet septa extending to the core from light brown to dark brown. Infected area of fruit surface exudes gum, becomes pinkish and sunken (Rohrbach & Schmitt, 2008). The stem becomes rosetted, curved, girdled and die. Control of Fusariosis is not economical because disease occurrence is irregular and random. Disease infection can be reduced when fungicides are applied three weeks after forcing (Joy & Sindhu, 2012)

Black Rot is also known as Thielaviopsis fruit rot, water blister or soft rot and is caused by *Chalara paradoxa*. This disease affects the fruit of pineapple, and is characterised by a glassy, brittle and water-soaked rot at the point of detachment of the fruit (Rohrbach & Johnson, 2003). Diseased tissues turn dark in later stage of infection. To reduce or prevent disease incidence, fruits are handled with care to avoid injuries and wounds that result from harvesting and post-harvest handling (Rohrbach & Johnson, 2003). Pineapple garbage and rejected fruits are removed from packing area/shed to avoid the spread of pathogen (Green & Nelson, 2015).

Leathery pocket and Interfruitlet corking are diseases caused by the same fungus, *Penicillium funiculosum*, which causes Fruitlet core disease. Interfruitlet corking is characterised by shiny patches on the skin in early development and corky tissues on the skin between the fruitlet. In severe cases fruit becomes malformed at one side. Fruits exhibit internal symptoms by forming corky tissues on the walls of the fruitlets. The corky tissues make the walls leathery and brown hence referred as Leathery pockets. Management of Interfruitlet corking is effective only when fungicides are applied into the

opening of the terminal leaves. For Leathery pocket, management is uneconomical since disease impact is very minimal (Joy & Sindhu, 2012).

White leaf spot is caused by *Chalara paradoxa.* The disease is characterised by the appearance of a tiny spot on the leaf. The spot lengthens and may reach more than 20 cm spreading to the leaf tip rapidly during wet season. In dry season, the affected area rapidly dries leaving a creamy to whitish papery spot. Because this disease is of no economic importance, management is not needed (Rohrbach & Johnson, 2003).

2.6 Phytophthora heart rot disease

Among the diseases of pineapple (*Ananas comosus*) Phytophthora heart rot (PHRD) is one of the most damaging and can cause up to 100% crop loss (Rohrbach & Johnson, 2003). This disease is caused by the oomycetes *Phytophthora cinnamomi*, *P. nicotiannae* and *P. palmivora* (Rohrbach & Johnson, 2008, 2013; Ratti *et al*., 2018).

It attacks pineapple plants of all ages including fruiting plants, suckers or ratoon plants. The most susceptible plants are 3-4 months old plants. The infection and spread of this disease depend on factors such as topography, drainage, rainfall and pH (Green & Nelson, 2015). The disease is characterised by a small change in the colour of heart leaves from green to yellow or light brown copper, followed by an advanced change in colour of heart leaves to deep copper brown. Heart leaves start to wilt, leaf edges roll over, outer leaves become limp and dieback from tip. Once symptoms are visible, leaves, especially young ones, easily pull out, dark bands separate rotted and healthy tissue, with white tissue on underside of leaves becoming water soaked and

rotten with foul smell. The entire plant dies and is easily uprooted (Rohrbach & Johnson, 2003)

2.6.1 *Causative organism*

The causative organism of PHRD belongs to the genus Phytophthora de Bary. It is an obligate parasite and has about 60 species (Erwin & Ribeiro, 1996). This number has doubled to approximately 120 described species (Martin *et al.*, 2014). The host range of *Phytophthora* spp. is very wide and causes major plant diseases (Drenth & Sendall, 2001). An example is the potato famine in Europe in the 19th century by *Phytophthora infestans* (Bourke 1964). *Phytophthora* spp. is one of the worst plant-destroying pathogens of all time (Agrios, 2005). Tropical and Temperate regions of the world are favourable environmental conditions for high levels of Phytophthora infection on many crop plants. Phytophthora is in the Kingdom Chromista, Order; Peronosporales and Class; Oomycetes (Hawksworth *et al*., 1995; van de Peer *et al*., 1996). The Oomycetes have similar characteristics to a true fungus (Drenth & Sendall, 2001). However, they are clearly different from Basiodiomycetes and Ascomycetes (Erwin & Ribeiro, 1996). They are placed in the Kingdom Chromista because they have a lot of characteristics such as differences in their metabolic pathways (Elliot, 1983), the presence of β-glucans instead of chitin in cell walls (Bartnicki-Garcia & Wang, 1983) and many more. Many species are found within the Phytophthora genus, such as *P. palmivora*, *P. botryose*, *P. heveae*, *P. nicotianae*, *P. meadii* and *P. infestans.* These species cause many important diseases like seedling dieback, black pod, root rot on cocoa; black stripe, patch canker, green pod rot and green twig blight in rubber plant and late blight of potato (Erwin and Ribeiro, 1996).

2.6.2 *Host range*

Phytophthora spp., vary greatly due to their wide host range: a species can infect only a single host plant; another can infect thousands of host plants; and there are those whose infection of plant is in-between the two extremes (Drenth & Guest, 2004). An example is *P. cinnamomi* which infects over 1000 different plant species (Erwin & Ribeiro, 1996) and *P. fragariae* which attacks just a single plant species (Kennedy & Duncan, 1995).

2.6.3 *Mating systems*

All isolates of *Phytophthora* are able to produce either male or female sexual structures (Galindo & Gallegly, 1960). Some *Phytophthora* spp. are homothallic and produce oospores in a single culture. Others are heterothallic and produce gametangia only when chemically induced by isolate of the opposite mating type (Ko 1978; Brasier 1992). The mating system of *Phytophthora* species influences its ability to outbreed (Drenth & Guest, 2004). However, both homothallic and heterothallic species have reproductive options (Drenth & Sendall, 2001). The primary mode of reproduction is asexual (Green & Nelson, 2015). The vegetative mycelium produces a fruiting body called the sporangia (Green & Nelson, 2015), which differentiates to produce zoospores, each of which goes through a process of dispersal and encystment before germinating (Drenth & Sendall, 2001). Sexual reproduction by two different mating types produces oospores. All the types of spores can cause diseases with chlamydospore and oospores functioning as resting structures (Drenth & Sendall, 2001).

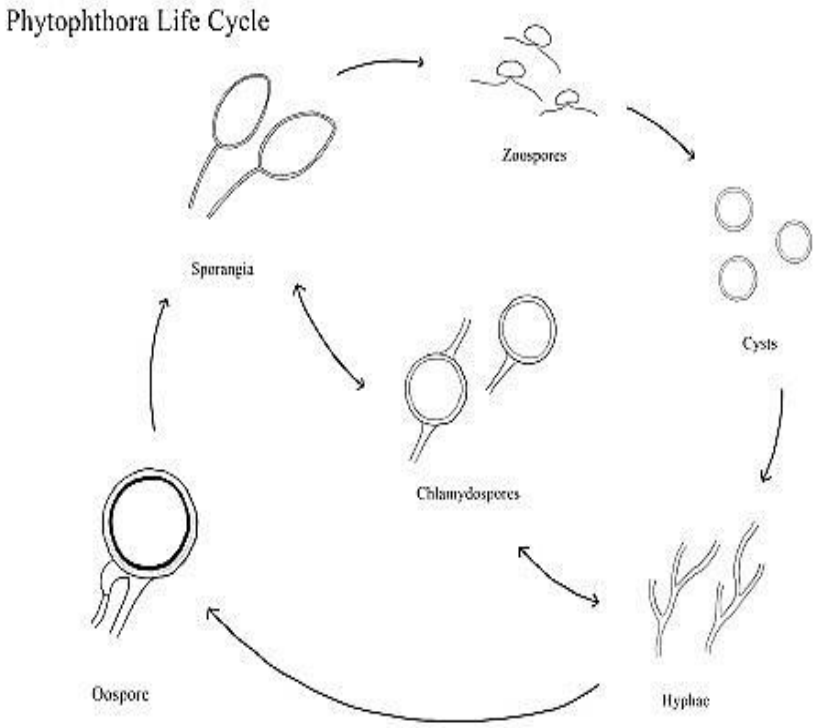


Figure 1: Life cycle of *Phytophthora* genus

2.6.4 *Distribution of Phytophthora cinnamomi, Phytophthora nicotianae and Phytophthora palmivora*

The geographical origin of *P. cinnamomi* is not known (EPPO, 2004). According to Centre for Agriculture and Bioscience International (1991), the pathogen was first described on *Cinnamomi burmannii* (Lauraceae) in Sumatra (ID) in 1922, but it can now be found worldwide. It has over 1000 host species (Zentmeyer, 1983) and it is the most distributed. The most significant food-crop losses due to *P. cinnamomi* include root rot in avocado, and heart rot in Pineapple (EPPO, 2004). It is primarily a root pathogen and causes rot of feeder roots leading to death of host plants. Depending on the severity of the rot leaves becomes yellow, wilted and dies back with rot extending into the base of the stem. It can also cause stem cankers in trees.

Other symptoms are decrease in yield, fruit size, gum exudation, heart rot (e.g. pineapple), and collar rot. *P. cinnamomi* infection can also occur together with other species of Phytophthora, like *P. cambivora* (EPPO, 2004).

Phytophthora nicotianae (Breda de Haan) is one of the major species of Phytophthora that causes heavy losses in host plant (Panabieres *et al.*, 2016). It was first identified and reported on Tobacco in 1896 (Erwin & Ribeiro, 1996). Since then it has been found on 255 plant genera in 90 families as *P. parasitica* Dastur (Cline *et al*., 2008). The name *Phytophthora nicotianae* was coined first because of it relation to Tobacco (Panabieres *et al*., 2016). *Phytophthora nicotianae* has been isolated in a lot of different ecological niches (Meng *et al*., 2014). It has a wide host range which includes vegetables (Prigigallo *et al.*, 2015), fruit trees (Moralejo *et al*., 2009), medicinal plant (Hulvey *et al.*, 2010) and forest trees (Beever *et al*., 2009). It can also be found at different places such as irrigation system (Hong & Moorman, 2005), farm tools, nurseries of potted plant and many more. This makes it more efficient in it spread (Olson & Benson, 2011). The differences in the host range, which result in high cost of disease management, growing alternate crops and productivity loss, is the reason why the economic impact of *P. nicotianae* cannot be estimated (Panabieres *et al*., 2016). Some diseases that are attributed to this pathogen are brown rot, foot rot, and black shank of tobacco (Gallup *et al*., 2006). *Phytophthora nicotianae* also causes gummosis and root rot in citrus (Erwin and Ribeiro, 1996) and heart rot in pineapple (Rohrbach & Johnson, 2008).

P. palmivora is an important species of Phytophthora and is found in the tropics and sub-tropics with a host range of 180 plant species (Erwin &

Ribeiro, 1996). It causes many different diseases and originated in Southeast Asia. *P. palmivora* causes significant losses in important tropical fruit, vegetables, ornamental and horticultural crops. It infects different parts of the plants; the roots, stem, branches, leaves, fruits and flowers (Erwin & Ribeiro, 1996). Some of the important horticultural hosts and infection caused are; black pod, Cherelle wilt and canker in cocoa; fruit rot in papaya; fruit rot and canker in durian; foot rot in black pepper; bud rot in coconut; canker in citrus and heart rot in pineapple (Australasian Plant Pathology Society, 2008).

2.6.5 *Epidemiology and transmission*

Chlamydospore is the primary inoculum for *P. cinnamomi, P. nicotianae* and *P. palmivora.* The chlamydospore can stay in the soil alone or in plant debris for many years (Erwin & Ribeiro, 1996; Ratti *et al.*, 2018)). It produces sporangia which is transported by wind, moving water and soil splash. *P. cinnamomi* infection starts from the roots through to the apex of the stem and then to the leaf axil, causing heart rot (Rohrbach & Johnson, 2003). Poor drainage and high moisture content in the soil promote infection (Rohrbach & Johnson, 2003). The chlamydospore of *P. cinnamomi* does not germinate when moisture levels are below 15% (McCain *et al.*, 1967; (Rohrbach & Johnson, 2003). The sporangia of *P. cinnamomi* is multinuclei and produce hyphal structures which enable it to survive outside the host for a long time. The sporangia can also produce a single nuclei zoospore with two flagella that enables it to swim and reach host via chemotaxis and electrotaxis (Walker & van West, 2007). Infection occurs in the leaf axils of the crown in the first four months after planting (Rohrbach & Johnson, 2003). Infection is less dependent on moisture (Hine *et al.*, 1964). The inoculum of *P. palmivora* spreads to host

plant through insect, rain splash and activities of human beings (Australasian Plant Pathology Society, 2008). The zoospores disseminate easily in hydroponic solutions and soil water (Stanghellini & Rasmussen, 1994). Asymptomatic plant is responsible for transferring the pathogen (EPPO, 2004).

2.6.6 *Detection and identification of PHRD*

It is essential that diseases are properly diagnosed in order to find methods to curb the spread of infection. Disease diagnosis involves detection, observation, and identification. Disease identification was initially based on the morphological characteristics of the pathogen but due to overlapping of morphological features of certain species of pathogens, molecular identification is now required as an addition approach to morphological identification. According to Martin *et al*. (2012) identifying *Phytophthora* spp. can be difficult due to the overlapping of morphological characteristics, and intraspecific variability.

Morphological identification requires the use of clean cultures and these are obtained from selective media such as V8 juice, Carrot agar (CA), Cornmeal agar (CMA), malt extract agar (MEA), potato dextrose agar (PDA) and many more depending on the species. *Phytophthora* spp. identification is based on the taxonomic keys developed by Waterhouse (1963) and Stamps *et al*. (1990). Characters used for species identification include: colony characteristics (pattern, colour, form, texture, margin, opacity, growth rate, and size), sporangium morphology; morphology of antheridia, oogonia and

oospores; presence or absence of chlamydospores, and size of hyphae (Drenth & Sendall, 2001).

Morphological characteristics of the three pathogens reported to cause PHRD have been described by Laundon and Waterston (1964), Waterhouse and Waterston (1966), and Gallegly and Hong (2008). According to Gallegly & Hong (2008), *P. cinnamomi* can be identified by its rosetted colony growth pattern, with colony edges undulating and overlapping each other. Its hyphae are coralloid, with grape-like cluster of chlamydospores. They further describe that the sporangiophore is thin, occasionally branched and often proliferating through the empty sporangium. The sporangia shape is spherical to ovoid with slight apical thickening and no papilla, averaging 57 x 53 µm in size. Waterhouse and Waterston (1966) further indicate that the sex bodies produced are long amphigynous antheridia, measuring 21-23 x 17 µm averagely; oogonia (40 µm), and an oospore nearly filling the oogonium. The maximum temperature required for optimum growth of *Phytophthora cinnamomi* is 33 ºC (Gallegly & Hong, 2008).

Phytophthora nicotianae can be recognized by its fluffy, irregular and rosetted, colony growth pattern (Laundon and Waterston, 1964). Its hyphae are tough with no typical swellings. Sporangia are slender, irregular and sympodially branched (Laundon & Waterston, 1964). The shape of the sporangia is described as ovoid orobpyriform to spherical with a few being ellipsoid (Gallegly and Hong, 2008). Gallegly and Hong (200) further show that the Sporangia are papillate and non-caducous, and Chlamydospore terminal and intercalary. The sex bodies produced are, round and smooth

oogonia (24-26 µm), spherical or ovoid antheridia (10 x 12 µm) and an aplerotic oospore (Gallegly & Hong, 2008). The maximum temperature necessary for growth of *Phytophthora nicotianae* is 35 ºC.

Morphological characteristics described by Gallegly and Hong (2008) for the detection of *Phytophthora palmivora* include, a stellate (star-like) colony pattern, lumpy branching hyphae with swellings, spherical, ovoid to ellipsoid Sporangia. The Sporangia is also conspicuously papillate, and caducous, with short pedicel. They described the sex bodies produced aplerotic oospores and amphigynous antheridia (Gallegly & Hong, 2008). The optimum temperature required for colony growth is 30 ºC.

There are several molecular techniques for species and sub-species level identification of pathogen. These include complex technologies and simple procedures that are less technical (Martin *et al*., 2012). Some of the procedures exploit nucleic acid sequence differences between species (Cooke *et al*., 2007).

The Polymerase Chain Reaction (PCR) is a very important and precise molecular technique available for plant pathogen detection (Ward *et al*., 2004). It is generally known as an efficient detection method for a lot of *Phytophthora* species. PCR is a method for amplifying copies of specific DNA sequences identified by specific primers using a thermostable enzyme. The procedure requires the use of DNA of the organism, DNA polymerase, primers, deoxyribonucleotide triphosphates (dNTPs), and a buffer. These items are incubated in a PCR machine. A pathogen is then detected at either

the genus, species or strain level. This is done depending on the type of primer used, which in turn influences selection at either a narrow or broad taxonomic level. The DNA amplified is checked using gel electrophoresis, but alternative detection formats include using colorimetric is available (Mutasa *et al*., 1996).

Another technique for pathogen identification and differentiation is the DNA micro and macro-arrays and chips. These are tools used for genes profiling of micro-organisms (Wu *et al*., 2001), and an alternative new method for the detection of many plant pathogens (Lievens and Thomma, 2005; Anderson *et al*., 2006). The DNA arrays work in a way that nylon and silicon are used to immobilize species specific oligonucleotides (Anderson *et al*., 2006; Lievens *et al*., 2005). Primers with species specific sequences are used to amplify the target DNA and exposed to the array. The ones that hybridize constitute the species present in the sample. This technique was first used for diagnosing disease and genetic disorders in human beings but now are used for detecting plant pathogens including the ones found in the soil, providing quantitative information on them (Lievens *et al*., 2005).

Enzyme-linked immunosorbent assay (ELISA), is a diagnostic tool based on the recognition of antigen by an antibody (Ali-Shatayeh *et al*., 1991). There are commercially available ELISA tests in a number of different formats for Phytophthora detection. These include an ImmunoStrips or Lateral Flow Devices (LFD) for single-use. Immuno-detection of *Phytophthora* sp. in various types of plant related samples has been recently adopted by a broad range of plant biologists due to test availability, simplicity, efficiency, cost, and speed with which results are produced (Martin *et al*., 2012). ELISA-based protocols have successfully detected *Phytophthora* spp. in numerous types of

samples, including plant foliage (Lane *et al.*, 2007), plant roots (Benson, 1991), soil (Schmitthenner, 1990), irrigation water (Ali-Shatayeh *et al.*, 1991; Cahill *et al.*, 1994), and hydroponic nutrient solutions (Grote & Gabler, 1999). In certain cases, these assays have not detected the presence of the pathogen, yielding false-negative results, due to type or quality of host tissue, (Martin *et al*., 2012).

There are several gel-based techniques that can be used to identify isolates to the species level using amplicons generated by PCR. PCR-restriction fragment length polymorphism (RFLP) is one of such techniques. This technique involves amplification of the ITS region followed by digestion with restriction enzymes which generates restriction profiles that could be useful for identification to a species level. This approach has been used by Camele *et al*. (2005) who characterized isolates from Italy. Bowman *et al*. (2007) differentiated *P. palmivora* from *P. nicotianae*, coupled with sequence analysis of the ITS region. Also, Roy *et al.* (2009) examined *Phytophthora* spp. in Eastern India and Drenth *et al*. (2006) improved the technique by developing primers that were specific for *Phytophthora* sp., allowing amplifications to be done directly from environmental samples.

2.6.7 *Management of PHRD*

An important part of plant disease prevention is to avoid the introduction of pathogen inoculum to an uninfected area. A number of strategies are employed in the management of PHRD, including cultural, chemical, biological and the use of plant extract (botanicals).

2.6.7.1 *Cultural control*

Cultural control is the use of cultural or farm practices to reduce or avoid pest and disease damage to crops. Cultural control methods include; good farm sanitation, time of planting, fertilizer application, irrigation, crop rotation, crop variety used and tillage. The use of cultural control method in controlling pathogens is hard to predict because of their varying effect on the host and the environment (Waller *et al*., 2001). Wet conditions are known to favour *Phytophthora* spp. leading to PHRD spread and infection (Green & Nelson, 2015) hence, pineapple fields should be well-drained and appropriately irrigated when necessary (Fulcher & Bowers, 2013). The inoculum of the pathogen can remain in the soil and diseased tissues for a long time (Erwin & Ribeiro, 1996), therefore crop rotation may be practiced though its benefits is limited (Green & Nelson, 2015). One of the main sources of PHRD is planting materials, tools, and farm machinery contaminated with soils from infected fields. Farm sanitation is therefore key to reducing inoculum levels by removing infected plant from fields, washing tools and machinery before preparing land for cultivation (Green & Nelson, 2015).

2.6.7.2 *Chemical control*

This is another approach to control PHRD. Fungicides used to control PHRD are Ridomil Aliette, Fosetyl Al, Metalaxyl and Fosphite (Green & Nelson, 2015). The effectiveness of the fungicide is dependent upon the method of application. Most fungicides are applied as pre-plant dips which has shown high efficacy (Rohrbach & Schenck, 1985). Some of these fungicides like Fosphite have shown some efficacy and have proved effective against *P. cinnamomi* (Desprez-Loustau *et al.*, 1998). Metalaxyl and Fosetyl Al were

highly effective in controlling PHRD when applied as pre-plant dips compared to foliar application on newly planted crowns (Rohrbach & Schenck, 1985). However, due to the detrimental side-effect of fungicides it is not the first management option for most farmers. Some fungicides tend to be very poisonous for other microorganisms like AMF (Arbuscular Mycorrhyzal Fungi), *Trichoderma*, useful bacteria, and algae which help in soil micro-biological processes like nitrogen transformation, organic matter decomposition, nutrient release and stabilizing soil structure (Edwards & Bater, 1990). Its application can also lead to water and soil pollution (Rani *et al*., 2017).

2.6.7.3 *Biological control*

Due to the many concerns raised against the use of pesticides, biological or bio control of plant diseases has become necessary. According to scientists, some biological organisms can antagonize and aggressively parasitize other organisms. These biological organisms are known to have some antifungal and antibacterial properties (Waller *et al*., 2001). Examples of such biological organisms are *Sporothrix flocculos*, a fungus that behaves like yeast which controlled rose powdery mildew, caused by *Sphaerotheca pannosa* var. *rosae* in commercial greenhouse (Bélanger *et al*., 1994) and non-aflatoxigenic strains of *Aspergillus flavus* which competitively eliminated aflatoxin-making strains of *A. flavus* to reduce aflatoxin contamination in crops like groundnut and maize. The application of the biological control method is largely in the form of foliar sprays, soil treatment, seed covering, protection of tree roots, stump treatments and many more (Waller *et al*., 2001).

The bio control fungus *Trichoderma* spp. of the order Hypocreales and division, Ascomycota is known for its ability to work as a bio-control organism since the 1920s (Samuels, 1996). The *Trichoderma* spp have diverse metabolic pathways that produce various enzymes and secondary metabolites and as such can grow in a great number of environments (Waghunde *et al*., 2016). According to Woo *et al*. (2006), varying species of *Trichoderma* are known for different interaction within plant and soil borne pathogens. *Trichoderma* spp can antagonize plant pathogens through different mechanisms including competition, colonisation, antibiosis, and mycoparasitism (Howell, 2003; Waghunde *et al*., 2016). It is also capable of inducing defence response in host plant (Waghunde *et al*., 2016). It is able to perform all these activities by growing faster and using its food sources efficiently than the pathogen, and may sometimes excrete certain compounds that slow down or completely inhibit growth of pathogen, or promote plant to produce a chemical that protects it from the pathogen. Different species of *Trichoderma* are used as bio- control agents but the most commonly used ones are the *T. viride* and *T. harzianum* (Waghunde *et al*., 2016). *Trichoderma* spp. have been used to control organisms including *Fusarium* spp. *Verticillium* spp. and *Pythium* spp. on flowers, fruits, vegetables and trees (Waller *et al*., 2001). According to Harman *et al*. (2004), *Trichoderma harzianum* gives a variation of antibiotic and antifungal peptides which interrelates with cell membranes of other plant fungal pathogens to inhibit their development. Saksirirat *et al*. (2009) reported that isolate of *T. harzianum* brought about resistance in tomato plant by dipping the occurrence of bacterial spot by 69.32% at 14 days post inoculation. The elicitor filtrate of *T. harzianum* (PDBCTh10 isolate) was

found effective against root rot in pepper plant by inducing resistance resulting in lower infection (Sriram *et al*., 2009). Also, on gray leaf spot, isolate *T. asperellum* brought about resistance and exhibited significant decrease in number of spots in 10 days after inoculation. *T. asperellum* was also able to progress the defense mechanism against infection of cucumber mosaic virus in *Arabidopsis thaliana* and also induced general resistance to establishment by SKT-1 and its cell free culture filtrate of *Pseudomonas syringae* (Yoshioka *et al*., 2012).

Diverse substrates are used for producing *Trichoderma* sp. to be used as a bio-control agent. They include sorghum grains, wheat, rice, neem cake, cow dung with neem cake, spent compost, farm yard manure, molasses, V8 juice, local cow urine, and butter milk (Waghunde *et al*., 2016). Solid substrates are however, better and are more required than liquid due to poor sporulation of *Trichoderma* sp. on liquid.

2.6.7.4 *Botanical control*

This approach of PHRD control involves the use of plant-based products. In recent times more efforts have been centred on this method considering the alarming effect of synthetic chemicals on the environment (Rani *et al*., 2017). Plants contain many active compounds with pesticidal properties e.g. alkaloids, steroids, tannins and others which are deposited in specific parts of the plant (Gupta *et al*., 2012). These phytochemicals contain antioxidant (Wong *et al*., 2009), antibacterial (Nair *et al*., 2005) and antifungal (Khan & Wassilew, 1987) used as natural pesticides. Botanicals such as *Tertrapleura tetraptera, Azadirachta indica and Khaya senegalensis* leaf extract, show antifungal and antimicrobial action against potato soft rot disease (Bdliya and

Abraham, 2010), *Callosobruchus maculatus* (Bamaiyi *et al*., 2006), late blight of potato (Ngadze, 2014), *Colletotrichum gloeosporioides*, and *Phytophthora cactorum* (Thobunluepop *et al.*, 2009).

Neem, *Azadirachta indica*, is a tropical evergreen tree in the family *Meliaceae*. It originates from Indo-malaysian region but also widely cultivated and naturalized outside its native region. The active constituent of the neem plant is azadirachtin and it exhibits anti-inflammatory, antihyperglycemic, antiulcer, antimalarial, antibacterial, antifungal and antiviral properties and all the plant parts can be used to prepare extracts (Subapriya & Nagini, 2005). Neem products have been extensively used in the control of insect pests (Vijayalakshmi *et al*., 2002), but there have been reports on it use against some fungal (Stoll, 1998) and bacterial pathogens (Emechebe & Alabi, 1997; Bdliya & Dahiru, 2006). The extracts prepared from neem may destruct the physiology of the insect, making it inactive and preventing it from causing damage to the plant. This disturbance is manifested through the following: In pest, it inhibits the growth of insect by suppressing moulting and causing malformation and sterility; it deters insect from eating by interfering in peristaltic movement during feeding and blocking its ability to swallow; disrupt sexual communication and mating between a male and a female; prevent females from depositing eggs, and repelling larvae and adult insect (Vijayalakshmi *et al*., 2002). Different extracts are gained from different parts of the neem tree and used as a bio-control agent. Neem kernel, leaf, cake and oil extracts are all different medium used in controlling pest and pathogens. As a fungicide, neem oil is mainly used as a preventative material when the

disease is just beginning to manifest symptoms (SGP, 2012). Study by Mahmoud *et al*. (2011), showed that the fungal pathogens such as *Aspergillus niger* and *Candida albicans* were inhibited in growth when 5% aqueous leaf extract of neem was tested on them. Ezeonu *et al*. (2018) also observed that both ethanol and water extract of different part of the neem tree inhibited the effect of fungal rot in yam and cocoyam.

Prekɛsɛ, *Tetrapleura tetraptera* is a flowering plant from the pea family Fabaceae. It is originating from Tropical Africa and found from Mauritania to Tanzania (Blay 1997). It grows in savannah woodlands, dry and riverine forests, and are mostly common in dense rainforests, (Kemigisha *et al*., 2018). It's a spice but has been found to have medicinal properties (Ojewole & Adesina, 1983). Its fruit which is mainly used, contains phytochemicals including alkaloids and tannins (Ebana *et al*., 2016). These phytochemical metabolites are antibacterial and antifungal and are effective in the management of yeast growth, bacteria, moulds and toxin production by microrganisms (Achi, 2006). Research conducted by Adeyini and Abiodun (2015) showed that, *T. tetraptera* had high efficacy against the mycelial growth of *Lasiodiplodia theobromae* at 80% concentration in contrast with extract of other plants. Furthermore, Ebana *et al*. (2016) observed that there was consistency in the inhibitory activity of ethanol extract of *T. tetrapleura* tested against the bacteria, *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and the fungus, *Rhizopus* sp.

African Mahogany is a deciduous evergreen tree which grows mostly within the native savannah woodlands of West and South Africa and some parts of Asia (Vietnam, Indonesia, Singapore) and South America (Cuba, Puerto Rico) (Orwa *et al*., 2009). The roots, bark and seeds are used as medicine for humans to treat headache, fever, allergies, infections and sterility, and in animals to treat liver fluke and ulcers in ruminants (Orwa *et al*., 2009; Thioune *et al*., 2003). The tree has some phytochemical properties that inhibit growth of some microorganism. These phytochemicals are saponins, tannins, aldehyde, flavonoids, terpernoids, and anthraquninones (Sylvanus *et al*., 2014). Audu-Peter *et al*. (2006) in a study subjected the extracted oil from the fruit of *Khaya senegalensis* to bacterial and fungal testing, and found out that the oil showed high activity against the bacteria and none at all against the fungal pathogen used. However, Shehu *et al*. (2016) in their work on the ethnomedicinal potential of *Khaya senegalensis*, tested the acetone, water and millet seeped water extract of the leaf and bark of *Khaya* on three pathogenic fungi; *Aspergillus* sp, Mucor sp. and *Rhizopus* sp. The mycelia of all three tested fungi were inhibited in their growth. van der Puije (2006) also reported efficacy of extracts of the bark of *Khaya senegalensis* in the management of Fusarium wilt of tomato either alone or in combination with *Trichoderma harzianum* and found it to inhibit mycelia growth and sporulation.

CHAPTER THREE

MATERIALS AND METHODS

The study was conducted in three main phases. They include surveys (household and field), pathogen identification and characterisation and *in vitro* disease management.

3.1 SURVEYS (DEMOGRAPHIC AND FIELD)

Surveys were conducted to identify farmers' knowledge of the Phytophthora Heart Rot Disease (PHRD), their management practices and to determine the incidence and severity of the PHRD infection in the selected area of study.

3.1.1 *Study Location*

Komenda – Edina - Eguafo-Abrem (KEEA), Abura-Asebu-Kwamankese (AAK) and Ekumfi districts were purposively selected for the study. These are districts known for pineapple production and characterised mostly by small holder farmers.

Figure 2: Map of Central region showing KEEA, AAK and Ekumfi districts where the study was conducted.

Komenda- Edina- Eguafo-Abrem District (KEEA)

The KEEA municipality covers an area of 452.5 square kilometres of which 86% is available as arable land. It is found between latitude 5º5N and longitude 1º21'W and bounded in the north by Twifo-Hemang-Lower Denkyira Municipality, in the east by Cape Coast Metropolis, in the south by the Gulf of Guinea and in the west by Mpohor-Wassa East District in the Western Region (Figure 1). Along the coast of this district are a member of lagoons and wetlands, and in the interior, steep slopes and hills. The rainfall pattern differs depending on the location; the areas close to the sea experience lower rainfall compared to the interior. The annual rainfall ranges between 750 mm -1000 mm for the coast and 1200 mm -1500 mm inland (MoFA, 2011). The temperatures are generally high ranging between 23ºC - 33ºC. The area is noted for the cultivation of cereals, vegetables, fruits and cash crops. Major crops produced in the district are maize, cassava, plantain, vegetables and pineapple. As at 2010, the estimated cropped area for pineapple was 168.9 ha with production levels at 5421.7 Mt (MoFA, 2011).

Abura-Asebu-Kwamankese District (AAK)

The AAK municipality covers an area of 380 square kilometres. It is located between latitude 5'05'N and 5º25N and longitudes 1º5W, and 1º20W. It borders the north by Assin South District, the east by Mfantsiman Municipality, the South-east by the Gulf of Guinea, on the south by Cape Coast Metropolis and the west by Twifo- Hemang- Lower - Denkyira District (Figure 1). It is low lying and undulating with elevation between 20 and 80 metre above sea level. It falls within the evergreen and semi deciduous forest zones and as such experience's double maximum rainfall between April –

June and October -November. Annual rainfall in the southern part of the district is generally lower, between 1000 mm -1100 mm, than in northern interior, with 1100 mm - 1700 mm. However, the area close to the Kakum valley experiences annual rainfall of about 1900 mm. Temperatures are generally warm and uniform throughout the year. Mean monthly temperature is about 26.9°C. The area is well noted for the cultivation of cassava, maize, plantain, yam, some vegetables and fruits mostly citrus (Ghana Statistical Service, 2014).

Ekumfi District

The Ekumfi district covers a land area of 276.65 square kilometres carved out of the Mfantsiman district in 2012 (Ghana Statistical Service, 2014). It is bounded to the north by the Ajumako- Enyan- Essiam District, to the east by the Gomoa West District, to the south by the Gulf of Guinea and to the west by the Mfantsiman Municipality (Figure 1). The area is low lying with elevations lower than 60 m above sea level. The district experiences double rainfall in May – June and October. Annual rainfall ranges between 900 mm - 1100 mm in the coastal parts and between 1100 mm and 1600 mm in the interior. The months of December – February and July – September are much drier than the rest of the year. The daily temperature ranges between 22ºC and 34ºC (Ghana Statistical Service, 2014). It has relative humidity of about 70% (Dickson & Benneh, 2001). The area is well noted for pineapple cultivation and has both small scale and commercial farms. However, most of the farmers are small scale producers and sell their produce to the local market. In addition, vegetables like okro, garden eggs and tomatoes thrive well; other

crops like cassava, plantain and maize also thrive well (Ghana Statistical Service, 2014).

3.1.2 *Demographic Survey*

In each of the three districts, four communities were selected using purposive sampling. The names of these communities are presented in Table 1. They are Akwanda, Atabadze, Brenu-Akyinim, Essaman, Amosima, Asebu-Ekroful, Asuansi, Ayeldu, Abor, Asokwa, Atwiaa, and Nanaben. From each of these communities, 10 farmers (respondents) totalling 120 were selected by the snowball technique and interviewed. The farmers interviewed were all small holder pineapple farmers.

Table 1: Communities selected for demographic and field surveys

District/ Municipality	Selected Communities	No. of farms
KEEA		
	Akwanda	5
	Atabadze	5
	Brenu-Akyinim	5
	Essaman	5
AAK		
	Amosima	5
	Asebu-Ekroful	5
	Asuansi	5
	Ayeldu	5
EKUMFI		
	Abor	5
	Asokwa	5
	Atwiaa	5
	Nanaben	5

A structured questionnaire containing both open and close-ended questions was used in collecting information from farmers by self-administration. This was pre-tested in Atabadze, a community within KEEA district.

The structured questionnaire was in four major parts seeking information on demographic characteristics of respondent (sex, age, occupation, level of education), Agronomic practices, Awareness of the disease and disease management practices by farmers.

3.1.3 *Field Survey*

A field survey was conducted for the incidence and severity of PHRD in all the selected communities in the three districts; KEEA, AAK and Ekumfi. The survey was conducted twice, in two cropping seasons: the first field assessment was done on 12th – 23rd July, 2018 and the second on 12th – 20th November.

Five farms were randomly selected in each of the selected communities' i.e.: Akwanda, Atabadze, Brenu-Akyinim, Essaman, Amosima, Asebu-Ekroful, Asuansi, Ayeldu, Abor, Asokwa, Atwiaa, and Nanaben (Table 1) for the study. At least 1000 plants were assessed per field along a diagonal excluding border plants for presence/ absence of PHRD. The farms that had disease incidence and belonged to respondents were selected and surveyed. On the average the area of farm surveyed measured between 1-3 acres.

Incidence of PHRD for various fields were calculated using the following formula by Imran *et al*. (2012):

$$\text{Disease incidence} = \frac{Number\ of\ infected\ plant}{Total\ number\ of\ plant} \times 100$$

Severity was scored using a key developed based on the progression of disease symptoms. The severity scale ranged from 0-5, with 0 representing no

symptom and 5 representing entire plant completely withered and all leaves pulled off from the heart.

The key used is as follows:

Score	Description
0	No symptom
1	Minimal change in colour of the heart leaves to yellow or light brown copper
2	Advanced change in colour of heart leaves to yellow and deep copper brown
3	Heart leaves wilted, leaf edges rolled over, outer leaf limp and dieback from tip
4	Leaves especially young ones easily pull out, dark band separates rotted and healthy tissue, white tissue under leaves is water soaked and rotten with foul smell, the root system is dead and plants can easily be pulled from the ground
5	Entire plant completely withers and all leaves pulled off from the heart

Severity was then calculated using the formula by Chester (1950) and Chiang *et al*. (2017). Thus,

$$Severity = \frac{\Sigma(Frequency\ x\ mean\ or\ score\ of\ rating\ category)}{Total\ no.\ of\ plant\ observed/examined}\ x\ 100$$

where Frequency = Number of infected plants.

Figure 3: (A) Symptoms of heart rot disease in pineapple showing dark band separating healthy tissues from water soaked rotted tissue; (B) leaves that have been easily pulled out; (C) entire plant completely withered

3.1.4 *Soil sampling*

Soils from the various fields where disease was present were collected and sent to the Soil Science Lab, School of Agriculture, University of Cape Coast to determine pH level, Organic matter content (OM), Cation exchange capacity (CEC), Percentage moisture content (%MC), C:N ratio, Organic carbon content (OC), Percentage Nitrogen (%N), available Phosphorus (P) and Potassium.

Stratified sampling was used in collecting soil samples. In areas where the land is sloppy, soils were collected at different points from the mid slope; where the land is flat, the land was divided into three sections and soil collected at different points from each section. At every sampling site, the litter on the surface of the soil was removed using a garden spade (Kulhare, 2012). With the help of an auger, soil samples were collected at a depth of 5 cm from the soil surface. Samples collected from each section of the land were thoroughly mixed in a bowl, and about a half to 1kg sample was taken using the quartering method (Kulhare, 2012). Samples were placed in polythene bags sealed and labelled.

Test for pH

Before measuring soil pH, the pH meter was calibrated using a standard buffer solution of known pH and the temperature of the pH meter adjusted to the temperature of the solution (Sawakar, 2012). The standard buffer solution was prepared by dissolving 10.21 g of analytical reagent grade of potassium phthalate in distilled water and diluted to 1 litre. One millilitre of crystal of thymol was added as a preservative (Sawakar, 2012). This procedure was used in the absence of a buffer tablet. The soil sample was sieved in a 2 mm mesh dish. An amount of 10 g of air-dried and sieved soil was weighed and put in a beaker, 20 ml of distilled water was added and mixed thoroughly and the mixture was allowed to stand for an hour. The pH meter was rinsed with distilled water, blotted with a filter paper and placed in the partially settled sample suspension (SERAS, 2002). The pH of the sample was displayed on the meter and recorded.

Test for % Moisture Content (MC)

The Gravimetric method (Tembe, 2012) was used to determine the moisture content of soil samples collected from the field. About 50 -100 g of soil was collected from each sample and placed in soil moisture cans with tight fitting lid. The soil samples were weighed immediately and dried to a constant weight in an oven at 105 ºC for about 24 hrs and reweighed after cooling in a desiccator. The moisture content was determined by calculating the loss in weight on drying and the weight of oven dry soil, using the formula;

$$MC = \frac{loss\ of\ weight\ on\ drying}{weight\ of\ oven\ dry\ soil} x\ 100.$$

Test for Organic Matter content

Organic matter content of sampled soil was determined using the Walkley-Black method (Walkley and Black, 1934). Two grams (2 g) of air-dried soil was weighed into a 500 ml Erlenmeyer flask and 10 ml of 0.167M potassium dichromate ($K_2Cr_2O_7$) was added by means of a pipette. An amount of 20 ml of Sulphuric acid was added using a dispenser and then swirled gently to mix. The suspension was allowed to stand for 30 minutes on an insulation pad and was further diluted with 200 ml of distilled water, after which 10 ml of 85% of Phosphoric acid (H_3PO_4), 0.2 g of Sodium fluoride (NaF) and 10 drops of ferroin indicator were added. The solution was titrated with 0.5M Fe^{2+} to a burgundy endpoint. A blank reagent without soil was run simultaneously. Percentage organic carbon was first determined and then multiplied by a factor of 1.72 and divided by 0.58 to obtain the percentage organic matter.

Easily oxidizable organic carbon (%C) = $\frac{(B-S)x\ M\ of\ Fe\ x\ 12\ x\ 100}{g\ of\ soil\ x\ 4000}$, where B= ml of Fe used to titrate blank, S= ml of Fe used to titrate sample, M of Fe = moles of Fe, 12/4000 = milliequivalent weight of C in g. Hence, % OM = $\frac{\%C\ x\ 1.72}{0.58}$

Test for Organic carbon (OC)

The organic carbon content of the soil was also determined using the Walkely and Black method. In this method an amount of the sampled soil (1 g) was weighed into a 500 ml Erlenmeyer conical flask and 10 ml of 1N potassium dichromate ($K_2Cr_2O_7$) and 20 ml of concentrated sulphuric acid (H_2SO_4) were added, mixed thoroughly and the reaction allowed to proceed for 30 min. The reaction mixture was diluted with 200 ml of distilled water, 10 ml of concentrated H_3PO_4, 10 ml of NaF solution and 2 ml of diphenylamine. The solution was titrated with a standard Ferrous Ammonium Sulphate (FAS) or Mohr's salt to a brilliant green colour. A blank without soil was run simultaneously (Sawarkar, 2012). The % Organic Carbon was calculated using the formula %OC = $\frac{10}{Blank}(blank - reading)x\frac{0.003\ x\ 100}{weight\ of\ soil}$

Weight of sample = 1g, Normality of $K_2Cr_2O_7$ used = 1 N, Volume of $K_2Cr_2O_7$ = 10ml Normality of FAS = 0.5 N

Test for % Nitrogen

Nitrogen was determined using the micro-Kjeldahl method per the procedure suggested by Association of Official Analytical Chemist (1995). An amount of 1g of air-dried and sieved soil sample was placed in a digestion tube, 10 ml of concentrated sulphuric acid and 5 g of catalyst mixture was added to the sample. The digestion tube was loaded into the digester and the digestion

block heated. The initial temperature was set at 100ºC till frothing was over. When the digestion process ended the sample turned colourless. The digestion tube was then cooled and loaded into the distillation unit. An amount of 20 ml of 4% boric acid with mixed indicator in a 250 ml conical flask was kept in the other hose of the distillation unit. An aliquot of 40 ml sodium hydroxide was automatically added by the distillation unit programme. The digested sample was heated by passing steam at a steady rate. The liberated ammonia was absorbed in 20 ml of 4% boric acid containing the mixed indicator solution turning it to green from its pinkish colour. One hundred and fifty (150 ml) of distillate was collected and a blank without soil was run simultaneously. The green distillate was titrated with 0.02N sulphuric acid and the colour changed to the original shade of pink. The blank and sample titre reading was noted and used to calculate for total nitrogen present in the soil using the following formula:

% Nitrogen =

$$\frac{R(sample\ titer - blank\ titer)x\ Normality\ of\ acid\ x\ Atomic\ weight\ of\ NItrogen\ x\ 100}{Sample\ weight\ (g)x\ 1000}$$

Test for C:N ratio

After determining the organic carbon content of the soil using the Walkely and Black method and the % Nitrogen using the Kjeldahl method a ratio of the two was calculated using the formula,

C: N Ratio = $\frac{OC}{TN}$ (SSLIM, 2011)

Where, C: N = Carbon to Nitrogen ratio

OC = Organic C (%) measured by Walkley-Black procedure

TN = Total Nitrogen

Test for Cation Exchange Capacity (CEC)

The CEC of the sampled soils was determined by the method described by Bower *et al.* (1952). Five grams (5 g) of the sampled soil was weighed and put in a centrifuge tube and 33 ml of Sodium Acetate (NaOAc) was added. The sample was centrifuged for 5 minutes at (Relative centrifugal force) RCF of 1000 until the supernatant liquid was clear. The supernatant liquid was decanted and discarded. The sample was centrifuged, decanted and discarded in the same manner three times. The residue was then treated with 33 ml of 95% ethanol, mixed thoroughly and centrifuged for 5 minutes at the same RCF for four times. The supernatant liquid was decanted and discarded each time. The electrical conductivity of the supernatant liquid from the third washing was less than 40 mmhos/cm as expected. Using the above centrifuging procedure, the absorbed sodium was replaced with 1.0N Ammonium acetate (NH_4OAc) solution. The residue was centrifuged with 33 ml of NH_4OAc three times and each time the supernatant was collected in a volumetric flask which was subsequently maintained to 100 ml by adding NH_4OAc. Sodium concentration was determined by flame photometer using a standard series of Sodium Chloride, (NaCl). The CEC was calculated using the formula;

$$\text{CEC } (meq/l) = \frac{Na\ concenration\ of\ extract\ x\ 10}{wt\ of\ sample\ in\ g}$$

Test for Available Phosphorus

Available phosphorus was determined using the Bray and Kurtz No. 1 method (van Reeuwijk, 2002). Two grams (2 g) of sieved and air-dried soil sample was weighed and poured into a 50 ml test tube, 14 ml of extracting solution

Bray 1 was added. The content in the test tube was shaken manually for one minute and filtered using Whatman No. 1 filter paper. The turbid filtrate was filtered again through the same filter paper into a test tube. An aliquot of 2 ml of boric acid, 1 ml of standard series and 3 ml of mixed reagent was pipetted into the extract in the test tube and homogenized. The solution was allowed to stand for at least an hour for the blue colour to develop to its maximum. Absorbance was measured on a spectrophotometer at 882 or 720 nm. Two blanks without soil were run simultaneously. Available Phosphorus was calculated as;

P (µg/g soil) = $(a-b)x\ \left(\frac{14}{1000}\right)x\left(\frac{1000}{s}\right)x\ mcf\ = (a-b)x\ \left(\frac{14}{s}\right)x\ mcf$

Where a = mg/l P in sample extract, b= ditto in blank, s = sample weight in gram, mcf = moisture correction factor.

Test for Potassium

The determination of Potassium in the sampled soils was done using the flame photometer. Five grams of air-dried and sieved soil was weighed into a 100 ml conical flask. An aliquot of 25 ml 1N Ammonium acetate solution was added to the content and mixed thoroughly for 5 minutes and then filtered through Whatman No. 1 filter paper. The extract was measured by flame photometer after calibration (Bagel, 2012).

Data Analysis

Data on household survey, percentage incidence, and severity index of PHRD from the various fields was subjected to the analysis of variance (ANOVA) using GenStat Statistical Software version 12 (VSN International). The means

were separated using least significant difference (LSD) method at 5% probability level.

Data from the various soil tests were also subjected to ANOVA using GenStat Statistical Software version 12 (VSN International). The means were separated using least significant difference (LSD) method at 5% probability level.

3.2 PATHOGEN IDENTIFICATION AND CHARACTERISATION

3.2.1 *Collection of disease samples*

Diseased pineapple samples consisting of whole plant and leaves were collected from the field and sent to the Crop Science Pathology Laboratory, University of Cape Coast, for isolation and identification of the causal agents of PHRD.

3.2.2 *Media preparation for pathogenic studies*

Carrot Agar (CA), Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) media were used for the isolation and growth of the pathogen and broth culture preparation. CA, PDA and PDB were prepared as described by Jeffers (2006).

PDA was prepared using 200 g of potato which was boiled in 500 ml of distilled water for an hour. The water was strained from the mixture to obtain a filtrate to which 20 g of Agar and 15 g of glucose were added and stirred till dissolved. The strained filtrate was topped up with distilled water to 1000 ml and autoclaved at 121ºC for 20 min.

PDB was also prepared using 200 g of potato which was boiled in 500 ml of distilled water for an hour. The water was strained from the mixture to obtain a filtrate and 15 g of glucose was added and stirred to dissolve. The strained

filtrate was topped up with distilled water to 1000 ml and autoclaved at 121ºC for 20 min.

Similarly, Carrot Agar was prepared using 200 g of grated fresh carrots boiled in 500 ml distilled water for 15 min. The mixture was strained and 20 g of Agar was added to the filtrate and stirred till dissolved. The filtrate was then topped up with distilled water to 1000 ml and autoclaved at 121ºC for 20 min. The CA and PDA were poured at 50ºC into 9 cm diameter Petri dishes and allowed to cool and solidify before they were used for isolation and growing of the pathogen.

3.2.3 *Morphological Identification*

Culturing of pathogen from disease samples

CA was used for the isolation and PDA for the growing and maintenance of the organism. The diseased leaf samples collected from the field were cut into 5 mm pieces and surface sterilised using 1% Sodium hypochlorite solution for 3 min and 70% ethanol solution for 1 min and then rinsed three times using sterilised distilled water. Samples were then blotted using sterilised 9 mm size Whatman No. 1 filter paper. Three pieces of samples were then placed on each Petri dish containing CA and incubated at 27 ˚C for 48 hrs for isolation of the causal agents of PHRD. The hyphal tips of the growing pathogen were cut and transferred onto fresh PDA for more growth. Isolates were maintained on PDA slants by periodical sub-culturing to obtain a pure culture and stored at 4 ºC. Isolates obtained from cultured diseased samples were designated as GF1, GF3, ABS and NB and used for subsequent experiments.

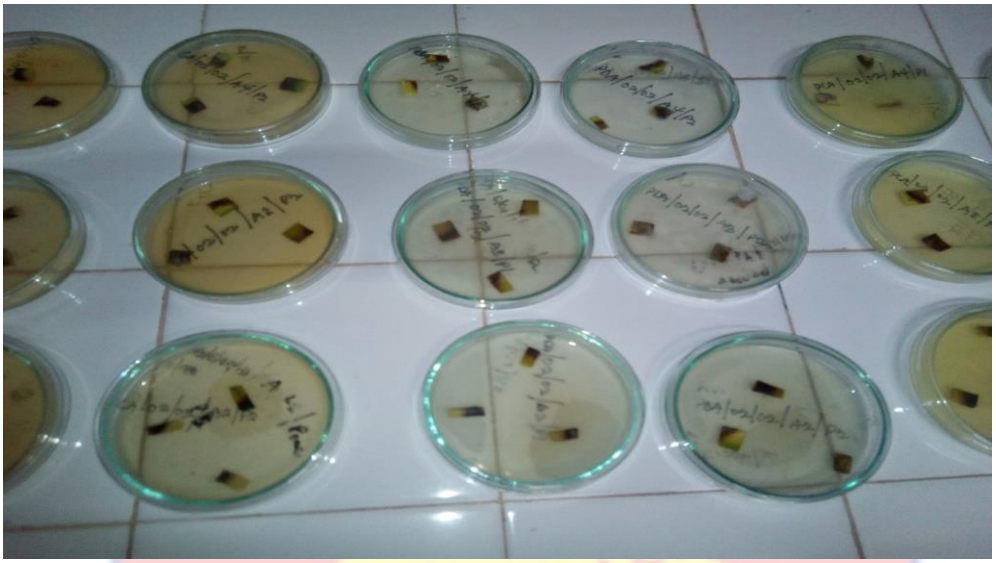


Figure 4: An image showing 5 mm pieces of diseased samples on 9 cm diameter petri dishes containing CA.

Data collection

Morphological growth was monitored daily and data collected on colony characteristics (pattern, colour, form, texture, margin, opacity, growth rate, size of mycelium), sporangia characteristics (shape, papillation) and hyphae characteristics. The colony diameter was measured as the mean of two perpendicular diameters, based on the method of Elad *et al*. (1981) and used to determine the growth rate of the isolates. Average Linear Growth Rates (ALGR) were calculated using the following equation:

$$ALGR\ (mm\ per\ day) = \frac{C7 - C1}{C1}$$

where, C7 is colony diameter in millimetre after seven days and C1 is colony diameter in millimetre after a day of incubation

Molecular Identification

DNA extraction of the pathogen was done at the laboratories of Molecular Biology and Biotechnology of the School of Biological Sciences, University

of Cape Coast and extracted DNA samples sent to Inqaba Biotechnological in Pretoria, South Africa for pathogen identification. DNA sequence data was subjected to the BLAST analysis and Phylogenetic tree drawn using the Maximum likelihood method.

DNA Extraction

DNA was extracted from the 7-day old cultures of the four isolates (GF1, GF 3, ABS and NB) using an extraction protocol by Gonzalez-Mendoza *et al*. (2010) with modification. Two hundred microlitres (200µl) of Extraction buffer (3% SDS (w/v) containing 0.5 mM EDTA, 1.0 M NaCl, and 0.1 mM hydroxymethyl-hydrochloride (Tris-HCl, pH 8.0)) was added to the fungal mycelia of isolates GF1, GF3, ABS and NB. The suspensions were shaken vigorously electronically for 1 min, 3µl of RNase and Proteinase K was added to the content and incubated at 65ºC for 10 min, after which 0.2 mL chloroform-phenol mix was slowly added and incubated again at 65°C for 10 min. The mixtures were cooled to room temperature and centrifuged at 10,000 g for 5 min. The supernatant was transferred to a new microtube and an equal volume of ice-cold absolute isopropanol was added. The contents were mixed gently and incubated at -20ºC for 30 min.; the mixture was then centrifuged at 10,000 g for 10 min again, after which the pellets were washed twice with 75% ethanol and further incubated at 37ºC for 15 min. The pellets were eluted in Tris EDTA (TE) buffer and stored at - 20ºC.

The pellets were checked for the presence of DNA using Gel electrophoresis method. An amount of 0.4 g of agarose gel was heated to dissolve in 40 ml of Trisboris TDTA buffer. Three microlitres (3 µl) of Ethidium Bromide was added and allowed to cool before pouring into a tray containing an inserted

comb and allowed to cool completely for 30 min. A Gel loader was added to the pellets and shaken. The pellets were pipetted into the well created by the comb in the tray. The setup was placed in a Gel tank to run at 90 V for 40 min. The DNA samples were then sent to be sequenced at Inqaba Biotechnological in South Africa.

Phylogenetic Analysis

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 17 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 205 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar & Tamura, 2016).

Data collection

Sequence data was edited using Chromas version 2.6.4 and generated consensus using the BioEdit version 7.0.5.3 (Hall, 1999). The sequence data was subjected to BLAST analysis in National Centre for Biotechnology Information (NCBI) data base (www.ncbi.nlm.nih.in) to identify the specific species of the pathogen.

3.3 *IN VITRO* DISEASE MANAGEMENT

Study on *in vitro* management of the causal agent of the PHRD was conducted in the laboratories of Department of Molecular Biology and Biotechnology, School of Biological Sciences, University of Cape Coast. This comprised the use of botanicals like extract from the African Mahogany (*Khaya senegalensis*), Prekɛsɛ (*Tetrapleura tetraptera*), and Neem (*Azadirachta indica*), a biological control organism *Trichoderma* and fungikill (fungicide). These treatments were used on the causal agent of the PHRD, with the fungicide as the positive control treatment. Each treatment was replicated three times. Samples of *Tetrapleura tertraptera* were obtained from Accra Central markets, bark of *Khaya senegalensis* was collected from the Botanical Garden of the University of Cape Coast, and the seeds of *Azadirachta indica* were collected from Bolgatanga in the Upper East region of Ghana. The *Trichoderma* spp. was collected from a collection of bio-control organisms belonging to Dr. Frank Kwekucher Ackah, a lecturer at the University of Cape Coast. The fungicide was obtained from an Agro-input shop in Kotokuraba. The identification and authentication of the plant materials was done by the Herbarium unit of the School of Biological Sciences, University of Cape Coast.

3.3.1 *Preparation of Extracts*

Extract preparation was done using Absolute ethanol and Sterilised distilled water for each of the botanicals.

Extract from Khaya senegalensis

The bark of the African mahogany obtained from the botanical garden was sun-dried for 7 days. Aqueous and Absolute ethanol extracts were prepared by

pounding 500 g of dried bark using mortar and pestle and then further grounded using a Brown Pestell Euromotor milling machine to a fine powder. The fine powder was totally submerged in 1 L of both solvents separately and allowed to stand for 24 hours or left overnight before filtering through a one-layer muslin cloth (1 mm mesh size) into sterilised labelled containers. The filtrates were evaporated using water bath at 60˚C to obtain the crude extract (Falah *et al*., 2008), and prevent phytochemicals from evaporating. An aqueous solution of the extract was made corresponding to different concentrations of 25/75, 50/50, 75/25 and 100 (g/ml).

Extract from Tertrapleura tetraptera (Prekɛsɛ)

Preparation of this extract was done using both Aqueous and Absolute ethanol. For the aqueous extraction 50 g of *T. tetraptera* was grounded into a fine powder using a Brown Pestell Euromotor milling machine. The grounded *T. tetraptera* was macerated in 500 ml of distilled water for 48 hours. The mixture was then filtered using a one-layer muslin cloth and evaporated at 55 ˚C to obtain crude extract. The crude paste was scraped into sterilised labelled tubes and stored in the refrigerator at 4 ºC and used for sensitivity test (Saague, 2019).

For the ethanol extraction method, 200 g of grounded *T. tetraptera* was soaked/ mixed in 1 litre of 70% ethanol and allowed to stay for 1 hour. Mixture was filtered using muslin cloth test (Saague, 2019). Filtrate was evaporated at 60 ˚C using a water bath to obtain the crude extract. The extract paste was scraped into sterilised labelled tubes and stored in the refrigerator at 4 ºC and subsequently used for sensitivity test. An aqueous solution of the

extract was made corresponding to different concentrations of 25/75, 50/50, 75/25 and 100 (g/ml).

Extract from Azadirachta indica

Aqueous extract of *A. indica* was prepared by pounding 25 g of dry neem seeds into fine powder. Powdered neem seeds were placed in a muslin cloth and soaked in 500 ml distilled water overnight. The muslin cloth containing the powdered neem seed was squeezed and the mixture filtered to obtain the filtrate. The filtrate was evaporated at 60 ºC using a water bath. The extract was scraped into sterilised labelled tubes and stored at 4 ºC and subsequently used for sensitivity test.

The fine powdered neem seed was weighed and totally submerged in absolute ethanol for effective extraction of phytochemicals. The mixture was sieved using a one-layer muslin cloth and filtrate evaporated using a water bath at 60 ºC. Extract was scraped into sterilised labelled tubes and stored at 4 ºC and subsequently used for sensitivity test. An aqueous solution of the extract was made corresponding to different concentrations of 25/75, 50/50, 75/25 and 100 (g/ml).

Preparation of Positive Control

Fungikill 50WP with the active ingredient Copper (II) hydroxide (35%) and Metalaxyl (15%) was used as a positive control. This fungicide was selected because it is used by most farmers on their pineapple crop. It was prepared using the manufacturer's dose of 75 g/15 L which translated to 0.5 g/100 ml. Different concentrations of the Fungikill 50WP was prepared and used for the sensitivity test. The concentrations were 25/75, 50/50, 75/25 and 100 (g/ml).

Preparation of Trichoderma metabolites

The *Trichoderma* sp used for this study was *Trichoderma* asperellum. *Trichoderma asperellum* was cultured on PDA for 7 days. An agar disc 5 mm size of *Trichoderma* sp. was transferred into a 500 ml PDB and incubated in the dark at room temperature for 10 days. The broth culture was then centrifuged. The supernatant was filtered using a membrane filter and placed in an Eppendorf tube and stored at 4 ºC for subsequent use in sensitivity test.

Figure 5: 7- day old *Trichoderma* sp. on PDA

Broth culture preparation of pathogen

Agar disc (0.2cm diameter) of 7-day old plate cultures of isolates were made using flame sterile cork borer. One disc of each isolate was transferred into 200 ml Potato Dextrose Broth in a conical flask. The broth cultures were incubated at room temperature for 48 hours and used for the *in vitro* management studies.

Figure 6: Broth cultures of the *Phytophthora* Isolates (GF1, GF3, ABS and NB)

3.3.2 Minimal Inhibitory Concentration Test (MIC)

Half a millilitre of (0.5 ml or 500 ul) of each broth culture was pipetted into sterile 9 cm diameter Petri dishes. A 20 ml aliquot of molten PDA of temperature 45 ºC – 50 ºC was added to the cultures and swirled clockwise and anticlockwise for a uniform mixture. Cultures were allowed to solidify and 4 agar wells of 0.8 cm diameter were created in the solidified agar in Petri dishes using a flamed cork borer. The agar wells were filled with 2 ml extracts from the Prekese, African Mahogany, Neem, the positive control (Fungikill) and *Trichoderma* sp. metabolites at 25/75, 50/50, 75/25 and 100 (g/ml) concentrations. The cultures were then incubated at a room temperature of 24 ºC for 24 hours. After 24 hrs of incubation, absence and presence of zone of inhibition were checked. Zones of inhibition observed in the extract were measured using a metric rule. The *Trichoderma* metabolites showed an antagonistic effect on all isolates. A confirmatory test was therefore performed using the Dual Culture Technique.

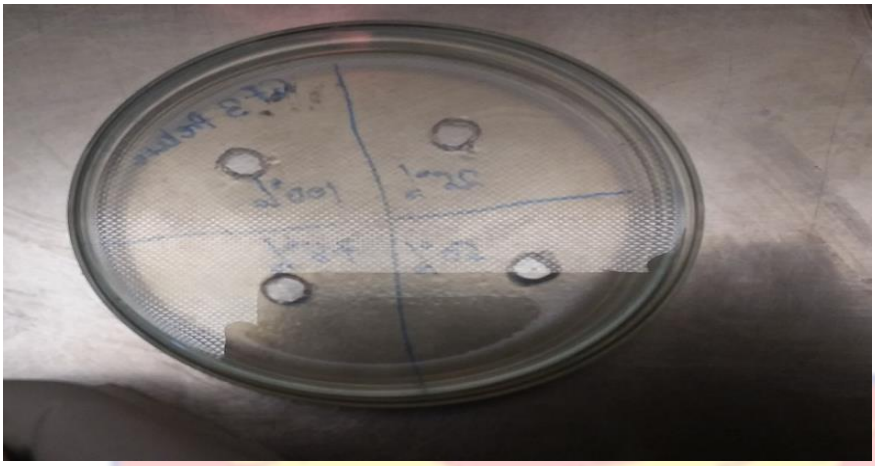


Figure 7: Agar wells in Petri dish containing broth culture of *Phytophthora* isolate.

3.3.3 *Dual Culture Test (DCT)*

The antagonistic effect of the *Trichoderma* asperellum against the isolates was done using the Dual Culture Technique (Dennis & Webster, 1971). A 5 mm mycelia plug of a 7-days old culture of *Trichoderma* was placed together with the *Phytophthora* isolates separately on PDA at an equidistance of 80 mm. The placement of the *Trichoderma* was done a day before, same day and a day after isolates were placed in Petri dishes. These were done separately and each replicated two times. For control treatment, a plug of *Trichoderma* sp. and isolates was placed on a PDA medium separately. Observation was made on the antagonistic activities against the Isolates and recorded daily. The type of colony interaction between the *Trichoderma* and isolates were visually assessed based on the interacting grade described by Skidmore & Dickinson (1976).

Grade	Description
1	Mutual intermingling without any macroscopic sights of interaction
2	Mutual intermingling growth, where the growth of fungus is ceased by the growth of opposed fungus
3	Intermingling growth, where the fungus under observation is growing on the opposed fungus either above or below
4	Sight inhibition of both the interacting fungi with narrow delineation line.
5	Mutual inhibition of growth at a distance of >2mm

Data collection

Data on Inhibition percentage from Minimal Inhibition Concentration and Dual Culture Technique was collected and subjected to the analysis of variance (ANOVA) using GenStat Statistical Software version 16 (VSN International). The means were separated using Turkey's test at 5% probability level. Inhibition percentage in DCT was calculated using the formula by Edgington *et al*. (1971).

$$Inhibition\ percentage\ (\%) = \frac{A1-A2}{A1} \times 100$$

A1= Colony area of uninhibited *Trichoderma* spp. in the control plate

A2= Colony area of inhibited *Trichoderma* spp. in dual culture plate.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of Respondents Farmers

The background information of the respondent farmers is shown in Table 2.

Out of the 120 pineapple farmers 105 farmers representing 87.5% were males, and 15, representing 12.5% were females.

Most of the farmers interviewed (33.3%) were between 26-45 years, followed by 20.0% farmers whose ages were between 36-45 years. Those whose ages were between 46-45 and 56-65 years contributed 17.7% each. Farmers, whose ages ranged between 18-25 years, represented 7.5%, whereas 2.5 % and 1.7% fell between 66-75 and 76- 85 years, respectively.

One hundred and one (101) farmers representing 84% grow only pineapples. The rest (19), work in other areas in addition to the pineapple farming. Nine (9) out of the 19 farmers representing 7.5% are fishermen, 4 farmers representing 3.3% are traders, also another set of 4 farmers representing 3.3% are carpenters, and 2 farmers representing 1.7% are Masons.

About 64 farmers (53.3%) have no formal education. Those with non-formal education (16) are 13.3%, some form of basic education 12.5% (15 farmers), basic education 9.2% (11 farmers), some secondary education 0.8% (1 farmer), secondary education 1.7% (2 farmers), MSLC 2.5% (3 farmers) and Tertiary education 6.7% (8 farmers).

Table 2: Demographic of respondents

	Frequency	Percentage (%)
Gender		
Male	105	87.5
Female	15	12.5
Total	120	100.0
Age		
18-25	9	7.5
26-35	40	33.3
36-45	24	20.0
46-55	21	17.5
56-65	21	17.5
66-75	3	2.5
76-85	2	1.7
Total	120	100.0
Occupation		
Farmer	101	84.2
Trader	4	3.3
Carpenter	4	3.3
Fisherman	9	7.5
Mason	2	1.7
Total	120	100.0
Level of Education		
No Formal education	64	53.3
Non-formal	16	13.3
Some basic education	15	12.5
Basic education	11	9.2
Some secondary education	1	.8
Secondary education	2	1.7
MSLC	3	2.5
Tertiary education	8	6.7
Total	120	100.0

(**Source:** Dermographic Survey, 2019)

From Table 3, majority of the famers (79.2%) have been in pineapple production for more than 5 years. About 15 farmers representing 12.5 % have been cultivating pineapple between 1-5 years. About 10 farmers (8.3 %) have been in pineapple farming for less than a year.

Over 63% made up of 76 farmers have farmlands between 1 - 3 acres. Farmers with land size above 3 acres were 41 representing 34.2%, and those with less than an acre were 3, representing 2.5%. About 85.8% (103) farmers rent the land they farm on, 10.8% (13 farmers) own the land and 3.3% (4 farmers)

practice sharecropping. Majority of the farmers 84.2% (101) use hired labour on their farms while 13.3% (16 farmers) make use of family members as a source of labour and remaining 2.5% (3 farmers) practice *Nnoboa*. Majority of the farmers 86.7% (104) use their own funds in the pineapple production. About 9.2% (11 farmers) obtain funds from their customers and produce for them, while 3.3% (4 farmers) go for loans from the bank. Only 1 farmer representing 0.8%, borrows from family members.

Table 3: Farming characteristics of respondents

	Frequency	**Percentage (%)**
Number of years in pineapple cultivation		
Less than 1 year	10	8.3
1-5 years	15	12.5
Above 5 years	95	79.2
Total	120	100.0
Size of land under cultivation		
Less than 1 acre	3	2.5
1- 3 acres	76	63.3
Above 3 acres	41	34.2
Total	120	100.0
Land tenure system		
Self-owned	13	10.8
Rent	103	85.8
Share cropping	4	3.3
Total	120	100.0
Source of Labour		
Hired	101	84.2
Family labour	16	13.3
Nnoboa	3	2.5
Total	120	100.0
Source of Finance		
Self	104	86.7
Bank	4	3.3
Family members	1	0.8
Customer	11	9.2
Total	120	100.0

(**Source:** Field Survey, 2019)

4.2 Agronomic Practices Employed by Respondents

Agronomic practices employed by farmers are presented in Table 4.

Most of the farmers 69.2% (83) make use of the slash and burn method of land preparation whereas 20.8% (25) do not till their land and only 10% (12) have adopted the use of tractor to plough their land. It is also evident from Table 4 that majority of the farmers 88 (73.3%) practiced mixed cropping. The remaining 32 (26.7%) farmers practised mono cropping. Out of the 88 farmers that practiced mixed cropping, 45.5% intercrop with cassava, 44.3% intercrop with plantain or banana and 10.2% intercrop with maize. All the 120 farmers (100%) do not practice crop rotation (Table 4).

Almost all the farmers 99 (82.5%) grow only the Sugar loaf variety of pineapple, 6 farmers (5%) grow only the MD2 variety, 5 farmers (4.2%) grow only the Smooth cayenne. The remaining farmers grow two or all the variety, 3 farmers (2.5%) grow both the MD2 and Sugar loaf, another 3 farmers (2.5%) grow both the Sugar loaf and Smooth Cayenne and the last 4 farmers (3.3%) grow all the varieties.

Majority of the farmers 95 (79.2%) obtain planting material from their own farm, 12.5% (15) take it from other farmers and (10) 8.3% buy it from an open market (Table 4).

Table 4: Agronomic practices employed by respondents - cultivation

	Frequency	Percentage (%)
Method of Land Preparation		
Zero tillage	25	20.8
Slash and burn	83	69.2
Tractor plough	12	10.0
Total	120	100.0
Farming practice used		
Mono cropping	32	26.7
Mixed cropping	88	73.3
Total	**120**	**100.0**
Kinds of crops respondents intercrop		
Cassava	40	45.5
Plantain/Banana	39	44.3
Maize	9	10.2
Total	88	100.0
Practice of crop rotation		
Yes	0	0.0
No	120	100.0
Total	180	100.0
Cultivar produced		
MD2	6	5.0
Sugar loaf	99	82.5
Smooth cayenne	5	4.2
MD2 and Sugar Loaf	3	2.5
Sugar loaf and Smooth cayenne	3	2.5
MD2, Sugar loaf and Smooth cayenne	4	3.3
Total	120	100.0
Sources of planting materials		
Open market	10	8.3
Farmer's own field	95	79.2
Other farmers	15	12.5
Total	120	100.0

(**Source:** Field Survey, 2019)

Almost all the farmers 90% (108) indicated that they do not use fertilizers on their farm while the remaining 10% (12 farmers) affirmed the use of fertilizer (Table 5). These 12 farmers (10%) make use of only chemical fertilizers. Out of these 12 that use chemical fertilizers, 6 (50%) use the fertilizer because it is more efficient, 4 (33.3%) use it because it is cheaper and 2 (16.7%) use it

Table 5: Agronomic practices employed by respondents – fertilizer application

	Frequency	Percentage (%)
Fertilizer usage		
Yes	12	10.0
No	108	90.0
Total	120	100.0
Type of fertilizer		
Chemical fertilizer	12	100
Organic-manure	0	0.0
Both	0	0.0
Total	12	100.0
Reason for fertilizer type		
Cheaper	4	33.3
More efficient	6	50.0
Easy to apply	2	16.7
Total	12	100.0
Type of chemical fertilizer		
NPK	8	66.7
Urea	3	25.0
Ammonia	1	8.3
Total	12	100.0
Method of fertilizer application		
Broadcasting	0	0.0
Spraying	12	100.0
Drilling	0	0.0
Total	12	100.0
Estimate quantity of chemical fertilizer usage per acre		
Less than 1kg	0	0.0
1 - 5 Kg	9	75.0
6 - 10 Kg	3	25.0
Total	12	100.0
Number of times respondents apply fertilizer		
Once	6	50.0
Twice	5	41.7
Others	1	8.3
Total	12	100.0
Stage of fertilizer application		
Vegetative stage	9	75.0
Flowering stage	3	25.0
Just before harvesting	0	0.0
Total	12	100.0

(Source: Field Survey, 2019**)**

because it is easy to apply. Majority of the farmers 66.7% (8) out of the 12 use the NPK fertilizer, 25% (3 farmers) use Urea and 8.3% (1 farmer) use Ammonia.

All the 12 (100%) farmers use the spraying method or technique to apply fertilizers on their farms. About 75% of the 12 farmers use between 1-5 kg of fertilizer per acre of farmland whereas the remaining 25% use between 6-10 kg of fertilizer per acre.

Half of the respondents representing 50% (6 farmers) use fertilizer once a year. Five farmers (41.7%) use fertilizer twice a year, the remaining farmer (8.3%) uses the fertilizer more than two times a year. About 75% (9) farmers apply the fertilizer at the vegetative stage while the remaining 25% (3) apply it at the flowering stage (Table 5).

4.3 Farmers' Awareness of Heart rot diseases

The awareness and knowledge of farmers in terms of the existence, cause, growth stage at which Heart rot disease occurs and the management strategies adopted on their farms are presented in Table 6.

About 88.3% (106) farmers observed the heart rot disease symptoms on their farm whereas 11.7% (14) farmers did not observe it on their farms.

Most of the farmers about 67% (71) observed the symptoms as rot and foul odour of the lower basal leaf and easy detachment of leaves, 14.1% (15) of the farmers observed the symptoms as yellowing of the leaves and giving off foul odour; the rest 18.9% (20) observed both symptoms on their farms.

While 30.1% (32) of the farmers attributed the cause of the disease to unfavourable soil conditions, 18.9% (20 farmers), attributed it to unfavourable climatic conditions and then 8.5% (9 farmers) said it was as a result of insect

attack. The remaining 42.5% (45 farmers) said they had no idea what causes the disease.

Table 6: Farmers' awareness of the PHRD disease

	Frequency	Percentage (%)
Observation of Heart rot disease		
Yes	106	88.3
No	14	11.7
Total	120	100.0
If yes, describe the disease		
Rot and foul odour from basal leaf and leaf detachment	71	67.0
Yellowing of leaves	15	14.1
All the above	20	18.9
Total	106	100.0
What causes the Heart rot disease		
No idea	45	42.5
Unfavourable soil conditions (poor drainage)	32	30.1
Unfavourable climatic conditions	20	18.9
Insects attack	9	8.5
Total	106	100.0
Stage disease is first encountered		
Vegetative stage	71	67.0
Fruiting stage	30	28.3
Flowering stage	5	4.7
Total	106	100.0
Season disease occur		
Dry season	15	14.2
Wet season	80	75.4
Both seasons	11	10.4
Total	106	100.0
Season disease is very severe		
Dry season	6	5.7
Wet season	100	94.3
Both seasons	0	0
Total	106	100.0
Estimated yield loss after infection		
< 10 %	65	61.3
11 – 20 %	35	33.0
21 – 30 %	6	5.7
Total	106	100.0

(Source: Field Survey, 2019**)**

Furthermore, majority of the farmers 67% (71) said the disease occur at the vegetative stage, while 28.3% (30) said it occurs at the flowering stage and 4.7% (5) said it occurs at the fruiting stage.

About 24.5% (26) of the farmers alluded that the disease occurs in the dry season, 63.2% (67) of the farmers also alluded that it occurs in the wet season and the remaining 12.3% (13) of farmers said the disease occurs in both seasons.

Most of the farmers 65 (61.3%) estimated the loss of crops after infection to be less than 10%, 35 (33%) of the farmers experience losses between 11-20% and 6 (5.7%) farmers experiences losses between 21-30% (Table 6).

4.4 Disease Management

Data on disease management of PHRD by the respondent farmers are presented in Table 7.

It was evident that a few farmers 10.4% (11) control the disease by applying chemical and 33% (35) manage the disease by removing infected plants. The rest of the farmers which are in the majority of 56.6% (60) do not control the disease. Approximately 7 (63.6%) out of the 11 farmers apply chemicals twice in the cropping season, while the remaining 4 (36.4%) apply the chemicals three times in the cropping season (Table 7).

All the 11 (100%) farmers who control disease on their farm use fungicides. Eight out of the 11 (72.7%) farmers buy their fungicides from Agro input shops, 2 (18.2%) obtain it from MoFA and 1 (9.1%) gets it form a fellow farmer. About 7 (63.6%) farmers take advice on choice of chemicals from Agric extension officer and the remaining 4 (36.4%) take advice from Agro input dealers. About 10 (90.9%) out of the 11 farmers make use of other

alternate chemicals whereas the remaining 1 (9.1%) farmer does not. About 8 (72.7%) farmers take advice on the alternate use of chemicals from Agric. extension officer, 2 (18.2%) take advice from Agro input dealers and the remaining 1 (9.1%) farmer takes advice from a fellow farmer.

About 8 (72.7%) farmers alternate the chemicals they use on their crops for protection and the 3 (27.3%) remaining farmers.' alternate chemicals for curative purpose. About 7 (63.6%) farmers said that the control measure is effective whereas the remaining 4 (36.4%) farmers said the control measure is not effective.

In addition to the PHRD, about 30 (25%) farmers encounter other diseases such as fruitlet core rot on their farms, 15 (12.5%) yellow spot, 55 (45.8%) Pineapple mealy bug wilt disease (PMWD) and 20 (16.7%) insect damage.

Table 7: Disease management[1]

	Frequency	Percentage
Control of disease		
Chemical app	11	10.4
Removal of infected plant	35	33.0
No control	60	56.6
Total	106	100.0
Frequency of application		
Once	0	0.0
Twice	7	63.6
Thrice	4	36.4
Total	11	100.0
Kind of chemical		
Fungicides	11	100
Total	11	100.0
Source of chemical		
Agro input shops	8	72.7
MoFA	2	18.2
Other farmers	1	9.1
Total	11	100.0
Source of advice on choice of chemicals		
Agric ext. off.	7	63.6
Agro input dealers	4	36.4

[11] Table 7 continued on page 71

Total	11	100.0
Alternate use of chemicals		
Yes	10	90.9
No	1	9.1
Total	11	100.0
Source of advice of alternate use of chemicals		
Agric ext. off.	8	72.7
Agro input dealers	2	18.2
Other farmers	1	9.1
Total	11	100.0
Why alternate the use of chemicals		
For Protection	8	72.7
For curative	3	27.3
Total	11	100.0
Is control measures effective		
Yes	7	63.6
No	4	36.4
Total	11	100.0
Other disease encountered on your pineapple farm		
Fruitlet core rot	30	25.0
Yellow spot	15	12.5
PMWD	55	45.8
Insect damage	20	16.7
Total	120	100.0

(**Source:** Field Survey, 2019)

4.5 Incidence and Severity of PHRD in the Districts

Table 8 shows the Mean Incidences and Severities of PHRD recorded for the selected communities in each of the three districts studied for the first survey. It can be seen that disease was not prevalent at all the communities. The disease was completely absent in Essaman, Asebu-Ekroful, Amosima, Ayeldu, and Atwiaa. Amongst the 7 communities where the disease occurred, the highest incidence occurred at Abrenu-Akyinim and the least at Atabadzi. ANOVA showed significant differences in the disease Incidence and Severity in the communities. The highest disease incidence was recorded at Abrenu-Akyinim (2.46%) which was not significantly different from incidence at Abor (1.8%). This is followed by Asofa (1.54%) which showed no significant

difference with Ankwanda (1.14%). There was no significant difference between Nanaben (0.9%), Asuansi (0.8%) and Atabadzi (0.3%). Ankwanda, Essaman, Asebu-Ekroful, Amosima, Ayeldu and Atwiaa did not record any incidence and as such showed no significant. Incidence at Abrenu-Akyinim (2.46%) was significantly different from incidence at Nanaben (0.9%), Asuansi (0.8%), Atabadzi (0.3%), Asebu-Ekroful (0%), Amosima (0%), Ayledu (0%), Essaman (0%) and Atwiaa (%).

The highest severity was recorded at Abrenu-Akyinim (1.06) which was significantly different from Abor (0.774). This is followed by Asofa (0.558) communities which was significantly different from Nanaben (0.342). Nanaben (0.342) and Asuansi (0.312) communities did not show any significant differences. Ankwanda, Essaman, Asebu-Ekroful, Amosima, Ayeldu and Atwiaa did not record any severity and as such showed no significant difference.

Table 8: Mean incidence and severity of PHRD at the communities – first survey

Communities	**Incidence (%)**	**Severity**
Abrenu-Akyinim	2.46c	1.06c
Ankwanda	1.14abc	0.18a
Atabadzi	0.3ab	0.13a
Essaman	0a	0a
Asebu-Ekroful	0a	0a
Amosima	0a	0a
Asuansi	0.8ab	0.31ab
Ayeldu	0a	0a
Nanaben	0.9ab	0.34ab
Asofa	1.54abc	0.56abc
Atwiaa	0a	0a
Abor	1.8bc	0.77bc
Mean	0.74	0.28
P. Value	0.02	0.01
Lsd(5%)	1.55	0.62

Table 9 shows Mean Incidences and Severities for the three selected districts. It is evident that disease was prevalent in all the districts. ANOVA showed significant difference in disease incidence but showed no significant difference in disease severity. The highest mean Incidence was recorded at Ekumfi (1.06%) and was significantly different from incidence at KEEA (0.97%) and AAK (0.2%). The highest mean severity was also recorded at Ekumfi (0.42) followed by KEEA (0.34) and AAK (0.08). There were no significant differences in disease severity in the three districts.

Table 9: Mean incidence of PHRD recorded for the three districts - first survey

District	**Incidence (%)**	**Severity**
KEEA	0.97ab	0.34a
AAK	0.20a	0.08a
EKUMFI	1.06b	0.42a
Mean	0.74	0.28
P. Value	0.09	0.13
Lsd (5%)	0.85	0.35

Table 10 shows the Mean Incidences and Severities of PHRD recorded for four selected communities in each of the three districts for the second survey. The disease was completely absent in Essaman, Asebu-Ekroful, Amosima, Ayeldu, and Atwiaa. ANOVA revealed significant differences in all 7 communities that showed disease incidence. The highest disease incidence was recorded at Abrenu-Akyinim (3.40%) and Asofa (3.40%) which was not significantly different from incidence at Ankwanda (2.80%). These were followed by Abor with incidence of 2.0% which was significantly different

from incidence at Asuansi (0.6%), Atabadzi (0.6%) and Nanaben (0.4%) which were not significantly different from each other. The remaining communities Asebu-Ekroful, Amosima, Ayeldu, Atwiaa and Essaman recorded no disease incidence.

In terms of disease severity ANOVA showed no significant difference between Abrenu-Akyinim (1.50) which recorded the highest severity, Asofa (1.29) and Ankwanda (1.26). Severity at Asuansi (0.31), Atabadzi (0.20) and Nanaben (0.15) were not significantly different from each other. Abor community recorded 0.90 and was not significantly different from all the communities.

Table 10: Mean incidence and severity of PHRD recorded for selected communities - second survey

Communities	**Incidence (%)**	**Severity**
Abrenu-Akyinim	3.40c	1.50b
Ankwanda	2.80c	1.26b
Atabadzi	0.60ab	0.20a
Essaman	0a	0a
Asebu-Ekroful	0a	0a
Amosima	0a	0a
Asuansi	0.60ab	0.31a
Ayeldu	0a	0a
Nanaben	0.4ab	0.15a
Asofa	3.40c	1.29b
Atwiaa	0a	0a
Abor	2.0bc	0.90ab
Mean	1.10	0.59
P. Value	0.001	0.001
Lsd(5%)	1.88	0.91

Table 11 shows the mean incidence and severity for three selected districts. At the district level ANOVA showed significant differences in all the three districts for both disease incidence and severity. The highest disease severity was recorded at KEEA (0.74) which was not significantly different from Ekumfi (0.58) which was also not significantly different from AAK (0.08).

Table 11: Mean incidence of PHRD at the three districts – second survey

District	Incidence%	Severity
KEEA	1.70b	0.74b
AAK	0.15a	0.08a
EKUMFI	1.45b	0.58ab
Mean	1.10	0.47
P.value	0.02	0.04
Lsd (5%)	1.14	0.52

4.6 Relationship between soil fertility status and PHRD incidence and severity

Table 12 shows the Pearson's correlation coefficient calculated to ascertain the relationship between soil fertility status and PHRD incidence and severity. Result obtained revealed no significant correlation between soil fertility levels (%MC, %N, %OC, %OM, CEC, CN, K, P and pH) and PHRD incidence ($p > 0.05$) and severity ($p > 0.05$)

Table 12: Correlation between soil factors and incidence and severity of PHRD

Variable	Incidence (%)	Severity
% MC	-0.1194	-0.1250
% N	-0.0185	-0.0431
% OC	-0.0666	-0.0765
% OM	-0.0666	-0.0765
CEC	-0.0891	-0.0690
C:N ratio	-0.0998	-0.0888
K	-0.0328	-0.0722
P	-0.1214	-0.0854
Ph	-0.0653	-0.0161

(**Source:** Field Data, 2019)

MC= Moisture Content, N= Nitrogen, OC= Organic Carbon, CEC= Cation Exchange Capacity, CN = Carbon Nitrogen, P= Phosphorus, pH= Power of Hydrogen

4.7 Morphology and molecular characterisation of *Phytophthora* isolates

4.7.1 *Morphological Characteristics*

Colony characteristics

The morphological characteristics of isolates are presented in Figure 7. The colony colour of all the four isolates i.e. ABS, NB, GF1 and GF3 on PDA was white. The shape and edge of the colony of all the isolates was irregular and undulate, respectively. The mycelium of all four isolates were translucent and have a cottony texture. They all had glistening surfaces and a rosetted growth pattern. The growth rate of GF1 and GF3 was relatively fast at 1.92 mm/hr and 1.81 mm/hr, followed by ABS at 1.58 mm/hr (Table 13). Isolate NB had a very slow growth rate at 0.30 mm/hr. The optimum temperature for mycelial growth was between 24 ºC – 32 ºC.

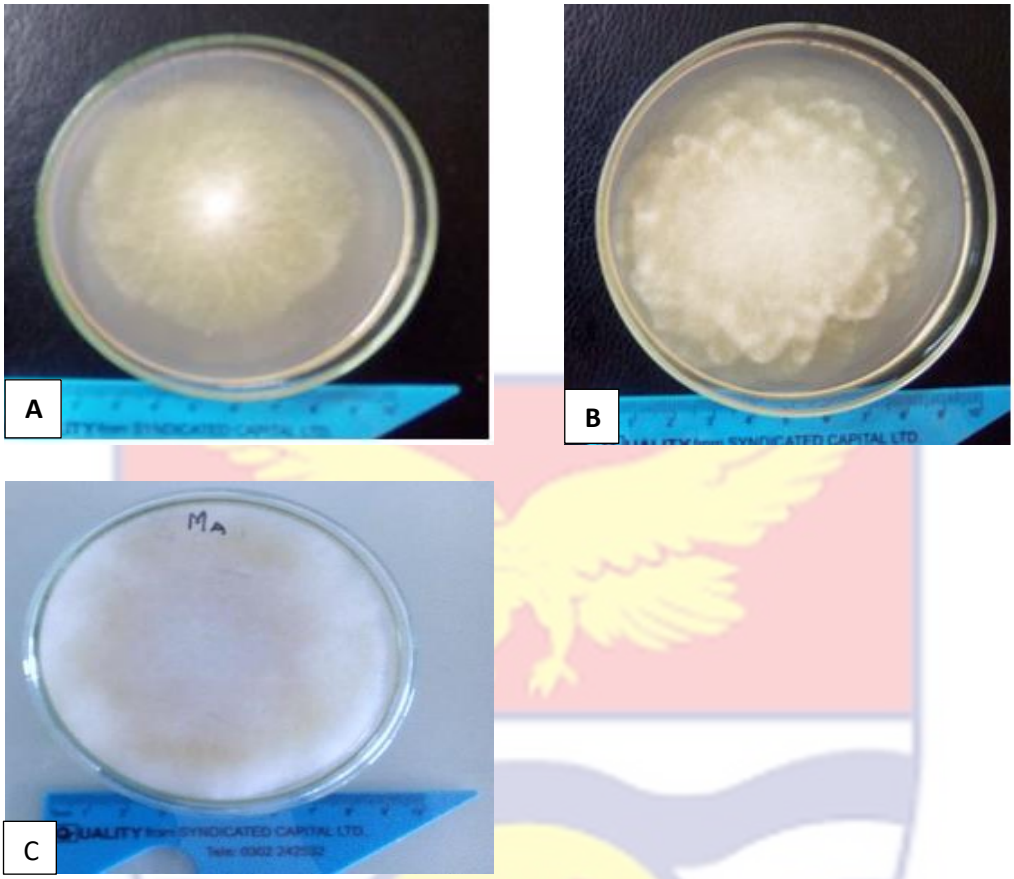


Figure 8: Colony growth of mycelium**;** A: 12 hrs old mycelium, B: 24 hrs old mycelium, 36 hrs old mycelium of GF1 on PDA.

Table 13: Growth rate of *Phytophthora* isolates

Isolates	**Growth rate (mm/hr.)**
GF3	1.81
GF1	1.92
ABS	1.58
NB	0.30

Sporangia characteristics

Sporangial characteristics are presented from Figures 9-11. On PDA, GF1 and GF3 have a papillate sporangium, ovoid in shape (Figure 9A). Isolates ABS and NB on the other hand have a papillate sporangium ellipsoid in shape (Figure 11B). All the isolates had caducous sporangia with no pedicel (Figure

9B). The mycelium for ABS and NB were non-septate, smooth and hyaline (Figure 9A) whereas GF1 and GF3 had coralloid hyphae (Figure 9C).

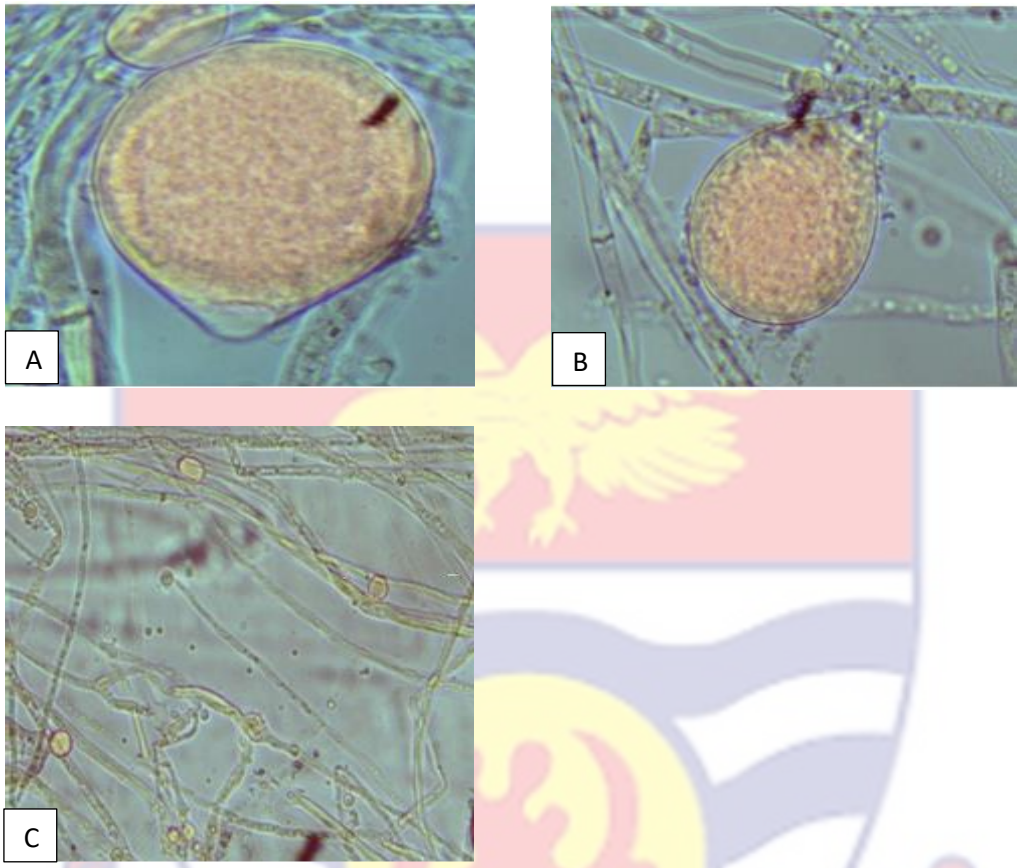
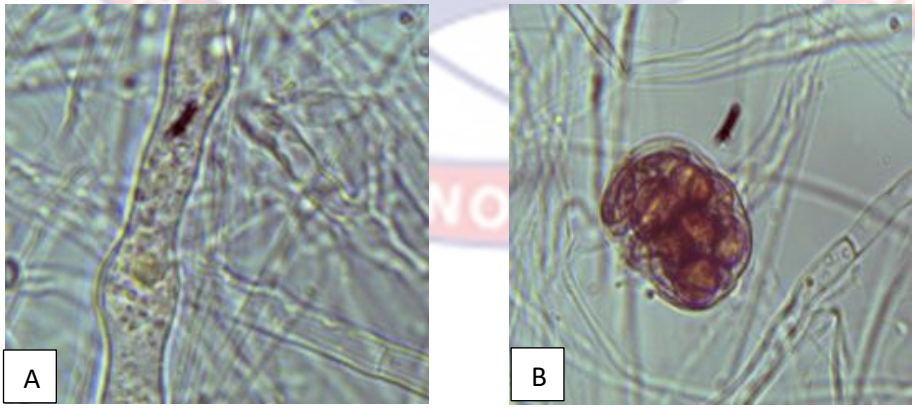


Figure 9: Sporangium morphology for GF1 and GF3; (A) papillate sporangium ovoid in shape, (B) caducous papillate sporangia with no pedicel, showing an exit pore releasing spores, (C) coralloid hyphae (400x)



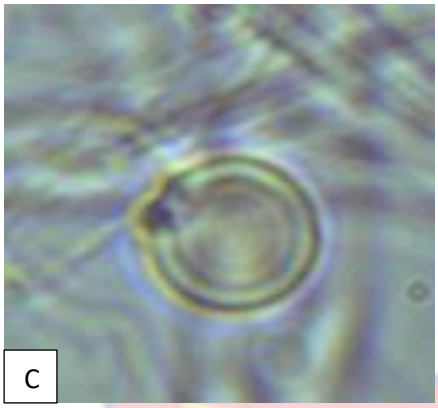


Figure 10: Sporangia morphology of ABS; (A) a swollen hyphae (400x), (B) papillate ellipsoidal sporangium showing spores within, (C) a thin walled oospore (400x)

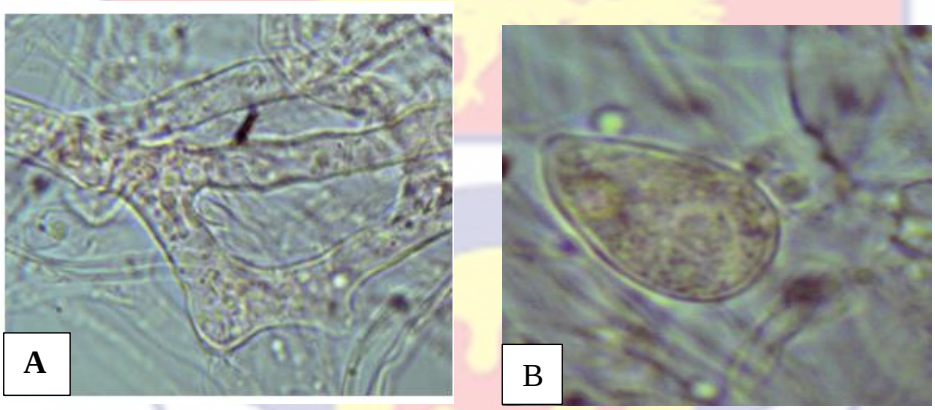


Figure 11: Sporangia and mycelium morphology of NB; (A) non-septate mycelium showing swellings on a hyphae, (B) papillate ellipsoid sporangium (400x)

4.7.2 *Molecular characteristics of Isolates*

After the blast analysis, the DNA sequence of all the four isolates in the NCBI database, had a percentage identity to the *Phytophthora* spp in the database ranged from 98-100%. The percentage identities of the various isolates were similar to the *Phytophthora* sp. indicated in the BLAST output column (Table 14).

Isolate ABS was closely related to *Phytophthora nicotianae* in the NCBI database with a percent identity of 98.0% and Accession numbers KY930644.1 and KF147901.1

Isolate NB was closely related to the *Phytophthora nicotianae* in the NCBI database with a percent identity of 100% and Accession numbers KY930644.1 and KF147901.1

Isolates GF1 and GF3 was closely related to the *Phytophthora cinnamomi* in the NCBI database with a percent identity of 100 and Accession numbers MN539998.1 and MN539997.1 (Table 14).

Table 14: BLAST output of fungal sequences

BLAST output	Percentage Identity (%)					Accession No.
	ABS	NB	GF1	GF3	TT	
Phytophthora nicotianae	98.0	100.0	–	–	–	KY930644.1
Phytophthora nicotianae	98.0	100.0	–	–	–	KF147901.1
Phytophthora cinnamomi	–	–	100.0	100.0	–	MN539998.1
Phytophthora cinnamomi	–	–	100.0	100.0	–	MN539997.1

The phylogenetic analysis tree (Figure 12) reveals two main clusters, Cluster I (CI) and Cluster II (CII). Cluster I consist of *Phytophthora* species in the NCBI database and Cluster II consist of the isolates.

Cluster II is phylogenetically distinct from the taxa in the NCBI database (CI) with a percent identity of 92 (Figure 12). In cluster II; ABS, GFI and GF3 are phylogentically similar with percent identity of 100 and phylogentically distinct from Isolate NB with percent identity of 57 (Figure 12).

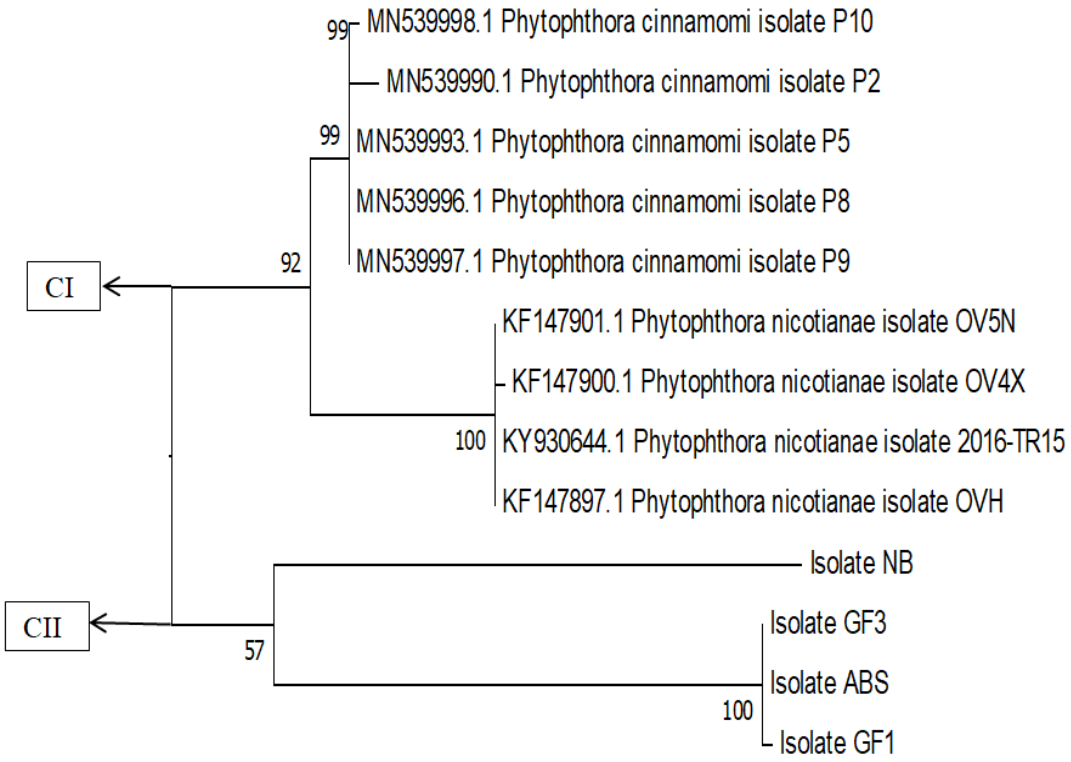


Figure 12: Maximum parsimony tree generated from reference sequences of ITS 6 ITS 7 with sequences of *Phytophthora* isolates from Pineapple.

Bootstrap = 1000 replicates (*Numbers next to branches represent bootstrap values of 1000 replicates calculated with maximum parsimony. Only bootstrap values higher than 50% are shown).

4.8 Evaluation of plant extracts and bio-control organism against isolates

In Figure 13, all treatments with the exception of mahogany, showed an inhibitory effect against Isolate GF1 with significant differences between them. The Prekese (*Tetrapleura tetraptera*) treatment showed the highest mycelial inhibition with an index of 3.00, followed by *Trichoderma* with 2.17, then Neem 1.48 and Control 1.03. All treatments with the exception of Mahogany, showed inhibitory effect against Isolate GF3 with significant differences between them. The Prekese treatment showed the highest mycelial inhibition with an index of 3.06 followed by *Trichoderma* with 2.40, Control

1.02 and Neem 0.91. Only *Trichoderma* treatment showed an inhibitory effect on isolate ABS and NB with an inhibition index of 2.06 and 2.36 respectively with no significant difference. The rest of the treatment did not show any inhibitory effect on ABS and NB.

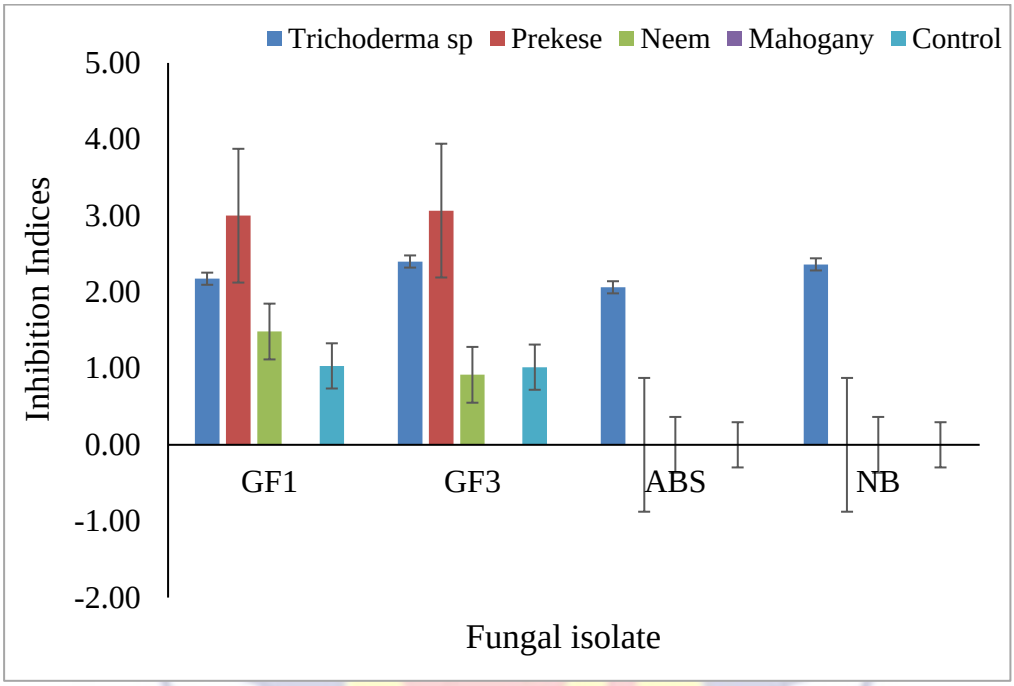


Figure 13**:** Mean inhibition indices for isolates after Treatment applications

Figure 14 shows results on the evaluation of *Trichoderma* sp. against all Isolates in a Dual Culture Technique test on different application days. There was a significant difference between NB and GF1, GF3 and ABS on all application days but there were no significant differences between GF1, GF3 and ABS. When the *Trichoderma* was applied a day (24 hrs) before the Isolates, the *Trichoderma* inhibited isolates GF1, GF3, ABS and NB with an inhibition indices of 54.00 (64.4%), 53.00 (65.6%), 52.5 (67.8%) and 81.5 (34.4%) respectively. When the *Trichoderma* was applied same day as Isolates, inhibition indices recorded on isolates GF1, GF3, ABS and NB are 42.5 (52.2%), 41.50 (54.4%), 44.50 (50%) and 79.00 (31%) respectively. When the *Trichoderma* was applied a day (24 hrs) after Isolates, *Trichoderma*

inhibited isolates GF1, GF3, ABS and NB with an inhibition indices of 32.00 (38.8%), 30.50 (38.8%), 29.00 (45.6%) and 59.00 (10%) respectively. Generally, inhibition index recorded on NB was poor compared to the others.

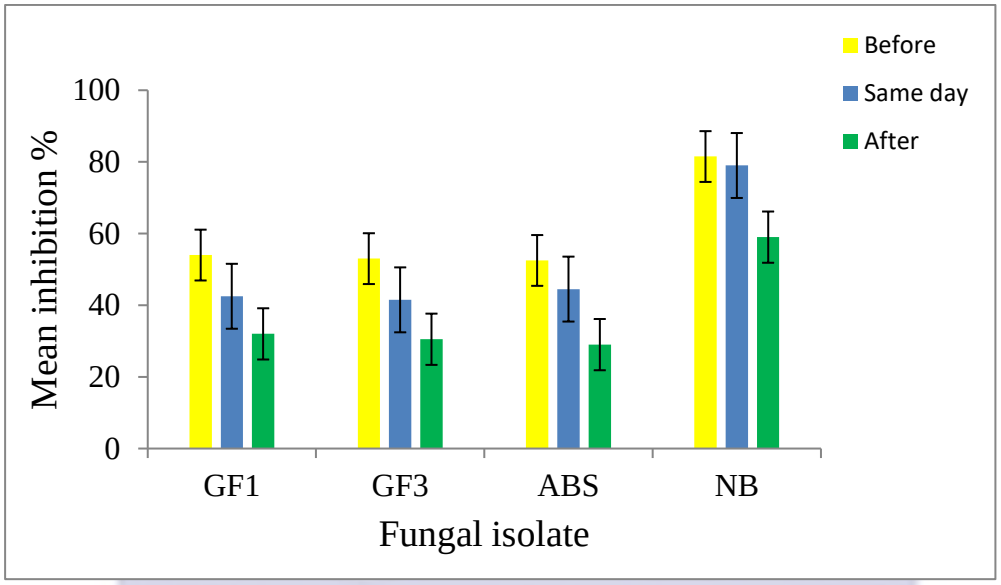


Figure 14: Mean inhibition percentages of isolates on the application of *Trichoderma* at different days.

Colony interaction of *Trichoderma* treatment and Isolates (GF1, GF3, ABS and NB) in Dual Culture Test at different application days

Figure 15 shows the interaction between *Trichoderma* treatment and isolates when *Trichoderma* was placed 24 hrs before isolates. *Trichoderma* ceased the growth of all four isolates; GF1 (A), GF3 (B), ABS (C) and NB (D) such that it occupied 1/3 portion of the Petri dish. It occupied the space the isolates could otherwise have occupied.

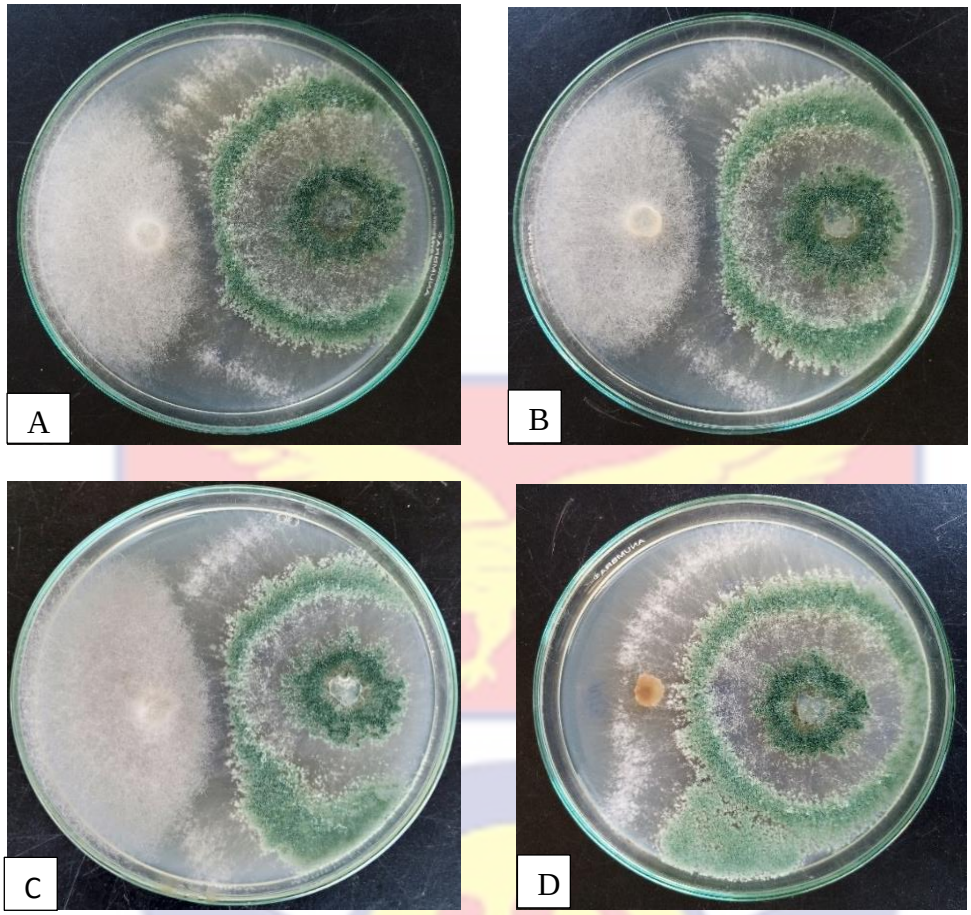
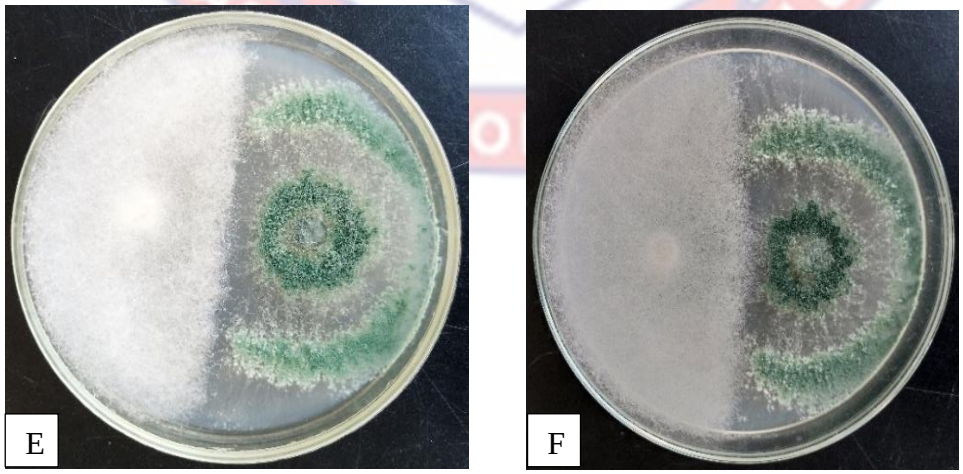


Figure 15: *Trichoderma* treatment placed 24 hrs before GF1 (A), GF3 (B), ABS (C) and NB (D).

In Figure 16, *Trichoderma* was placed same day as GF1 (E), GF3 (F), ABS (G) and NB (H). With the exception of NB (H), the rest of the isolates and the *Trichoderma* occupied half each of the Petri dish.



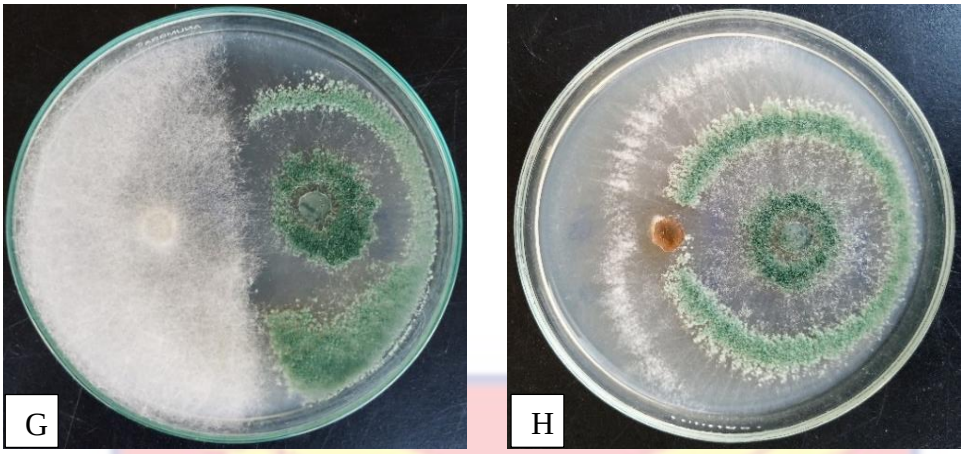


Figure 16: *Trichoderma* treatment placed same day as GF1 (E), GF3 (F), ABS (G) and NB (H)

Figure 17 shows the interaction between the *Trichoderma* treatment and isolates when *Trichoderma* was placed 24 hrs after the isolates. GF1 (I), GF3 (J) and ABS (K) grew and occupied 2/3 portion of the Petri dish but could not grow any longer because it was stopped by the *Trichoderma* which occupied 1/3 of the Petri dish. For the NB (L) the *Trichoderma* grew over it.

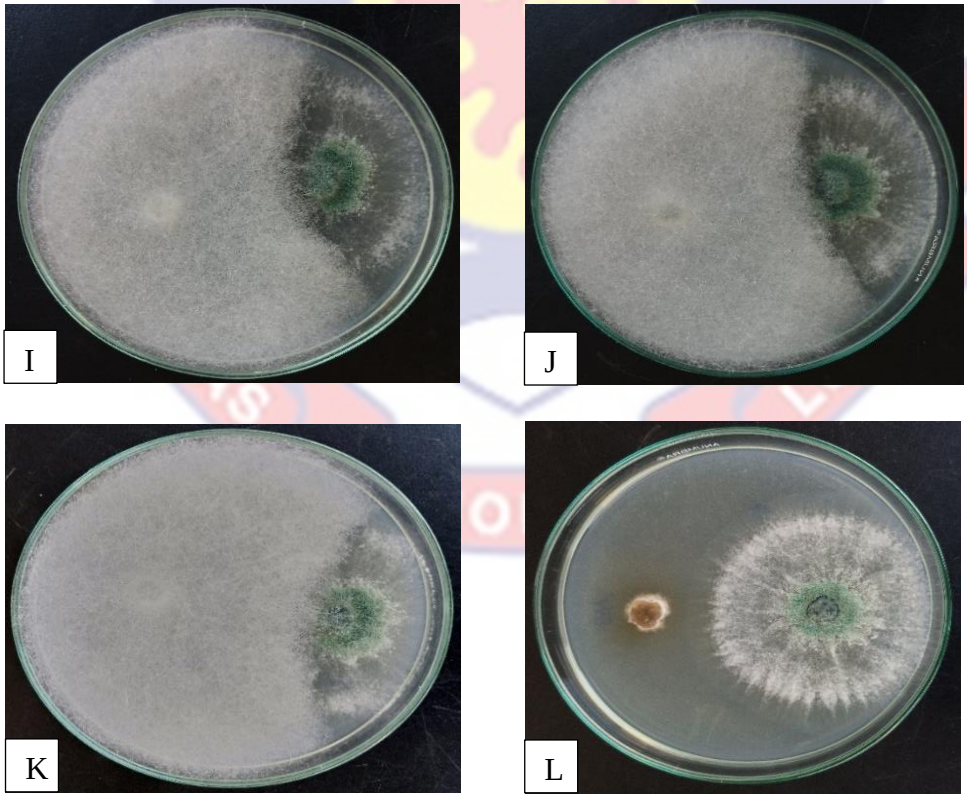


Figure 17: *Trichoderma* treatment was placed 24 hrs after GF1 (I), GF3 (J), ABS (K) and NB (L).

CHAPTER FIVE

DISCUSSION

5.1 Farmers Awareness, Knowledge and Management of the Disease

The study revealed that majority of the respondents had encountered PHRD on their farms. The disease was well known to farmers in mostly 2 out of the 3 districts in the Central Region as most of them were able to give the description of the disease. Farmers awareness of the disease could be attributed to their experience in pineapple production as most of them have been producing pineapple for more than (5) five years. A study by Donkoh & Agboka (1997) on constraints of pineapple production in Ghana, made mention of the disease occurring on a limited scale, this shows the disease has been in the country for some time. However, the few farmers who were not aware of the disease (Table 6) could in part be attributed to the fact that they are cultivating pineapples for the first time. This agrees with Nagarajuet *et al*. (2002), who reported that apart from formal education, experience in farming can also be a source of information.

Most of the farmers observed the symptoms as rot of the basal heart leaves and foul odour (Rohrbach & Johnson, 2003). The foul odour associated with the rot is a characteristic symptom of the disease (Joy & Sindhu, 2012), in addition to the easy removal of basal leaves (Rohrbach & Johnson, 2003). The rest of the farmers observed either the initial or all of the symptoms.

Even though farmers were aware of the disease, most of them did not know the exact cause but could only assume causes. Some attributed it to climatic factors such as rainfall/moisture and humidity. This is confirmed by Hine *et al*. (1964), Bartholomew *et al*. (2003), Rohrbach & Johnson (2003), and Sarpong

(2016), where the incidence and severity of the disease was linked to moist/wet environment. The rest of the respondents attributed it to unfavourable soil conditions like poor drainage and poor sanitation. According to Green & Nelson (2015), the disease spread is limited to poor drainage. Bakengi (2014) confirms this in Pest Management Guide: PHRD, in which she suggested that pineapple plant should be cultivated on a well-drained soil and residue/debris be removed from field as a preventive measure in reducing disease incidence. PHRD is known to be caused by a pathogen instead of insect pest (Rohrbach & Johnson, 2003) as speculated by some farmers.

Most of the farmers indicated that the disease occurs at the vegetative stage, followed by fruiting stage with flowering stage being the least. This agrees with work by Shen *et al*. (2012) and Ratti *et al*. (2018) who suggested that younger plants are more susceptible though matured plant can also be infected as well as fruit-bearing plants. Thus, confirming that the disease can attack all stages as the farmers indicated but at varying degrees.

From this study it can be seen that most farmers observe the occurrence of the disease only in the wet season, some experience it on their farms in the dry season and the rest indicated both seasons. According to Rohrbach & Johnson (2003) and Green & Nelson (2015), PHRD is most prevalent in a wet environment even though it can also occur during warm seasons (Rohrbach & Schenck, 1985; Rodriguez *et al*., 2012; Ratti *et al*., 2018). Dry season disease occurrence could possibly be due to poor drainage on the field. Poor drainage has been reported to contribute to poor growth due to stagnation of water and cause severe losses (Joy & Sindhu, 2012).

Farmers, who reported the presence of the disease in their farms, experienced some form of losses. This has been confirmed by Rohrbach & Johnson (2003) and Ratti *et al*. (2018) who reported that plant mortality can be up to 100% depending on edaphic factors and humidity.

Very few farmers controlled the PHRD by chemical application. This is because chemical control is one of the easiest disease control measures farmers adopt. Fungicides are used extensively in modern agriculture to control disease caused by fungi and 17% - 20% of applied pesticides are fungicides (Mitchell *et al*., 2002). Most farmers applied the fungicides twice during the cropping season. This confirms work by Rohrbach & Johnson (2003) who reported that for excellent control of PHRD fungicide application should be done twice; first as a pre-plant dip and then as foliar application at 3-6 months old.

Some farmers controlled the disease via cultural method, including the removal of infected plants. According to Bakengi (2014) and Satyagopal et al. (2014) one of the effective cultural methods of controlling PHRD is collection and destroying of plant infected with disease. However, only a few farmers used this method of cultural control.

Most farmers did not control the disease and this could be attributed the fact that conditions did not stay favourable for a long time and as such disease spread was curbed. This can be confirmed by Rohrbach, (1980) and Rohrbach & Johnson (2003) who through personal observation noticed that disease development will occur within 1-2 weeks if environmental conditions are favourable.

The data indicated that the advice on the choice of chemical used and its alternative was mostly by extension officers and sourced from Agro-input shops. This is confirmed by Kwakye *et al.* (2018) who reported that farmers get most information and update on pesticide usage, mode of application and safety, through extension officers.

From the data most farmers indicated that they alternate the fungicide when it does not work. In Kwakye *et al*. (2018) farmers are willing to change pesticides if the other alternatives are effective, affordable and available. Most of them go for fungicides that will protect and kill the pathogen. This can be confirmed by Mueller & Robertson (2008) who reported that curative fungicides are not effective against more advance latent infections but protective fungicides can be used when disease incidence is at low levels and also to prevent new infections from occurring.

The effectiveness of the control measure by farmers can be attributed to the fact that fungicides are highly effective on fungal pathogen when all necessary protocols are adhered to (Waller, 2001). The ineffectiveness of the fungicide on some farms could be attributed to late application (Mueller & Robertson, 2008) and resistance development of the pathogen (Singh & Dwivedi, 1987; Erwin & Ribeiro, 1996; Waller, 2001).

All the other diseases that farmers reported to have encountered on their farms are also diseases of pineapple (Rohrbach & Johnson, 2003).

5.2 Incidence and Severity of PHRD in the Selected Communities

PHRD was observed in 7 out of the 12 communities selected for the prevalence study. From the 7 communities mean incidences and severities of disease ranged from 0.3 – 3.4 % and 0.1 – 1.50 % in both surveys,

respectively. Figures do not suggest high prevalence but according to Bakengi (2014) when symptoms appear on 5 – 7 plants per quarter of an acre which contains 5,500 plants, the action threshold is reached and pathogen is already in the soil and can cause significant damages. This suggests that in spite of the low values, the action threshold is reached and farmers need to apply control measures, which majority of the farmers did not adhere to.

Prevalence of the disease may be due to soil factors such as moisture content and pH (Green & Nelson, 2015) and environmental factors such as Temperature. According to Green & Nelson (2015), PHRD is associated with wet soils and high pH, but, in contrast to this study, soil analysis conducted and temperatures taken, showed low pH values between 4.07 – 6.68, optimum moisture content of 18% and temperature range of 22 ºC – 35 ºC. This agrees with Hine *et al*. (1964), Rohrbach & Johnson (2003), Shen *et al*. (2012) and Ratti *et al*. (2018) who reported that, PHRD can occur in areas of less moisture content and temperature range of 25 ºC – 36 ºC.

Significant differences in the mean incidences and severity of PHRD disease recorded in the study can be attributed to land preparation, source of planting materials, and cropping system. From the household survey in this study, it was realised that only few farmers ploughed their land. Little or no tilling can lead to soil compaction thereby increasing water retention which increases infection and multiplication rate of the pathogen (Bartholomew *et al*., 2003; Green & Nelson, 2015). Also, majority of the farmers use planting materials from their own farms. The primary inoculum of PHRD originates from the soil and infected plant material (Joy & Sindhu, 2012; Green & Nelson, 2015). Farmers do not practice crop rotation and this can lead to pathogen build up;

the causal agent of PHRD is soil borne and can persist in the soil for several years (Erwin & Ribeiro, 1996). Also, according to Australian Government (2008) when pineapple is grown as a monocrop it becomes susceptible to many fungal diseases and as such recommends crop rotation as a means of controlling pathogen build up.

The low rate of incidence in some communities (Atabadze, Asuansi and Nanaben) can be attributed to the age of plant and farm sanitation. Even though plants of all ages are susceptible to PHRD, 1 - 3 months old plants are most susceptible (Rohrbach & Johnson, 2003). The ages of the plant in some of the farms were between 6 – 11 months old whereas farms with high incidence rate ranged from 1 - 4 months old. Some of the farms had previous debris on the farm which they use as mulch. The pathogen that causes PHRD is also known to persist in plant debris for many years and as such can cause infection rate to increase when conditions become favourable (Bartholomew *et al*., 2003).

High disease prevalence in second survey as compared to first survey can be attributed to favourable climatic conditions such as rainfall and temperature. Due to recent changes in the climate conditions in Ghana, there was very little rainfall during the time of taking the first survey which was in the month of July. But during the time of conducting the second survey which was in November, there was a good amount of rainfall in the preceding months and the month the survey was conducted. The continuous rainfall resulted in very wet soils, and temperature range during that time was between 24 ºC – 35 ºC. These conditions created a conducive environment for disease infection to increase as indicated by Hine *et al*. (1964) and Rohrbach & Johnson (2003).

Five communities (Essaman, Asebu, Amosima, Ayeldu and Atwiaa) recorded no infection and 3 out of the 5 communities were found in the Abura-Asebu-Kwamankese municipality. This can be attributed to the fact that most of the farmers were cultivating pineapple for the first time and as such had virgin lands and planting materials sourced from the open market which usually have clean planting materials. The pathogen of PHRD is soil borne; therefore, their absence meant no infection.

5.3 Soil fertility and disease incidence and severity

Nutrients are vital for growth and development of plant and microorganism (Agrios, 2005) and also an important factor in the ability of plant to tolerate or resist pathogen attack (Graham & Webb, 1991). There are contradictory reports on the effect of nutrients on plant disease i.e. the presence of some nutrients in the soil can either increase or decrease disease incidence or severity and vice versa (Dordas, 2008). An example is nitrogen (N), for obligate parasites a high N increases severity in infection, however in facultative parasites high N supply decreases the severity of disease infection (Dordas, 2008).

The soil analysis conducted however, showed a very weak relationship between all the soil factors and disease incidence and severity. It can therefore be said that nutrients in the soil had little or no influence on PHRD disease incidence and severity in the study location.

Even though there have been reports on nitrogen and phosphorus enhancing disease incidence and severity (Rohrbach & Johnson, 2003; Sarpong, 2016) and high soil exchangeable calcium and magnesium cation concentration positively correlating with disease severity (NSCU, 2010), soil data collected

stated otherwise which according to Dordas (2008) indicated that there are contradictory reports on disease incidence by soil nutrients.

According to Rohrbach & Johnson (2003), PHRD is associated with heavy wet soils but sometimes can be associated with less moist soils when caused by *P. nicotianae.* This was confirmed by the incidence of the disease in both wet and dry areas.

It is known that the pathogen that causes PHRD, which is a soil-borne, thrives in low acidity soils or high pH soils (Rohrbach & Johnson, 2003). From the soil data, quite a number of the communities studied had soil pH of 6 which is in confirmation to the report of Rohrbach & Johnson (2003).

5.4 Morphology and Molecular characterisation of the *Phytophthora* isolates

The whitish colour, shape, undulating edge, translucent and cottony texture of the mycelium of all the isolate were the same. These characteristics are similar to that of *Phytophthora* as indicated by Microbiology module (2012).

The mycelium of all the isolates were non- septate with ABS and NB having a smooth and hyaline surface and GF1 and GF3 having a coralloid surface, suggesting a possibility of isolates being *Phytophthora* (Gallegly & Hong, 2008) but of different species. The growth rate of the mycelium of GF1, GF3 and ABS were faster compared to NB and this could also be attributed to differences in species of *Phytophthora*.

The sporangia shape of GF1 and GF3 are same and that of ABS and NB are also same but all are similar to *Phytophthora* species (Gallegly & Hong, 2008) but suggest possible differences at the species level. These differences were

confirmed by the molecular test when DNAS of isolates were subjected to ITS sequence analysis

Morphological characteristics alone can lead to misidentification of *Phytophthora* species due to overlapping features and intra-specific variability (Martin *et al*., 2012), therefore analysis of sequence of gene of rDNA gives near accurate identification of pathogen and helps to separate *Phytophthora* isolates into species. The analysis of ITS sequences of the isolates confirmed the identification of *Phytophthora* spp. based on the morphological features of mycelia, colony and sporangia characteristics. The study showed that the causal agent of PHRD in the selected area of study was P*hytophthora cinnamomi* and *P. nicotianae,* though *P. palmivora* is also a known causative agent elsewhere (Ratti *et al.,* 2018) but was not found in the study area. Isolates GF3, GF1 and ABS were found in the Ekumfi district whereas NB was isolated from KEEA. The differences in the occurrence of the species could be attributed to climate and soil factors. As indicated by Rohrbach & Johnson (2003) PHRD caused by *Phytophthora cinnamomi* is associated with wet or cold environmental conditions, high elevated soils and high pH soils whereas *Phytophthora nicotianae* is less dependent on moisture (Hine *et al*., 1964; Rohrbach & Johnson, 2003). Ekumfi district experiences more rains than KEEA, hence has a more suitable condition for the prevalence of *P. cinnamomi* prevalence.

5.5 Evaluation of Plant Extracts and Bio-control organism against isolates

The fungicide which was used as a control treatment has metalaxyl as the active ingredients and it is mostly used by some farmers in the study area. When the botanicals were compared to the fungicide, two of them (Prekɛsɛ

and Neem) performed better than the fungicide with Prekɛsɛ giving the highest inhibition followed by the Neem.

Prekɛsɛ extract gave the highest mean inhibition index for GF1 and GF3 out of the four isolates. This could be attributed to the phytochemicals and essential oils found in Prekesɛ which are known to have antimicrobial, antibacterial and antifungal effects on pathogens (Burt, 2004). Ilondu (2011) also reported that some essential oils and phytochemicals of some plant extracts have shown remarkable antifungal effect exhibited by the inhibition of mycelia growth. In addition, antimicrobial activity of plant extracts may not be due to the action of a single active compound but rather the concert or synergistic effect of several active compounds in minor proportions in plants (Davicino *et al.*, 2007).

Neem extracts inhibited GF1 and GF3 but at a low mean inhibition. This could be attributed to concentration levels of the extract. Singh *et al*. (2019) showed that at higher concentration levels the extract of *Parthenium* and *Calotropis* plant used on certain fungal strains in a study gave a better inhibition result than a low concentration.

The positive control treatment which is a fungicide with metalaxyl as an active ingredient gave a very low mean inhibition on GF1 and GF3 and no inhibition on ABS and NB. This could be attributed to resistance build-up in the pathogen against the fungicide and mode of action of the fungicide. Even though fungicides with metalaxyl as active ingredients are effective on all *Phytophthora* spp. *in vitro* (Drenth & Guest, 2004), resistance has developed in many *Phytophthora* spp. particularly *P. infestans* (Cohen & Coffey, 1986). Also, Coffey & Bower (1984), reported that low concentrations of metalaxyl

inhibited the germination of chlamydospore of *P. cinnamomi* and *P. parasitica*. According to FERA (2018) most fungicides used against *Phytophthora* tend to check its development rather than kill it, and once its activity has declined the progression of the disease may continue.

Mahogany extract treatment showed no inhibition on all four isolates. This could be attributed to the chemical characteristics of solvent and method of extraction (Pineloo *et al*., 2004), phenological age of plant material (Felix, 1982), concentration levels of extract and the synergistic effect of active compounds (Dellavalle *et al*., 2010). Different methods of extraction release different phytochemicals and essential oils. It is possible that the ethanol extraction of Mahogany could not release the necessary phytochemicals which include flavanoids, glycosides and resin that have antimicrobial and bacterial properties (Kubmarawa *et al*., 2008). Also, concentration levels may have been too low to exhibit any antifungal activity to cause an inhibition in pathogen growth as reported by Banso *et al*. (1999) who observed that higher concentrations of antimicrobial substances showed more growth inhibition.

The failure of the Prekesε and Neem extract treatments on Isolates ABS and NB could be attributed to the concentration levels of the extracts. According to Dellavalle *et al*. (2010) concentration of crude extract and their respective dilutions have an effect on the growth inhibition of pathogen and that antifungal activities of extract dilutions are weaker compared to crude extract. Also, Banso *et al*. (1999), reported that higher concentrations of antimicrobial substance showed more growth inhibition on fungal pathogens.

Trichoderma metabolites inhibited the mycelia growth of all four isolates. This agrees with work done by Harman (2006), who reported that

Trichoderma spp. has the ability to control, antagonize and rapidly colonize especially soil-borne pathogens such as *Phytophthora* spp. This is further confirmed by an experiment conducted by Sriram *et al*. (2010), where *Trichoderma harzianum* inhibited the growth of *P. capsici* which causes damping off disease in chilli pepper by 50% *in vitro*.

5.6 Dual Culture Test of *Trichoderma asperellum* and isolates

The type of interaction depicted in the dual culture test of *Trichoderma* and isolates GF1, GF3, ABS and NB at different application days were all Grade 2. This means that, there was a mutual intermingling of *Trichoderma* treatment and isolates such that the *Trichoderma* inhibited the growth of the isolates (Skidmore & Dickinson, 1976).

In all three application days, applying the *Trichoderma* treatment 24 hrs before the isolates (Fig. 14) showed the *Trichoderma* occupy a larger area on the Petri dish than all the isolates. When the *Trichoderma* treatment and isolates were placed same day (Fig. 15), *Trichoderma* slightly outgrew GF1, GF3 and ABS and largely crowded NB. When the *Trichoderma* was placed 24 hrs after the isolates (Fig. 16), the *Trichoderma* treatment occupied a small area of the Petri dish of GF1, GF3 and ABS whilst it totally outgrew NB.

From this study and observation made, the *Trichoderma* operates on the mechanism of Competition. This is where the *Trichoderma* grows faster and uses up the food sources more efficiently than the isolates thereby overcrowding and out competing it (Waghunde *et al*., 2016). It is therefore best that in using *Trichoderma* as a treatment, it is applied to the planting material before planting. This helps achieve best result.

CHAPTER SIX

SUMMARY CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The study was conducted in three (3) pineapple growing districts in the Central region. It sought to investigate PHRD prevalence in pineapple through field and household survey; pathogen identification and characterisation and evaluation of botanical and a biological control organism against the identified pathogen.

The field survey revealed prevalence of the disease in all three (3) districts studied. Seven (7) out of twelve (12) communities studied in the 3 districts recorded the disease. The highest incidence and severity were recorded at Ekumfi (1.06% - 1.45%; 0.42 - 0.58) followed by KEEA (0.97% - 1.70%, 0.42 - 0.74) and the least at AAK (0.2% - 0.15; 0.08) respectively.

The household survey of 120 farmers revealed that 83.3% of the farmers have observed the disease on their farms. Majority of the farmers had no idea what causes the disease, noting that it occurs mostly at the vegetative stage. Regardless, farmers recorded some losses; some were significant others were not. Majority of the farmers (56.6%) left the disease uncontrolled; some (33%) adopted the use of cultural method where they uproot diseased plants whereas a few (10.4%) used chemical means of control.

Diseased pineapple samples were collected and taken to the lab for pathogen isolation and identification. Four pathogens were isolated and labelled as, isolates ABS, NB, GF1 and GF3. These were identified morphologically using colony colour, shape, texture, growth pattern, growth rate and septation of mycelium; sporangia shape, presence of pedicel, papillation, and caducity.

DNAs of these isolates were extracted and sent to Inqaba Biotech in South Africa for further identification using primers ITS6 and ITS7.

The DNA sequence of all four isolates were blasted in the NCBI database. The percentage identity of the isolates to the *Phytophthora* spp. in the database ranged from 98% - 100%. Isolate ABS was closely related to the *Phytophthora nicotianae* in the NCBI database with a percent identity of 98.0 and an accession numbers KY930644.1 and KF147901.1. Isolate NB was closely related to the *Phytophthora nicotianae* in the NCBI database with a percent identity of 100 and accession numbers KY930644.1 and KF147901.1. Isolates GF1 and GF3 were closely related to the *Phytophthora cinnamomi* in the NCBI database with a percent identity of 100 and accession numbers MN539998.1 and MN539997.1. Isolates GF3, GF1 and ABS were from the Ekumfi district but different farms whereas NB was from KEEA.

Plant extracts from mahogany, prekɛsɛ, and neem seed; biological control organism (*Trichoderma sp.)*, and chemical control, (Fungikill metalaxyl fungicides as positive control) were evaluated against all four isolates using the Minimum Inhibitory Concentration (MIC) test. All treatments with the exception of mahogany extract had an inhibitory effect against isolates GF1 and GF3. The prekesɛ extract treatment gave the highest inhibition ratio (3.0), followed by *Trichoderma* sp. (2.17), then neem extract (1.48) and positive control (1.03) for isolate GF1. For isolate GF3 the prekesɛ extract treatment gave the highest inhibition ratio (3.06), followed by *Trichoderma* sp. (2.40), then positive control (1.02) and then neem extract (0.91). Isolates ABS and NB were only inhibited by *Trichoderma* sp. with ratio of 2.06 and 2.36 respectively

Because the *Trichoderma* sp. treatment was effective on all Isolates, a Dual Culture test was performed for confirmation at different application days. The test showed that, when *Trichoderma* sp. was placed 24 hrs (a day) before the isolates, it inhibited the mycelia growth of the pathogen better than when placed same day and 24 hrs (a day) after the Isolate.

6.2 CONCLUSION

From the study it can be concluded that

- Many of the farmers in the study area were aware of symptoms of PHRD but not the cause. They attributed the causes to unfavourable soil and climatic conditions.
- The management practices they employed were chemical and cultural control but majority do not control the disease.
- PHRD is prevalent at all the three districts studied though at a generally lower rate, with Ekumfi and KEEA districts having the highest incidence and severity, and AAK the least. Even though it was observed that the incidence of the disease was low, however the whole incidence is also above the action threshold.
- *P. cinnamomi* and *P. nicotianae* were identified as the species responsible for the disease in the study area.
- Amongst the plant extracts, Prekese was the most efficient in controlling all the isolates whereas mahogany was inefficient.
- *Trichoderma asperellum* had varying effects on the isolates and was best at controlling isolates when placed 24 hrs before the isolates.

6.3 RECOMMENDATIONS

- Intensive education on the causes and management on PHRD disease should be carried out in pineapple growing areas in Ghana.
- Field trials should be carried out to assess the effectiveness of the Prekesɛ extract and *Trichoderma* sp. treatment since laboratory conditions provide test pathogens conducive environment like same nutrition, temperature and humidity which is not same in a natural environment.
- A combination of treatments would have to be used in the management of *Phytophthora* heart rot disease due to variations in the responses of *Phytophthora* isolates to the various treatments.

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APPENDIX

SURVEY QUESTIONNAIRE

DEPARTMENT OF CROP SCIENCE, SCHOOL OF AGRICULTURE, UNIVERSITY OF CAPE COAST

This questionnaire is designed to elicit information from **Heart rot disease** on pineapple farms in some selected districts in the Central region of Ghana. This questionnaire is strictly for research purposes and any information given will be treated with all confidentiality.

District code…….

Name of community…….....

Serial number of respondent…….

Demographic Characteristics of Respondent

1. Sex a) Male b) Female
2. Age……
3. Occupation……
4. Level of education reached:
   a) None b) Non-formal c) Some basic education e) Basic education f) Some secondary education g) Secondary education h) MSLC i) Tertiary
5. How long have you been cultivating pineapple?
   a) < 1year b) 1-5 year c) above 5 years
6. 6. What is the size of your pineapple farm?
   a) < 1 acre b) 1-2 acres c) above 3 acres
7. 7. What land tenure system do you practice?
   a) Self owned b) Rent c) Share cropping d) Others……
8. What is your source of labour?
   a) Hired b) Family labour c) Nnoboa
9. 9. How do you finance your work?
   a) Self b) Bank c) Susu operator d) Family members e) Others……

Agronomic Practices

10. What method of land preparation do you employ?

a) Zero tillage b) Slash and burn c) Tractor plough d) Others… ….

11. Do you use fertilizers on your farm? a) Yes b) No

12. If yes, which type? a) Chemical fertilizer b) Organic-manure c) Both

13. Why this type of fertilizer?

a) Cheaper b) More efficient c) Easy to apply d) Other.....

14. Which types of chemical fertilizers do you use?

a) NPK b) Urea c) Ammonia d) Others, specify.....

15. What method of fertilizer application do you use?

a) Broadcasting b) Spraying c) Drilling d) Others specify……

16. Estimate quantity of chemical fertilizer usage per acre on your farm.....

17. How many times do you apply fertilizer before you harvest your pineapple? a) 0nce b) Twice c) Other, specify.....

18. When (what stage) do you apply your fertilizer after planting? ……

19. What farming practices do you use?

a) Monocropping b) Mixed cropping c) Others……

20. If mixed cropping, what kinds of crop do you intercrop?

a) Bean b) Groundnut c) Others…….

21. Do you practice crop rotation? a) Yes b) No

22. What variety do you cultivate?

a) MD2 b) Sugar loaf c) Smooth cayenne d) Others specify ….

23. What is the source of your planting material?

a) Open market b) Farmers own field c) Other farmers d) Agro input dealer e) MoFA

Farmers' awareness of the disease

24. Have you observed the **Heart rot disease** on your farm before?

a) Yes b) No

25. If yes, describe the disease?

……

26. What causes the Heart rot disease?

……

27. At what growth stage do you first encounter the disease?

a) Seedling b) flowering c) Fruiting

28. Which season does the disease occur?

a) Wet season b) Dry season b) Both seasons

29. At which season is the disease very severe?

a) Wet season b) Dry season b) Both seasons

30. What is the estimated yield loss after infection?

a) <10% b) 11-20% c) 21-30% c) 31-40% d) 41-50%

e) above 50%

Disease management

31. How do you control these diseases?

a) Chemical application b) Removal of infected plants c) Botanicals

d) No control e) Others…….....

32. If chemical, how often do you apply? Please specify……

33. If chemical is used, mention the kind of chemical (s)?

…….

34. What are the sources of your pesticides? Specify……..

35. Who advises you on the choice of chemical?

a) Agric extension officers b) Agro-input dealers c) Other farmers

d) Others……

36. Do you alternate the use of pesticides? a) Yes b) No

37. If you alternate, who advices you? Specify……..

38. Why do you alternate?

…….....

……

39. How long do you wait after spraying before harvesting? Please specify…….

40. Is the control measure effective? a) Yes b) No

41. What other diseases do you encounter on your pineapple farm?

……

……

42. What type of damage do they cause to the plants? Please describe

……

……

……

43. What is the estimated yield loss after infection?

a) <10% b) 11-20% c) 21-30% c) 31-40% d) 41-50%

e) above 50%