KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

MORPHOLOGICAL CHARACTERISATION AND *in vitro* MANAGEMENT OF THREAD BLIGHT PATHOGEN(S) OF COCOA (*Theobroma cacao* L.)

BY

EMMANUELLAH LEKETE (BSc. HONS.)

AUGUST, 2012

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THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE, MASTER OF PHILOSOPHY IN PLANT PATHOLOGY

AUGUST, 2012

DECLARATION

I declare that, except for references to other people's work which have been duly cited, this work is the result of my own original research and that this work has neither in whole nor in any part been presented for a degree elsewhere.

Emmanuellah Lekete …… ……..
(Student; PG: 2091049) Signature Date

Certified by:
Dr. Charles Kwoseh …… ……
(Supervisor) Signature Date

Certified by:
Dr. Enoch Adjei Osekre …… ……..
(Co-Supervisor) Signature Date

Certified by:
Dr. Charles Kwoseh …… ……
(Head of Department) Signature Date

DEDICATION

I dedicate this work to the Almighty God for his mercy, guidance and protection throughout my period of study**.**

I also dedicate this work to my beloved lecturer and a father, Dr. Enoch Adjei Osekre and to his wife, Mrs. Lawrenda Osekre and to my father, Rev. Godson Lawson whose love I cherish so much. To my dearest mother, Dr. Mrs. Rosailine Lawson and my Aunty Mrs. Agassi Matilda and all the Lawsons and Missebukpor families in Togo, for their love, care and moral support.

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ABSTRACT

Cocoa (*Theobroma cacao* L.) is the most important cash crop in Ghana, supporting the livelihood of a significant number of the Ghanaian populace, mostly farmers and rural dwellers. The increasing demand with concomitant increase in the price of cocoa on the world market is contributing significantly to raising the living standards of farmers. In recent years significant attention has been given to food security and the realization of the Millennium Development Goals (MDG). Decline in crop productivity as a result of increased rate of disease prevalence is one of the major constraints to agricultural productivity and realization of the MDGs in sub-Saharan Africa. A survey was conducted to assess the disease problem and to determine farmers' knowledge about the disease. The disease was present on every cocoa farm surveyed and the incidence was between 50-55%. The disease is believed to be associated with some food crops. Six different isolates of thread blight were recovered. Mycelial growth rate, colony character and sporulation pattern of the fungal isolates, grown on seven different culture media viz., Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), V-8 Juice Agar (V8), Oatmeal Agar (OMA), Plantain Agar (PA), Banana Agar (BA) and Green Cocoa Mucillage Agar (GCMA) were observed for seven days of incubation at 28±1°C. The cultural characteristics and sporulation pattern were greatly influenced by the type of medium used. Seventy different ages of cocoa seedlings and three alternative hosts were inoculated with each fungal isolate obtained from the farmers' fields and symptoms expression observed. Symptoms appeared whitish on the stem and the leaf of the test plant. The pathogenicity test showed that white thread blight is responsible for causing most of the thread blight disease in cocoa. Eight different fungicides recommended on cocoa were used *in vitro* on the fungal isolates. Bioassay revealed that all the fungicides used inhibit mycelial growth of the test fungi to various degree, that increased in concentration of fungicides will increase percentage inhibition in the growth of the organism. The results showed Metalm 72WP and Fungikill were most effective on the thread blight pathogen.

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CHAPTER ONE

1.0 INTRODUCTION

Cocoa (*Theobroma cacao* L.) is the most important cash crop and the backbone of the economy of Ghana. The cocoa industry supports the livelihood of a significant number of Ghanaians, especially cocoa farmers and rural dwellers. The increasing demand with concomitant increase in the price of cocoa on the world market is contributing significantly to the Ghanaian economy and to raising the living standards of the farmers.

Production of cocoa more than doubled from 395,000 tons in 2000 (19 %) to 740,000 tons in 2005, contributing 28% of agricultural growth in 2006(Bogetic*et al.*, 2007). According to Breisinger*et al*. (2008), cocoa will remain the most important agricultural export commodity, and will be accounting for about 60 % of agricultural exports by 2015 and thus contribute about 20%of foreign-exchange earnings to the economy of Ghana.

One factor limiting the attainment of food security in Africa is high crop loss through insect pests and diseases. Therefore, significant attention has been given to problems associated with pest and disease control to ensure food security. The cocoa industry is thus threatened by these biological factors.

There are different types of diseases that affect cocoa in Ghana and worldwide. The most important fungal disease that affects cocoa is the Black pod disease, resulting in the browning, blackening and rotting of cocoa pods and beans. The disease is caused by *Phytophthora* species. Other important fungal diseases are witches broom and

stem cankers. The most important viral disease of the crop is cocoa swollen shoot caused by a virus (Cocoa Swollen Virus).

Among the most important diseases of cocoa reported recently from all cocoa growing areas in Ghana is the thread blight of cocoa (Opoku *et al*., 2007). This disease is gradually emerging as a major threat to the cocoa industry. Thread blight is a disease of a number of tropical and semitropical woody plants including cocoa. It is a serious foliar disease facilitated by certain environmental conditions such as rainfall and high humidity and causes significant losses of crops (Mohanan and Kaveriappa, 1983).

Thread blights of cocoa are believed to be caused by species of *Pellicularia* and *Marasmius* and they form filamentous mycelia on the surface of twigs and leaves (Wood and Lass, 1985). High relative humidity and frequent rainfall favour the development of thread blight, whereas warmer areas that have low humidity and little rainfall seem to have relatively low levels of this disease (Wood and Lass, 1985). According to Leston (1970), the incidence of this disease in Ghana was as high as 6-48%, thus affecting the livelihood of a significant number of the Ghanaian populace that depends on cocoa production.

This disease causes network of mycelial threads spreading all over leaves, and causing die-back, death of leaves and branches and, if not controlled, leads to the death of the entire plant (Opoku *et al*., 2007). Thus, indirectly, the disease causes losses of pods because of defoliation that is associated with the infected leaves and

removal of affected plants. Therefore, measures need to be put in place to reduce the rate of spread of the disease.

Although reports indicate that the incidence of thread blight is escalating, little studies have been done to identify the actual causal agent(s) of the disease and its prevalence in order to formulate effective management strategies. The identification and characterization, and development of management strategies of thread blight pathogen(s) of cocoa will ensure improved crop yield and stability of Ghana's economy which will go a long way to meet the Millennium Development Goals (MDGs). With these perspectives, the present study aimed at identifying and describing colony growth characteristics and to observe the influence of eight different culture media on the mycelial growth, colony characters and sporulation patterns of thread blight fungi isolated from cocoa trees at different incubation periods in light, darkness and alternation of light and darkness conditions.

The main objective of this study was to determine cocoa farmers' perceptions about the disease problem and re-affirm the causal agent(s) of thread blights pathogen(s) of the crop and to identify appropriate management strategies for the disease.

The specific objectives were to:

(i) determine the prevalence and farmers' perception of the thread blight disease of cocoa

(ii) isolate, identify and characterise the causal agent(s) of the disease and

(iii)evaluate fungicides for the management of the disease pathogen(s).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Production and growth conditions of cocoa

Cocoa is very suitable for adoption as part of a mixed cropping system with food crops, especially those that provide shade to the young plant, and so can be adopted with minimal disruption to traditional systems (Ashby, 1991).

In West Africa, planting in thinned forest is the most common method and in Ghana, cocoa is grown in forest areas of Ashanti, Brong Ahafo, Central, Eastern, Western and Volta Regions where the rainfall is between 1100-3000mm (45-80 ins) per annum. In Volta Region, cocoa cultivation is limited to the river valleys, the rest is too dry. In Ashanti and Brong Ahafo too, some parts are unsuitable for planting cocoa because they are too dry (Ashby, 1991). Young cocoa plants usually need shades which are often provided by crops such as banana and plantain. Cocoa is susceptible to pests and diseases and for this reason there has been a tendency to open up new areas of forest to the industry, where there is enough fertile soil and no buildup of pests and diseases.
(http://www.antislavery.org/includes/documents/cm_docs/2008/c/cocoa_report_2004.pd).

Intercropping cocoa plantations with other plants may help reduce costs and increase cocoa yields in Ghana. Study revealed that, growing cocoa with other crops on the same plot boosts the productivity of cocoa farms, compared with growing cocoa alone (IITA, 2007).The research showed statistically that on average, cocoa farms that have other crops yield more cocoa per hectare than plots with only cocoa.

The reports cited additional benefits such as biodiversity, sustainability and increased farmer incomes. Report from FASDEP (2002), indicate that, multiple cropping is good for biodiversity, conservation and income generation for farmers.

2.2 Suitable Soils for Cocoa

The best soil for growing cocoa must have satisfactory organic matter content, reasonable nutrient reserves and pH not less than 4.5. Land with deep soil (about 1.2 m), loams and clay loams, well-drained soils and light loam, loam, heavy loam and light clay soils are mostly recommended for growing cocoa(MOFA, 2007).

2.3 Benefits derived from cocoa and its production

Cocoa is a key economic crop and a major source of export and fiscal earnings (Bulir 1998; McKay and Aryeetey, 2004) and accounts for 3.3% of GDP and its sub-sector employs 24% of labour force (FASDEP, 2002). It also accounts for 55% of the total household income among cocoa farmers in Ghana (IITA, 2002). Therefore, a significant growth of the economy depends, to some extent, on the growth of the cocoa sector.

Coulombe and Wodon (2007) reported that cocoa production has enabled national poverty rate in Ghana fall from 51.7% in 1991/1992 to 28.5% in 2005/2006. Both rural and urban poverty declined by about 10 to 10.8 % and 39.2%, respectively. Poverty rate among cocoa farmers which was 60.1 % in 1991/1992 declined significantly to 23.9%, in 2006(Breisinger *et al*., 2008).

Cocoa exports, the second most important export goods for Ghana, have more than doubled between 2002 and 2006 (Bogetic *et al.*, 2007). In 2005, cocoa beans (24.3%) and cocoa products (3.8%) accounted for about 28% of total exports (Breisinger *et al*., 2007).Its exports also constituted 28% of foreign exchange earnings, 57% of overall agricultural exports and 87 % if forestry and fishery are excluded (Breisinger *et al*., 2007). It has been estimated by Breisinger *et al.* (2007) that cocoa will remain the most important export agricultural commodity (60% of agricultural exports by 2015).

2.4 Pests and Diseases of cocoa in West Africa

The incidence of cocoa pests and diseases as a cause of low yields has been known and documented by many researchers over the years. Insect pests such as mirids, shield bugs, and diseases such as Black pod and Swollen shoot have received extensive research attention (Thorold, 1975; Wood and Lass 1985; Acquaah, 1999; Wilson, 1999).

The importance of insect involvement in the development of pathogen-induced plant disease is so great that it can hardly be exaggerated (Agrios, 2005). Though over 1500 different insects are known to feed on cocoa, only about 2 % are of economic importance (Wood and Lass, 1992).High level of yield loss to pests and diseases is a major problem in cocoa production.

Cocoa is known to be affected by a number of infectious diseases caused by fungi, bacteria and viruses and noninfectious or abiotic problems caused by poor soil

nutrients. Some of the diseases are *Phytophthora* black pod disease, *Phytophthora* canker, *Phytophthora* seedling blight, *Thielaviopsis* pod rot, Cocoa swollen shoot virus(CSSV)disease, Cherelle wilt, Charcoal pod rot and Collar crack disease (Adegbola, 1972). However, those of economic importance in Ghana are Black pod disease, Swollen shoot virus and Cherelle wilt (Opeke, 1987) and new emerging Thread blight disease. In economic terms, black pod disease is regarded as the most important disease of cocoa in Ghana and other West Africa countries including Nigeria and Cote d'ivoire (Abdulai, 1995).

2.5Cocoa disease control problems and yield loss

The most important problem faced by cocoa farmers is pest and disease control. Globally, yield losses due to diseases are estimated at about 30% (Anon., 2003). In West Africa, it ranges from 10 to 80%, with Ghana recording 30 to 50%and 50 to 80% in Cameroon (Abdulai, 1995).

The situation has been similar for the control of other diseases of cocoa. The majorities of farmers either do not spray their farms, or make only one or two applications instead of the recommended six to seven applications per year for black pod control, thus incurring heavy crop losses every year. In 2001/2, the Government of Ghana reintroduced state controlled and funded pesticide spraying scheme, in order to ensure better disease control in the cocoa industry and to protect its reputation for high quality. As a result of that, cocoa production hit an all time high of 3.6 millionmetric tons in 2005/6 (ICCO 2007). West African countries, including

Ghana, accounted for most of this growth, with more than 70 percent of total world cocoa production in 2006 (ICCO 2007).

2.6 Importance of disease diagnosis

According to McCartney *et al.* (2003), the ability to identify the causal organism(s) responsible for specific crop diseases is the cornerstone of plant pathology. Thus, without this ability, it will be difficult to characterize the disease or, be able to recommend effective and appropriate control measures. All aspects of plant disease epidemiology, from studies of disease spread to estimation of yield-loss/disease relationships, require the ability to identify the pathogen. Implementation of plant disease regulations through quarantine also requires the ability to diagnose plant disease, as in seed testing. Pathogen or disease diagnosis is, therefore, fundamental to virtually all aspects of Plant Pathology (McCartney *et al.*, 2003).

2.7 Thread blight as emerging important disease of cocoa

Thread blight disease is caused by a fungus, *Marasmius/Marasmieullus* spp. which occur during both the wet and dry season but more prevalent in wet season (Barros, 1981). It is mainly characterized by thick, threadlike strands of mycelium on the undersides of the leaves and branches. The disease is, therefore, worse in areas of heavy rainfall and poor management of farms (Wood and Lass, 1985). Major damage from the disease is the death of the leaves and branches of the affected trees. The disease is recognized by the appearance of creamy-white and black or brown network of mycelial threads spread over leaves, petioles and branches (Adedeji, 2006). Under certain circumstances, thread blight can affect large numbers of cacao trees (Wood and Lass, 1985).

Gregory (1978) suggested that, the pathogens of cocoa at that time of causing minor economic loss should be considered in detail, because they may in future become important in areas where they are present but unimportant. Thread blight is one of such diseases. These pathogens may even be transported to new areas, as new locations are developed for growing cocoa.

Wood and Lass (1985) reported that thread blights occur in most of cocoa growing countries. Two types of thread-blights; the white thread blight caused by *Marasmius scandens* and Horsehair blight caused by *M. equicrinus* have been identified (Wood and Lass, 1985), although taxonomy of these species has not been satisfactorily resolved. Opoku *et al*. (2007) also reported two kinds of thread blight disease of cocoa in Ghana, namely white thread-blight caused by *Marasmius scandens* as observed by Wood and Lass (1985) and black thread blight caused by *M. byssicola.* But they were considered to be of little economic importance and later found to be far more prevalent in certain areas than hitherto reported (Leston, 1970).

Another type of thread-blight of cocoa caused by *Koleroga noxia* (Muller) has been reported as important in some parts of Colombia (Barros, 1981). This fungus is also named as *Pellicularia koleroga* (Muller). All these three thread blight pathogens occur in Malaysia where thread-blight is quite common, especially after branch damage from cocoa or coconut fronds from the shade trees (Turner, 1968).

Dade (1927) reported that the damage caused by thread blight of cocoa in Ghana was probably economically unimportant. The incidence and effect of thread blight of cocoa on yield have been little studied, but in some years, especially 1981/1982, its

incidence became conspicuous and caused much concern when it caused massive defoliation of trees, resulting insignificant crop losses. Invariably, the trees outgrow the infection after a period but then succumb to it again (Asare-Nyarko, 1997).

2.8. Thread blight of apple

Thread blight of apple is a fungal disease caused by *Corticium stevensii* Burt. (Kornerup and Sutton, 1978). Primarily, the disease is a problem in poorly managed orchards in the southeastern United States. In well-managed orchards, the disease usually does not occur until after harvest, when growers have discontinued their fungicide spray programmes.

2.9Thread blight Tea [Camellia sinensis (L.) O. Kuntze]

Thread blight disease of tea is caused by *Marasmius pulcher* (Berk & Br.). The disease attacks both young and old leaves and makes it a limiting factor to tea production in the low land areas of South Western Nigeria (Adedeji, 2006).Adedeji (2006) observed that symptoms were quite different from the previously reported blights, in that, it has network of threads and infects through the lower surface of the leaf blade. Also, fungal hyphae infecting through the stomata pores were found only on the abaxial surfaces of the leaves.

2.10 Signs and symptoms of thread blight of cocoa

The mycelial strands of the causal organism ramify the leaf blade of the affected cocoa leaves, forming network of threads (Opoku *et al*., 2007). Thus, the creamy-white or brown mycelium can be clearly seen as it runs along the affected twigs and

leaves. The leaves are killed, turn dark brown in colour and remain suspended from the twigs by thread of mycelium. Severe infection can result in broken canopies (Opoku *et al*. 2007).Wood and Lass (1985) also reported that white thread-blight kills leaves and a network of mycelial thread spreads over leaves, petioles and remain hanging from the branches by strands of mycelium for a long time.

Horsehair blight, on the other hand, forms a tangle of black fungal threads through the canopy, but it is believed not to kill foliage. Thus, though leaves are not killed, after natural dehiscence, they are held to the tree by the fungal threads and thus tend to smother new growth (Dade, 1927). The symptoms of *Koleroga* thread-blight are similar to white thread-blight except that the fungal threads are brown (Wood and Lass, 1985).Thread blight was found to be far more prevalent in certain areas than earlier reported with an incidence varying between 6 and 48%. (Leston, 1970; Opoku *et al.,* 2007).

2.11Induction of growth and reproduction of the thread blight pathogens of cocoa

Several workers have recognized the importance of reproductive structures for inocula production and identification of fungi. The fungal systematics is still based mainly on morphological criteria as observable characteristics. Hence, fungi are recognized and identified basically by their phenotypes (Zain *et al*., 2009). Moreover, studies have been conducted on the effects of various media components along with important physiological parameters that lead to maximum sporulation (Saxena *et al.,* 2001; Kim *et al*., 2005; Saha *et al*., 2008).

Fungi grow on different habitats in nature and are cosmopolitan in distribution, requiring several specific elements for growth and reproduction (Saha *et al*., 2008). In the laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopic examination and biochemical and physiological characterization. Culture media play important roles for the optimum mycelial growth of different fungal species. In case of Thread blight species, different types of media are used for isolation, identification and preservation. Therefore, a range of nutritionally poor to rich agar media at different intervals of incubation period of thread blight pathogens of cocoa are important for their growth characteristics.

A wide range of media are used for isolation of different fungi that influence vegetative growth and colony morphology, pigmentation and sporulation, depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding mixture of atmospheric gas (Northolt and Bullerman, 1982; Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008). Preference of certain media over others as a result of identification of the best medium support can be made for fulfilling the growth requirements of thread blight pathogen(s) during its regular culture, sub-culture and long-term *in vitro* preservation. In addition, medium experiments can be helpful to describe phenotypic characters of pure isolates of thread blight pathogen(s) in defined growth conditions of medium.

Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell, 1996). In addition,

findings for one species are not readily extrapolated to others, particularly for threadlike fungi where significant morphological and physiological variations exist (Meletiadis *et al*., 2001).

2.12. Mushroom production

One of the major advances in agriculture is the development of cultivation techniques for mushroom production. Generally, mushrooms occur naturally in nature, especially in the circumstances where spores of fungi responsible for growing into mushroom are deposited on the surface of a suitable substrate (Aneja, 2001).The life cycle of the mushroom starts with the microscopic spores produced from the underside or gills of the fully opened mushroom. Spores can be obtained by cutting the stalk down on a clean paper for about 10 min (Sawyerr, 2003). Spores obtained from mushroom are usually microscopic and are carried by air currents which are deposited on a favourable substrate with enough nutrient, sufficient moisture and temperature to germinate (Sawyerr, 2003).

2.13.0Management of thread blight disease of cocoa

Various management strategies have been used to control thread blight disease. Thread blight, once established, is difficult to control with fungicides (Adedeji, 2006). Applying preventive fungicide sprays to trees prior to infection may help protect the plant from thread blight infections.
(http://www.caf.wvu.edu/kearneysville/disease_descriptions/threadblight.html)

Thread blight disease may be prevented through certain cultural practices. Pruning of the infected twigs and leaves have been effective since the disease spreads from the infected to healthy twigs through direct contact (Asare-Nyarko, 1997). According to Adedeji (2006), pruning should be done immediately the disease is noticed and the pruned parts should be burnt outside the farm to avoid re-infection of healthy trees. Pruning blighted twigs and branches promote better penetration of sunlight and air. It is also recommended to undertake regular monitoring to detect any further re-infestation of the healthy twigs

There are several biological control agents used on pests and diseases. These include the use of microbial organisms such as *Trichoderma harzanium* (Peri), botanicals, parasites and pheromones (Frais and Garcia, 1981). Bio-control method is self-sustaining and, once introduced, continues to persist in the environment. Biological control agents may be used wisely as a complement to chemical application and cultural practices. Thus, compatibility with the synthetic fungicide would be desirable, as it is often possible to schedule both in control programmes (Coffey, 1991).

CHAPTER THREE

3.0 MATERIALS AND METHODS

A survey and three laboratory experiments involving (i) identification and characterisation of causal agent(s), (ii) testing of pathogenicity of thread blight pathogen(s) and (iii) *In vitro* management of thread blight pathogens were conducted. The laboratory work was conducted at the Plant Pathology Laboratory of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3.1 Field survey: Assessment of thread blight disease incidence, severity and farmers' perception of the disease in selected major cocoa growing areas in Eastern and Ashanti regions of Ghana

The survey targeted selected cocoa farmers and their farms. A total of 270 cocoa farmers were selected randomly from the major cocoa growing areas in four and three districts in the Eastern and Ashanti regions, respectively. Each farmer was interviewed one-on-one, using questionnaires (Appendix 1). Farmers' perception on the disease occurrence, the disease severity and the management options, among others, were obtained. Cocoa on experimental fields as well as research stations were not included in the study.

3.2 Survey and sampling sites

3.2.0The survey was conducted in seven cocoa growing districts; three districts in Ashanti region and four districts in the Eastern region

The districts in the Ashanti region are Asante-Akim South, Asante-Akim North Municipality and Sekyere East District. These areas lie within the range of latitude 6°30N-7°32 N and longitude 0°15 W-1°20 W. They are within the moist semi-

deciduous forest zones of Ghana. They are characterized by two rainy seasons and dry season in a year. The rainfall patterns and temperature vary within the districts (http://ghanadistricts.gov.gh/).

The districts visited in the Eastern region are Atiwa, East Akim, Fateakwa and New Juabeng municipality. These areas lie within the wet semi-equatorial climatic zone, characterized by two maxima rainfall with mean annual rainfall between 150.0mm and 2000mm, followed by a prolonged dry season. Atmospheric temperature is fairly uniform throughout the year ranging from 26-30°C, while the lowest is 22°C. Relative humidity is generally high, from 65-80%.

Disease sampling was done during the major raining season (April-June, 2011) and minor raining season (September-November, 2011).

Personal observations were also made on the disease symptoms on the cocoa plants and other suspected hosts of the pathogen(s) in farmers' fields. Farm maintenance and various possible ways of the spread of the disease were also noted. Samples of diseased twigs and leaves were collected from three randomly selected farms at three different locations per each district. The diseased samples collected were put into paper envelopes, labeled and brought to the laboratory for pathological analysis.

Thread blight disease incidence was assessed from 60 cocoa trees (including thread blight infected and non-infected trees) per farm plot along two diagonals in each randomly selected farm.

Disease incidence per selected farm was computed as follow:

% Thread blight disease incidence on the field=

$$\frac{\text{Number of infected cocoa plants}}{\textit{Total number of selected cocoa plants}} \times 100$$

Disease severity was scored on a scale of 0-4following (CRIG Annual Report, 2007)

0: - no infection of thread blight

1:1- 25% of the total canopy of the plant infected with thread blight

2: 26- 50% of the total canopy of the diseased plant infected with thread blight

3: 51-75% of the total canopy covered with thread blight

4: 76-100% the entire canopy of the diseased plant covered with the thread blight on the twigs and thread blight infected dried hanging leaves.

3.3. Isolation and identification of thread blight pathogen(s) and other associated fungi

3.3.1Preparation of culture media

Eight different culture media categorized into poor; (Water agar, Green cocoa Mucilage), general purpose (Potato Dextrose agar) and rich media such as: Oat meal agar, V-eight Juice, Malt Extract agar, Unripe Plantain and Banana agar were employed for the isolation, and observation of colony growth character.

Media compositions (Appendix 3) were adopted from different sources (Tuite, 1969; Stevens, 1981; Johnston and Booth, 1983; Atlas, 1993) and modified to suit the purpose of this study. pH of the media varied between 5.5 and 6.0 (Stevens, 1981).

Preparation of PDA from scratch; Potato was peeled and chopped with a sterile knife. The peeled and chopped potatoes were boiled in 500 ml of distilled water in a pyrex beaker for 30 min. With the aid of a cheese cloth, the potato suspension was sieved into a beaker,30 g of glucose and 20 g of agar was added. The pH was checked and adjusted to 5.5(Stevens, 1981). The mixture was then amended with

500 mg of chloramphenicol to suppress bacteria growth on the media and topped with extra distilled water to 1 l. The resultant suspension was stirred thoroughly with magnetic stirrer, transferred into a conical flask, stoppered with non-absorbent cotton wool and autoclaved at 0.98 kg/cm² 121 °C for 20 min.

Oatmeal agar was prepared by using 75 g of white oats in 1 l distilled water and heated to boiling until the oats was completely dissolved. The pH was checked and15 g of agar was then added to it. The mixture was amended with 500 mg of chloramphenicol to suppress bacterial growth, and transferred into conical flask which was then stoppered with non-absorbent cotton wool and autoclaved as previously described.

Water agar was prepared by adding 20 g of agar to 1 l of distilled water. The resultant suspension was again stirred thoroughly with magnetic stirrer, and transferred into conical flask which was then stoppered with non-absorbent cotton wool and autoclaved. The composition and preparation of other media are captured in Appendix 3. The pH of the media was adjusted before being autoclaved. The media, after autoclaving, were poured into 9-cm-width sterile Petri dishes (10 to15ml per plate).

3.4 Fungal culturing, isolation and identification

The diseased samples collected were cut with scalpel blade into pieces and surface-sterilized with 10% sodium hypochlorite solution, rinsed three times in sterile distilled water and blotted on tissue paper (Gothier *et al*., 2001). The sterilized samples were aseptically transferred onto PDA plates and incubated at 28±1 °C with

photoperiod of 12h: 12h (Light: Darkness) for six days and sub-cultured till pure cultures of thread blight from different locations were obtained.

Mycelial, obtained from the various cultures, were mounted on slides and observed under a Laborlux S Leitz compound microscope. Spores from some of the thread blight fungi were also observed under compound microscope. Identification was done using identification keys by Hibbett *et al*. (1997) and Humber (2005). Macroscopic (pigmentation and key characters; radial diameter, growth morphology, texture and colony density) and microscopic (spore shape, fruiting body/sporulation and pigmentation) characters were used in the identification, grouping and coding.

3.5 Determination of the best culture medium for thread blight pathogens

Pure cultures of thread blight isolates obtained were multiplied by sub-culturing them on different media and incubating at 28±1 °C and photoperiod of 12h: 12h (Light: Darkness, (L:D)) for seven days (Tuite, 1969).Colony diameter, rate of growth, mycelial density and sporulation were taken at two-day intervals for one week to determine the best culture media support for the growth of thread blight fungi.

3.6.0Morphological characterisation of thread blight pathogen(s)

3.6.1Growth conditions and observations

Mycelial disc agar plug was taken from a 7-day-old(an actively growing) colony of pure culture of each of the seven isolates of thread blight fungi using cork borer of 5 mm diameter. The disc plug was placed at the centre of sterile Petri dishes

containing each of the eight different culture media, including Water agar. Petri plates were then incubated under different incubation conditions such as constant light, total darkness and alternation of light and darkness with time. The set-ups were placed in incubation room at 28±1 °C. Colony character of each isolate was recorded every two days for six subsequent days. In case of dark incubation, colony character was recorded after six days period. This was because agar plates, once examined under light, were discarded. Colony surface colouration was described using the description by Shrestha *et al*. (2006). There were three replications per each isolate.

3.7. Determination of mycelial growth and colony character of thread blight pathogen(s)

Radial growth bioassay and colony characterisation were used to determine the morphological features of thread blight isolates from different locations. Colony diameter and rate of growth of all the six isolates were also taken to confirm the best medium support. Radial growth was taken every two days for eight days and rate of growth (cm/day) was taken daily for seven days. Colony pigmentation was described based on the method by Sharma and Pandey (2010).

3.8 Determination of the density of the isolates on the culture medium

Two methods were used in determining mycelial density. The method of Shrestha *et a*l. (2006) was modified and used in the first method. This involved the culturing of 5 mm agar plug of mycelial colonies of thread blight pathogens on the surface of a cellophane membrane overlaying agar of the different culture media with constant volume of 20 ml per Petri dish.

Each culture medium was replicated three times and was incubated in the transfer chamber at a temperature of 28±1 °C for 10 days. Mycelium was scraped off and its biomass measured using sensitive electrical scale.

Mycelium density per each culture was computed as:

Density ρ (g/ml) = $\frac{\beta(g)}{v(ml)}$

Where ρ is the mycelial density in g/ml, β represents the mycelial biomass in g and v (ml) is the constant volume of each culture medium used.

In method two, Shrestha *et al*. (2006)'s method of 'comparative description' was modified and used. Thus; characteristics of thread blight related to mycelial density and texture have been defined in terms of comparative description.

Mycelial discs (5 mm) diameter were cut from the edge of each of the growing pure colonies of all the seven isolates of thread blight grown on PDA and were inoculated on each of the eight different agar media including PDA. Inoculated plates were incubated separately under both light and dark conditions at 28±1 °C. Colony growth characteristics such as mycelial density and mycelia texture of all the isolates were recorded every two days for 10 subsequent days for each of the test medium. Mycelial density was visually grouped into poor (0-0.1), moderate (0.2-0.4) and abundant (>0.4) depending upon the density of aerial mycelia while extremely poor mycelia density was denoted 0.0. Similarly mycelial texture was categorized into light, cottony sparse and dense.

3.9Induction of sporulation in thread blight pathogen(s)

Thread blight fungi are from class Basidiomycetes; they do not sporulate readily on artificial agar medium. Experiment was conducted to identify conditions under which sporulation could be induced in thread blight fungi of cocoa. Agar plug of 5 mm obtained from pure culture of each isolates was used to inoculate sterile Petri plate containing each of the eight different media.

The inoculated Petri plates were divided into three sets. One set of inoculated plates was placed under constant fluorescent light at 28±1°C temperature for 20 days. The second set was wrapped with aluminium foil and put into a thick wooden box to provide total darkness at temperature of 28±1°C for 20 days. The third set of inoculated plates was also wrapped with aluminium foil and placed in the thick wooden box for 12 h, then moved to the incubation room at 28±1°C, 12 h photoperiods for 20 days. Based on the results obtained from previous preliminary study, cultures were examined for sporulation after five days intervals. Cultures under dark conditions were observed after 20 days and discarded. Sporulation was assessed on glass slides by mounting a small portion of mycelia or the fungal fruiting body in sterile distilled water with blue stain and observed under microscope.

3.10Production of thread blight pathogen(s) by the mushroom production technique for identification

Thread blight isolates are believed to have come from class Basidiomycetes and are mostly fossil mushroom (Hibett *et al*., 1997). Therefore, mushroom production method was employed to help with the identification of the thread blight**.** Mushroom production using modified box method (Sawyerr, 1991) was used to grow the

pathogen(s) to aid morphological identification of the pathogen(s). The method involved the following:

(i) Spawn preparation: Pure cultures of each thread blight pathogen were grown on Oatmeal agar. Bits of each of the pure cultures were separately transferred to sterilised sorghum grain in a half-filled 1.5 l coca cola bottle. The entire set-ups were kept in an incubator at the temperature of 10°C for 14 days. This was to allow the fungal pathogen to grow well on the grain. The spawn was used as planting material for growing the mushroom.

(ii) Composting of bedding materials: Rice husk and sawdust of Odum (*Mellisa excelsa* L.) were composted and used as the bedding materials for growing the mushroom. Plantain and banana suckers were also used as hosts since they are suspected to be alternative hosts for the thread blight pathogens.

(iii) Bagging and sterilisation of composted materials: Composted sawdust and rice husk were packed into heat resistant polypropylene bags; bottle necked and covered with cotton and newsprint for proper sterilisation. The bags containing composted materials were autoclaved at 0.98kg/cm², 121 °C for 45 min. This was done to kill any micro-organism that might be present in the compost bag.

(iv) Inoculation/spawning: Spawns were ready after two weeks. The autoclaved compost bags were left to cool, and later transferred to an inoculation room. The bags were inoculated with the grown spawn. Some of the trunks of plantain and banana were also used as the mushroom substrate. For the purpose of this study, 30 grains of spawn were introduced into each bag through the bottle neck and the cotton wool and newsprint were replaced immediately after inoculation. In all, 24 bags containing two composts (Odum sawdust and rice husk) were inoculated.

(v) Incubation: The inoculated bags were then arranged on the shelves in the incubation room with spaces of 15 cm between them to allow for good aeration. The bags were kept in the incubation room at the temperature of 28±1 °C and relative humidity of 65 % for 10 days.

The bags containing fully grown mycelia of the test fungi were transferred to the crop house for watering and flushing. The bags were watered once in the wet season and two times in the morning and evening during the dry season.

The entire set-up were left for 23 days for the mushroom to sprout and harvest

The stalk and the cap of the fruiting body of the mushroom were measured and spore print were also analysed for the identification of the pathogen, using identification manuals such as mushroom picture descriptive books by Hibbett *et al*. (1997) and Binder and Hibbett (2002).

3.11Proof of pathogenecity of the thread blight pathogens on cocoa, banana, oil palm and plantain seedlings

Pathogenicity test based on Koch's postulates (Agrios, 2005) was done on one-year-old and six months-old established hybrid cocoa seedlings and one year old of three alternative host plants of thread blight pathogen(s). The alternative host crops used were plantain, banana and oil palm seedlings. Pathogenicity test was used to analyse the growth patterns and the spread of the disease pathogen. This was also used to determine the susceptibility of cocoa seedlings and the suspected alternative host crops to thread blight pathogen(s).This was done to determine the suitability of these crops as intercrops for cocoa farms in the face of the threat posed by thread blight pathogens.

3.12Sterilisation of soil and bagging of soil

Top soil was thoroughly sieved to remove stones, plastic materials and plant debris. The soil was sterilised, using a modified steam sterilizer and left to cool before use. The sterilised oil was dispensed into 100 plastic nursery bags. Each nursery bag contained 1500 ml of the soil. The soil in bags were then watered and left for three hours to allow for drainage.

3.13 Raising of cocoa seedlings and three suspected alternative host crops for pathogenicity test

Eighty hybrid cocoa seedlings and three each of banana, plantain and oil palm were raised in the nursery bags at one seedling per bag to avoid overcrowding and competition. The Oil palm seedlings, plantain and banana suckers obtained from famers' field were also raised in the plastic bags with a sucker per bag. The bagged seedlings were watered every two days. The established cocoa seedlings were grouped into eight groups. Each group consisted of five bags of one-year-old and five of six-months-old bags of cocoa seedlings, making each group of ten bags. Seven groups of the bagged seedlings were tested for their susceptibility to the fungus, using the stem and both hardened and fresh tender leaves.

3.14 Preparation of inocula of fungal isolates and inoculation for pathogenicity

For each of the fungal isolates,1 cm diameter of mycelial discs of one-week-old culture growth from the various districts on their respective best growth medium was used. Seventy cocoa seedlings and four of each of three other alternative host crops identified were inoculated, using sterilised absorbent cotton wool, Scissors, forceps, surgical blade and masking tape.

The surfaces of the stem and the leaves of individual plants were sterilized with 70 % ethanol prior to inoculation. The stem and the two surfaces (adaxial and abaxial) of four leaves per plant were inoculated with 1 cm disc of each of seven pathogens. Inoculation was done by attaching the disc of mycelia (inoculum) to the test plant part with moistened cotton wool and taped with masking tape to establish a good contact between the test plant part and the inoculums to ensure higher infection. Inoculated plants were moistened, covered with transparent polythene bag to provide good and ambient environment for infection. The set up was kept in the plant house at the Department of Crop and Soil Sciences, KNUST.

Ten of un-inoculated plants of each test crop served as control. After 10 days, samples were collected from the infected parts for re-isolation and examination in the laboratory. The re-isolated pathogens were then compared with the previously isolated ones.

3.15 Evaluation of fungicides bioassay *in vitro*

The bioassay technique of Sharvelle (1961) was modified and employed to evaluate the effects of eight copper-based fungicides on mycelial growth of the thread blight isolates *in vitro*. The selected fungicides were: Champion77%WP (Copper hydroxide), Ridomil Plus (copper 60 plus 10% metalaxyl, Mefenoxam + copper), Funguran-OH (Copper hydroxides), Kocide 2000 (53.8 DF), Metalm 72 WP, Nordox 75 WG (Cuprous oxide), Agro Comet and Fungikill. These fungicides are recommended by CRIG for the treatment of black pod disease of cocoa. All the

fungicides used were wettable powder. Radial growth of the test organisms was determined in various concentrations of the fungicides.

3.16Preparation of fungicides

Recommended weights of individual fungicides were used. A weight of 0.66g each of Champion, Funguran-OH and Kocide 2000, 0.33g of Metalm and Ridomil Plus, and 0.5 g of Agro comet, Fungikill and Nordox were each suspended in100ml of sterile distilled water to give stock solution (absolute concentration). Serial dilutions of 25, 50, 75 and 100 ppm concentrations of each fungicide were used. Thread blights grown on Oatmeal Agar (OMA) without any fungicides served as control. Each fungicide concentration was computed as follows:

$$\text{AD (g)} = \frac{RD(g)}{RV(ml)} \times AV(ml)$$

Where AD = Application dosage weight (g), RD = Recommended dosage weight (g), AV = Constant application volume (ml), RV = Recommended volume (ml)

Fungicides dilutions were calculated as: C1V1 = C2V2Where:

C1 is the stock solution, V1 is volume of stock solution required, C2 is the final solution, and V2 is the final volume.

3.17. Poisoned food technique

Ten millilitres of each of the fungicide concentrations was mixed with 250ml of Oat meal molten sterile agar medium.The mixture was poured into sterile Petri plates and allowed to solidify. Agar plates were later inoculated with 1 cm-diameter disc of myceliataken withflame-sterile cork borer from one-week-old culture of thread

blight isolate(s). Inoculated plates were incubated for six days at 28±1 °C in the incubation chamber.

3.18 Effects of fungicides on radial growth of thread blight pathogen(s)

The radial growth of colony of thread blight isolates was recorded when maximum growth was observed in controlplates. It was calculated using 'Vincent's formula' by Jamadar and Lingaraju (2011) shown below:

$$I = \frac{C - T}{C} \times 100\ \%$$

Where: I = Percentage inhibition

C = Radial growth in control plate

T = Radial growth in fungicide plates

Radial growth was measured to assess the toxicity of each fungicide concentration. Each set of treatments was replicated three times. The treatments were analysedin completely randomized design (CRD).

3.19 Effect of *Trichoderma* species on thread blight pathogens *in vitro*

Trichoderma sp. was among the fungi associated with thread blight pathogen during isolation. It occurred as a contaminant of the pure culture of thread blight pathogens and caused suppression in the growth of thread blight organism.

The dual plate culture technique was followed to determine the bio-control potential or efficacy of *Trichoderma* sp. on thread blight pathogen. For this, *Trichoderma* sp.

and each of the thread blight pathogen were inoculated equidistant on OMA medium aseptically and incubated at 28±1 °C in incubation chamber. Each treatment was replicated three times. Thread blight of the same culture grown equidistant in the separate plate served as control. After obtaining the maximum growth in the control, themeasurement on radial growth of thread blight pathogen(s) was taken and percentage inhibition was computed using 'Vincent's formula' as stated previously.

3.20 DATA ANALYSIS

Data collected were subjected to Analysis of variance using Genstat statistical package (5th ed. 2011). Least significant difference (lsd) at 5 % was used to separate means in fungal radial growths on various media.

CHAPTER FOUR

4.0 RESULTS

4.1 Field survey: Assessment of thread blight disease incidence, severity and farmers' perception of the disease in selected major cocoa growing areas in the Eastern and Ashanti regions of Ghana.

Majority (78 %) of the 270 farmers' interviewed in the seven districts in the Ashanti and Eastern regions were males. The ages of the farmers ranged between20 and 85 years; with those between the ages of 41-60 years being the majority (Fig. 4.1).

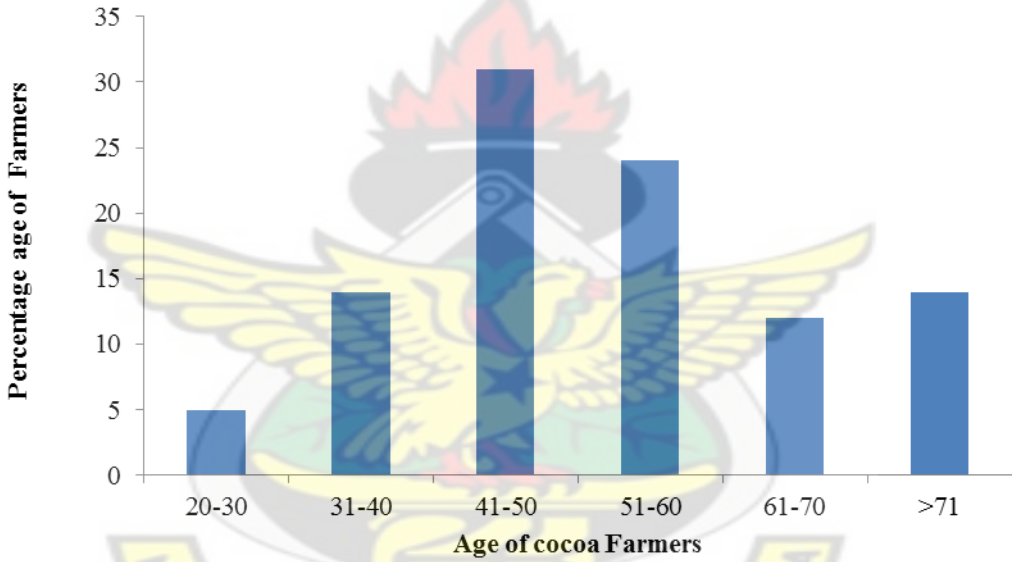


Figure4.1: Age distribution of the farmers interviewed in the seven cocoa growing districts

4.2. Identification of the alternative host crops of thread blight pathogen(s)

Surveys on selected farms in the selected districts detected thread blight symptoms on oil palm seedlings, plantain and banana plant; similar to those found on cocoa (Plate 4.1). It was observed that these plants were mostly used to intercrop with cocoa plants.

A: Thread blight on plantain sucker

B: thread blight on oil palm leaves

C:Thread blight on banana plant

D: Thread blight infection from banana to cocoa

Plate4.1. Alternative host plants infected with thread blight disease on the field

4.3. Cropping system practiced by cocoa farmers interviewed

About 37% of the farmers owned their farms while the rest were migrants who engaged in sharecropping. It was observed that thread blight disease was more pronounced in the farms that were managed by farmers engaged in sharecropping. All the farmers interviewed practice multicropping. About73% of them intercropped their cocoa with plantain, banana, oil palm and yam (*Dioscorea paren* L.) whilst 27% practiced cocoa monoculture (Table 4.1). All cocoa farms visited were located at rainforest corridors with high relative humidityexcept few areas in the Ashanti Region that were dry during the time of visit.

Table 4.1: Cropping system practiced by cocoa farmers in the seven selected cocoa growing districts in Eastern and Ashanti regions of Ghana

Cropping system	Percentage number of farmers/cropping system
Sole Cocoa	27.0
Multi cropping	73.0
Total	100.0

Table 4.2: Cocoa cultivars planted by cocoa farmers in the selected districts in Ashanti and Eastern regions of Ghana

Region/District	% Cultivars cultivated by farmers						
Districts	Hybrid	Tetteh-Quashie	Amazonia	Tetteh-Quashie + hybrid	Amazonia+hybrid	Tetteh-Quashie +Amazonia	All (3)
Asante Akim-South	32	11	16	5	21	5	10
Asante Akim-North	64	0	9	18	0	0	9
Fanteakwa	56	6	6	0	19	0	13
Atiwa	45	7	7	7	17	10	7
East Akim	31	15	3	5	20	18	8
Effiduase	56	0	33	0	11	0	0
New Juabeng	45	9	9	9	9	0	18

The field survey revealed that most farmers cultivated hybrid cocoa followed by Amazonia (Table 2). Few farmers cultivated the cocoa cultivar Tetteh-Quashie, one of the oldest cocoa breeds. Some farmers also indulged in cultivation of more than one cocoa cultivar in their farms. It was observed that the disease affected all the cocoa cultivars, both the young and the old cocoa plants alike. However, the disease was more pronounced in farms with older plants and a mixture of old and new cocoa plants than farms with young plants alone and mono cultivar. This was observed in some cocoa growing areas in the East Akim and Fanteakwa districts in Eastern region and Sekyere East district in Ashanti region.

4.5 Major problems faced by the cocoa farmers

Table 4.3: Major problems encountered by cocoa farmers on the fields surveyed in seven selected districts in Ashanti and Eastern regions of Ghana

Problems encountered	Percentage of farmers' response/problem
Diseases of cocoa	52.0
Insects pests of cocoa	23.0
Parasitic plants (mistletoe)	9.0
Excessive rainfall	7.0
Unavailability of cocoa seedlings	5.0
Weeds	3.0
Inadequate rainfall	1.0

The major problems encountered by cocoa farmers, apart from finance, included parasitic plants, unavailability of cocoa seedlings, excessive rainfall leading to flooding the farm land and making it inaccessible, weeds, inadequate rain in some parts of Ashanti region and diseases and pests. About 75% of the farmers ranked diseases and pests as the major constraint to cocoa production (Table 4.3).

4.6 Diseases identified in selected cocoa farms

Table 4.4: Types and occurrence of diseases identified in cocoa farms in the selected districts in Ashanti and Eastern regions of Ghana.

	Types and diseases occurrence in selected cocoa farms surveyed per district (%)				
Districts	Blackpod	CSSVD	Threadblight	Pink disease	Stemcankers
Asante-Akim-S.(Ashanti)	87.5	0.0	11.0	0.0	25.0
Asante-Akim-N(Ashanti)	72.5	0.0	65.5	0.0	45.5
Sekyere East (Ashanti)	67.5	0.0	57.5	0.0	30.0
Atiwa (Eastern)	66.0	13.3	93.0	69.7	56.3
East Akim (Eastern)	80.4	9.2	85.0	17.0	28.0
Fanteakwa (Eastern)	28.0	42.7	90.3	40.7	50.0
NewJuabeng (Eastern)	55.0	18.0	73.0	27.0	54.0

Five major diseases of cocoa were identified in cocoa farms surveyed in the seven selected districts. Among the diseases, Black pod disease,thread blight and stem canker occurred in all the seven districts visited (Table4. 4). Although the occurrence of thread blight disease in some of the farmsin some districts was more severe than that of black pod disease, farmers considered black pod disease as the mostimportant disease problem affecting cocoa production.

Pink disease was found in all the four districts surveyed in Eastern region, with incidence of 69.7% and 40.7% in Atiwa and Fanteakwa Districts, respectively (Table 4.4). All the 90cocoa farms visited in the three districts in Ashanti region recorded no incidence of pink disease. This studyalso showed stem canker occurring in all the visited cocoa farms in the seven selected districts, with the Atiwa District recording the highest percentage occurrence of 56.3 %, followed by New Juabeng Municipality (54.0 %) and Fanteakwa (50.0 %) (Table 4.4).

4.7 Insect pests identified in selected cocoa farms

Table4.5: Types and occurrence of pests identified in cocoa farms in the selected districts in Ashanti and Eastern regions of Ghana.

Regions and Districts	Types and % occurrence of insect pests identified in selected cocoa farms per district (%)				
	Mirids	Mealybug	Ants(black/red)	Stem borer	Parasitic plants
Asante-Akim-South (Ashanti)	66.0	7.0	2.5	50.0	39.5
Asante- Akim-North (Ashanti)	41.5	35.5	5.0	20.0	14.5
Sekyere East (Ashanti)	80.0	20.0	10.0	32.5	45.0
Atiwa (Eastern)	42.3	23.0	25.0	3.7	56.7
East Akim (Eastern)	52.6	14.2	30.0	15.6	71.4
Fanteakwa (Eastern)	31.3	8.3	15.0	75.3	21.0
New Juabeng (Eastern)	27.0	18.0	18.0	0.0	18.0

Four major insect pests and a parasitic plant were identified in cocoa farms surveyed in the seven selected districts. Among the pests ranked by the farmers interviewed, mirids (Akate) were the most important insect pests, affecting cocoa production across all the districts visited. Sekyere East District recorded the highest mirids infestation of 80.0 %, followed by Asante-Akim-South with 66.0 % in Ashanti region (Table 4.5). East Akim District recorded 52.6 % as highest mirids infestation and the New Juabeng recorded the least, (27.0 %) in Eastern region. For stem borer infestation, Fanteakwa District in Eastern region recorded the highest; followed by Asante-Akim-South in Ashanti region. East Akim District in Eastern region recorded the highest infestation of mistletoes (parasitic plant) with incidence of 71.5 % while Asante-Akim North in Ashanti region recorded the least of 14.5 % (Table 4.5).

It was reported by some of the cocoa farmers interviewed that black ants as well as some of the parasitic plants found on the thread blight infected cocoa tree served as agents for spreading thread blight pathogen(s).

4.8Types of thread blight pathogens based on colour of mycelia or Rhizomorphsin the selected cocoa growingdistricts

Four types of thread blight based on the colour of mycelia or rhizomorphswere found on cocoa in the farms visited. These were the white, brown, cream and black mycelia. White thread blight was of two types; the thread-like stranded mycelia type (Plate 2A) and the broad-stranded mycelia type (Plate 2B).The cream mycelia colour was also of two types; the cream thread-like stranded (Plate 2C) and the cream thread-like strands with fruiting bodies (Plate 2D). The brown thread blight (Plate 2E) and the black thread blight (Plate 2F) were the least prevalent of the four.White thread blight occurred most in all the selected cocoa farms visited.

Table 4.6. Occurrence of thread blight disease in cocoafarmers fields in the seven selected districts

Type of thread blight pathogens	(%) Occurrence in cocoa farms
White thread	70.0
Cream	20.0
Brown	7.0
Black	3.0

About 70.0 % of the farms visited in the seven selected districts were infected with white thread blight whilst the brown and black types were each present in less than 10 % of the farms (Table 4. 6).

i)White thread blights1 ii) white thread blight 2 iii) Black threadblight

iv) Cream thread blight v) Brown thread blight

vi)Cream thread blight with fruiting bodies

Plate4.2: Types of thread blight identified in the seven cocoa growing districts

Out of 270 farmers interviewed, 227 farmers did not know that the disease is called thread blight.Thirty of the farmers called it in twias "ahomafitaa" (White thread). Eight of them also called it "Ananse" (web-like) to denote how it entangles the dried

leaves and the twigs like a spider web (Plate 2D) and Five people called it thread blight.Table 4.7 illustrates the various local names of thread blight pathogen.

Table4.7: Local names of thread blight pathogen known by the farmers interviewed

Local Names	Number of farmers naming it
Ahomafitaa	30
Thread blight	5
Ananse	8
No local name	227
Total	270

4.9Field observation and disease assessment by the cocoa farmers

The farmers reported that the disease attacked both young and old cocoa plants and thatthe disease occurred in both wet and dry seasons. However, the disease spreads faster in the rainy season than in the dry season. They also indicatedthat, in the initial stage of the disease development in the field, the host plant manages to outgrow the infection after a period by growing new leaves to replace the defoliated ones.This makes it difficult for them to detect the initial damage caused by the disease and only noticed its effect when the plant succumbs to the infection of the pathogen at its advanced stage, thus making the disease difficult to control.

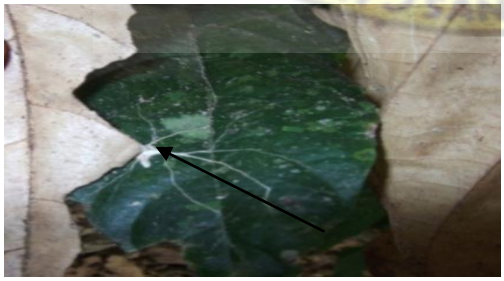
It was observed that, the disease spreads mostly through direct contact with the infected plant parts.Majority of the thread blight pathogens affected the branch or the twig of the cocoa plant, showing signs like threadlike white, cream, brown or black sticky hyphae (Plate 4.2) above. Thethreadlike myceliagrew from the point of infection mostly on the branches, and then extended to the leaves of the infected plant (Plate 3A), thus causing necrosis in the leaves and eventually the leaves die (Plate 4.3B and D). It was also observed that the disease spread faster when the infected cocoa leaf was in contact with healthy leafand that the disease could infect the leaf through either adaxial or abaxial surfaces of the cocoa leaf (Plate 4.3E and F).

A. Signs of thread blight on cocoa

B. White thread blight symptom on cocoa

C. Thread blight pathogen causing necrosis on infected young plants

D. Advanced stage of thread blight infection



E. Thread blight pathogen spreading Through adaxial surface of the cocoa leaves

F. Thread blight pathogen spreading through abaxial surfaces of the leaves

Plate4.3: Signs and Symptoms of thread blight and their mode of spread

Although, the disease starts on the stem of the host plant in most cases, necroses were seen mostly on the leaves of the host plant with part or the entire leaf covered with the mycelialstrands (Plate 4.3F). Field study also showed that whenever necrosis forms on the leaf, it spreads within14-21 days to other parts of the leaf blade.According to some farmers, the diseased plants generally produced smaller cocoa pods, and in severe infections, the infected plants do not produce any pods at all, resulting in yield reduction.

4.10Causal agents of thread blight perceived by cocoa farmers interviewed in the seven selected major cocoa growing districts in Eastern and Ashanti regions of Ghana

Of the farmers interviewed, 18% had various views about the cause of the disease (Fig. 4.2). Some believed that the disease was caused by environmental factors such as too much rainfall and prolonged dry season. About 82% of the farmers had no idea about the cause of the disease (Fig.4.2).

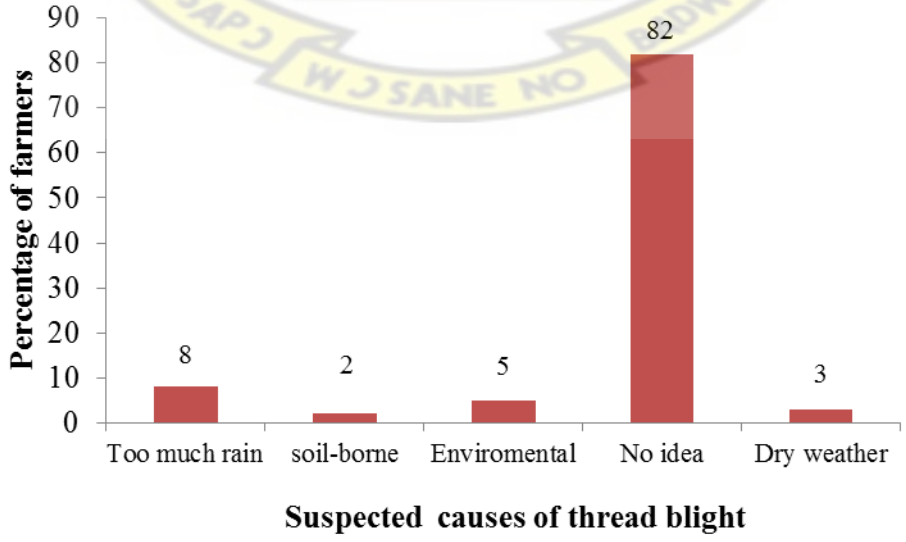


Figure4.2: Causes of thread blight disease of cocoa perceived by farmers interviewed in major cocoa growing districts in Eastern and Ashanti regions

4.11Control measures employed bycocoa farmers interviewed in the seven districts for controlling thread blight diseases

All the cocoa farmers interviewed do not manage thread blight disease in any way. However, 96% of the farmers indicated they use chemicals to control other major pests and diseases of cocoa on their farms (Table 4.8). About 4% of the farmers do not use chemicals on their farms due to financial constraint. Distribution of farmers and agro-chemical they use are presented in Table 4.8.

Table4.8: **Distribution of farmers and the agro-chemicals used in the districts surveyed**

	% Chemicals used per district				
	Insecticide	**Fungicide+Insecticide**	**Insecticide+Sidalco**	**Fungicide+Sidalco+Insecticide.**	**No Chemical**
Districts					
AsanteAkimSouth.	4.0	7.0			
Asan-AkimN.	4.0	1.0	1		
Fanteakwa	1.0	3.3	2	4	1
Atiwa	2.0	8.0	2	4	1
East Akim	1.0	8.0	18	12	2
Effiduase	3.0	2.0	1	1	
N.Juabeng	1.0	2.0	2	2	
Total (%)	16	31	26	23	4

Of the farmers that use agro-chemicals on their farm, most of them have their farms covered by the Mass cocoa Spraying scheme organized by the Government of Ghana, whilst the remaining apply these chemicals themselves .

Table4.9. List of agro-chemicals used by the cocoa farmers interviewed

Fungicides	**Insecticides**	**Fertilizer**
Ridomil Plus	Confidor	Foliar fertilizer(Sidalco)
Kocide	Akate master	
Champion	Akatesro	
Nordox	Atara	

4.12.0 Incidence and severity of thread blight disease on cocoa in seven districts in Eastern and Ashanti regions of Ghana

4.12.1 Percentage Incidence of thread blight disease

Table4. 10.Distribution of thread blight pathogen(s) on cocoa in seven selected districts in Eastern and Ashanti regions of Ghana

		Type of thread blight infected/farm					
Location	**No. farms visited**	**White**	**Brown**	**Black**	**Cream**	**No. farms infected**	**Severity score**
Asante-Akim S							
Juaso	15	7			2	4	1
Aboaboso	15	1			4	3	1
Asante-AkimN							
Mensakrom	15	5			4	9	2
Okaikrom	15	5	4		3	7	2
Sekyere East							
Adediaja	15	7	1		4	12	3
Effiduase	15	10			1	11	2
Atiwa							
Anyinam	15	7			6	13	3
Kwabeng	15	6			5	11	3
AkyemSekyere	15	7			5	12	2
Fanteakwa							
Osino	15	11			4	11	3
Mapontenu	15	10			7	15	4
Manyiaso	15	9			5	13	4
East Akim							
New tafo	15	8			2	14	4
Old Tafo	15	9			6	12	2
Osiem	15	7	1	4	5	14	4
Bunso	15	8			4	12	3
Kukurantumi	15	7			6	13	4
New Juabeng							
Jumapo	15	8			4	11	3

Out of the 19 villages/towns visited in seven cocoa growing districts in Ghana, Mapontenu in Fanteakwa District had all the 15 farms visited infected with thread blight disease. This is followed by Osiem and New Tafo in East Akim District with each recording14 out of 15 infected farms. Anyinam in Atiwa District, Osino in

Fanteakwa and Kukurantumi in East Akim Districts all recorded 13 infected farms each. All the above towns/villages mentioned are located in Eastern region of Ghana.Aboaboso and Juaso-Nkwantain Asante-Akim South recorded the least number of infected farms in Ashanti region (Table4.10).

Again, Mapontenu and Manyiaso in Fanteakwa District recorded the highest severity score of 4 of thread blight infection. Similar severity was scored in farms at Kukurantumi, New Tafo and Osiem in East Akim District (Table 4.10). Adediajain Sekyere East District in Ashanti region, Anyinam and Kwabeng in Atiwa District, Osino in Fanteakwa District and Bunso in East Akim District in Eastern region scored 3 whilst Juaso and Aboaboso in Asante Akim South recorded the least score of 1.

The result revealed that more farms in Eastern region had thread blight pathogens and higher disease severity than in Ashanti region. Thus, thread blight disease was more pronounced in the Eastern region than in Ashanti region. In the Ashanti region, it was observed that, more farms in Sekyere East District were infected as compared to other districts visited in the region.This was because; most of the cocoa trees found in the farms visited in Sekyere East District were of older breeds such as Tetteh Quashie and Amazonia.

A similar situation prevailed at Osiem in East Akim District and Mapontenuin Fanteakwa District. However, most of cocoa farms visited at Anyinam, Bunso and New Tafohad new cocoa hybrid cultivar and yet are infected with thread blight disease.Plate 4.5 provides pictorial presentations of the various severity scores.

A). Severity 25 % scored 1 **B). Severity 50 % scored 2**

C). Severity@ 75 % scored 3 **D). Severity @ 100 % scored 4**

Plate4.4. Direct estimation of severity of thread blight disease

4.13.0Isolation and identification of the thread blight pathogen(s)

4.13.1 Occurrence of various fungi isolated from diseased samples

Diseased samples taken from cocoa and the three suspected hosts from each district had thread blight mycelia on them. Twenty-one fungi were isolated from the diseased samples collected on cocoa trees from all the seven selected cocoa growing districts in Ghana. Eighteen of them were identified with characteristics of thread blight pathogen species and were grouped in six, based on their morphological features (Plate 6A, B, C, D, E, F and G) with their respective microscopic features (Plate 6 A' B' C' D' E' F' and G'). The pathogen(s) were coded as TB1, TB 2, TB 3, TB 4, TB 5 and TB 6 with their respective percentages of occurrence (Fig4.3). *Aspergillusniger* and *Trichoderma* species were identified to be associated with the above pathogens. One fungal species was unidentified (Fig.4.3).

Fungi isolated in this study belonged to two genera, Ascomycotina (*Aspergillus* sp., and *Trichoderma* sp.,) and Basidiomycotina (Thread blight sp.).

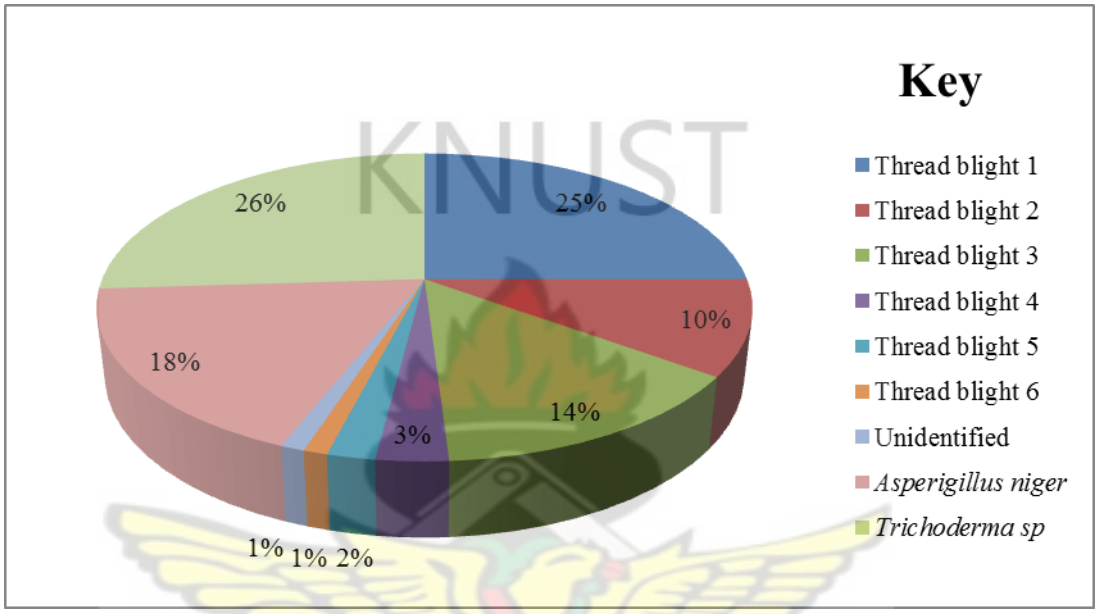
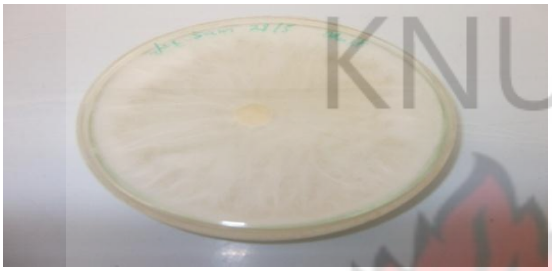


Figure4.3:(%) Occurrence of fungi isolated from diseased cocoa samples collected from seven selected cocoa growing districts in Ashanti and Eastern regions of Ghana

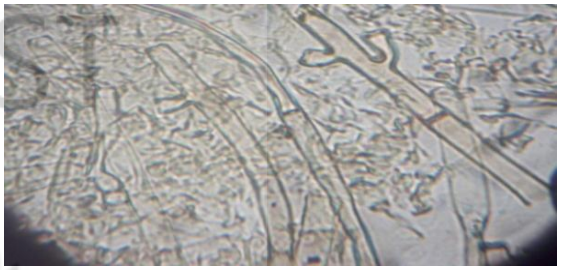
4.14. Microscopic and Macroscopic characteristics of thread blight pathogens isolated

Different fungal pathogensisolated from diseased cocoa samples collected from farmers' fields from the seven districts in Ashanti and Eastern regions are listed in Table 4.10. The fungi identified were white thread blight type one (TB 1; Plate 6A, 6A') isolated from East Akim, Atiwa and part of Asante-Akim South Districts. White thread blight type two (TB 2; Plate6B, 6B'), represent isolates from part of East Akim District, New Juabeng Municipality and Fanteakwa District.Thread blight type three (TB 3; Plate 6C, 6C') which was brown thread blight was isolated from

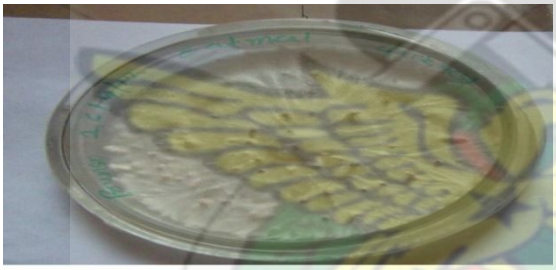
East Akim and Sekyere East districts, while type four (cream thread blight) (TB 4; Plate 6D, 6D')was isolated from Asante-Akim North, Sekyere East, Atiwa and East Akim Districts. Type five (TB 5; Plate 6E, 6E') which was black thread blight wasisolated from East Akim and SekyereEast Districts, whilst type six (TB 6Plate 6F, 6F') and the unidentified one were isolated from Atiwa District (Plate 4. 6G, 6G').



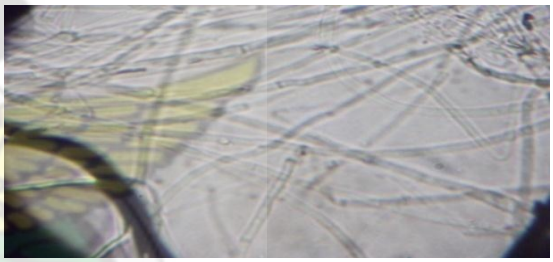
A) TB 1 isolate in culture



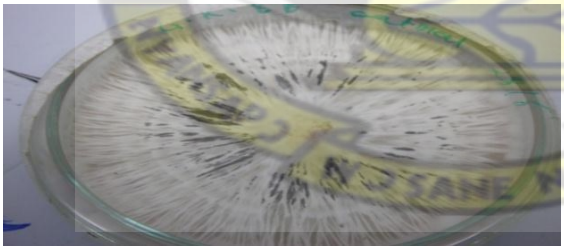
A') Micrograph of TB 1 isolate



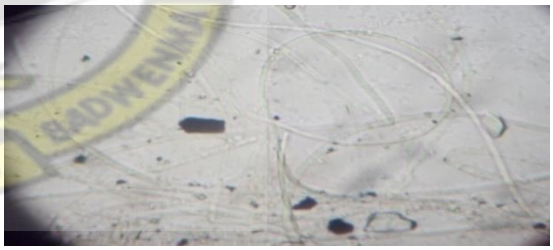
B) TB 2 isolate in culture



B') Micrograph of TB 2 isolate

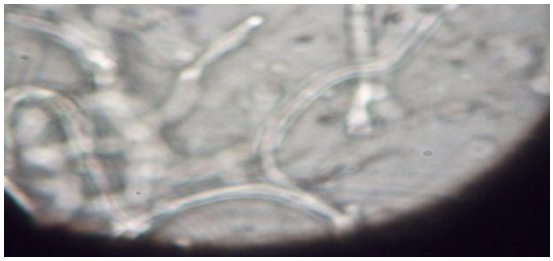


C) TB 3 isolate in culture

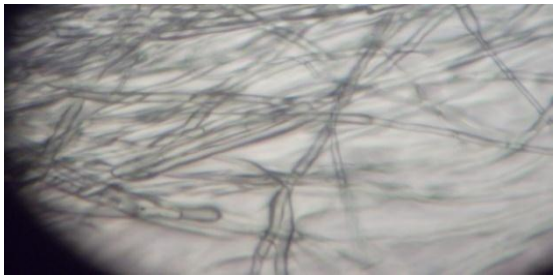


C') Micrograph of TB 3 isolate

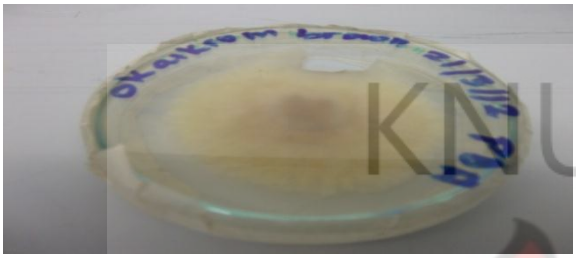
D) TB 4 isolate in culture

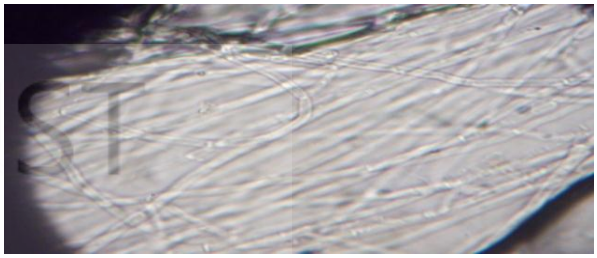


D') Micrograph of TB 4 isolate



E) TB 5 isolate in culture **E') Micrograph of TB 5 isolate**





F) TB 6 isolate in culture **F') Micrograph of TB 6 isolate**

G) Unidentified isolate in culture **G') Micrograph of unidentified**

Plate4.5: Cultures and micrographs of thread blight pathogens isolated from diseased samples collected from the farmers' field in seven districts in Ashanti and Eastern regions

Thread blight isolates (TB 1, TB 2, TB 3, TB 4, TB 5and TB 6) appear to differ from their macroscopic and microscopic features. For instance, thread blight isolates 3 and 5 which were brown and black thread blight gave whitish to cream mycelia with black pigmentation background on the culture medium and differed in microscopic features (Plate 4.6). Thread blight TB 1 and TB 2 are all white thread blight. However, they differ from each other in morphological features. TB 1 is whitish,

fluffy, smooth and cottony texture with no black pigmentation and no spore and mostly infect through 'vegetative' means (Table 4.11). Assessment of its microscopic features revealed branched-binding and generative hyphae with septa which also look like *Rhizotonia* (Plate4. 6A').

TB 2, is whitish with black pigmentation background anddevelop fruiting bodies known as setaein culture which produce spores. It has thick, rough, velvety mycelia texture.Its microscopic features include thick-walled, non-septate, skeletal hyphae and with or without sparsely clamp connections. Other thread blight pathogens were also identified in the same characteristics (Table 4.11and 4.12). However, furtheridentification of these fungi were not possible because their reproductive structures (especially spore) could hardly be seen. Preliminary sporulation test conducted showed that all the TBs isolates could not readily produce spore on the agar medium except TB 2 isolate.

Table4.11: Identification and characteristics of fungi isolated from the diseased samples collected from seven cocoa growing districts in Eastern and Ashanti regions of Ghana

Isolate code	Growth morphology	Colony colour	Key characters	Spore shape	Identity of fungus
TB 1	Fast growing, Cottonny, Fast growing and fluffy	White	Distinct radial strands and no pigmentation of agar medium	Spore lacking	Thread blight sp.
TB 2	Thick with fruity bodies	White	Strands of hyphae and various types spore bearing structures, pigmentation of agar medium	Elliptical and smooth	Thread blight sp.
TB 3	Strands of mycelia and fluffy	White with black pigmentation background	Strands of mycelia	Spores lacking	Thread blight sp.
TB 4	Concentric growth strands and fluffy	Cream with yellowish background	Network strands of mycelia	Spores lacking	Thread blight sp.

Table 4.11: Identification and characteristics of fungi isolated from the diseased samples collected from seven cocoa growing districts in Eastern and Ashanti regions of Ghana.

TB 5	Concentric and slow growing	Blackish-white with fruiting bodies	Strands of hyphae with scattered blackspots	No spore	Thread blight sp.
TB 6	Slow growing strands of mycelia	Creamish-yellow	Strands of mycelia	No spore	Thread blight sp.
Unidentified	Fast growing	Brown	Strands of mycelia	No spore	Unidentified

4. 15 Identification of the causal agent(s) of thread blight disease on cocoa, oil palm (*Elaeis guineesis* Jacq), banana and plantain (*Musa* spp.)

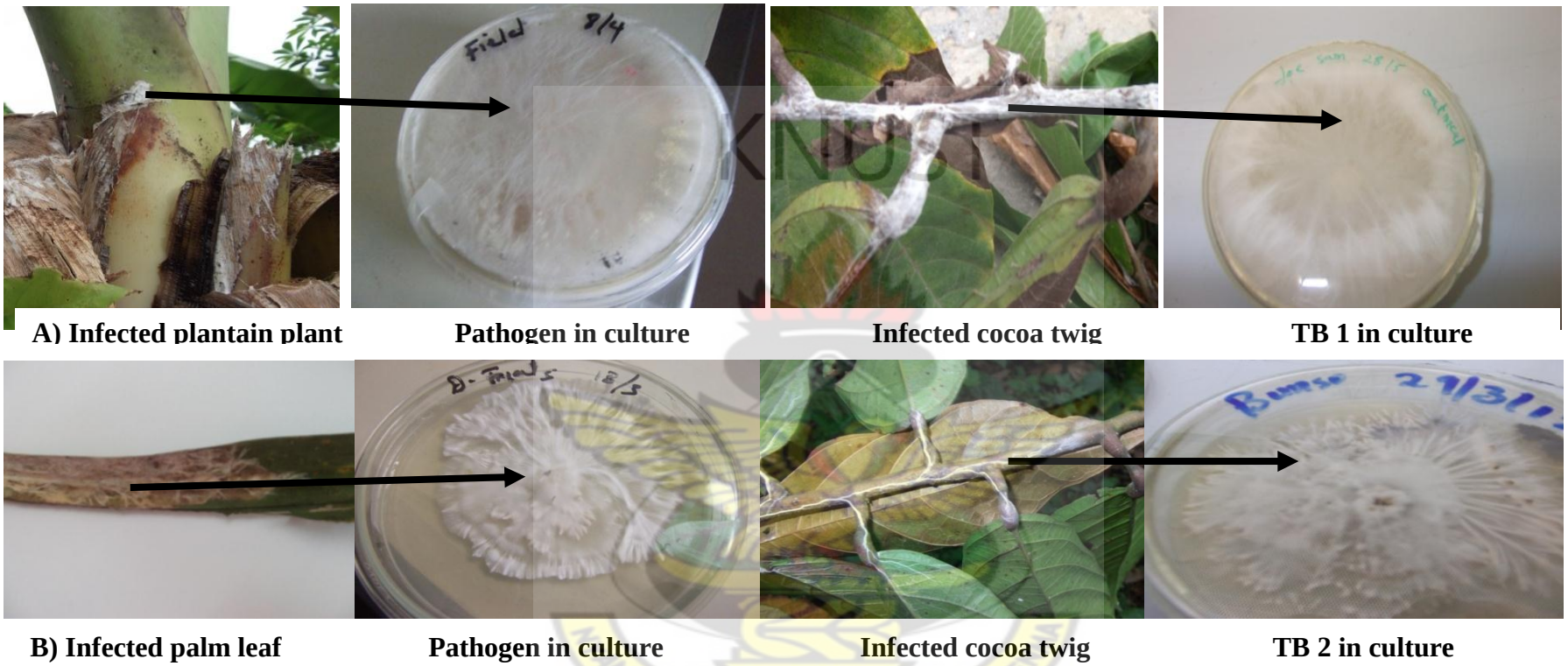


Plate 4.6: A and B**:** Similarities between cultures obtained from infected cocoa trees and other alternatives host crops identified

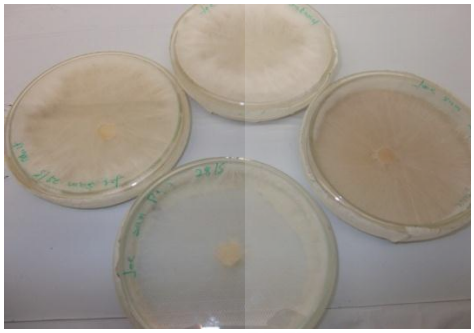
Plate 4.6 shows similarities in the morphological features of cultures of thread blight pathogen s obtained from the suspected host crops (banana and plantain; *Musa* spp. and oil palm; *Elaeis guineesis* Jacq.) and some of the thread blight pathogens isolated from the infected cocoa tree. This shows that features of thread blight pathogen type one (TB 1) from cocoa was the same as the pathogen isolated from the infected plantain plant (Plate 4.6 A) Features of thread blight pathogen type 2 (TB 2) was also similar to the pathogen obtained from the infected oil palm Plate 4.6 B.

4.16. Colony characterization and best mycological medium support of thread blight pathogens isolated from seven districts in both Ashanti and Eastern regions

All the eight culture media supported the growth of thread blight pathogen(s) to various degrees. Four of the isolates namely TB 1, TB 2, TB 3 and TB 4 showed maximalmycelial growth on Oatmeal agar, with the mean colony diameter of 8.4, 4.7, 5.2 and 3.9 cm, respectively (Table 4. 12). Thread blight type 5 (TB 5) and TB 6 also showed maximum mycelial growth on Malt Extract Agar medium with their respective colony diameter of 4.2 and 3.6 cm, while the unidentified fungus showed higher colony growth of 8.7 cm on PDA (Table 4.12). Other media such as Plantain Agar (PA) and V-8 juice agar also supported thread blight mycelial growth to a certain degree. However, Green Cocoa Mucilage Agar and Banana Agar (BA) poorly supported the mycelial growth of thread blight fungi which resulted in shorter length of colony diameter (Table 4.12).

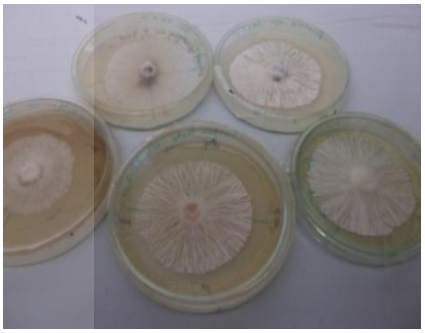
Differences in surface pigmentation of fungal colonies were shown on two growth media. Thread blight type 5 (TB 5) wascreamish to yellowish-brown with scattered black spots at the centre on PDA and whitish cream with yellow background on OMA

(Plate 4.7A, Table 4.12). TB 6 cultures were yellowish-brown with dark brown exudates at one sideon PDA, whitish- cream with distinct strands of mycelia on OMA (Plate 4.7B, Table 4.12). However, the surface colony colour of TB 1, TB 2 and TB 3 was white on all the media used except TB 2 and TB 3 that produced black pigmentation background which started from the point of inoculation on both PDA and OMA medium after seven days of incubation (Plate 4.7C).



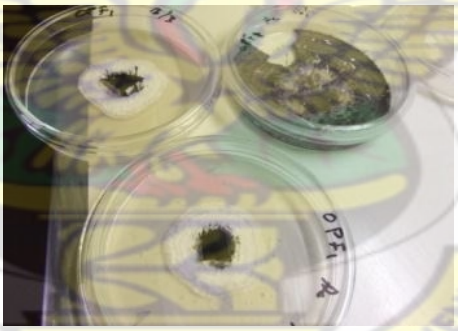
A) TB 1 isolate

B) TB 2 isolate

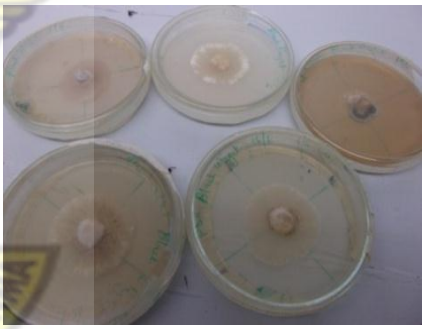


C) TB 3 isolate

D) TB 4 isolate



E) TB 5 isolate



F) TB 6 isolate

H) Unidentified isolate in culture

Plate 4.7: Different types of thread blight isolates from cocoa on different culture media

In this study, the zonations observed (Table 4.12) in fungal colonies were found to be influenced by the culture media used. In PDA, almost all isolates tested were characterized with distinct stranded mycelial growth and concentric zones TB 2 isolate but had in addition prominent scattered greyish setae (Plate 4.7B; Table 4.12). On the other hand, none of the fungal colonies exhibited concentric radial zones on GCMA medium (Table 4.12, Plate 4.7I). However, majority of thread blight pathogens tested exhibited concentric zonation and cottony texture on OMA and PA (Table 4.12). The unidentified isolates showed no concentric zonation but rather stranded mycelial growth on all the media used (Plate 4.7H).

Table 4.12:Mycelial growth, colony characters and sporulation pattern of thread blight isolates on seven culture media

			Colony Character		
Isolates	Media	Colony diameter(cm)	Mycelial texture	Surface Colour of mycelia	Zonation
TB 1	PDA	7.5	Velvety thick	Whitish	Radially stranded
	OMA	8.4	Velvety thick	Whitish cottony	Concentric radialstranded
	MEA	6.0	Fine	White	Radially stranded
	GCMA	8.1	Fine	White	Radially stranded
	V8	7.6	Fine	White	Radially stranded
	PA	7.5	Velvety	White	Concentric zones
	BA	6.4	Fine	White	Concentric zones
TB 2	PDA	3.9	Velvety thick with citae	Creamish-white	Concentric zones with setae
	OMA	4.7	Thick,elongatedcitae	Dirty white greyish spores	Concentric zones of setae
	MEA	4.2	Fine with setae	White	Concentric zones
	GCMA	3.1	Light with scatered setae	White	Scattered whitish setae
	V8	3.0	Thick closed short setae	Dirty white	Concentric zones of setae
	PA	3.7	Thick with setae	White	Concentric zones
	BA	4.7	Velvety thick	Whitish-cream	Concentric zones
TB 3	PDA	4.8	Velvety thick; strand	White	Distinct threadlike radial strands
	OMA	5.2	Velvety thick strand	White,black pigments	Distinct threadlike radial strands
	MEA	4.2	Velvety thick strand	White	Distinct threadlike radial strands
	GCMA	4.6	Fine	White	Radial furrows

Table 4.12.Mycelial growth, colony characters and sporulation pattern of thread blight isolates on seven culture media cont'd

	V8	3.1	Fine	White	Distinct marginal zones
	PA	4.1	Cottony	Whitish-black	Concentric radial zones
TB 4					
	PDA	3.2	Velvety thick with strand	Cream	Distinct marginal zones
	OMA	3.9	Velvety thick	Creamish white	Distinct radial strands
	MEA	3.2	Fine	Creamish-brown	Concentric radial strands
	GCMA	3.1	Velvety	Llight brown	Radial furrowed
	V8	1.9	Velvety thick	Brown	Concentric zones
	PA	2.1	Fine and cottony	Brownish-cream	Radial concentric
	BA	3.1	Fine	Cream	Concentric zones
TB 5	PDA	3.0	Velvety thick cottony	Cream, scattered black-spot	Concentric zones,radial fine strands
	OMA	3.9	Velvety	Blackish-white	Distinct white to cream marginal zones
	MEA	4.2	Velvety light	Black	Concentric radial strands
	GCMA	3.5	Velvety thick	whitish-black	Radial furrowed
	V8	2.3	Fine	Black	Concentric zones
	PA	2.0	Velvety thick	Black	Concentric zones
	BA	2.2	Velvety, light	Blackish-white	Radial furrowed

Table 4.12.Mycelial growth, colony characters and sporulation pattern of thread blight isolates on seven culture media cont'd

			Velvety thick, stranded		
TB 6	PDA	3.5		Brown	Radial furrowed
	OMA	2.8		Cream	Concentric radial strands
			Velvety light andstrands		
	MEA	3.6	Velvety light	Creamish brown	Concentric zonations
	GCMA	3.0	Velvety	dark brown	Radially stranded
	V8	2.0	Velvety	brown	Radially furrowed
	PA	3.5	Fine	light brown	Concentric
			Smooth, strand		Concentric, radial stranded
	BA	3.2		brown	
	PDA	8.4	Fluffy		Concentric radial strand
Unidentified				Darkish brown	
	OMA	8.3		Dirty white to slightly	Concentric zones,
			Fine strands	brown	
	MEA	7.9	Fine	Slightly brown	Concentric zonations
	GCMA	8.1	Fine	Slightly brown	Concentric zonations
	V8	8	Fine and light	Colourless to brown	Concentric zonations
	PA	8.1	Fine strand	Colourless to light brown	Concentric strands
	BA	7.3		Deep brown	Radial strands

4.17Mycelial density, texture and sporulation under light, darkness and alternate of light and darkness

Mycelial density of all the six isolates of thread blight in light and darkness incubation wasextremely poor on Water Agar, poor on GCMA, and moderately poor on Banana Agarand V-8 juice Agar media (Table 4.13, Plate 4.8). However,mycelial density varied from moderate to abundant on the rest of the media, except Oat Meal Agar and Plantain Agar which always supported abundant mycelial density growth of all the isolates (Table 4.13).

OMA medium showed semi-cottony to cottony texture and moderate to abundantmycelial density of all the isolates. It was observed that mycelium of TB 1 could grow in a wide range of media including Water agar.All the six tested isolates could not sporulate readily on all the culture media used except TB 2 isolates (Plate 4.8) which sporulated heavily on OMA medium after the provision of light, temperature and darkness with the latter being an essential condition to induce maximum sporulation in some of the fungal organisms.

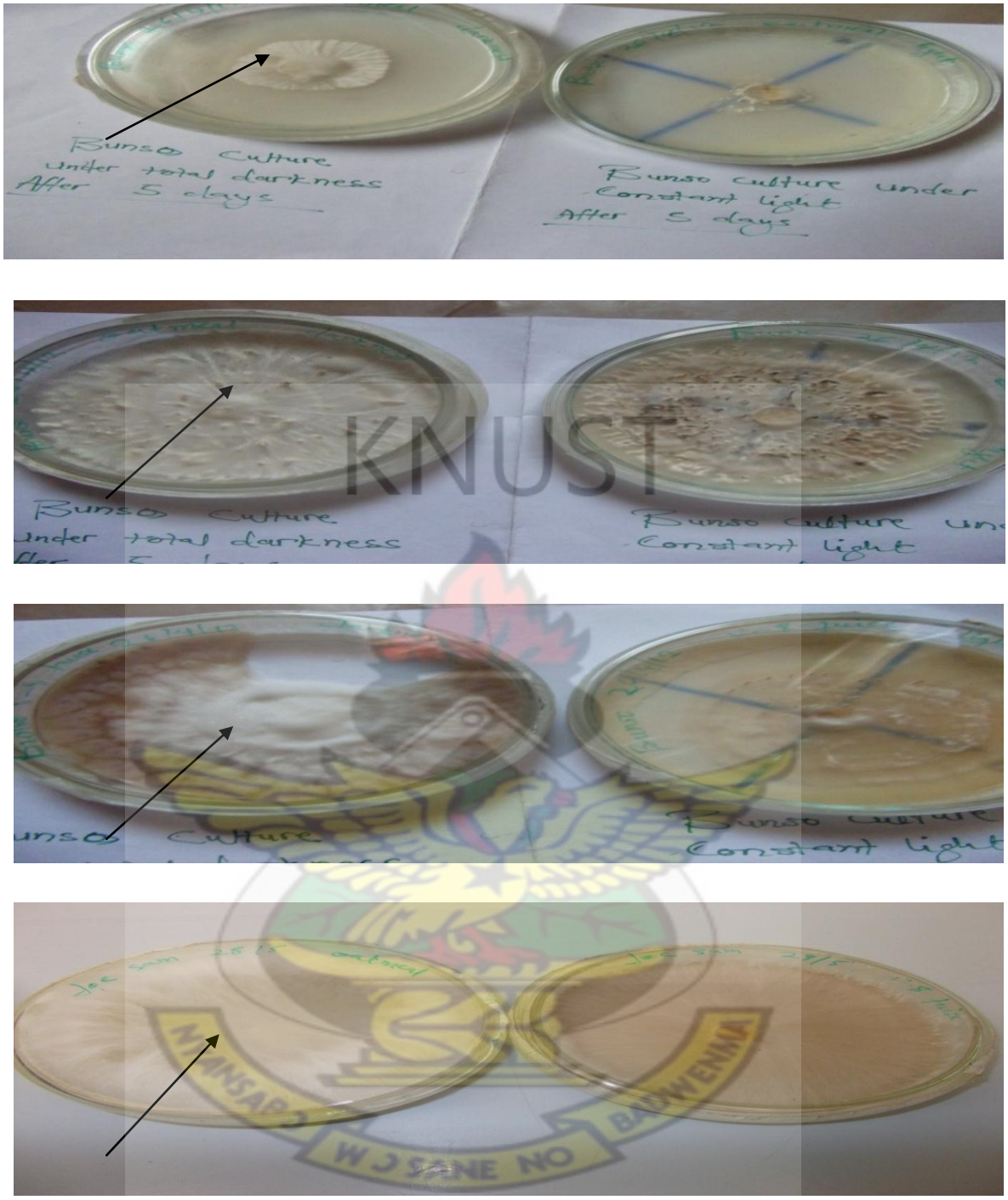


Plate 4.8: Mycelial density of various arrowed thread blight isolates on agar medium placed under darkness and light

Under light incubation, mycelial texture of all the six isolates was flat in most of the agar media except on OMA which induced both fine and semi-cottony texture in some of the isolates with mycelial density ranging from 0.2 to 0.4 g/ml³ (Table 4.13). MEA,

PA and BA, however, appeared to induce semi-cottony texture in TB 1 and TB 5 isolateswithmycelial density of0.3 g/ml^{3}during the late incubation periods. In this study, thread blight isolates were found to be light on nutritionally less rich media, while light to semi-cottony and cottony textures were observed in highly rich media such as OMA, MEA, PA and BA (Table 4.13).

Under dark incubation,mycelial density in WA was 0.0-0.1g/ml^3,which was very poor with no trace of sporulation andlight textured mycelial growth (Table 4.13). Mycelial density was between 0.1 to 0.5 g/ml^3, with poor sporulation on all media except OMA which exhibitedhighest myceliadensity of between 0.5 and 0.8 g/ml^3 across all the thread blight isolates.Mycelial density wasalso cottony texture with moderate sporulation pattern in thread blight Type 2. However, there was no spore or setae formation in the rest of the thread blight isolates under the dark treatment. Rather, high mycelial densitieswere observed under the dark incubation period in all the isolates cultured on the tests media (Table 4.13).

Mycelial growth characteristics differed less amongst the different media under dark incubation as compared to those grown under light incubation. Isolates incubated under the alternation of both light and dark also showed a combination of similar colony characteristics observed under light and dark incubation with less difference in their sporulation pattern except OMA and PA culture media which produced heavy spores in TB 2 isolate (Table 4.13). Various degrees of colony pigmentation ranging from black, cream to brown were produced by TB 2, TB 3, and TB 5 on OMA, PA, BA and PDA media under light incubation. No pigmentation was, however, produced by any of the six isolates incubated in the darkness (Table 4.13).

Table 4.13: Mycelialgrowth characteristics of thread blight pathogen(s) on various mycological culture media under different incubation periods

Isolates		Light condition of mycelia			Dark condition of Mycelia			Light and Dark alternate		
					Density			Density		
	Medium	Density(g/ml³)	Texture	Sporulation	(g/ml)	Texture	Sporulation	(g/ml)	Texture	Sporulation
TB 1	PDA	0.3	Light	Nil	0.5	Cottony	Nil	0.5	Cottony	Nil
	OMA	0.4	Cottony	Nil	0.8	Cottony	Nil	0.7	Cottony	Nil
	MEA	0.4	Cottony	Nil	0.5	Cottony	Nil	0.5	Cottony	Nil
	GCMA	0.2	Light	Nil	0.2	Sparse	Nil	0.2	Light	Nil
	V8	0.2	Light	Nil	0.5	Cottony	Nil	0.2	Light	Nil
	WA	0.1	Light	Nil	0.1	Light	Nil	0.1	Light	Nil
	PA	0.1	Light	Nil	0.4	Dense	Nil	0.2	Light	Nil
	BA	0.2	Light	Nil	0.4	Cottony	Nil	0.4	light	Nil
TB 2	PDA	0.1	Light	Nil	0.4	Dense	Nil	0.3	Sparse	Moderate
	OMA	0.3	Cottony	Heavy	0.7	Cottony	Mild	0.4	Cottony	Heavy
	MEA	0.3	Cottony	Moderate	0.4	Cottony	Mild	0.2	Light	Nil
	GCMA	0.1	Light	Nil	0.2	Light	Nil	0.2	Light	Nil
	V8	0.1	Light	Nil	0.5	Cottony	Nil	0.2	Light	Nil
	WA	0.0	Light	Nil	0.1	Light	Nil	0.1	Light	Nil
	PA	0.3	Cottony	Heavy	0.5	Cottony	Mild	0.3	Sparse	Heavy
	BA	0.3	Cottony	Heavy	0.4	Dense	Nil	0.5	Cottony	Mild

Table 4.13: Mycelial growth characteristics of Thread blight pathogen(s) on various mycological culture media under different incubation periods

TB 3	PDA	0.3	Cottony	Nil	0.4	Cottony	Nil	0.3	Light	Nil
	OMA	0.4	Cottony	Nil	0.8	Cottony	Nil	0.4	Light	Nil
	MEA	0.2	Light	Nil	0.5	Cottony	Nil	0.2	Light	Nil
	GCMA	0.1	Light	Nil	0.2	Light	Nil	0.1	Light	Nil
	V8	0.2	Light	Nil	0.5	Cottony	Nil	0.2	Light	Nil
	WA	0.1	Light	Nil	0.1	Light	Nil	0.1	Light	Nil
	PA	0.2	Light	Nil	0.5	Dense	Nil	0.2	Light	Nil
	BA	0.2	Light	Nil	0.4	Cottony	Nil	0.3	Light	Nil
	PDA	0.1	light	Nil	0.2	Light	Nil	0.2	Light	Nil
TB 4	OMA	0.4	Cottony	Poor	0.5	Ramified	Nil	0.5	Cottony	Nil
	MEA	0.3	Cottony	Nil	0.2	Light	Nil	0.4	Cottony	Nil
	GCMA	0.1	Light	Nil	0.2	Light	Nil	0.2	Light	Nil
	V8	0.2	Light	Nil	0.2	Light	Nil	0.3	Light	Nil
	WA	0.0	Light	Nil	0.1	Light	Nil	0.1	Light	Nil
	PA	0.2	light	Nil	0.2	Light	Nil	0.2	light	Nil
	BA	0.2	light	Nil	0.4	Cottony	Nil	0.1	light	Nil
TB 5	PDA	0.1	Light	Nil	0.2	Light	Nil	0.4	Cottony	Nil
	OMA	0.3	Cottony	Nil	0.5	Cottony	Nil	0.6	Cottony	Nil
	MEA	0.1	Light	Nil	0.3	Light	Nil	0.3	Cottony	Nil
	GCMA	0.1	Light	Nil	0.2	Light	Nil	0.2	Light	Nil
	V8	0.1	Light	Nil	0.3	Light	Nil	0.2	Light	Nil
	WA	0.0	Nil	Nil	0.1	Light	Nil	0.1	Light	Nil
	PA	0.3	Cottony	Nil	0.2	Light	Nil	0.2	Light	Nil
	BA	0.3	Cottony	Nil	0.2	Light	Nil	0.2	Cottony	Nil

Table 4.13: Mycelial growth characteristics of thread blight pathogens on various mycological culture media under different incubation periods

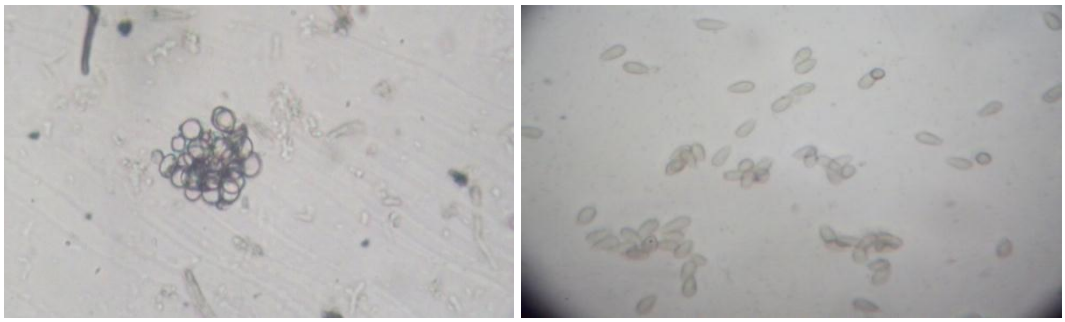
TB 6	PDA	0.1	Light	Nil	0.4	Cottony	Nil	0.4	Light	Nil
	OMA	0.3	Cottony	Nil	0.7	Cottony	Nil	0.5	Cottony	Nil
	MEA	0.1	Light	Nil	0.5	Cottony	Nil	0.4	Cottony	Nil
	GCMA	0.1	Light	Nil	0.2	Light	Nil	0.2	Light	Nil
	V8	0.1	Light	Nil	0.2	Light	Nil	0.2	Light	Nil
	WA	0.0	Light	Nil	0.1	Light	Nil	0.1	Light	Nil
	PA	0.1	Light	Nil	0.4	Cottony	Nil	0.2	Light	Nil
	BA	0.1	Light	Nil	0.2	Light	Nil	0.2	Light	Nil

4.18 Induction of sporulation in thread blight pathogen(s)

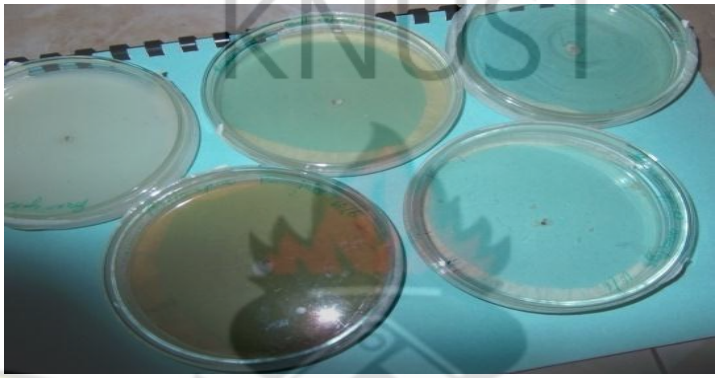
There were differences in the sporulation patterns of the test fungal isolates on the eight culture media including Water Agar (Table 4.13). Five of the isolates did not sporulate on any of the media used.Fungal isolates ofthread blight Type two (TB 2) produced sporesafter 20 days of incubation on Oatmeal Agar and Plantain Agar(Table 4.13). The shape of the spore produced by thread blight Type 2 was oval, bearing minute apiculus, smooth thin walled, usually guttulate, slightly greyish or colourless, 10-13 × 5.5–7µm and scattered when viewed under compound microscope (Plate 4.9B). Thread blight Type 1 produced spores after forming into mushrooms (fruiting bodies) (Plate 4.10 C; Plate 4.9A). Its spores were roundish and clustered, bearing minuteapiculus, smooth thick double layers walled and slightly darkish when viewed under compound microscope.

However no sporewas observed when thread blight Types1, 3, 4, 5 and 6 were cultured on other media and placed under the same conditions. Instead, minimalmycelial growth was observed in them when they were placed under constant light for the purpose of induction of spores.

4.19 Shapes/forms of spores obtained from the two types of white thread blight pathogens



A) Spores of thread blight isolate Type 1 **B)** Spores of thread blight isolate Type 2



C) Germination of individual spores after 24 h

Plate4.9 Spores of thread blight Type 1 and 2

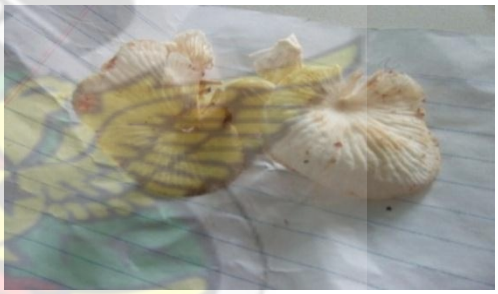
4.20 Identification of thread blight pathogens through the mushroom production technique

The results obtained from this technique showed thatisolates TB 1, TB 2, TB 3, TB 4, TB 5 and TB 6 fungi were from the class of Basidiomycetes. The morphological characteristics depicted by the test fungi when they were inoculated on both the sorghum in the bottle and on the substrates in the polythene bags (Plate4.10 A and 4.10 B) confirmed that.Isolates TB 1, TB 2, TB 3, TB 6 were able to grow into mushroom after 21 days of inoculation in the mushroom bag (Plate 4.10; C, D, E and F).

Pure cultures transferred to sterilized grain in a bottle became completely overgrown with the mycelium after two weeks. Species of thread blight pathogens were categorized into two, based on their colony growth; the fast growing ones and the slow growing ones. The fast growing ones (TB 1and TB 2) filled the grain bottle within one week while the slow growing ones (TB 4, TB 6, TB 3 and TB 5) filled the bottle after two weeks.

A)Thread blight isolates on sorghum

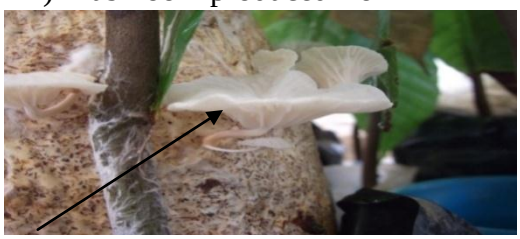
B) thread blight isolates on substrate



C) Mushroom produced from TB 1 Isolate

D) Mushroom pin head from TB 2 isolate H) Mushroom produced from TB 3 isolate

E) Mushroom produced from TB 4



F) Mushroom produced from TB 5

G) Mushroom produced from TB 6 isolate

Plate4.4. Thread blight isolates growing into different types of mushrooms

The present study revealed that, various fruiting bodies of thread blight pathogens with some consisting of a complete pileus with stipe (Plate 4.10; C, D,E and H), and some with fragments of a pileus (Plate 4.10 F and G). All thread blight isolates inoculated on the sterilised grain (sorghum) and the substratesexhibited the same morphological features of the agar medium. TB 3 isolates with white mycelia strand which exhibited black pigmentation at the background on Oatmeal Agar medium, showed the same colony character on the grain in the bottle and on the sawdust and rice husk substrates as well (Plate 4.10G and Plate 4.11).However, TB 5 and TB 6 took longer time to grow into mushroom.

In addition to phenotypic differences among species, it appears that, their nutritional requirements also differ. TB 1 grows well on wide varieties of substrates including different types of artificial culture agar with varied nutrient composition, whilst TB 2 and other TBs identified grow on limited substrate. Thus, TB 1 showed to be more pathogenic on wide varieties of plant species as compared to other thread blight pathogens identified. Results obtained under this technique confirmed that thread blights of cocoa are caused by different species of fossil mushrooms.

Thread blight isolate Type two (TB 2) with white velvety thick mycelia and setae with black pigmentation background on Oatmeal Agar medium showed similar morphological features when inoculated on the sawdust and rice husk substrates composted in the mushroom bag (Plate 4.10E). However, TB 2 did not show similar features when inoculated on the grain in the bottle.Similar mushrooms were produced from TB 1 isolates when plantain and banana suckers were used as substrates in mushroom production technology after 21 days of inoculation (Plate 4.12).

Plate 4.11. Thread blight type 3(arrowed) with black pigmentation in composted bag together with other isolatespacked in a box in an incubation room

A B

Plate 4.12 Thread blight isolates growing into mushroom on plantain sucker

A) Plantain sucker inoculated with TB isolate from cocoa

B) Isolate growing into a mushroom

4.21. Confirmation of pathogenicity of isolated fungi on one-year-old and six-months-old cocoa seedlings and three suspected alternative host of the disease in a moist chamber

Out of nine fungal isolates (6 TBs and 3 associated fungi) tested for pathogenicity, six of the TBs isolates (TB 1, TB 2, TB 3, TB 4, TB 5 and TB 6) produced symptoms between 14-21 days after inoculation with thread blight isolates (TBs). Pathogenicity tests carried out indicated that white to cream mycelia produced by some of the TBs isolates were similar to those observed on the infected samples collected from the farmers' field (Plate 4.13). New fresh leaves of the test cocoa seedlings were also infected (Plate 4.13a). Three of the associated fungi; unidentified isolate, *Trichoderma* sp. and *Aspergillus niger* did not produce any signs and symptoms of thread blight disease.

The study revealed that, TB 1 isolate and inocula isolated from banana, plantain and oil palmcaused infection on the test plants after seven daysof inoculation, whilst the rest of the TBs isolates took longer time (between 14 and 21 days) to cause infection.

The thread blight disease progressed from the point of inoculation to the leaf andcovered the entire leaf surface and subsequently, caused necrosis on the leaf which resulted in its death (Plate 4.14).

Plate 4.13. Necrosis caused by thread blight pathogen on younger cocoa leaf

Re-isolation of these pathogens from the polythene-covered-infected test plants produced pure cultures of thread blight pathogen(s) with the morphological features similar to those inocula used for theinoculation.

All cocoa seedlings used were found to be susceptible to all the thread blight pathogens except the three associated fungi (unidentified one, *Trichoderma* sp. and *Aspergillusniger* (Table 4.14a). Thread blight Type one caused 100 % infection on all the test plants including the alternative host crops (Plate 4.15). Inoculation of TB 2,TB 3, TB 5and TB 6 recorded 0 % infection on the alternative hosts. However, TB 4 recorded 33 % of the infection on the plantain sucker but caused no infection on the banana and oil palm seedlings. An unidentified fungus isolated did not cause any

infection on all the test plants including, banana, plantain and oil palm seedling indicating that the organism is probable not a thread blight pathogen.

It was observed that infection was established on the stems of the test plants earlier than the inoculated surfaces of the leaves; however, the infected leaves were necrotized and died-off earlier as comparedto the infected stemsthat were inoculated at the same time with the leaves (Plate 4.15).

a) TB 1 infection on cocoa seedlings

b) TB 2 infection on cocoa seedlings

c)TB 3Infectionon cocoa seedlings

d) TB 4Infection on cocoa seedlings

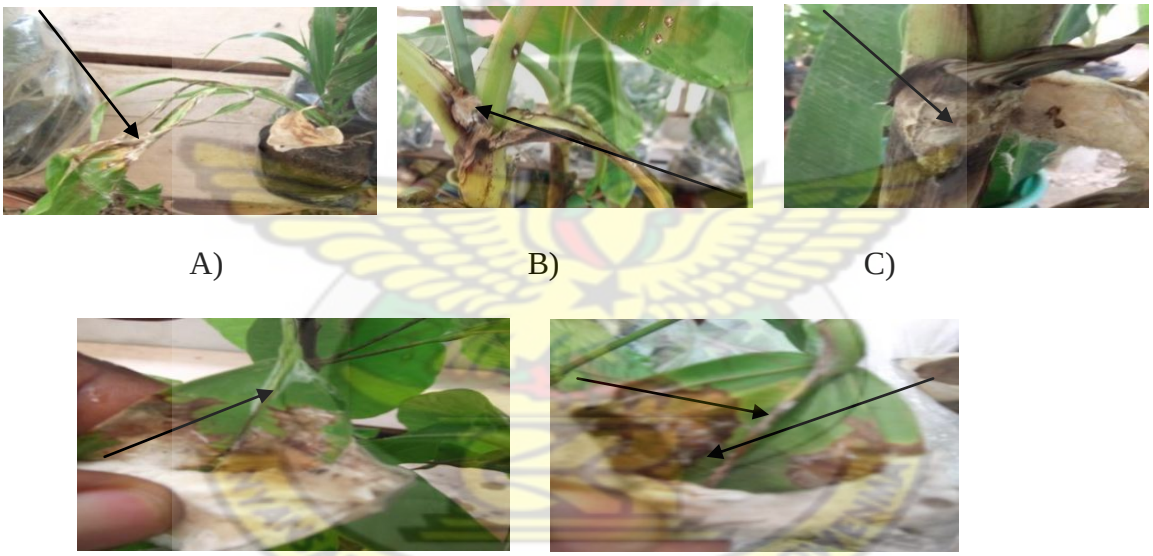
e) TB 5 Infection on cocoa seedlings

f) TB 6 Infection on cocoa seedlings

g) TB no Infection (0 %) on cocoa seedlings (Control)

Plate 4.14: Point of infection (arrowed) caused by thread blight pathogens

However, pure cultures of TB 3 (Brown thread blight) and TB 5 (Black thread blight) produced white thread-like mycelia on the individual test plant in the initial stage and later turned brown and black respectively as they aged on the test plant. It was observed that pathogen isolated from plantain/banana had the same morphological features just as TB 1 pathogen and was also virulent on both plants species (Plate 4.I5A and B). Also, no infection was caused by TB 5, TB 6and TB 3 on the alternative hosts (Table 4.14g). Infection was detected earlier on the stem than on both sides of the leaf (Plate 4.14a; Plate 4.15 D).



A) B) C)

D) Symptoms of thread blight pathogens on both the adaxial and abaxial surfaces of the leaf

Plate 4. 15: A) Advanced thread blight infection on oil palm

B) Thread blight infection on plantain plant

C) Thread blight infection on banana plant

Table4.14: Percentage infections of seven different inocula of thread blight on test plants

Test plant		**Thread Blight isolates**						
one year								Unidentified
	Replications	TB 1	TB 2	TB 3	TB 4	TB 5	TB 6	fungi
	1	+	+	+	+	+	+	-
	2	+	+	-	+	+	+	-
	3	+	-	+	-	+	+	-
	4	+	-	+	+	-	-	-
	5	+	+	-	+	-	-	-
% infection		100	60	60	80	60	60	0
Six months old								
	1	+	+	+	+	-	-	-
	2	+	+	+	-	+	-	-
	3	+	-	+	-	-	+	-
	4	+	-	-	+	+	-	-
	5	+	-	+	+	+	-	-
% infection		100	40	80	60	60	20	0
Oil Palm	1	+	+	-	-	-	-	-
Plantain	2	+	+	-	-	-	-	-
Banana	3	+	-	-	+	-	-	-
% infection		100	66	0	33	0	0	0

Where *+ =infected, - = uninfected

4.22 Fungicide bioassay *in vitro*

Among the fungicides used, Metalm was significantly superior to all other fungicides against thread blight isolates(TB 1,TB 2, TB 5 and TB 6) with respective percentage inhibition of (94.7, 87.5, 94.1 and 85.9 %) except thread blight Type 3 and 4 (TB 3 and TB 4). This was followed by Nordox (89.7, 76.9, 84.6 and 90.4 % inhibition) and was comparable toFungikill (88.9, 86.8, 76.9, 86.4 and 84.5 % inhibition) (Table 4.15).

On TB 3 and TB 4, however, Fungikill was significantly superior (89.9 and 85.4% inhibition, Plate 17A), followed by Metalm (88.4 and 80.0 % inhibition) and was at par with Nordox 70W (87.9 and 80.0 % inhibition, Plate 17C). Funguran-OH and Champion were found to be the least effective among the fungicides evaluated against all thread blight isolates tested (Table 4.15, Plate 17E and 17F). The different fungicides concentrations (25, 50, 75 and 100 ppm) provided increasing percentage radial growth inhibition of the thread blight pathogens along concentration gradient, thus increased inhibition with increased concentration of each fungicide.

There was significant difference ($P<0.05$) in the growth of all the thread blight isolates used. It was observed that TB 1 and TB 5 isolates required least amount of Metalm 70W fungicide (0.6mg/ (ai)/l) to achieve 100 % inhibition of mycelial growth (Table 4.15). The mycelial growth of TB 2 and TB 3 isolates were not initially affected by the treatment of Agro comet, Champion and Kocide 2000 fungicides at 25 and 50 ppm, but were effective at 75 and 100 ppm (Table 4.15).

Agro comet and Ridomil Plus fungicides were more effective in suppressing the mycelial growth of TB 5 isolate (Plate B) than Funguran-OH, Champion and Kocide 2000. Also, thepercentage inhibition of RidomilPlus fungicide was moderate on the mycelial growth of all thethread blight isolates (TBs). Higher percentage inhibition of radial growth in thread blight Type1 isolate was achieved, using all the fungicides (Table 4.15, Plate 4. 17 D).

Table 4.15:*In vitro* evaluation of fungicides against thread blight pathogens

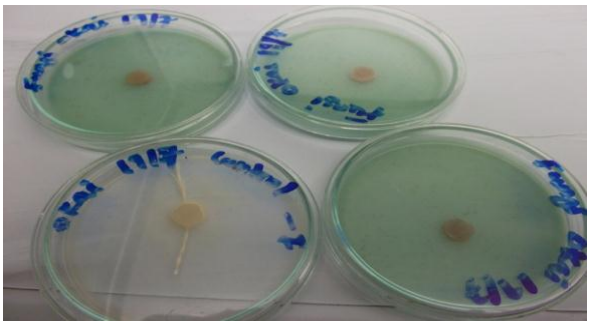
Treatments:/Mean colony diameter and percentage inhibition of mycelial growth of thread blight pathogens on PDA poisoned with different fungicides (cm/%) after 6 days of inoculation

Thread blight isolates	Concentration of fungicide(ppm)	Agro comet	Champion	Funguran-OH	Fungikill	Kocide	Nordox	Metalm	Ridomil Plus
TB 1	25	2.1 (74.0)	3.5 (56.8)	4.8 (40.7)	2.0(75.3)	3.7(54.3)	1.4(82.7)	1.2(85.2)	1.4(82.7)
	50	1.3 (83.9)	2.8 (65.4)	3.0 (63.0)	1.2(85.2)	2.3(71.6)	1.1(86.4)	0.1(98.8)	1.1(86.4)
	75	0.2 (97.5)	0.9 (88.9)	1.7(79.0)	0.0(100.0)	1.6(80.2)	0.0(100.0)	0.0(100.0)	0.5(93.8)
	100	0.0(100.0)	0.4(95.1)	0.7(91.4)	0.0(100.0)	0.7(91.4)	0.0(100.0)	0.0(100.0)	0.1(98.7)
LSD (5%)		1.2	1.4	1.1	1.3	1.2	1.5	2.3	1.6
Distilled water (cm)	0000	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
TB 2	25	3.2 (55.5)	3.3(54.2)	5.1 (29.2)	3.5(51.4)	4.2(41.7)	2.4 (66.7)	1.5 (79.2)	2.2(69.4)
	50	1.9 (73.6)	1.7(76.4)	4.7 (37.5)	2.9(59.7)	2.8(61.1)	1.6(77.8)	1.2 (83.3)	1.5(79.2)
	75	1.1 (84.7)	0.6 (91.7)	2.8 (61.1)	1.1(84.7)	1.2(83.3)	1.0 (86.1)	0.0(100.0)	1.2(83.3)
	100	0.5 (93.1)	0.1 (98.6)	1.3 (81.9)	0.4(94.4)	0.3(95.8)	0.1(98.6)	0.0(100.0)	0.3(95.8)
LSD(5%)		1.3	1.4	1.1	1.5	1.6	2.0	1.9	1.7
Distilled water (cm)	0000	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
TB 3	25	4.2 (39.1)	4.0 (42.0)	5.5 (20.3)	1.2(82.6)	4.5(34.8)	1.4 (79.7)	1.3(81.2)	1.5(78.3)
	50	2.8 (59.4)	2.7(60.9)	4.4 (36.2)	0.9(87.0)	3.1(55.1)	1.1(84.1)	1.1(84.1)	1.1(84.1)
	75	1.2 (82.6)	1.3(81.2)	1.6 (76.8)	0.0(100.0)	1.3(81.2)	0.0(100.0)	0.0(100.0)	0.1(98.6)
	100	0.5 (92.8)	0.3 (95.7)	0.9 (87.0)	0.0(100.0)	0.4(94.2)	0.0(100.0)	0.0(100.0)	0.0(100.)

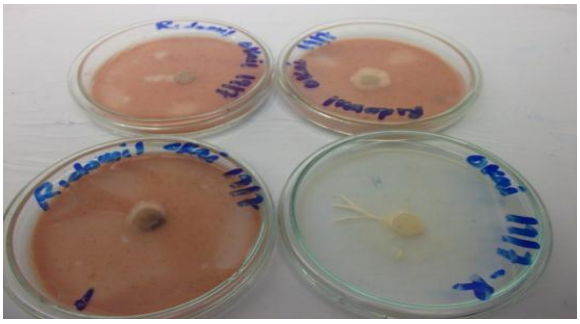
Table4.15:*In vitro* evaluation of fungicides against thread blight pathogens

LSD (5%)		1.2	1.0	1.3	1.6	1.4	1.8	2.1	1.7
Distilled water (cm)	0000	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
TB 4	25	2.7(50.9)*	3.5 (36.4)	4.0(27.3)	1.4 (74.5)	3.6(34.5)	1.5(72.7)	1.7(69.1)	2.7(50.9)
	50	1.9 (65.5)	2.1 (61.8)	2.8 (49.1)	1.0 (81.8)	2.4(53.8)	1.3(76.4)	1.3(76.4)	2.1(61.8)
	75	1.2 (78.2)	1.0 (81.8)	1.9(65.5)	0.0(100.0)	1.8(65.4)	0.5 (90.9)	0.3(94.5)	1.2(78.2)
	100	0.7 (87.3)	0.3 (94.5)	0.8 (85.5)	0.0(100.0)	1.0(81.8)	0.0(100.0)	0.0(100.0)	0.4(92.7)
		1.2	1.4	1.1	1.7	1.0	1.8	2.0	1.4
LSD (5%) Distilled water (cm)	0000	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
TB 5	25	1.6 (74.2)	4.1 (32.3)	4.0 (35.5)	1.4 (77.4)	3.7(40.3)	2.1 (66.1)	1.1(82.2)	2.2(64.5)
	50	1.0 (83.9)	2.8 (54.8)	2.8 (54.8)	1.1 (82.2)	2.5(59.7)	1.4 (77.4)	0.0(100.0)	1.5(75.8)
	75	0.0(100.0)	1.1 (82.2)	1.7 (72.6)	0.0(100.0)	1.5(75.8)	0.6 (90.3)	0.0(100.0)	1.3(79.0)
	100	0.0(100.0)	0.5 (92.0)	1.0 (83.9)	0.0(100.0)	0.8(87.1)	0.0(100.0)	0.0(100.0)	0.6(90.3)
LSD (5%)		1.4	1.6	1.2	1.7	1.1	1.9	2.0	1.6
Distilled water (cm)	0000	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
TB 6	25	2.3 (55.8)	1.4 (73.1)	4.4 (15.4)	1.2 (76.9)	3.8(26.9)	1.4(73.1)	1.4 (73.1)	2.0(61.5)
	50	1.5 (71.2)	0.9(82.7)	2.8(46.2)	0.6 (88.5)	2.7(48.1)	1.0(80.8)	0.8.(84.6)	1.7(67.3)
	75	0.5 (90.4)	0.2 (96.2)	1.5(71.2)	0.0(100.0)	1.2(76.9)	0.0(100.0)	0.0(100.0)	1.1(78.8)
	100	0.0(100.0)	0.0(100.0)	0.6(88.5)	0.0(100.0)	0.4(92.3)	0.0(100.0)	0.0(100.0)	0.2(96.2)
LSD (5%)		1.5	1.4	1.1	2.1	1.7	2.0	2.2	1.3
Distilled water (cm)	0000	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2

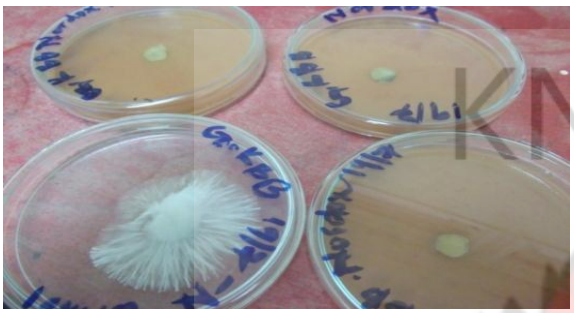
*Percentage inhibition in parenthesis

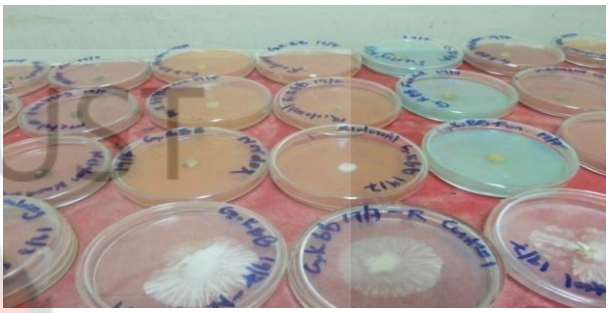


A) Fungikill-treated TB4 isolates

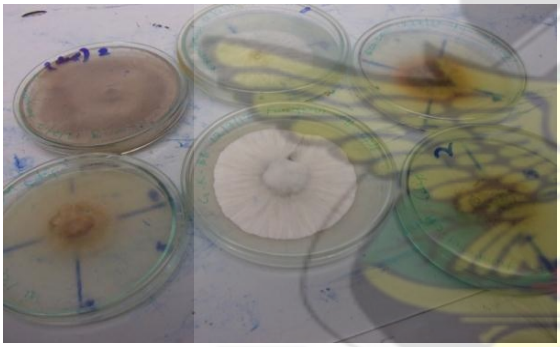


B) Ridomil-treated TB 5 culture





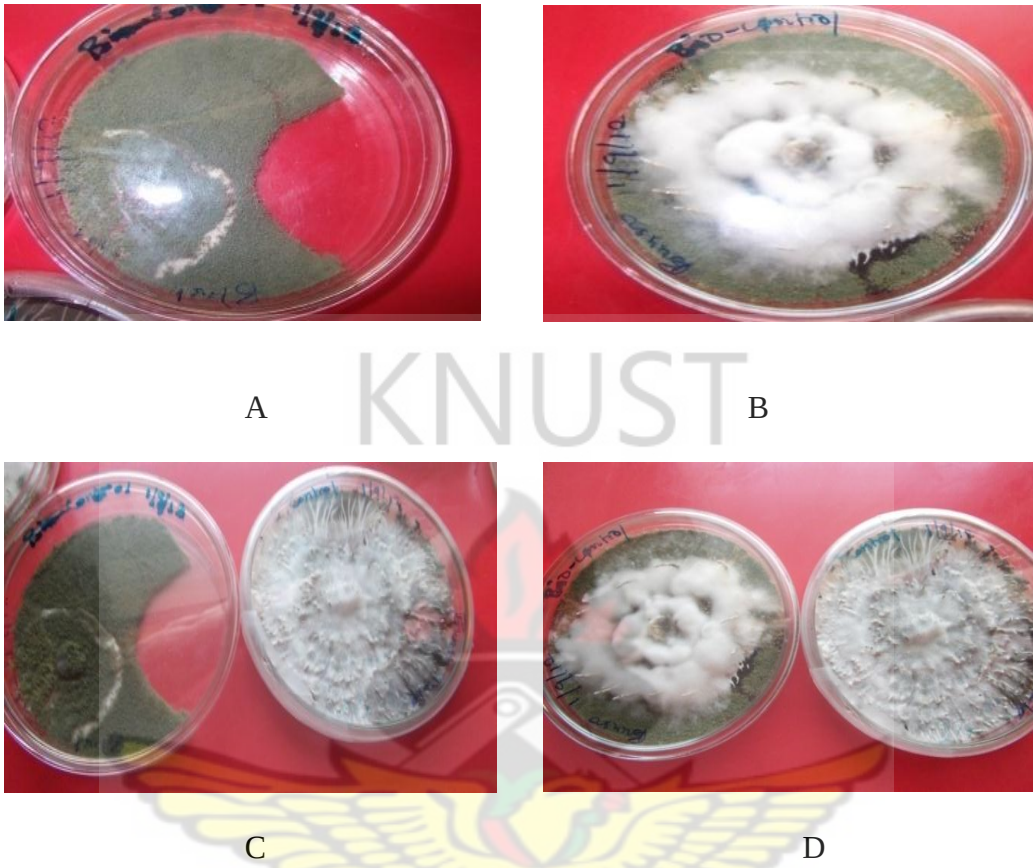
C) Nordox-treated TB 3 cultureD) Fungicide-treated TBs isolates



E)Funguran-OH-treated TBs isolates **F) Champion-treated TB 2 isolate**

Plate 4.16. Fungicides-treated cultures at various stages with control

4.23Biological control of thread blight pathogen using *Trichoderma*sp



A B

C D

Plate4.17 (A, B, C and D): Bio-control of thread blight pathogenwith *Trichoderma* sp.and control

The result obtained as shown in Plate4.18 indicated that, *Trichoderma* sp. was able to effect varying degrees of suppression of the mycelial growth of thread blight pathogens. *Trichoderma* sp. significantly suppressed TB 1 (89.40 % inhibition) as compared to other thread blight pathogens treated under the same condition (Table4.16). There was significant difference between TB 2(69.40 % inhibition) and TB 6 (65.27 % inhibition). TB 4 (44.22 % inhibition) and TB 5 (39.80 % inhibition) were found to be least affected by the *Trichoderma* sp. treatment (Table 16).

Table4.16:*In vitro* evaluation of *Trichodermaharzianum* against thread blight pathogens

Thread blight pathogens	Percentage Inhibition
TB 1	89.5
TB 2	69.4
TB 3	51.8
TB 4	44.2
TB 5	39.8
TB 6	65.3
S.E	1. 5
LSD (5 %)	4.5

Further morphological identification suspected the *Trichoderma* sp. to be *Trichoderma harzianum*. This organism has been worked on by early researchers (Lo *et al*. 1997) and has been identified as apotential bio-control agent for the control of pathogens that cause foliar diseases such as thread blight pathogen of cocoa. Interestingly, there was no infection which followed the pathogenicity test on all the test plants using *Trichoderma harzianum*. This showed that the organism identified was not pathogenic but only acts to suppress the radial growth and proper development of thread blight pathogens.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Field Survey: Assessment of thread blight disease incidence, severity and farmers' perception of the disease in the selected major cocoa growing areas in Eastern and Ashanti regions of Ghana

Majority of cocoa farmers interviewed were malewho fell within the active group. This could be due to the fact that, the work load involved in cocoa production is too heavy, hence attracting mostly men. Most cocoa farmers ranked diseases and pests as the major constraints to the production of cocoa. This observation is in line with the report by many researchers including Wood and Lass (1985), Acquaah (1999) and Wilson (1999) have published documentary evidence on incidence of cocoa pests and diseases as the major cause of low yield in cocoa production and the major problem confronting cocoa farmers in Ghana.

Thread blight disease observed on the farmers' fields affected mainly the stem but mostly caused necrosis in the leaves, thus killing the entire plant (Opoku*et al*., 2007). There was indication that necrosis only formed after the pathogen's hyphae have penetrated the leaf blade.This could be due to release of toxic exudate by the pathogen into the leaf tissue. Infected cocoa plants probably outgrow the thread blight infection after a period by replacing dead leaves with new ones but the plant succumbs to the infection again when the plant becomes overwhelmed by the aggressiveness of the pathogens. This results into massive defoliation in the infected plant, probably contributing to the low yields in cocoa production. This observation agrees with the findings of Asare-Nyarko(1997).

The survey revealed that thread blight disease was more pronounced in the farms that were managed by farmers engaged in sharecropping than farmers that managed their own farms. Inadequate cooperation on the part of farm owners to purchase inputs such as chemicals and fertilizers to manage the farm could be the cause of this problem. It was also revealed that majority of the cocoa farmers practiced mixed cropping, usinghost crops such as plantain, banana and oil palm as intercrops, aggravating the thread blight problem since these crops serve as alternative hosts.Benchimol*et al*. (2001) conducted a survey and detected damages caused by white-thread blight pathogen (*Ceratobasidiumstevensii* (Burt)) on more than 40 important agricultural and forestry plant species including coconut (*Cocosnucifera* L.)and banana (*Musa* sp. cv. yangambi), making evident the importance of that pathogen to the Amazon region. This might have exacerbated the incidence and severity of the disease on farmers' fields.

Disease incidence and severity were lesser in cocoa farms whose owners engaged in mono-cropping and practiced constant pruning of the cocoa trees. In mixed-cropping, the alternative host plants harboured the disease but showed little symptoms of the disease compared to cocoa trees. This probably explains why thread blight disease manifested more on cocoa plant due to its' susceptibility to the disease than any of the alternative host plants as observed on the farmers' fields.

The infection of healthy leaves of cocoa was mostly through direct contact with infected ones. Thus, the leaves stop to develop as soon as necrosis sets in andbecome dry and detached from the branch. The fungal hyphae can be easily removed without leaving any sign of the disease pattern on the leaf or on the infected

stem if necrosis had not started. This finding confirmed the report by Adedeji (2006) on thread blight disease on tea plant that necrosis only formed after the pathogen hyphae had penetrated the blade of tea leaf.

This study revealed six different types of thread blight pathogens on cocoa, and this is contrary to the four types that have been reportedearlierby Asare-Nyarko(1997). Among the six types of thread blights identified on the field, two types of white thread blight occurred most in all the farms. Apparently, they all cause defoliation symptoms in cocoa plant. Meanwhile, white thread blights (TB 1) appeared to be the most virulent, thus, causing massive defoliation in the cocoa plants.

5.2 Incidence and severity of thread blight disease

The high incidence of infection at Mapontenu in Fanteakwa District was unexpected. Although, this farming area is closer to the river Birim and lies within the wet semi-equatorial climatic zone with humidity as high as 65-80 % (File on Eastern region/district report, 2012), the presence of the river or rainfall alone cannot be the cause. This is because areas such asOsino, Mankyiaso, Anyinam, Kwabeng, and Bunso, are all closer to the river Birim and fall within the same rainfall belt, yet the difference between the infection figures for Manpontenuwas significantly ($\rho<0.05$) different as compared withthat of the five cocoa growing areas lying within the same rainfall belt.

The percentage infection at Old Tafo and Osiem in the East Akim District were also unexpected. This is because these two farming areas also fall within the same

rainfall belt with New Tafo and Kukurantumi, and here again the difference between the infection figures for Old Tafo and Osiem and that of New Tafo and Kukurantumi are significantly different. Rainfall pattern in the Eastern and Ashanti regions in 2010/2011 might have influenced the spread of the thread blights pathogen.This is because it is generally known that, most fungal pathogens strive well in the present of moisture, but the low percentage infection recorded at Juaso (1%) and Aboboaso (1%) in Asante-Akim South in the Ashanti region do not support it. Leston (1970) was amazed when he found thevast difference between the percentage infection of 6 to 48 % of thread blight pathogen in four cocoa farming communities in the same district.

It was revealed that thread blight disease was very severe with high incidence in more districts in the Eastern region than in Ashanti region. This could be due to the fact that the disease was first found in the Eastern region by the early researchers (Bunting and Dade in 1924). At that time, the disease was regarded as not economically important hence, little study was done on it to curb its spread in the region. The low percentage infection of thread blight in Ashanti region could be due to the fact that the numbers of farmers that cultivate one type of cocoa cultivar were more than those engaged in multi-cultivar cropping.

Contrary to the findings of Asare-Nyarko (1997), the disease was observed to infect both young and old cocoa plants, including the young cocoa seedlings, causing necrosis in both young and old leaves. However, necroses show early on the older leaves, which might be due to the direction of the spread of the pathogen from the affected branch to the leaves thus, the older leaves first get infected with the disease before it gets to the younger leaves.

Although the type of breed and age of infected plant might not be a factor in the determination of the disease incidence and severity, it was observed that the disease was more pronounced in the farms with matured plants than the farms with younger plants irrespective of the type of breeds. This could alsobe due to the fact that, the stomata of the older leaves on matured cocoa plants open wider and are easily penetrated by the hyphae of thread blight pathogen, thus causing a more severe necrosis in the older leaves than in the younger ones.This observation was also documented by Adedeji(2006) on thread blight on tea plant. Low percentage infection and severity in the Asante-Akim South and North indicated that the infection had just started in few farms in the districts. Thus, there is a need to help curb the spread of the disease in these districts.

5.3 Farmers' perception about the thread blight disease and disease control

Majority of the farmers had no local name for the thread blight disease and also had no idea about the cause of thread blight disease in their farms. Probably, most of them did not know the disease and its effect on their farms.

Although the effect of thread blight disease in some of the farms was more severe than the effect of black pod disease, farmers see black pod disease as the major and most important disease problem affecting cocoa production. This is because unlike thread blight disease, black pod disease affects the cocoa pod directly, resulting in low yield and quality of cocoa beans. This agrees with the observations by Oluye and Lawal (2008) who reported that black pod disease is the most serious disease of cocoa.

Defoliation associated with thread blight disease also may be contributing to low yield and in severedeath of the cocoa plants. However, because farmers have little knowledge about the disease, its importance is not much realized.As a result of that, most farmers did not have any form of control measures for the disease. Moreover, majority of them sprayed their farms against other forms of diseases and pests such as black pod and mirids but not against thread blight disease.

5.4 Identification of fungi from diseased samples

Thread blight of cocoa has been thought to be caused by *Marasmius*sp.(Wood and Lass, 1985; Asare-Nyarko, 1997). According to Opoku*et al*. (2007), white thread blight of cocoa in Ghana is caused by *Marasmiusscandens* (Murrill) and the black thread blight is caused by *Marasmius bysicolar(*Petch). Dennis and Reid (1957)also reported that white thread blight on cocoa is caused by *Marasmiellusscandens* which is the only preferred name, whilst *Marasmiusscandens*(Massee) and *Marasmiusbyssicolor*(Petch) are used as synonym.

This study has shown that white thread blight on cocoa is caused by two different genera, *Marasmius* and *Marasmiellus* and not by onegenus. This is contrary to the findingsof Opoku*et al*. (2007) and Denis and Reid (1957). According toSinger (1986),there are differences between genus *Marasmius* and *Marasmiellus*.He stated that‘‘*Marasmiellus*differs from *Marasmius"*in the structure of theepicutis of the pileus and the inamyloid (nondextrinoid) hyphae(in response to potassium iodide, amyloid cells stain blue, whereas dextrinoid cells stain reddish-brown).

The present study also revealed that,there are differences in both the structure and the mode of formation of the spores of *Marasmius* and *Marasmiellus*. Though both species formed fruiting bodies prior to their spore formation, the structure of the fruiting bodies of *Marasmius* and *Marasmiellus* are also different. One consists of a complete pileus with stipe, and the other consists of a fragment of a pileus. This agrees with the observation made by Hibbett*et al*. (1997) who reported on the differences between the two genera *(Marasmius* and *Marasmiellus*)and went further to establish differences between theirspores;that the former has clustered form of spores whilst the later has the spores scattered when viewed under stereo microscope.

Morphological and physiological studies have shown that, white thread blight TB 1is caused by*Marasmius* sp. and white thread blight TB 2 is caused by *Marasmiellus* sp. Brown thread blight TB 3 and thetwo types of cream thread blights (TB 4 and TB 6) are believed to belong to genus *Marasmiellus* but may differ in species. Black thread blight which was thought to *be* caused by *Marasmius bysicolar* (Petche) may also have differentview based on its morphological features. This might be caused by *Marasmiellus* and not *Marasmius*as stated by early researchers.

However, due to the lack of informative morphological characters on the thread blight pathogens of cocoa and the present controversy over the genera *Marasmius* and *Marasmiellus* with regard to the genetic compatibility and relationship among the thread blight fungi. It is difficult to correctly identify the remaining four types of the thread blight pathogens (TB 3, TB 4, TB 5 and TB 6) as *Marasmius* or *Marasmiellus,* unless molecular techniques settle their differences.

5.5 Colony characterization and best mycological medium support of thread blight pathogens isolated from seven selected cocoa growing districts in both Ashanti and Eastern regions

Mycelium can adopt light or cottony type of mycelial growth, on the basis of availability of nutrition, although it differs from species to species and strain to strain (Maheshwari*et al*., 2001)

Poor mycelial density observed is as a result of poor media used, thus GCMA medium and WA did not support the mycelia growth of thread blight fungi, because they are poor in nutritional value (Sharma *et al*., 2010). PDA served as general purpose medium and supported mycelial growth to a certain degree, but could not depicts othercolony features including the texture and colony pigmentation among the cultures hence could not be described as the best medium for thread blight pathogens. Although MEA, V8 and BA did support mycelial growth and helped depicts some of the colony features, they could not support mycelia density of the tested fungi. However, PA and OA media were able to support all the colony characters with high mycelial density, thus were described as the rich and the best mycological media for thread blight pathogens. This agrees with the observation made by Sharma *et al.* (2010) that, rich medium best supports the growth and the mycelial density of a fungal pathogen.

It was observed that dark incubation led to rapid radial growth compared to the light incubation. Light might have exerted some degree of inhibitory effects on mycelial growth rate of thread blight isolates. Mycelial growth in other organisms were negative phototropism (degree of light sensitivity) under constant light, and that of TB 2 isolate was positive phototropism hence they are able to produce spore under the constant light.

5.6 Mushroom production technology.

Thread blight of cocoa include mushroom forming-fungi, thus could best be described as homobasidiomycete (Hibbett*et al*., 1997). This is based on the morphological characters observed from the fruiting bodies that were formed from the thread blight pathogen of cocoa. This agrees with the observation made by Hibbett*et al.* (1997) who reported that, formation of fruiting bodies are the most conspicuousphase of the homobasidiomycete life cycle and encompassa broad spectrum of morphological variation. Thus, this technique re-affirmed that, thread blight pathogen of cocoa is caused by different species of fossil mushrooms that are closely related but exhibit different morphological characters (Singer, 1986). Mushrooms grow well when the right conditions are provided. Thus, delay in TB 5 and TB 6 to growth into mushroom could be as a result of differences in their genetic make-up.

5.7 Confirmation of pathogenicity test of isolated fungi on one year old and six-months-old cocoa seedlings and three suspected alternative host of the disease in a moist chamber

Pure cultures of *Aspergillus* sp. and *Trichoderma* sp. did not produce thread blight symptoms on both the stem and the leaves of cocoa seedlings, suggesting that, they are secondary pathogens and are not responsible for the thread blight disease. Pure culture of unidentified fungus was frequently isolated from the necrotic parts of infected leaves and stem when PDA was used. However, its pure culture could not give the well-marked threadlike strand of mycelial symptom observed in the field. Thus, it could be possible that, this organism was a saprophyte. The same observation was made by Adedeji (2006) on tea plant.He reported about two fungi (*Botryodiplodiatheobromae*and *Fusarium sp.*) that were frequently isolated from the necrotic parts of infected leaves on tea plant when potato dextrose agar was used.

However, none of them gave the well-marked thread network symptom observed in the field. Thus, it is possible that these organisms were saprophytes.

The whitish thread-like strands of mycelia produced on the test plants by the test fungi and brownish necrosis on the leaves similar to those found on the field suggest that these organisms are responsible for the thread blight disease on the cocoa plant. Although Brown thread blight (TB 3) and Black thread blight (TB 5) produced white thread-like mycelia on the individual test plant at the initial stage of the infection, they, however, turned brown and black thread-like mycelia as they aged on the test plant.

The high percentage infection produced by thread blight pathogen type one (TB 1) on all the test plants indicated that pathogen TB 1 was more virulent and could cause massive defoliation in the field. Again,100 % infection caused by TB 1 on all the suspected host crops also suggested that, these crops were alternative hosts for this particular pathogen. However, zero percent recorded by other thread blight pathogens on the suspected host crops could be due to other factors such as host plant resistance, low levels of pathogen virulence, low moisture content or low humidity leading to the failure of the disease establishment with the alternative host crops as documented by Agrios (2005). Arguably, it may bebecause these suspected host crops were not alternative hosts for these types of thread blight pathogens.There was no infection on the control plants. Meanwhile, pure cultures were produced when re-isolation was done thus, Koch's postulates helped to obtain the six different types of pathogen causing thread blight disease on cocoa.

Cocoa seedlings that were inoculated with thread blight inoculum from plantain, banana and oil palm plant produced similar symptoms to those found on the plantain, banana and oil palm. Alternatively, thread blight inoculum from cocoa that were inoculated on plantain, banana and oil palm also showed similar symptoms. Re-isolation of these isolates gave the same fungal features used for inoculation. Therefore, pathogenicity test based on Koch's postulates produced positive results.

5.8Fungicide bioassay*in vitro*

The results revealed that the efficacy of the fungicides varied with concentrations. Higher concentration of each fungicide inhibited to a certain degree, radial growth in all the six organisms (Oke, 1986). The significant difference ($\rho<0.05$) observed in the inhibition of mycelial growth of the test organisms revealed higher efficacy in the fungicides with increasedconcentration. Thus, Metalm, Fungikill and Nordox were found to be the more effective than all other fungicides used.These observations agreed with those of Jamadar and Lingaraju (2011), who reported inhibition of mycelial growth of *E. ampelina*-Ea3 with various concentrations of chlorthalonil, propineb, mancozeb and Copper oxychloride. They also found out that chlorthalonil was more effective than the other fungicides used, whilst Copper oxychloride was less effective among all the fungicides used.

It is possible that, some of the thread blight pathogens might be more equipped in terms of ability to metabolise and detoxify toxic substances in the fungicides.Thus, three of thread blight pathogens (TB 2, TB 3, and TB 4) were found to exhibit mycelial growth over a wide range of fungicide concentrations than in TB 1, TB 5

and TB 6 pathogens. This may explain why more than 50 % of the fungicides used *in vitro*in this study, which are mostly used by the farmers could not suppress the growth and the spread of the thread blight disease on the field.This may also, have its explanation in the type and efficiency of metabolism occurring in the different organisms.Amadi (1998) reported that most fungicides used directly*in vitro* tend to match their performance *in vivo*.

The survey conducted revealed chemicals used by thetargeted cocoa farmers on their farms.Unfortunately, the three promising fungicides evaluated *in vitro* in this study werethe least mentioned among the fungicides used by these farmers. This may be the reason why thread blight pathogens were not effectively controlled.However, farms that were frequently sprayed with fungicidestogether with other cultural practices recorded low percentage of the thread blight disease incidence as compared to farmsthat were not frequently sprayed.Pruningof infected twigs and leaves may be the most effective control measure in controlling this disease, as observed by Adedeji (2006),who studied white thread blight on tea plant and suggested that the effective way to control white thread blight on tea plant is by pruning.

5.9 Biological control of thread blight pathogens*in vitro*

The laboratory study of thread blight pathogen revealed that *Trichodermaharzianum*affected the growth of thread blight pathogens, indicating this organismas a potential bio-control agent for the control of thread blight pathogens.Cook and Baker (1983) broadly defined biological control of plant pathogens as "any control achieved through a living system". Spurr (1985) proposed that, the concept to identify and develop one bio-agent to control one pathogen is

usually retained in succeeding research, thus, the idea of identifying and developing suspected bio-agent (*Trichodermaharzianum*) as bio-control agent for thread blight pathogen is in the right direction.

According to Lo *et al*. (1997), *Trichodermaharzianum* has been shown to significantly inhibit disease severity of some plant diseases during the initial stage of disease development. This study revealed that thread blight pathogens are mostly spread from the infected plant to healthy plant through leaf contact. Thus, its control requiressuppression of the initial infectionto enhance reduction of the infections rate. This agrees with the observation made by Lo *et al*. (1997) thatmany plant pathogens spread readily in the foliar parts, and control of these diseases requires both suppression of initial plant infection and reduction of infectious rate.

Due to the problems associated with chemical application to control plant disease, biological control of plant diseases has been a core mark of numerous research projects in recent years ((Heydari and Pesseraka, 2010). Thus, the development of non-chemical alternative strategies to control plant diseases, including thread blight pathogens, is important. However, successful development and application of thisstrategy requires more knowledge and intensive management. Researchers like Baker (1987), Cook (1993) and Heydari (2004) have all stated in their various reports that successful application of biological control techniques requires more knowledge-intensive management.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Allthe cocoa farmers interviewed on thread blight disease acknowledge the disease problem. This study has shown that thread blight disease is emerging as one of the major diseases of cocoa. The disease is prevalent throughout the year and is more severe in farms with older cocoa plants.Farms with poor cultural and sanitary farming practicesare likely to beinfected with the disease.

The study reviewed that the infection of healthy plantswas either through direct contact with infected leaves or twig or through the sticky hyphal clump, which extends from the dead detached and loosely hanging leaves.It also revealed three alternative hostsnamely banana, plantain and oil palm as contributing to the spread of the disease.

The study showed the current incidence/percentage infection of thread blight disease of cocoa in Ghana and that rainfall alone cannot be the cause. The thread blight pathogen was found to infect both young and old leaves; however, necrosis appeared early on the leaves. Sixdifferent types of thread blight were found to affect cocoa of which some sporulatedon agar medium and others did not.Further techniques revealed that all thread blight pathogens produced spore when they form fruiting body. White thread blight of cocoa was shown to be caused by two genera *Marasmius* and *Marasmiellus* and are closely related in their mode of infection; but are different, based on their physical and morphological features.

Differentculture media were found to influence the growth, colony character and sporulation of thread blight pathogens. OMA and PA were the most suitable for heavy sporulation in all the thread blight isolates in group two (TB 2). MEA and

PDA reproduced most visible colony morphologically.Study showed that, combination of two or more of the media is more appropriate for routine cultural and morphological characterization to observe different colony features.

The pathogenicity test conducted revealed that, the disease affects both the young and old cocoa plants, including seedlings.The disease can be prevented throughthree different means, culturally; through effective pruning, chemically; application of Metalm and Fungikillfungicides and biologically; use of *Trichodermaharzianum*.

6.2 RECOMMENDATIONS

- Farmers should be educated on the signs, symptoms and effects of thread blight disease on cocoa
- Farmers should be advised not to intercrop their cocoa plants with suspected alternative host crops suchas plantain, banana and oil palm
- Pruning should be done immediately the disease is noticed and the pruned parts should be burnt outside the farm to avoid re-infection of healthy cocoa trees.Regular monitoring should be carried out to detect any further re-infection of the healthy twigs and leaves
- Further studies should be conducted on the alternative hosts of the thread blight pathogen in other cocoa growing areas in Ghana
- The role of insects (black ants) in the transmission of the causal agent(s) of the disease should be investigated
- Further study on economic effect of thread blight disease on yield should be conducted
- Further identification of the pathogen through mushroom production technique should be undertaken

- Field trials should be done to evaluate the effectiveness of the fungicides Metalm, Nordox andFungikill and *Trichodermaharzianum*, the biocontrol agent.

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APPENDICES

Appendix 1 QUESTIONNAIRES

PERSONAL DATA

1. Name of the Farmer ……

2. Age……

3.Sex……

4. Marital status ……

5. Household size ……

6. How long have you been farming? ……..…..

7. Apart from cocoa, what other crops do you produce? …….

8. What is the size of your farm? …….....

9. How old is your farm? ……

10. Where do you get your seedlings? ……

11. What type breed? ……

12. What is your planting distance?……

13. Apart from money, what other problems do you face in cultivation? .....

14. What diseases affect your cocoa farm? …….....

15. What about thread blight? ……...

16. How is it called locally?..……

17. Have you been observing white or black thread blight symptoms?……..

18. When do you start noticing the disease?……

19. Do you see it on other plants/tree around?…….

20. White or black thread which one is common? ……

21. How does it start? ……

22. What do you think causes that disease?.....……

23. Do you think environmental condition contributes to this disease incidence?…..

24. Do you see the symptoms on the nursery plant as well? .....

25. Do you think the disease is important? ……Yes/No……

26. If important how do you control it? ……

27. What type of chemicals do you use? …….....

28. How often do you apply pesticides to control pests and diseases? …….

29. Is the spraying helping with the disease control? ……Yes /No……..

Appendix 2: Categories of different agar media used in this study

Category	Full names of media along with their abbreviations	Reference
Poor media:	Water agar (WA)	Stevens (1981)
General purpose media	Oatmeal agar (OMA)	Stevens (1981)
	Malt extract agar (MEA)	Atlas (1993)
	V8 Juice agar (V8)	Johnston and Booth (1983)
Rich media	Potato Dextrose agar	Johnston and Booth (1993)
Others	Green cocoa mucilage agar (GCMA)	KNUST
	Plantain agar (PA)	Lekete (2012)
	Banana agar (BA)	Lekete (2012)

Appendix 3: Composition and concentration (g/l) of culture media used in this study

	Media							
Components	WA	PDA	MEA	V8	OMA	GCMA	PA	BA
Dextrose		30	20					
Malt extract			20					
Potato		200						
Oatmeal flake					100			
V-8 juice				200				
$CaCO_3$				3				
NaCl(%)						10		
Fresh cocoa mucilage						400		
Fresh plantain							200	
Fresh banana								200
pH	6	6	5.5	6	6	6	5.5	5.5
Agar	20	20	20	20	15	20	15	15
Chloramphenicol(mg)	250	500	500	500	500	500	500	500

Refer to Appendix 2 for the abbreviation of media

Appendix 4: The severity score range of thread blight disease in seven cocoa growing Districts

Districts	Severity score (range)	Disease classification
Ash.akim-S	0-1	Very mild
Ash.akim-N	1-2	Mild
Effiduase	1-3	Severe
Atiwa	2-4	Very severe
Fanteakwa	2-4	Very severe
East akim	2-4	Very severe
N. Juabeng	1-3	Severe

Appendix 5:Diseases and pest found in cocoa farms based on Location

Areas visited	**Percentage of Disease found (%)**						
	Blackpod		CSSV	Thread blight	Pink disease	Stem cankers	Others
Ashanti-Akim S	**87.5**	**Farmers**		**11**		**25**	**53.5**
Nkwanta	75	12	-	8	-	50	50
Aboboaso	100	7		14	-	-	57
Ash. Akim N.	**72.5**			**65.5**		**45.5**	**34.5**
Mensahkrom	85	7		71	-	71	29
Okaikrom	60	4		60	-	20	40
Effiduase	**67.5**			**57.5**		**30**	**45**
Senyre east	60	5	-	40	-	60	50
Adadieja	75	4	-	75	-	-	40
Fanteakwa	**28**		**42.7**	**90.3**	**40.7**	**50**	**25.7**
Osino	14	7	43	71	57	-	57
Mapontenu	20	5	60	100	40	100	20
Makyiaso	50	4	25	100	25	50	-
Atiwa	**66**		**13.3**	**93**	**69.7**	**56.3**	**22**
Anyinam	56	9	22	89	78	89	44
Kwabeng	78	9	-	100	67	44	22
Akyem-Senyre	64	11	18	90	64	36	-
East Akim	**80.4**		**9.2**	**85**	**17**	**28**	**33.6**
Kukuratumi	62	13	23	77	-	31	38
New Tafo	93	12	8	93	-	42	33
Old Tafo	93	14	-	93	-	21	36
Osiem	77	13	-	85	-	23	38
Bunso	77	13	15	77	85	23	23
N.Juabeng							
Jumapong	55	11	18	73	27	54	18