AETIOLOGY, ECONOMIC IMPORTANCE AND CONTROL OF MANGO (*Mangifera indica* L.) TREE DECLINE DISEASE IN NORTHERN REGION OF GHANA

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY (MPHIL) CROP SCIENCE DEGREE

JULY, 2016

DECLARATION

I, Fred Kormla Ablormeti do hereby declare that except for references to other people's work which has been duly acknowledged, this work is the result of my original research and that this thesis has neither in whole nor part been presented for another degree elsewhere.

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DEDICATION

This thesis is dedicated to the glory of the Almighty God and to my lovely and caring mother Elizabeth Gedze and my siblings as well as those who live in my mind, and my heart throughout the whole span of my life and are nearest, dearest and deepest to me. Lastly, to my lovely future wife Elizabeth Akpene Agenah.

ACKNOWLEDGEMENT

Praise and glory be to the most-high God for the successful completion of this work. My success in this research work is by the grace and mercies of El-Shaddai. I am most grateful to you my Lord.

A number of individuals have made valuable contributions towards the successful execution of this study. Although time and space may constraint me in mentioning every Tom, Dick and Harry, I believe I owe some few people a moral duty to appreciate.

My heartfelt appreciation goes to my relentless supervisors: Dr. J. O. Honger and Dr. E. W. Cornelius for their keen scholastic supervision, constructive suggestions, useful criticisms, kind cooperation and inspiration in planning and execution of this research work. To this end, I say thank you all.

My heart also goes out to my entire family especially my lovely mum, Miss Elizabeth Gedze and all my siblings as well as my lovely wife to be Miss Elizabeth Akpene Agenah for their constant encouragement and motivation given me. Unto them I say “Ayiko”.

I am highly grateful to The Office of Research and Innovation Development (ORID) for sponsoring this research work.

I would also like to use this opportunity to express my unfettered gratification to Mr. Isaac Badu, Mr. Kinsley Ochar and Mr. Eric Twum for their immense contributions towards this work. May you guys never lack.

To many others whose names have not been mentioned but played diverse roles towards the completion of this research work, I say may God bless you all.

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LIST OF ABBREVIATIONS

BLAST:	Basic Local Alignment Search Tool
CPDA:	Carbendazim Potato Dextrose Agar
CRD:	Completely Randomized Design
df:	Degree of Freedom
DI:	Disease Incidence
DNA:	Deoxyribonucleic Acid
DNTP:	Deoxynucleoside-triphosphate
EDTA:	Ethylenediaminetetraacetic acid
EU:	European Union
FAO:	Food and Agricultural Organisation
FAOSTAT:	Food and Agricultural Organisation Statistics
FPDA:	Funguran Potato Dextrose Agar
GENSTAT:	General Statistics
GEPC:	Ghana Export Promotion Council
GFPED:	Ghana Fresh Produce Exporters Directory
GNA:	Ghana News Agency
HR:	Relative Humidity

ITS:	Internal Transcribed Spacer
JHS:	Junior High School
LSD:	Least Significant Difference
MPDA:	Mancozeb Potato Dextrose Agar
MS:	Mean Square
MSLC:	Middle School Leavers Certificate
MT:	Metric Tonnes
MTDD:	Mango Tree Decline Disease
PCR:	Polymerase Chain Reaction
PDA:	Potato Dextrose Agar
PPDA:	Prochloraz Potato Dextrose Agar
PXE:	Pseudoxanthoma Elasticum
RCBD:	Randomized Complete Block Design
rDNA:	Recombinant DNA
S80PDA:	Sulphur 80 Potato Dextrose Agar
SI:	Severity Index
SIREC:	Soil and Irrigation Research Centre
SPSS:	Statistical Package for Social Science

SS: Sum of Square

TAE: Tris-Acetate-EDTA

TIPCEE: Trade and Investment Program for a Comparative Export Economy

USAID: United State Agency for International Development

WA: Water Agar

ZPDA: Zamir potato dextrose agar

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ABSTRACT

This research was conducted from May, 2015 to July, 2016 in the three mango growing districts of the Northern Region of Ghana to obtain baseline information on farmers' knowledge and perception on prevalence, spread, economic importance and control of mango tree decline disease; determine disease incidence and severity; identify the causal agent(s) of the disease and to develop a chemical control regime for management of the disease. Questionnaire survey and interviews were used to gather information from eighty-four (84) mango farmers selected at random in the three mango growing districts in the Northern Region of Ghana. Disease incidence was determined by counting both healthy and infected mango plant. Disease severity was determined across seven mango farms in each of the experimental communities using a 0 to 5 disease assessment key. Isolation of the causal organism(s) from advancing margins of diseased tissue was done first on Water Agar (WA) and sub-cultured onto a Potato Dextrose Agar (PDA). Identification was carried out using both morphological and molecular techniques. The pathogenicity of three fungal isolates were tested with an incision made on ten months old mango seedlings and mycelia plugs of each isolate inserted singly into the incision made on the stem. There were five mango seedlings per fungal isolate with control. Six fungicides with three levels were evaluated *in vitro* for their inhibitory effects on mycelial radial growth of the disease causal organism on Potato Dextrose Agar amended with three recommended rates of each fungicide in Completely Randomized Design with three replicates. Field evaluation of three fungicides selected from the *in vitro* trial was carried out in Randomized Complete Block Design with five plants per fungicide treatment with four replicates. Data was collected on disease incidence and severity before each fungicidal application at two (2) weeks intervals and vegetative growth of plants. The study revealed that, all the farmers (100%) were aware of the occurrence of the mango tree decline disease but were unable to identify the causal agent(s) as well as the agents for spread of the disease within and among farms. Again, mango farmers were replacing their infected mango trees with arable crops such as groundnut, maize, yam and cassava. Disease incidence was generally high in all the three mango growing districts: Karaga (77.9%), Savelugu-Naton (77.1%) and Kumbugu (63.9%). However, Savelugu Nanton municipality recorded the highest disease severity (2.57) followed by Karaga district (2.0) and then Kumbugu district (1.42). Disease symptoms such as necrosis of leaves on small branches, gum exudation, bark splitting, vascular discolouration as well as defoliation of leaves were observed during the field visits. The pathogen causing the disease in the study area was identified as *Lasiodiplodia theobromae.* During the pathogenicity test, *L. theobromae* produced characteristic symptoms of mango tree decline disease. All the fungicides evaluated during the *in vitro* trial completely inhibited mycelial radial growth of *L. theobromae* except for Sulphur 80. Application of urea fertilizer to boost tree growth alongside spraying of Carbendazim (50 g/15L water) at two weeks spraying interval was effective in controlling the disease. The study revealed that mango tree decline disease is prevalence in the three mango growing districts where the study was carried out.

CHAPTER ONE

1.0 INTRODUCTION

Mango (*Mangifera indica* L.) is an important tropical fruit crop belonging to the family Anacardiaceae in the order spindales and commonly cultivated in many tropical and sub-tropical regions of the world. It is the second most important tropical fruit crop in terms of production and acreage (Muchiri *et al*., 2012). Over 1000 mango varieties exist globally and only a few assume commercial scale production and are therefore traded (Solís-Fuentes and Durán-de-Bazúa, 2011). Approximately 77 % of the world's mangoes are produced in Asian countries while 9% and 13% are produced in African and American countries respectively. The estimated world mango fruit production is over 26 million and approximately 912 853 metric tonnes of mangoes were exported worldwide in 2005 (FAOSTAT, 2007).

Globally, commercial production of mangoes occurs in more than 103 countries and production is increasing each year due to increasing consumer demand and its outstanding sensory therapeutic and nutritional properties (Bicas *et al*., 2011). It has been well recognized that mango fruits are of an imperative source of micro-nutrients, vitamins and other phyto-chemicals. Mango fruits are also noted for their provision of energy, proteins, dietary fibre, carbohydrates, fats and phenolic compounds which are crucial to normal human growth, development and health (Tharanathan *et al*., 2006).

The prominence of mango to many Ghanaians results in the description of the crop as “Golden tree”, “gold mine”, “next cash crop”, “Ghana’s future”, amongst others (GFPED, 2007). Despite these accolades, Ghana’s mango industry is still in an infant stage and has not developed extensively with the times. The productivity and exports of mango are low hence contributed only 0.3 % of total agricultural exports in 2009 which was valued at US$ 234,950 (Abu *et al*., 2011).

Between January and December 2007, Europe's import of mango from Ghana was 1 071 tonnes, signifying only 1 % as Ghana's stake on the EU market. Nevertheless, the country recorded the highest annual growth (73 %) as well as the highest total growth (79.9 %) amongst the exporters of fresh mango fruits within the period suggesting that mango has the potential as a foreign exchange earner in Ghana (Abu *et al*., 2011).

The crop is found all over Ghana but commercial production occurs in the Guinea Savannah (Northern Region) and Coastal Savannah zones of the country (Greater Accra, Volta and Eastern Regions) (FAO, 2009). Commercial mango production is also carried out in the Brong-Ahafo and the Ashanti Regions (Jaeger, 2008). The potential for the Northern Region of the country to play a leading role in the mango industry is very paramount due to its favourable climatic conditions. In recent years, over 2000 mango farmers were financially and technically supported to access quality mango seedlings to establish and expand the mango plantation in Northern Ghana. Interventions such as this would go a long way to increase mango production in the Northern Region and Ghana as a whole (Tettey, 2008).

Mango production in Ghana over the years is beset with myriad of economically important diseases such as anthracnose caused by *Colletotrichum gloeosporioides*, die-back caused by *Botryosphaeria theobromae* and fruit spot caused by *Gloesporium mangiferae* (Oduro, 2000). Currently, many mango trees or parts of trees began wilting and dying and spreading steadily in some mango farms in the Northern Savannah zone of Ghana. The disease is characterized by sudden collapse of severely affected mango trees. The disease symptoms initially appear as gummosis from the stem bark and branch decline on affected trees followed by vascular discolouration beneath the bark. This disease sometimes shows no easily recognized external symptoms except stunting which may be apparent on the field. This description befits mango tree decline disease caused by a fungus called *Lasiodiplodia theobromae*. Masood *et al*. (2010) observed that mango tree decline disease usually causes the death of affected trees within six months of first symptom appearance. Al-Adawi *et al*. (2006) and Saeed and Masood (2008) further noted terminal and minimal necrosis of leaves which finally lead to the death of leaf blade of diseased mango trees. The above-mentioned symptoms may be found alone or in combination in different mango orchards (Ploetz *et al*., 1996; Iqbal *et al*., 2007)

The appearance of the disease in the country and its subsequent threat to local mango production requires that effort should be made to control the disease. Currently, no management measures have been put in place by farmers and Agriculture Extension Agents because the causal agent(s) and epidemiology of the disease are not known (Honger personal communication, 2015)

The devastating effect of this disease on mango production calls for a comprehensive research to identify the cause(s) and economic importance in order to develop sustainable disease management practices to increase productivity, raise incomes of farmers and alleviate poverty. This will also facilitate further studies on the epidemiology of the disease. The objectives of this study therefore were to:

- assess farmers' knowledge and perception on prevalence, spread, economic importance and control of mango tree decline disease.
- determine the incidence and severity of mango tree decline disease.
- identify the causal organism(s) of the disease.
- develop a chemical control regime for management of the disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. The mango plant, origin and distribution

2.1.1 The mango plant

Mango (*Mangifera indica* L.) plant, a member of Anacardiaceae family is reported to have originated from India and found to be widely dispersed throughout the world. Mango evolved as a canopy layer species in the tropical rainforest regions in South and South-East Asia (Kaur *et al.*, 1980). Typically, the crop is cultivated in the tropical and the sub-tropical regions of the world. It is predominantly produced for the fresh consumption of its fruits as well as a raw material in the processing industries where the juice, flavour, fragrance and colour are highly exploited. Mango fruits are large, fleshy drupe and contain edible mesocarp which is variable in thickness. They are also variable in size, shape and colour as a result of cultivar differences. Mature and ripened fruit colour may be yellow, orange, red or green as a result of genotypic differences (Abu, 2010).

As a perennial tree species, the tree can grow to a height of about 30-40 m and a crown radius of about 10 m (Litz, 2003). A surviving tree can stay with fruit bearing for over 100 years. The canopy is oval, elongated or dome shaped and the flowers are positioned on the terminal inflorescence which is described as being broadly conical. Two flower forms (hermaphrodite and male) can be observed on the same inflorescence (Abu, 2010).

2.1.2 Origin and distribution of mango

With uncertainty of the origin of mango, historical and palaeobotanical records show enough evidence that mango originated from Indo-Burma-Malay region. The availability of wild form of mango and its related species and numerous cultivated varieties in India were some of the principal reasons in favour of mango to have originated from the Indo-Burma region (Vavilov, 1926). The presence of maximum number of allied species growing in malaysia, made some school of thought to have believed that Malaysian region is the original home of Mango (Pavani, 2009).

Man has been noted as the chief promoter of the spread of mango. Watson and Winston (1984) attributed the distribution of mango outside their origin to three historical events: (i) the movement of monoembryonic or Indian varieties along the trade routes of the Portuguese to Africa and South America; (ii) the spread of southeast Polyembryonic which is Asian varieties through the Pacific Ocean to Central and South America by the Spaniards; and (iii) the formation of a secondary centre of mango diversity in South Florida due to the systematic introduction of mango germplasm from India and Southeast Asia. Records have it that a Chinese traveler (Hwen Chang) who visited India between 632-645AD was the first person to have carried mango from its origin to the outside world. Mango became established in Somali land in the eastern coast of Africa before 1331. It reached the Persian Gulf in the 16th century and in 1960 it was cultivated under Greenhouse in England (Pavani, 2009).

2.2 World mango production

Mango production continues to increase in terms of acreage across all mango growing regions in order to meet the demands for the fresh consumption of the fruits at both local and international markets due to the attractive nutritional component present in the fruits. The crop is under cultivation in over 103 countries globally and that there is an increasing demand for the fruits which has resulted in expansion of production areas (Bicas *et al*., 2011). Though, several varieties of the crop have been reported to be under cultivation, only a few are used for commercial production and trade (Solis-Fuentes and Duran-de-Bazua, 2011). FAOSTAT report indicates that about 77 % of the global mango production occurs in Asian countries, 13 % in the American countries and 9 % in the African countries (FAOSTAT, 2007). It is estimated that 26 million tonnes of mango fruits are produced annually (FAOSTAT, 2007). The world's major mango producing countries are India, China, Thailand, Pakistan, Mexico, Indonesia, Brazil, the Philipines, Kenya, Egypt, and Nigeria (Table 2.1).

Table 2.1 Major mango producing countries in the world

Producing Country	Production (MT)
India	16,337,400
China	4,351,593
Thailand	2,550,600
Pakistan	1,784,300
Mexico	1,632,650
Indonesia	1,313,540
Brazil	1,188,910
Philippines	825,676
Kenya	553,710
Egypt	505,731
Mali	470,800
Peru	454,330
Dominican Republic	299,650
Colombia	243,375
Cote d'Ivoire	42,500
Ghana	7,000

Source: FAOSTAT (2010a) MT: Metric tonnes.

2.3 Export and import of mango

2.3.1 Mango exporting countries

The global demand for mango fruits continuous to surge and in 2005, an estimated figure of 912 853 MT of mango fruits were exported (FAOSTAT, 2007). Mexico, India, Brazil, Netherlands, Pakistan, Peru, Ecuador, Philippines, Belgium and La Côte d'Ivoire were the top ten (10) mango exporting countries in the world (Table 2.2).

Table 2.2 Major mango exporting countries of the world

Producing country	Amount exported (MT)
Mexico	232,643
India	175,467
Brazil	110, 355
Netherlands	81,932
Pakistan	73,575
Peru	69,191
Ecuador	47,591
Philippines	21,472
Belgium	14,614
Cote d'Ivoire	13,763
Spain	8,524
Costa Rica	7,117
Senegal	6,650

FAOSTAT, 2010b). MT=Metric tonnes.

2.3.2 Mango importing countries

The world's major importing countries of mango fruits from 2003 to 2005 include USA, Netherlands, the United Arab Emirates, Saudi Arabia and China. Currently the global imports of mangoes stand at 137 million tonnes in 2010 with an astronomical increase by 50 % between the years 2006 and 2010. Globally, the United States of America is noted as the hub of importing destination of mango followed by China and Holland. Approximately 90 % of Holland's imported mangoes are re-exported to other European countries such as Germany. The United States of America equally imports over 75 % of mango from Mexico (Abdallah, 2012). Table 2.3 below provides a summary of the world mango importing figures in tonnes between the years 2006 and 2010.

Table 2.3 Major mango importing countries of the world

Importing country	Amount imported (MT)
1 Brazil	73,709
2 Peru	68,232
3 Israel	13,997
4 Pakistan	11,738
5 Cote d'Ivoire	9,768
6 USA	8,475
7 Costa Rica	6,873
8 Dominican Rep.	6,345
9 Senegal	5,338
10 Mexico	5,254
12 Burkina Faso	2,081
13 Mali	1,781
14 Gambia	1,503
25 Ghana	197
31 Guinea	93
32. Others	10,513

Source: Abdallah (2012).

2.4 Uses and nutritional value of mango

2.4.1 Uses of mango

The mango plant is popularly cultivated for its many important uses in its growing areas of the world. The fruits which are consumed in their fresh state serve as an important food source during the season of production. In the processing industry, fruit juice is extracted from the pleasant and tasty fresh fruits. The flesh or pulp is sometimes processed into canned products such as fruit salads, purees, jams, canned slices, pickles, frozen mango and dehydrated products (GEPC, 2005). The leaves of the crop are also reported to be used in some cultures as decorations during public celebrations such as weddings and religious ceremonies (McGovern and LaWarre, 2001).

Another most important use of mango that has recently received research attention has to do with the beneficial leaf extract which is used as a natural preservative in food industries (Morsi *et al.*, 2010) and as anti-ageing compound incorporated in cosmetic products (Charrier *et al.*, 2006). In the pharmaceutical industries, extracts exploited from mango leaves are used as chemo-preventive agent capable of counteracting the negative effects associated with reactive oxygen species or oxidative stress. This property arises from the presence of the abundant antioxidant and health supporting properties of the extracts (Mohan *et al.*, 2013).

2.4.2 Nutritional properties of mango

The mango fruit is highly nutritious and serves as an important source of many essential nutrients including micronutrients, dietary fibre, proteins, carbohydrates, fats, vitamins and other phytochemical constituents such as carotenoids, anthocyanin, chlorophyll and xanthophylls. As the fruit advances in maturity or approaches ripening stage, the chlorophyll content reduces while carotenoids and anthocyanin contents increase (Tharanathan *et al.*, 2006). Other important nutritional component present in mango includes oxalic, malonic, succinic, pyruvic, adipic, galasturonic, tartaric, glucolic and mucic acids. It is also known that soon after fruit set, starch accumulates in the mesocarp. Thus sugars including fructose and sucrose levels in ripened fruits greatly accumulate as a result of starch hydrolysis.

The peels serve as a rich source of cellulose, hemicellulose, fibre, proteins, enzymes, lipids and pectin which plays an essential role in human health (Iqbal *et al.*, 2009; Kim *et al.*, 2012; Sogi *et al.*, 2013). A study by Ajila *et al*. (2007) indicated that mango peels have received much attention in scientific research in recent times as a result of the presence of certain phytochemical compounds including polyphenols, ascorbic acid, vitamin E, carotenoids and enzymes which have antioxidant value. In addition, reports indicate that mango peels are currently being used as vital ingredient in most food products. For instance, mango peel flour has been reported by Ajila *et al*. (2007); Ajila *et al*. (2010); Aziz *et al*. (2012) as ingredients in such products as bread, noodles, sponge cakes, biscuits and other related bakery products. Again, Ajila, *et al*. (2010) pointed out in their study that addition of mango peel powder to macaroni improved its nutritional properties without any associated effects on the textural, cooking, sensory and the nutraceutical characteristics. The mango seeds have been reported to contain 7.1–15 % crude fats and could play a similar role as that of shea and cocoa butter (Solís-Fuentes and Durán-de-Bazúa, 2011; Muchiri *et al.*, 2012; Jahurul *et al.*, 2013; Jahurul *et al.*, 2014).

2.5 Distribution and production of mango in Ghana

In Ghana, the mango crop is well adapted for cultivation in all the agro-ecological zones and thus can be found in almost every community across the country. However, report by TIPCEE (2009) estimated mango production to be at 40,000 tonnes per annum and spread over 17,000 hectares. Commercial production of the crop is mainly concentrated in the Northern Region (around Tamale) and also Greater Accra, Eastern and the Volta Regions which are in the Southern belt of the country (Saeed *et al.*, 2012; FAO, 2009). Commercial mango production is being carried out in the Brong-Ahafo and the Ashanti Regions (Jaeger, 2008). A major constraint to mango production in the country is the high incidence of pests and diseases with major impacts experienced in the southern part of the country where humidity levels are higher. The Keitt variety is predominantly cultivated in the Southern part of the country due to its early maturity period and the attractive export attributes (Jaeger, 2008). A case study conducted by Okorley *et al.* (2014) in the Dangme West district reveals that mango farming business in Ghana is male dominated and predominantly practised as monoculture by small to medium scale holders with kent and keitt as the major varieties grown. Fig. 2.1 shows mango production in Ghana from 2009- 2010.

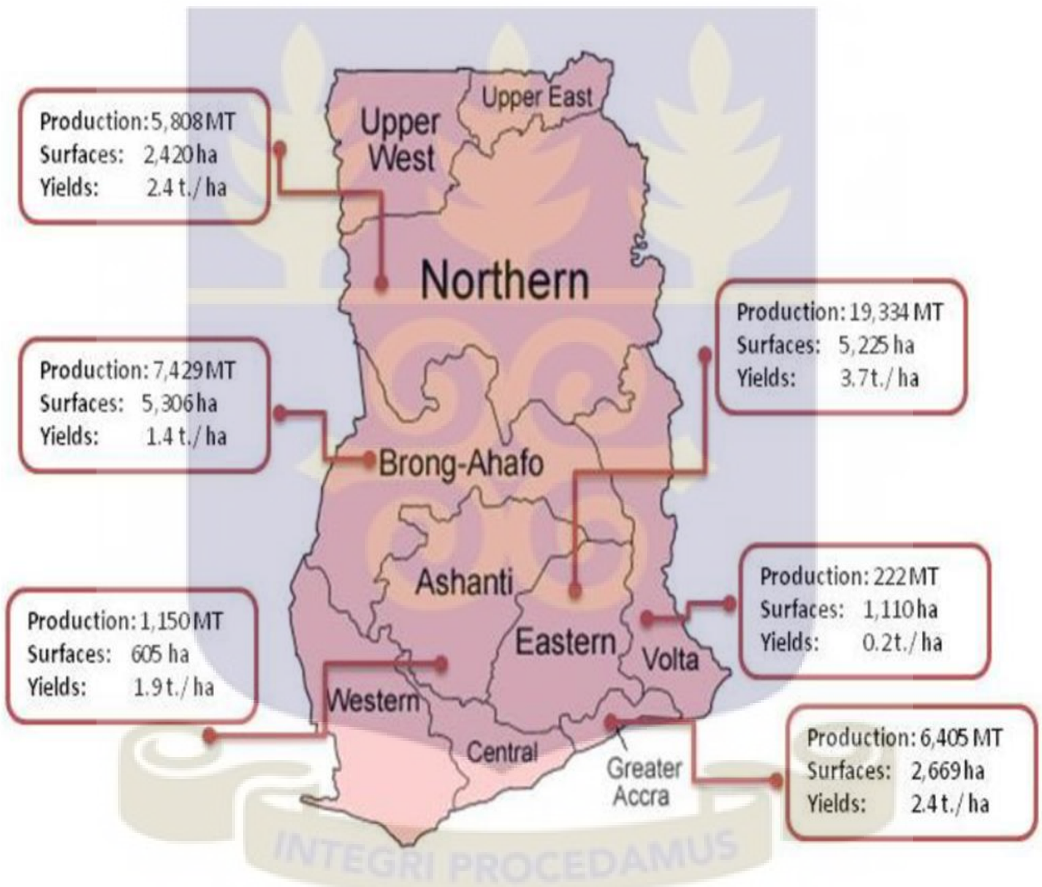


Fig. 2.1 Mango production in Ghana (based on 2009-2010 figures)

Source: Abdallah (2012).

2.6 Mango varieties grown in Ghana

2.6.1 Kent

Kent is one of the most popularly produced mango varieties in Ghana. This variety originated from Miami, Florida and was later brought to Africa where major cultivation was carried out at the Foulaya Experimental Station in Guinea around the 1950s (Vanniere *et al*., 2011). It was later spread to other stations in the Western and Central Africa. The variety which is a descendant of Brooks, a Sandersha-derived seedling was developed in the year 1944 (GEPC, 2005). It is characterized by its erect and slender growth habit with moderate size stem. Though it is a prolific fruit bearer, it requires ethylene treatment in order to enhance its colour for a better market price (Morton, 1987). The fruit is ovate in shape, firm, fleshy and fibreless with a pleasant taste. The fruits are relatively large and weighs between 500-900 grams (Vanniere *et al*., 2011). The maturity process is gradual though harvested fruits which are near maturity can be stored over a longer period at lower temperature. In terms of its fruit qualities, especially the sensory properties, kent has gained a higher recognition in both local and international markets (GEPC, 2005). The variety is prone to attack by insect pests with piercing mouth parts causing damage to the fruits as a result of its thin skin. Under favourable conditions that aid in their expression, anthracnose disease is likely to affect them. Kent mango variety has the tendency for alternate bearing (Knight, 1997).

2.6.2 Keitt

Keitt is another common variety of mango introduced to Africa and for that matter Ghana around the 1950s (Vanniere *et al.*, 2011). The distinguished features of the tree include an upright and medium-sized stem, open canopy and high fruit bearing (Morton, 1987). The fruits are oval in shape, longer and relatively flatter in length as compared to that of Kent. Keitt is a late maturing variety whose fruits weight range between 500 g and 1000 g. The fruit colour may be deep pink or brightly red thus making it very attractive. It has a greenish-yellow colour with pink or red blush and lavender bloom. There are abundant white or yellow or red lenticels on the thick and fairly tough skin. Unlike Kent, this variety is less prone to attacks by pest such as fruit flies, mango stone weevil and diseases such as anthracnose (Vanniere *et al.*, 2011) but highly vulnerable to bacterial black spot and affected by interior breakdown of the flesh as well as sunburn (Knight, 1997).

2.6.3 Haden

Haden or Hayden is a mango variety believed to have been introduced to Africa in the early 20th century (1902) through Mulgoba seedlings grown in South Florida and known to be popularly cultivated globally. The seeds of this variety are relatively large and the trees are characterized by its undesirable alternate year bearing habit. Studies have indicated that Haden is the most likely parent of other mango varieties later developed in Florida (Knight, 1997). It is characterized by a rapid growth, large and dispersed canopy. The variety has a bright yellow colour with deep crimson or red blush and many but large yellow spots all over the fruits. The fruits of this variety are oval in shape, round base and it is 10.5 to 14 cm long by 9 to 10.5 cm broad. Average mature fruit weight ranges from 520-680 g (Litz, 1997). The fruit has a thick, tough and adherent skin which encloses the firm, juicy and fibrous pulp and the fibrous pulp tends to separate from and deteriorate around the seed leading to a limited quality and poor fruit shelf life. The pulp is deep yellow, excellent in nutrient and has a desirable aroma, making this variety stand tall in terms of quality. This variety is highly susceptible to antracnose disease but relatively prone to powdery mildew (Griesbach, 1989; Griesbach, 1991).

2.6.4 Tommy Atkins

Tommy Atkins are vigorous variety of mango reported to have originated from Fort Lauderdale Florida. It has a dense and rounded yellow canopy and the distinctive features of the fruits include an orange to yellow fruit colour, crimson or dark red blush with several but small white dots on the fruit surface. The fruits are oval to oblong in shape with a broadly rounded base and medium to large fruit size (Campbell and Malo, 1968). The length of the fruits ranges between 12-14.5 cm long, 10-13 cm broad and 8.5-10 cm thick. Mature fruit weight of Tommy Atkins ranges between 450-700 g (Litz, 1997). The fruit has a smooth surface with a thick, tough and adherent skin which confers on it a mechanical resistance to injury. Besides, the fruits are resistant to anthracnose disease causing fungus (*Colletotrichum gloeosporioides*). The relatively juicy, firm and fibrous flesh or pulp has a deep yellow colour, sweet taste and a strong lovely flavor (Popenose, 1957; Ledin, 1958; Campbell and Malo, 1968).

2.6.5 Zill

This variety is known to have originated from Lake Worth, Florida in 1930 and has its trees being tall, rapid in growth and a noticeable open spreading canopy. The fruit colour is greenish yellow to yellow with deep red or crimson blush. The fruits are characterized by oval to ovate shape, a slightly flattened base, rounded to bluntly flattened apex. The length and width of mature fruits range between 8.5-10 cm and 7.5-8.5 cm respectively. Single mature fruit weight is about 230-370 g (Litz, 1997). Fruit skin of this variety is thin, tender and adherent and the fibreless pulp is pale yellow, soft and juicy. It has a sweet taste and pleasant aroma, thus giving it an outstanding eating quality. However, the thinner nature of the skin makes fruits of this variety susceptible to stresses associated with storage and shipping. This property places a limitation on Zill for commercial production (Campbell, 1992).

2.6.6 Julie

The mango variety Julie is also known as “St. Julienne” and originated from the West Indies. The trees have a compact growth habit with a dense canopy. The mature rounded fruits have flattened apex and known for their green-yellow colour with pink to maroon blush and several minor white spots. The fruit length ranges between 7-9.5 cm long while the fruit width is in the range of 4-7.5 cm. The fruit weight ranges between 200-325 g with a thin, tender skin and soft juicy pulp (Tettey, 2008).

2.7 Contribution of Mango to the Economy of Ghana

The economic contribution of mango fruits to the major producing countries of the world is enormous. The potential contribution of mango fruits to the economy of Ghana is quite predictable as the prevalent conditions of the various agro-ecological zones of the country are capable of greatly supporting its successful production. The potential of mango in Ghana as a non-traditional export crop is expected to earn higher revenue and even serve as a replacement for cocoa (GNA, 2008; Ambele *et al*., 2012),

Mango production for export has the potential to offer employment opportunity to individuals engaged in it. About 5000 people in the Eastern Region alone are employed in the cultivation of improved exportable mangoes (USAID, 2005). This consequently reduces rural-urban migration. Similarly, there is also employment opportunity for individuals engaged in the agro-based processing industry where mango fruits are supplied as raw materials. Consumption of mango fruits is a source of essential nutrients required for healthy livelihood (Saeed *et al.*, 2012).

2.8 Constraints of Mango Production in Ghana

2.8.1 Exportation of Fresh Fruits

A number of challenges hinder mango production in Ghana. A study conducted by Abdallah (2012) identified the following as the major constraints facing mango exportation in Ghana. Strict sanitary and phytosanitary requirements by destination countries, frequent change in taste and preferences of the import countries, bad faith between importers and exporters and no reliable entity to represent the interest of exporters in the destination market. Others include inadequate market information systems, lack of unity among farmers leading to unnecessary competition between mango farmers producing for the same clienteles and major cultural diversification between Ghana and importing countries.

2.8.2 Inadequate Logistics

Logistics are one of the bottlenecks hindering mango production in Ghana. There have been interventions by Government to improve the infrastructural deficits such as roads linking the markets and the production centres, improving access and stability of power among others. However, logistics still remain a major challenge facing the mango industry. Poor infrastructure, especially bad nature of the road network, still impacts negatively on the quality of mangoes, rackety trucks used in conveying mangoes from farms to storage houses, poor or no cooling facilities all compromise the quality of the produce and high freight charges as a result of low export volumes (Awafo and Dzisi, 2012).

2.8.3 Production and Post-harvest Operations

In Ghana, it has been estimated that the average loss of mango fruits after harvesting is between 20 and 50% (Abdalla, 2012). Post-harvest losses of mango in Ghana have been blamed on the presence of fruit flies, poor or no cold storage rooms and long transportation time. The production challenges include, cost and accessibility of farm inputs including fertilizers, high cost of seedlings, poor or no intervention from extension officers, reliance on rainfall by farmers due to lack of irrigation facilities (GNA, 2008; Jaeger, 2008).

2.8.4 Diseases

Mango production in Ghana is beset with myriad of economically important diseases caused by fungi, bacteria and nematodes. Even though distribution of the diseases is not known, some of the important ones are reported in Ghana and other mango growing countries. Some mango diseases reported in Ghana and elsewhere are described in Table 2.4.

Table 2.4 Some Mango Diseases in Ghana

Causal Agent	Disease	Symptom	Pathogen
Colletotrichum gloeosporioides	*Anthracnose	Symptoms occur on leaves, twigs, petioles, flower clusters (panicles) and fruits. On leaves, lesions start as small, angular, brown to black spot that can enlarge to form extensive dead areas. The lesions may drop out of leaves during dry weather. The first symptoms on panicles are small or dark-brown spots which can enlarge, coalesce and kill the flowers before fruits are produced, greatly reducing yield. Petioles, twigs and stems are also susceptible and develop the typical black, expanding lesions found on fruits, leaves and flowers.	Fungus
Gloeosporium mangiferae	Fruit spots	Sunken spots and streak on the ripe fruits	Fungus
Capnodium citri	Sooty mould	The disease is characterized by the presence of black velvety thin membranous covering on the leaf lamina. In severe cases, the tree completely turns black with mould on the entire surface of twigs and leaves. The affected leaves curl and shrivel under dry conditions.	Fungus
Rhizoctonia solani	Leaf blight	Minute yellowish spots on the upper surface of the mature leaves. Spots often coalesce forming big irregular patches	Fungus
**Xanthomonas campestris pv. Mangiferea indica.*	*Bacteria Black spots	Leaves produce angular water soaked spots of 1-3 mm in diameter which are delimited by veins. These may coalesce, become black and slightly raised and can exude gum under very humid conditions. Fruit lesions develop as water-soaked halos around lenticels or wound and soon become raised, blacken and crack open with gummy infection. Fruit drop occurs especially when infected start from young fruits and when fruit stalks are infected.	Bacteria

Source: Modified after Oduro (2000). *Important mango diseases in Ghana

2.9 The mango tree decline disease (MTDD)

2.9.1 Overview of mango tree decline disease

Mangoes are among the most economically important tree crops cultivated all over the world. However, mango production in Ghana has been hit by a new disease known as mango tree decline disease (MTDD) which has been reported elsewhere but outside Ghana. It is a complex disease and believed to have caused several yield losses in countries such as Pakistan, Oman and Brazil. It is characterized by tip die back, gum exudation, and wilting of the whole leaves. The disease on the tree may be noticed at any time of the year but it is most prevalent during October to November. The disease is characterized by drying of twigs and branches followed by complete defoliation which gives the tree an appearance similar to that affected by scorching fire (http://horticultureworld.net/mango-india2.htm).

2.9.2 Origin, history and distribution of mango tree decline disease

Mango tree decline disease was first reported in Brazil in 1945 where about 60 % of mango trees were reported to have been affected (Gupta and Zachariah, 1945). The decline disease was later reported in several other mango producing countries including India (Al-Adawi *et al*., 2006) Mexico (Alvarez- García and López_García, 1971), USA (Ploetz *et al*., 1996), and Oman (Asad *et al*., 2010), Pakistan (Fateh *et al*., 2006) and Jordan (Al-Adawi *et al*., 2006). Table 2.5 shows the chronological spread of mango tree decline disease.

The mango tree decline disease is also known by various names such as "Rapid decline disease", "Quick/ sudden decline disease" or "Complex decline syndrome" but technically referred to as "Collar rot" or "Stem rot" disease. The disease is reported to be one of the most devastating diseases of mango capable of causing severe yield losses through rapid death of trees within few days after appearance of the initial symptoms (Mahmood and Gill, 2002; Sial, 2002). Though the disease had been reported in other countries, it is yet to be reported in Ghana.

Table 2.5 Chronological spread of mango tree decline disease

Country	Year	Reference(s)
Brazil	1945	Gupta and Zachariah, (1945)
India	1970	Al-Adawi *et al*. (2006)
Mexico	1971	Alvarez-Garcia and López-Garcia (1971)
USA	1996	Ploetz *et al*. (1996)
Oman	1999	Asad *et al*. (2010)
Pakistan	1999	Fateh *et al*. (2006)

2.9.3 Causative agents of mango tree decline disease

The mango tree decline disease (MTDD) is a disease complex that involves several different fungi. The principal pathogen in most countries has been identified to be the fungus called *Lasiodiplodia theobromae* (Al-Adawi *et al.*, 2003; Iqbal *et al*., 2004; Malik *et al*., 2005; Al-Adawi *et al.*, 2006). Three other fungal pathogens (*Ceratocystis omanensis, C. fimbriata, and L. theobromae)* have been associated with the disease (Adawi *et al*., 2006 and Al-Subhi *et al*., 2006). Studies by Van Wyk *et al*. (2007) identified *C. manginecans* as the causal organism associated with the disease in Pakistan and Oman. *Fusarium* species has also been reported to be associated with the disease (Kazmi *et al*., 2005).

The occurrence of mango tree decline disease is highly promoted by beetle (*Xeleborus offinis*) at a relative humidity above 80 % and temperature of 25 – 31 °C (Rawal, 1998). A mango bark beetle, *Hypocryphalus mangiferae* has been implicated as a vector of the mango tree decline disease in Pakistan (Al Adawi *et al*., 2006; Saeed and Masood, 2008; Masood *et al.*, 2010). Schaffer *et al.*

(1988); Ploetz (2003); Nafees *et al.* (2010) and Masood *et al.* (2012) identified in their study that nutritional deficiency, drought, temperature fluctuations and mechanical injuries are the principal abiotic factors which speed up mango tree decline disease. Moreover, poor orchard management practices such as improper irrigation, intercropping, root damages caused by deep ploughing and the presence of infected plants are the predisposing factors for development of the disease (Saeed *et al.*, 2006).

2.9.4 Symptoms of mango tree decline disease

The symptoms of the disease at the early stage of infection have been reported by Masood *et al.* (2010) where trees are noticed to have gummosis from the bark, bark splitting, streaking and vascular discolouration beneath the bark. Rotten Canker is commonly observed on severely affected trunks which sometimes results in exudation of liquid with offensive aroma (Masood *et al.*, 2010). Under similar conditions, the vascular bundles became blocked, preventing the translocation of nutrients and high mortality of trees (Khuhro *et al.*, 2005).

In a study conducted by Pernezny and Ploetz (2000), Symptoms of Mango tree decline disease identified included blight, canker, gummosis, twig blight, tip die-back and stem bleeding. Studies about mango tree decline disease of mango in Oman revealed that the disease was principally characterized by such symptoms as trunk gummosis, wilting, tree death and wood staining (Al-Adawi *et al.*, 2006).

In a related study by Ploetz *et al.* (1996), leaves of trees affected by mango decline disease were observed as defoliation, vascular discolouration and marginal chlorosis. Leaves found on the small branches of affected trees exhibit signs of necrosis of leaves but remains attached to the dying trees. Eventually this leads to die-back symptom which progresses to the larger branches and consequently a reduction in the number of secondary roots (Rasmos *et al.*, 1991; Al-Adawi *et al.*, 2006; Saeed and Masood, 2008). Symptoms observed in affected trees may be found alone or in combination as well as differing across different mango orchards (Iqbal *et al.*, 2007).

2.9.5 Economic importance of mango tree decline disease

Mango tree decline disease, technically referred to as collar rot has been currently noted as the most destructive disease of mango plantations globally since it kills mango trees after a short exposure to the disease (Iqbal and Saeed, 2012). It is becoming a worrying threat since young and matured mango trees are dying at an alarming rate (Mahmood and Gill, 2002; Masood *et al.*, 2011).

Threat to mango production and the global market as a result of mango tree decline disease has been reported from Australia (Sakalidis *et al.*, 2011), Brazil (de Oliveira Costa *et al.*, 2010), Egypt (Ismail *et al.*, 2012) and Taiwan (Ni *et al.*, 2012). Incidence of the disease was found to be 20 % in Punjab, over 60 % in Sindh Provinces of Pakistan and 60 percent in Al Batinah region of Oman (Mahmood *et al.*, 2002 Al-Adawi *et al.*, 2006; Saeed *et al.*, 2006). Again a study conducted by Mahmood (2008) in a mango plantation found in seven (7) districts of the Punjab province revealed 100 % prevalence of the mango tree decline disease. A similar survey conducted on ten (10) mango trees sampled in Punjab and six (6) surveyed in the Sindh districts in Pakistan revealed the disease incidence to be 3.2. It is also reported that twig blight, tip die back, gummosis and bark splitting (cracking) were noted with 55.0, 50.0, 25.0 and 25.0 % disease prevalence respectively in a mango orchard found in Punjab province (Iqbal *et al.*, 2007).

2.10 *Lasiodiplodia theobromae*

2.10.1 Aetiology and description of *Lasiodiplodia theobromae*

The species *Lasiodiplodia theobromae* is synonymously used to refer to the fungus *Botryodiplodia theobromae* (Khanzada *et al*., 2004; Pitt and Hocking, 2009) which is a cosmopolitan soil-borne fungus (Domsch *et al.*, 2007) responsible for causing both field and storage diseases on more than 280 host plant species including mangoes (Abdalla *et al.*, 2003; Pitt and Hocking, 2009; Abdollahzadeh *et al.*, 2010). The fungi belong to the genus *Botryodiplodia* of ascomycetous family which have parasitic and pathogenic effects on various host plants. As ascomycetes which are also heterotrophs, these range of pathogens obtain their nutrients from dead or living organisms (Carroll and Wicklow, 1992; Griffin, 1994).

The species *L. theobramae* induces both pre-harvest and post-harvest disease symptoms such as die back, root rot, fruit rot, blight gummosis, stem necrosis, leaf spots, canker and witches' broom disease (de Oliveira Costa *et al*, 2010; Sakalidis *et al.*,2011; Ismail *et al.*,2012). Being opportunistic pathogens, they are capable of remaining as latent infections on healthy plants, but cause diseases when the host plants are stressed-up (Denman *et al.*, 2000; Slippers and Wingfield, 2007).

2.10.2 Geographical distribution of *L. theobromae*

The fungus is a common soil-born saprophyte or wound parasite distributed throughout the tropical and sub-tropical regions of the world where temperatures are relatively high (Khanzada *et al*., 2004; French, 2006; Hseu *et al.*, 2008). The fungus *L. theobromae* is capable of growing in such conditions where temperatures range between 20 °C and 45 °C or under optimum temperature range of 30 °C-40 °C. Nonetheless, lower temperature (below 15 °C) can inhibit the growth of the organism. The fungus is reported to be well adapted to areas where the altitude is approximately 40° N and 40° S of the equator (Punithalingam, 1976; Pitt and Hocking, 2009).

2.10.3 Host range of *L theobromae*

Susceptibility of plants to *L. theobromae* is dependent on the sucrose level of host plant and reducing sugars present. The higher the sucrose content, the higher the susceptibility. It has also been identified to be pathological to human and has the ability in causing keratomycosis and phaeohyphomycosis (Punithalingam, 1976) and can be found in association with approximately 500 host plants (Punithalingam, 1980). Table 2.6 shows some of the susceptible plants and the diseases caused by the organism.

Table 2.6 Susceptible host plants and their disease caused by *Lasiodiplodia theobromae*

Plant	Disease caused	Reference
Cashew	Gummosis	Cardoso *et al*. (2004)
Peach trees	Gummosis	Li *et al.* (1995)
White cedar	Canker	Sandrock *et al*. (1999)
Peanut	Collar rot	Phipps and Porter (1998)
Kumquat	Die back	Ko *et al.* (2004)
Blueberry	Stem blight	Cline and Milholland (1992)
Mango	Dieback	Ragab *et al*. (1971) and Abdalla *et al*. (2003)
Pawpaw	Soft rot	Anthony *et al*. (2004)
Guava	Soft rot	Wall and Cruz (1991)
Lemon fruits	Die-back	Alam *et al*. (2001)

2.10.4 Colony characteristics of *L. theobromae*

Colonies of *L. theobromae* have often been reported to be greyish sepia, to mouse grey to black, fluffy with abundant aerial mycelium. Matured cultures have black pigmentation (Philip, 2007). Celiker and Michailides (2012) studied *L. theobromae* causing canker and shoot blight of fig tree in Turkey and observed that fungi colonies occupied 85 % of the plated plant tissues. It was also noticed that the fungi colonies had profuse area mycelia that eventually turned from grey to black with age which formed a black pycnidia. The pycnidiospores were found to exhibit oval greenish brown with one septum in the middle.

2.10.5 Morphology of *L. theobromae*

In a culture media, mature cultures of *L. theobromae* can develop in a scattered, clustered or centered with noticeable pycnidia which will eventually sporulate. Pycnidia which may be up to 5 mm wide may be seen covered with bristles beneath or within the mycelium (Pitt and Hocking, 2009). Although, a temperature of 28 °C will give a better sporulation (Meah *et al.*,1991). Khanzada *et al.* (2006) reported that a temperature range of 35 °C-40 °C is ideal to produce a maximum number of pycnidia. Environmental factors such as exposure to irradiation by fluorescent light and ultraviolet radiations as well as culture media constituents are known to influence the process of sporulation (Ekundayo and Haskins, 1969; Saha *et al.*, 2008). The formation of *L. theobromae* spores occurs endogenously in the pycnidia from the inner surface layer of cells lining the pycnidial cavity through which spore or conidiophores exude visibly as pink pigmented or blackish fluid into the culture (Meah *et al.*, 1991; Mascarenhas *et al.*, 1996). The conidiophores are characterized by being simple, cylindrical, translucent and sometimes presence of noticeable septations (Mascarenhas *et al.*, 1996).

Conidia formed may initially be observed as unicellular and translucent, sub-ovoid to ellipsoid oblong, thick-walled, granulose and having truncated base. Conidia are two-celled (one-septate) and cinnamon to dark brown, thick-walled, ellipsoidal and more commonly striated longitudinally (Alvarez and Nishijima, 1987; Phipps and Porter, 1998). In the presence of paraphysis, conidia become translucent, cylindrical or may be septate (Alvarez and Nishijima, 1987).

2.11 Disease cycle and epidemiology of mango tree decline disease

Knowledge about the infection process and epidemiology of pathogens is required to establish an efficient and effective control approaches as well as quarantine regulations. The mode of entry into mango tissues by Botryospharia fungus is not well understood (Ohene- Mensah, 2012). The fungus has therefore been reported to take advantage of natural openings (stomata, lenticels, hydathodes), and wounds resulting from pruning and attacks by other organisms (Mass and Uecker, 1984; Johnson, 1992). As an opportunistic pathogenic fungus, *L. theobromae* can inhabit healthy plant tissues endophytically as well as plant residues, planting materials, insects and soil (Denman *et al.*, 2000; French, 2006; Slippers and Wingfield, 2007). Restricted infections, which normally occur in the form of sunken or necrotic lesions result when the fungus invades natural openings. This eventually leads to exudation of gum from the trunk and branches of the affected mango trees (Jacobs, 2002). The pathogen is capable of entering the vascular tissues of affected plants though the movement may be slow (Ramos *et al.*, 1991). Environmental stresses including drought, freezing and mineral deficiency limits the ability of mango trees to resist or tolerate infections with the pathogen leading to a rapid development of disease symptoms. A study by Jacobs (2002) revealed that fruiting structures of the fungus are normally produced on diseased plant parts during the advanced stage of infection. Fungal spores or conidia may easily be discharged through openings or ostioles on affected plants and get spread via splashes of raindrops, wind and pruning tools to other healthy host plant tissues (Sutton, 1981; Mass and Uecker, 1984). Fig. 2.2 shows disease cycle for tree decline.

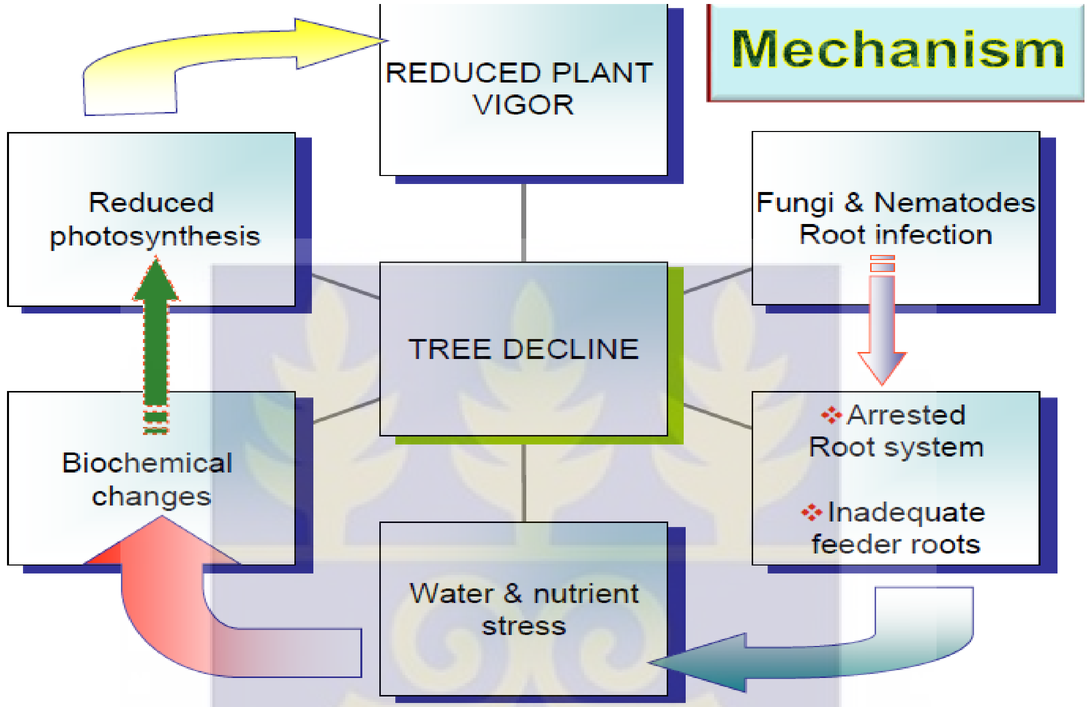


Fig. 2.2 Disease cycle for a tree decline

Source: Safdar, www.uaf.edu.pk/golden

2.12 Control of mango tree decline disease

2.12.1 Non-chemical control/proper cultural practices

Ensuring proper cultural practices in tree plantations has the potential of helping to control the spread of the disease pathogen. Practices that prevent shaking of pedicels of fruits as well as those that cause injuries to plant parts could minimize the spread of the fungal pathogen (French, 2006). Since the pathogen takes advantage of entry points such as wounds or natural openings on the plant, careful handling that avoids damages are required in order to avoid disease spread. Attack by the pathogenic fungi could also be reduced via the control of pest damage to plants. Rotation techniques have been reported to be effective in breaking the disease cycle (French, 2006). Mango planting materials should be sourced from accredited suppliers, clean and preferably, materials that are certified. Regularly check on mango plantations for the presence of new pests and unfamiliar symptoms can help to curtail the decline disease (Anonymous, 2005).

2.12.2 Integrated disease management

For sustainable integrated disease management, the use of systemic fungicides and insecticides being injected into the stem of the affected mango plants combined with good farm hygiene, proper pruning and balanced use of macro and micronutrients have been suggested (Poland *et al*., 2006). Other reported control measures recommended include avoidance of deep ploughing and intercropping, plant protection measures based on the situation of pest as well as effective pruning of the affected tree parts followed by spraying the wounded areas with 5:5:50 Bordeaux mixture (Parkash and Raoof, 1989).

2.12.3 Chemical control (fungicides)

The use of fungicides in managing plant diseases is a common phenomenon all over the world. There have being several reports available in which fungicides have proved to be effective in controlling plant diseases caused by soil borne pathogens (Ilieseu *et al*., 1985; Rajput *et al*., 2006; Iqbal *et al*., 2010; Govindappa *et al*., 2011; Ahmed *et al*., 2012). A study conducted by Muhammad *et al*. (2005) reports Carbendazim to be more effective in inhibiting the growth of *L. theobromae* mycelia as compared to Thiophanate-methyl and Alliete under both *in vitro* and field conditions in suppressing gum exudation, dieback and wilting. This resulted in significant improvement in the vegetative growth of diseased plants. Mahmood *et al*. (2002) reported 1st foliar spray of Topsin-M (Thiophanate-methyl) at 1.0 gL^{-1} showed reduction in the infestation of *L. theobromae* to 10% and 2nd spray of the same fungicide completely inhibited the fungus as no tissue yielded this fungus.

The injection method of controlling mango tree decline disease has also been reported to be effective in managing the disease when infection is less than 25 %. The drilling technology package involves drilling 3-4 holes with the help of manual or motorized drill at an angle of 45°, inserting a piece of sponge in the hole; injecting fungicides (Thiophenate methyl) as per recommended dose (5 g/20 ml water) with the help of syringe. This is followed by closing the hole with plaster and repeating this process in the same hole after recommended interval (15 days) till the symptoms vanish.

Mancozeb was found to be slightly effective in inhibiting the mycelial growth of *L. theobromae (*Sahi *et al.*, 2012). Narasihudu and Reddy (1992) recommended a paste of Bordeaux mixture or Carbendenzim to be one of the effective means of controlling gummosis of the disease.

Fungicides viz. thiophanate- methyl, Carbendazim, diethofencarb and copper oxychloride at 50 and 100 μg ml^{-1} showed 100% inhibition over control, while copper oxychloride revealed only 20.20% inhibition (Shahbaz *et al*., 2009). In a related study, Rawal (1998) observed that die-back of mango caused by *L. theobromae* was controlled by spraying Carbendazim at 0.1%, Methylethiophanate at 0.1% or Chlorothalonil at 0.2% over a fortnightly interval. Lonsdale and Kotze (1993) reported that broad-spectrum systemic fungicides are useful in controlling mango die-back disease.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental sites

The survey was carried out in three mango growing districts in the Northern Region of Ghana where symptoms similar to the mango tree decline disease reported elsewhere were found on some mango trees. The laboratory experiment was undertaken in the Plant Pathology Laboratory of Crop Science Department and the Molecular Biology Laboratory, Biotechnology Centre, College of Basic and Applied Sciences, University of Ghana, Legon.

3.2 Survey to assess mango farmers' perception and reactions to mango tree decline disease

A survey was conducted in May, 2015 to find out the perceptions and reactions of mango farmers to the mango tree decline disease, a new disease in the experimental communities. Preceding the survey, a preliminary focus group discussion was held with the various Mango Farmers Associations with the aid of the various Chairmen of the associations. Questionnaires were administered during the survey to obtain a baseline data on mango farmers' knowledge, perception, experiences, spread and economic importance of the new disease. Twelve communities were selected at random (Table 3.1) and seven mango farmers were randomly selected from each of the experimental community. In all, eighty-four (84) mango farmers were engaged from the experimental communities. A well-designed questionnaire read out and interpreted to the farmers at the various Mango Farmers Association's Meeting and their responses were documented in each experimental community. Subject matters considered in the survey questionnaires are shown in Appendix 1. Data collected were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 16.

Table 3.1 Districts and communities selected for the study

Community	District/ Municipal
Gushie	Savelugu
Diare	Savelugu
Tunayili	Savelugu
Pong-Tamale	Savelugu
Kumbungu	Kumbungu
Zugu	Kumbungu
Zangbalung	Kumbungu
Gbullung	Kumbungu
Tamaligu	Karaga
Zankali	Karaga
Maligunayili	Karaga
Bagurugu	Karaga

3.3 Farm visit

Some of the mango farms where symptoms of the disease had been reported were visited occasionally to study the nature of the disease symptom. Mango trees that were found exhibiting the disease symptoms in the farm visited were also inspected and the nature of the diseased symptoms recorded. In some cases, farmers on whose plantations the diseased mango trees were found were questioned to ascertain the observations made.

3.4 Determination of incidence and severity of mango tree decline disease in the three mango growing districts of the Northern Region

Field visits to determine the disease incidence and severity of the mango tree decline disease was carried out between August, 2015 and December, 2015 in the three mango growing districts in the Northern Region. In each locality, seven mango farms were selected at random and 10 mango trees per farm were inspected for the symptoms of mango tree decline disease. In order to have better coverage of the mango farms, systematic sampling method with a 'W' shaped sampling path was carried to determine incidence and severity. To eliminate selection bias of the units (trees), counts of every 10th tree was made between samples. The number of mango trees that were found exhibiting the decline symptoms within a locality was used to determine the disease incidence using the formula:

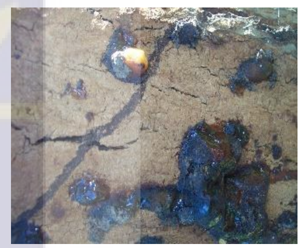
$$\text{Disease incidence (DI)} = \frac{\text{Number of trees showing disease symptom}}{\text{Total number of trees infected and uninfected.}}\ x\ 100$$

Disease severity on the other hand was determined on 10 mango trees selected at random from each farm. Each selected tree was inspected and disease severity was rated visually on a scale of 0-5 (Table 2) modified after Panhwar *et al*. (2007**)** (Fig. 3.2). The disease severity index for each assessed mango trees per farm was calculated using the formula:

$$\text{Severity Index (SI)} = \frac{\sum fx}{\sum x}$$

Where, x=a particular rating and f=frequency of occurrence of the particular rating.

Table 3.2 Assessment key for severity of mango tree decline disease

Index	Qualitative rating	Pictorial rating
0	No signs of the disease	
1	Gum traces oozed out/few smaller branches dried	
2	Oozing of gums/bark splitting and few dead branches	
3	Up to 35% of tree dead	
4	More than 35% of the tree dead	
5	Foliage of whole tree wilted	

Modified after Panhwar *et al*. (2007).

3.5 Isolation and identification of microorganism(s) associated with mango tree decline disease

Diseased plant parts (barks, leaves, roots and branches) collected from some of the diseased mango trees observed on the field were placed in polyethene bags, labelled and transported in an ice chest to the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana, Legon for the isolation of likely causal agent(s) of the disease. The collected diseased samples were stored in a refrigerator at 4°C in the laboratory until required.

3.5.1 Preparation of water agar (WA)

Three grams of dehydrated agar (Oxoid Ltd., Basingstoke, Hampshire, England) was weighed into 100 ml distilled water in a 250 ml conical flask. The conical flask was then pluged with non-absorbent cotton and covered with aluminium foil to prevent wetting of cotton wool during condensation. The mixture was autoclaved at 1.05kg/m^2 pressure and 121^{o}C for 15 minutes. After which the cotton wool plug was removed, the neck of the conical flask was flamed and about 10 ml water agar poured into sterilized 9 cm petri dishes and allowed to solidify.

3.5.2 Preparation of potato dextrose agar (PDA)

Potato Dextrose Agar (Oxoid Ltd., Basingstoke, Hampshire, England) was prepared by dissolving four grams of the powder in 100 ml of distilled water in a 250 ml conical flask. The conical flask together with the content was shaken, covered with aluminium foil and autoclaved at 1.05kg/m^2 pressure and 121^{o}C for 15 minutes. The PDA was then poured in 9 cm Petri dishes and allowed to cool.

3.5.3 Isolation, percentage occurrence and morphological identification of causal organisms associated with mango tree decline disease

Disease samples selected for the isolation of the causal organisms were first washed under running tap water and air-dried in the laminar flow. Tissue segments were excised from advancing margins of diseased samples with flamed scalpel and surface sterilized in 1% sodium hypochlorite for1 minute. The tissues were allowed to air-dry and put on water agar. The Plates were incubated at 23 to 31^{o}C and 60 to 70% RH for up to 5 days. Using a sterile cork borer, growths on the Water Agar (WA) plates were taken and sub-cultured on a Potato Dextrose Agar (PDA) plates. Slants of isolates on PDA were prepared and kept in a refrigerator for storage until required. Percentage occurrence of fungi was calculated according to a formula by Hyde and Jones (1988).

$$\text{Percentage occurrence} = \frac{\text{Number of occurrence of a particular fungus}}{\text{Total number of diseased samples examined}} x\ 100$$

Pure culture plates of isolates were incubated at 28°C under flourescent light for 20 days to enhance sporulation. With the aid of an inoculation needle, matured pycnidia produced after 20 days of incubation were transferred into sterile distilled water on a sterilized glass slide and then teased to diffuse spores. The solution on the slide was then observed under a compound microscope to confirm the presence and even distribution of spores. Slides with the spores of the isolates identified were then inverted and pressed onto the surface of solidified Water Agar (WA) media to obtain their growth.

Mycelia plug of seven day old cultures were plated on PDA and incubated at temperature of 27°C and 65% RH on benches in the laboratory.

3.5.4 Morphological characteristics

Bits of mycelia were fixed on a slide and observed under low and high power magnification under light microscope. The nature and colour of the hyphae and any sporulating structures found were observed and recorded. Micrographs were then taken using a Kodak digital camera. For isolates that did not sporulate easily, the cultures were maintained in the laboratory and inspected daily for sporulating structures, by fixing bits of mycelia every other day till the mycelia dried out.

3.5.5 Molecular identification of fungus associated with the disease

Globally, fungus has been associated with mango tree decline disease. The isolated organisms were identified using the cultural and morphological methods in section 3.5.4 in this thesis. Polymerase chain reaction (PCR) was further run to confirm the identity of the organism with species specific primers. Other isolates that were similar to the *L. theobromae* but failed to sporulate during the period of the experiment were also included in the molecular studies.

3.5.5.1 DNA extraction

Extraction of DNA (deoxyribonucleic acid**)** from isolates of *L. theobromae* and the other selected isolates was carried out in the Molecular Biology Laboratory, Biotechnology Centre, University of Ghana, Legon. A plug of the isolates was plated on PDA and allowed to grow for 5 days. Using a sterile loop, the mycelia of the pathogen was scrapped from the surface of the PDA and dried overnight in the laminar flow hood. The dried mycelia were mashed using mortar and pestle under liquid nitrogen after which the powdered mycelia were poured into microcentrifuge tube.

DNA extraction was performed using the Sigma's GenFlute Plant Genomic DNA Miniprep Kit following the manufacturer's instructions as follows. First, 350 µl of Lysis solution part A and 50 µl of lysis solution B were added to the mycelia in the microcentrifuge tube after which a pestle was used to gently mash the mycelia in the solution. The mixture was then vortexed and incubated at 65°C for 10 minutes. After that 130 µl of precipitation solution was added to the mixture and mixed thoroughly and then placed on ice for 5 minutes after which the sample was centrifuged at 12000 x g for 5 minutes. The supernatant was pipetted onto a Genflute filtration column and centrifuged at 12000 x g for 1 minute after which the filtration column was discarded and 70µl of binding solution was added to the flow through liquid in the collection tube and the mixture mixed thoroughly by inversion. A binding column was then prepared by pipetting 500 µl of the Column preparation solution to a miniprep column and centrifuging at 1200 x g for 30 sec after which the flow through liquid was discarded. After that 700µl of the flow through liquid in the collection tube obtained prior to the preparation of the binding column was pipette onto the column prepared and was centrifuged at 12000 x g for one minute after which the flow through liquid was discarded and the collection tube retained. The column was returned to the collection tube and the rest of the lysate prepared prior to column preparation was added and the solution was centrifuged at 1200 x g for one minute after which the flow through liquid and collection tube were discarded while the column was retained. The binding column was placed in a clean 2 mL collection tube after which 500 µl of alcohol-diluted Wash Solution was added to the column and the solution centrifuged at 1200 x g for 1 minute after which the flow through liquid was discarded and the collection tube retained. Another 500 µl of the diluted Wash Solution was added to the column and centrifuged at 1200 x g for 3 minutes after which the binding column was transferred into a fresh 2 ml collection tube. 100 µl of pre warmed (65°C) Elution Solution was added to the column and centrifuged at 1200 x g for 1 minute.

3.5.5.2 Storage of extracted DNA of isolates

A prepared buffer was added to the extracted DNA contained in a 1.5 ml micro-centrifuge tube. The individual tubes containing the DNA of each isolate were labeled to indicate the source of isolate, i.e., place of collection of sample. The tubes with DNA were stored in a refrigerator at 4°C for later use. Frozen DNA was thawed on ice before use.

3.5.5.3 Agarose gel electrophoresis, loading dye preparation and DNA ladder and primer reconstitution

Agaros gel (0.8% w/v) was prepared by weighing 0.912 g of hydrated Molecular Biology agarose (BIORON, Germany) into a 114 ml 1 x TAE buffer. The mixture was heated in a microwave oven and allowed to cool to about 50°C after which 2.5 µL of Ethidium bromide (10 µg) was added and the flask swirled to ensure that the ethidium bromide mix thoroughly with the agarose solution. The resultant solution was poured into a horizontal electrophoresis tray that has been mounted in a gel casting tray fitted with 10 or 20 teeth comb. The preparation was allowed to stand to allow the solution to polymerise after which tray was removed from the gel caster and placed in an electrophoresis tank containing 1XTAE buffer. Fresh loading dye was prepared by mixing 0.5 g of 0.25% bromophenol blue, 0.5 g of 0.25% xylene FF and 6 ml of 30% glycerol to 20 ml of ultra-pure water. To aid dissolution of the reagents in the water, few drops of 0.5 M EDTA were added and the mixture agitated carefully after which the preparation was stored till needed. A 1.0 kb DNA ladder was constituted by mixing 10µ l of stock solution, 20 µl of loading dye and 170 µl of sterile distilled water and the preparation stored until needed to be used. Primers were reconstituted into 100 picomoles by adding sterile distilled water and resuspending overnight. This was also stored till needed to be used. Quality of extracted DNA was assessed by loading 5µl of DNA into each well and electrophoresis carried out at 45 volts for 30 minutes.

3.5.5.4 Polymerase chain reaction

DNA extracted from isolates of the pathogen was used as templates in polymerase chain reaction with the *L. theobromae* species specific primer Lt347-F (AACGTACCTCTGTTGCTTTGGC) and

Lt347-R (TACTACGCTTGAGGGCTGAACA) (Xu *et al*., 2015) was used. A second set of PCR was performed using the universal primer pair ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) to amplify the internal transcribed spacer (ITS) region of the isolates. PCR was carried out in a total reaction volume of 50µl. The reaction mixture was made up of 34.25 µl of double distilled water, 5 µl of 10X PCR buffer (Invitrogen, Carlsbad, CA), 2.5 µl of deoxynucleoside-triphosphate (DNTP) mix (2.5 mM each), 0.25 µl bovine serum albumin (20 mg/ml), 2 µl each of the forward and reverse primer, and 0.2 µl of taq polymerase, 1.8 µl of magnesium chloride (50 mM) and 2 µl target DNA. The reaction was carried out in a Thermo Hybaid PXE Thermal Cycler. The reaction cycles were denaturing for 2 min at 94°C followed by 35 cycles of 1min at 94°C, 1 min at 55°C, 2 min at 72°C and a final of 10 min at 72°C. Amplification products were separated by 1.5% w/v agarose gel stained with Ethidium bromide or gel red alongside 1.0 kb marker at 80 V for about 1 hour. Bands were observed under UV light and Polaroid photographs taken or viewed using the Gene Flash Documentation System (Snygene Bio Imaging).

3.5.5.5 Purification and sequencing of amplified product of the ITS region

The PCR amplified product of the ITS region were sent to ETON Bioscience Laboratory at Raleigh in North Carolina for purification and sequencing. 10 picomole of each primer was used to sequence the product directly from both directions.

3.5.5.6 Basic local alignment search (BLAST)

The sequences of the entire ITS region obtained were used in a BLAST (www.ncbi.nlm.nih.gov) search and the most identical isolate was recorded to further aid in the identification of isolates. The expected value (E-value) which indicates the probability of finding a correct match by chance was recorded together with the percentage similarity. Hits (sequences in the data base that matched the query or yet to be identified sequence) with E values less than 10-4 is considered very significant.

3.6 Pathogenicity studies

Pathogenicity trial was evaluated in a greenhouse by inoculating 10-month old grafted mango seedlings in fulfillment of Koch's postulates.

3.6.1 Transplanting of mango seedlings

Mango seedlings of pencil thickness were sourced from the mango nursery of the Soil and Irrigation Research Centre (SIREC) and sent to the Sinna's garden of the Department of Crop Science for transplanting into plastic pots half filled with garden soil.

3.6.2 Inoculum preparation

Using a 5 mm-diameter cork borer, mycelia plugs were cut from the margins of actively growing pure cultures of isolates obtained from the diseased mango plants. The mycelial plugs were then transferred onto PDA media and incubated at 28°C for five days. Inocula from five-day old actively growing pure cultures were used for the inoculations.

3.6.3 Inoculation of mango seedlings

Incisions were carefully made on the stem of each seedling using a sterile razor blade. Plugs of mycelia of each fungal isolates were then taken with sterile cock borer and inoculating pins and inserted into the incisions on the stems of five (5) seedlings. For control seedlings, only agar slant was placed without any fungal mycelia in five seedlings. The peeled portion of the stem was replaced and held in place with a cellotape. The inoculated seedlings were left in a screen house in the Sinna's garden. Seedlings were observed daily till symptoms were seen. An isolate was considered pathogenic when it was able to cause disease symptoms.

3.6.4 Re-isolation of the organism

The infected plants from the artificially inoculated plants were sent to the Plant Pathology laboratory, Department of Crop Science, University of Ghana, Legon and the suspected causal organism was re-isolated in fulfillment of Koch's postulate on water agar and later sub-cultured on PDA and observed under a compound microscope.

3.7 Determination of the inhibitory effect of some fungicides on mycelial radial growth of *L. theobromae*

A preliminary study for field testing of some fungicides was carried out in the laboratory to determine the efficacy of five protectant fungicides and one systemic fungicide on the mycelial radial growth of *L. theobromae.* The mycelial radial growth of the *L. theobromae* isolate used in this study was evaluated using food poisoned technique (Kiran *et al.*, 2010) using potato dextrose agar (PDA). The *L. theobromae* isolate was collected from diseased mango materials from the field.

PDA was prepared as described in section 3.5.2. The effectiveness of five protectant fungicides namely Prochloraz, Zamir, mancozeb, Sulphur 80 funguran, and one systemic fungicide namely Carbendazim were assessed in the laboratory by plating the fungal pathogen on the fungicide amended PDA which were Prochloraz potato dextrose agar (PPDA), Carbendazim potato dextrose agar (CPDA), Funguran potato dextrose agar (FPDA), Mancozeb potato dextrose agar (MPDA) and Zamir potato dextrose agar (ZPDA), Sulphur 80 potato dextrose agar (S80PDA). Unamended PDA was used as a control treatment. The mode of action, active ingredient and the respective concentrations of the fungicides were given in Table 3.3. Completely randomized design (CRD) with three replicates per fungicide of three concentrations each were incubated in the laboratory at

23°C to 31°C and 60-70% RH. The plates were observed daily and colony diameter measured using a ruler for four days.

The percentage reduction in radial growth (I) over control was calculated using the formula proposed by Kiran *et al.* (2010)

$$I = \frac{C - T}{C} x\ 100$$

Where,

I = Percent reduction in growth of test fungi

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment.

Table 3.3 Type of fungicides and application rates used in the *in vitro* experiment

Trade name	Active Ingredient	Mode of action	Various levels of concentration		
			Lower	Standard	Higher
Bendazim	Carbendazim	Systemic	0.085g/50ml	0.17g/50ml	0.26g/50ml
Prochloraz	Prochloraz	Contact	0.075ml/50ml	0.15ml/50ml	0.23ml/50ml
Zamir	Prochloraz and Tebuconazole	Contact	0.075ml/50ml	0.15ml/50ml	0.23ml/50ml
Mancozeb	Mancozeb	Contact	0.13g/50ml	0.25g/50ml	0.38g/50ml
Funguran	Copper Hydroxide	Contact	0.17g/50ml	0.33g/50ml	0.50g/50ml
Sulphur 80	Sulphur	Contact	0.085g/50ml	0.17g/50ml	0.26g/50ml

3.7.1 Field evaluation of three fungicides for control of mango tree decline disease

The field trial was conducted at a mango orchard close to Tunayilli a village in the Savelugu Nanton Municipality in the Northern Region. The farm had a history of mango tree decline disease with mango plants showing higher disease severity. The experiment was designed using the Randomized Complete Block Design (RCBD) with four replications. Mancozeb, Funguran, and Carbendazin which gave a satisfactory inhibition of *L. theobromae* in the *in vitro* evaluation were used in the field control of the disease. The rates for the fungicides are shown in (Table 3.4). Before the first spray, the plants were tagged and the affected trees selected for the trial were pruned to ensure the removal of dead branches and all plant debris from the field.

There were five plants per fungicide treatment. The plants were copiously sprayed four times with the fungicide after every 14 days interval. Before the first spray, treated and control plants were provided with the necessary urea fertiliser at a rate of 870 g per tree based on the manufacturer's recommendation. The ring method was used during the fertiliser application and plants irrigated every two (2) days until the end of the trial.

The effects of the fungicides on mango plants were evaluated by assessing the disease severity before and after treatment. Disease severity was evaluated with the help of a method used by Panhwar *et al.* (2007). Data were collected on the vegetative growth comprising new shoots and leaves for every two weeks after each fungicidal treatment and these were analyzed using the GENSTAT (Version 12).

Table 3.4 List of fungicides, mode of action and their recommended concentration used in field control

Trade Name	Mobility in the plant	Concentration
Bendazim	Systemic	50g/15 L water
Mancozeb	Contact	50g/15 L water
Funguran	Contact	100g/15 L water

CHAPTER FOUR

4.0 RESULTS

4.1 Mango Farmers' Knowledge and Perceptions on Mango Tree Decline Disease (MTDD)

The outcome obtained from interviews and questionnaires administered to 84 mango farmers from mango growing communities in the Northern Region of Ghana to obtain baseline data on the prevalence, spread, economic impact and control of mango tree decline disease are presented below:

4.1.1 General background of mango farmers

Most of the mango farmers at the three mango growing districts in the Northern Region were males (82%) with females constituting only (18%) and about a third of the farmers (29.76%) were young adults between the ages of 31 and 40 years. One in four farmers (25%) were in middle adulthood between (41 and 50 years) whiles 28.5% of them were beyond 50 years. Thus, majority of the farmers were at least 40 years old (Fig. 4.1).

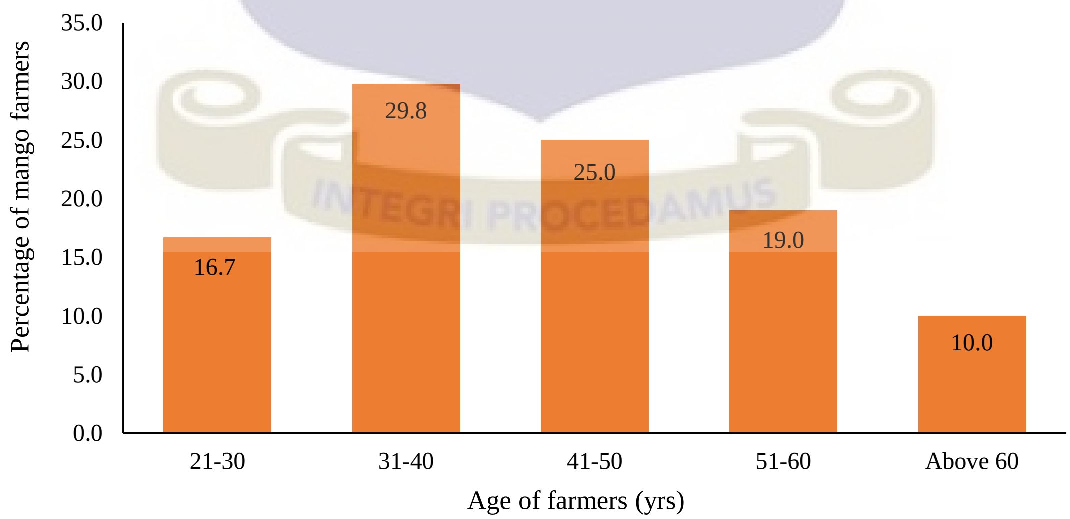


Fig. 4.1 Age distribution of mango farmers in the three mango growing districts in the Northern Region.

A sizable proportion of famers had no formal education (48%) whereas a little over 20% had been educated up to the basic education level (Table 4.1). The percentage of farmers who had obtained a basic level education was not so different from their cohorts with a secondary or vocational qualification (19%). Thirteen percent (13%) of the mango farmers have had their education up to tertiary level.

Table 4.1 Educational attainment of mango farmers

Educational level	Percentage of mango farmers
No formal education	48
Primary/JHS/MSLC	20
Senior high school	19
Tertiary	13

In terms of the age of the mango plantations, 67% of the farms were at most 12 years. In the same vein, 27% of farmers had farms that were between the ages of 13 to 16 years whiles a smaller proportion of farmers (4.8%) had plantations which are more than 16 years old (Fig. 4.2).

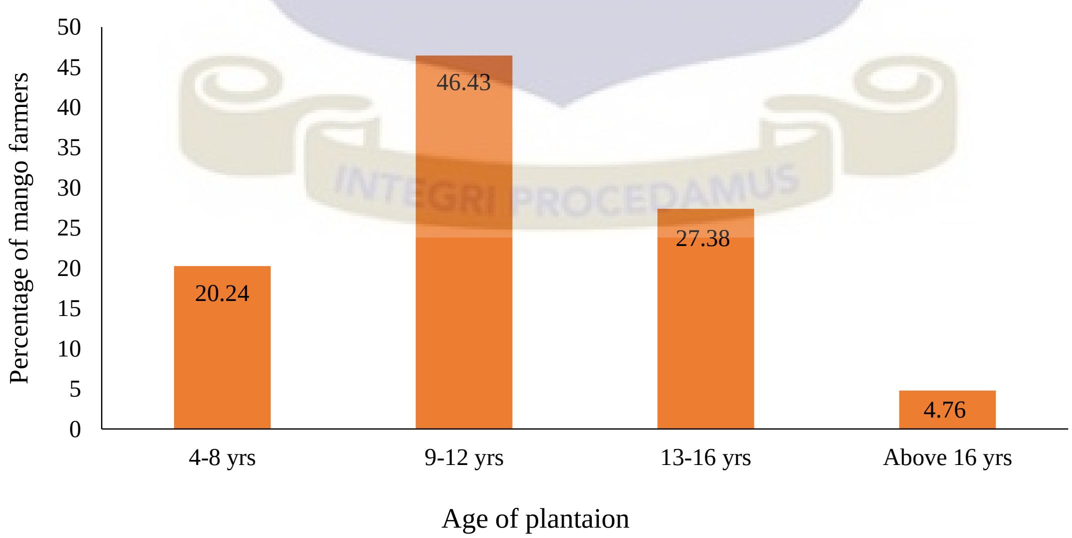


Fig. 4.2 Age of mango farms

4.1.2 Farmers' knowledge, perception and experiences on mango tree decline disease prevalence

All the mango farmers surveyed had observed the new disease on their farms. Farmers' description of symptoms exhibited by diseased mango plants are shown in Table 4.2. Symptoms relating to dying of trees was the most dominant description given by farmers (64%). Gum exudation from trees (6%), dry dead branches (9.5%), bark cracking (6%) and both bark cracking and dry branches (3.6%) were also experienced by diseased plants.

Table 4.2 Farmers' description of symptoms of the new disease

Description of the disease(s)	Percentage of mango farmers (%)
Drying trees	28.6
Stunted growth	2.4
Bark cracking	6.0
Trees dying gradually	35.7
Gum exudation	6.0
Dry branches	9.5
Others	12.8
Total	100

4.1.3 Perceptions of the mango farmers on cause(s) and spread of mango tree decline disease

Diverse causes of the disease were perceived by the farmers. Most farmers (44%) believed that draught is the main cause of the disease followed by poor soil (24%), pathogen (8%) and poor management practices (7%). However, 18% of the farmers had no idea of the cause of the mango tree decline disease. Furthermore, all the farmers attest to seeing or hearing other farmers complaining about the disease (Fig. 4.3).

More than half of the farmers (56%) observed the disease between 2011 and 2015 whiles about 30% of the mango farmers detected the disease between 2006 and 2010. Almost 11% of the farmers noticed the disease between 2001 and 2005. On the other hand, few of them (3.6 %) had no idea on the exact period the disease was first observed (Fig. 4.4).

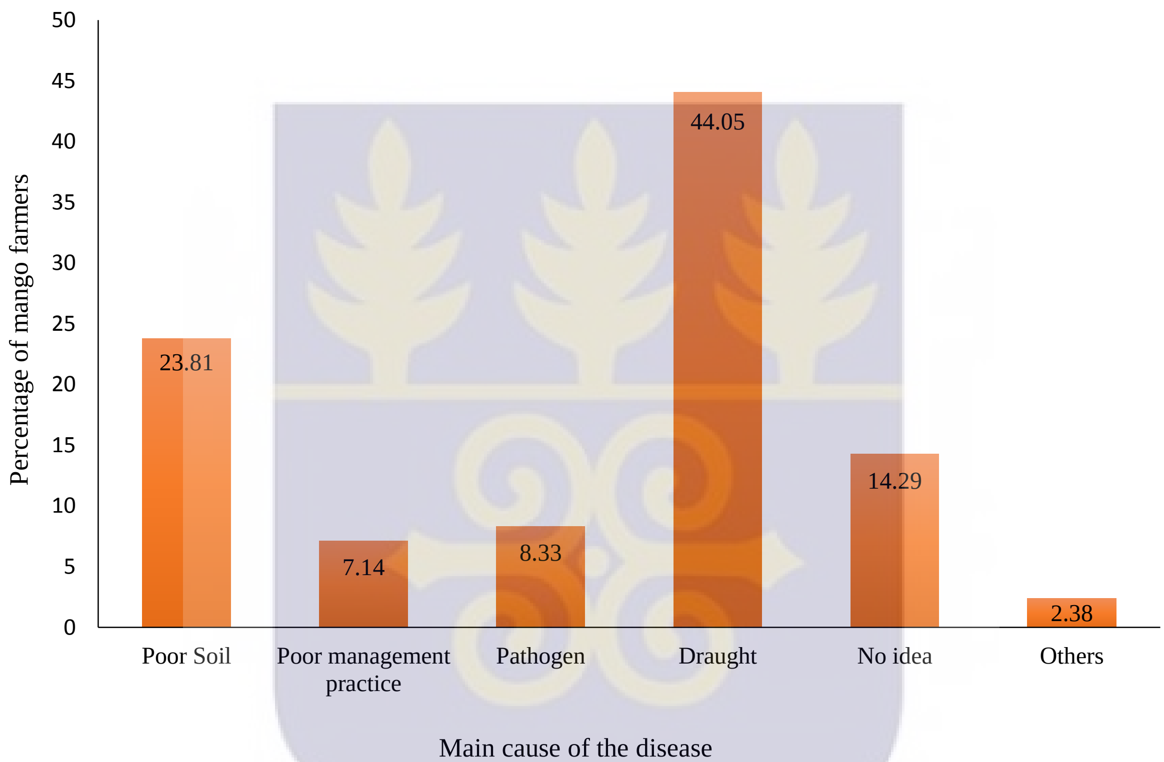


Fig. 4.3 Perception of farmers on cause of mango tree decline disease

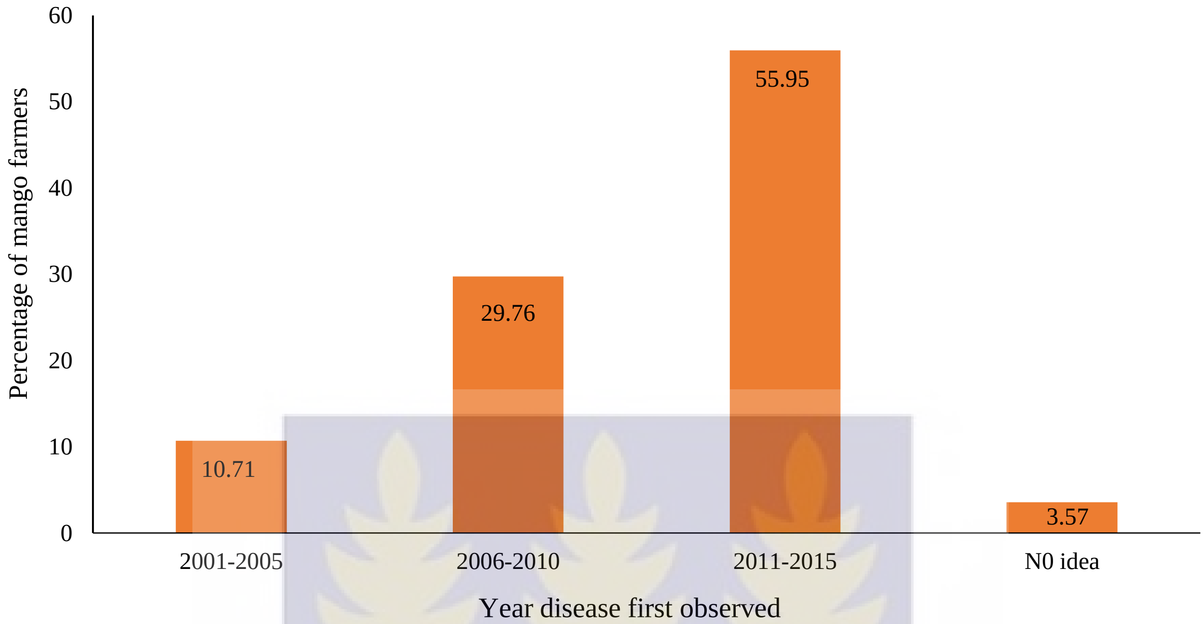


Fig. 4.4 Period (yrs) mango farmers first observed the occurrence of tree decline disease in their farms

About 62% of the farmers often observed the disease in the dry season with almost 37% of them observing it in both rainy and dry seasons. The rest (1%) of the farmers indicated sighting the disease in the rainy season. All the farmers, however, observed a higher prevalence of the disease during the dry season (Table 4.3).

Table 4.3 Season(s) of the year when mango tree decline disease is observed by farmers

Season	**Percentage of mango farmers**
Rainy season	1.2
Dry season	61.9
Both rainy and dry seasons	36.9
Total	100.0

Two-thirds (66.7%) were ignorant about how the disease spread on the same farm whiles twenty-three percent (23%) of mango farmers attributed the spread of the disease on the same farm to edaphic factors. However, 6% of the farmers attributed the spread of the disease to contact with other diseased plants whiles about 5% believed the disease was spread by insects (Table 4.4).

Table 4.4 Perceptions of farmers on means of spread of mango tree decline disease within the same farm

Means of spread of the disease on the same farm	Percentage of mango framers
Insects	4.8
Soil	2
Contact with diseased plant	6.0
No idea	66.7
Total	100.0

Most mango farmers (87%) in the three mango growing districts were ignorant about how the disease spread from one farm to another whiles few farmers (8% and 5%) attributed it to infected planting materials and insects respectively (Table 4.5).

Table 4.5 Perceptions of mango farmers on agents responsible for dissemination of mango tree decline disease among farms

Means of spread of the disease among farms	Percentage of mango farmers
Insect	4.8
Infected planting material	8.3
No idea	86.9
Total	100.0

4.1.4 Farmers' experience on economic impact of mango tree decline disease

Most mango farmers (73%) indicated that diseased trees produced less fruits compared to healthy trees and almost 24% specified that affected trees do not produce any fruits at all. However only a few (3.6%) of the farmers believed the decline diseased trees produced normal fruits like the healthier ones (Table 4.6).

Table 4.6 Farmers' experiences of the effects of mango tree decline disease on yield of fruits

Nature of the impact of the disease on yield	Percentage of mango farmers
Produces less fruits	72.6
It does not produce fruits at all	23.8
Produces healthy fruits	3.6
Total	100.0

Thirty-one (31%) percent of the respondents experienced 41-60% yield (fruit) loss as a result of the disease whereas a quarter of the farmers witnessed 61-80% reduction in yield. Moreover, 24% and 20% of the mango farmers estimated that the percentage loss in yield was at least 81% and 21-40% respectively. In terms of percentage reduction in farmers' income due to the disease, 32% of the farmers experienced 41-60% reduction in their income per annum. No difference was observed for the proportion of farmers who indicated that they have witnessed 61-80% and 81% and above. About 20% of the farmers had a reduction in income of at most 40%. Percentage reduction in income and yield is shown in Fig.4.5.

Reduction in farmers' income contributed to their inability to cater for their families (64%). Approximately, 17 % of the farmers had difficulties in financing the education of their wards and 13 % were unable to pay their hospital expenses (Table 4.7).

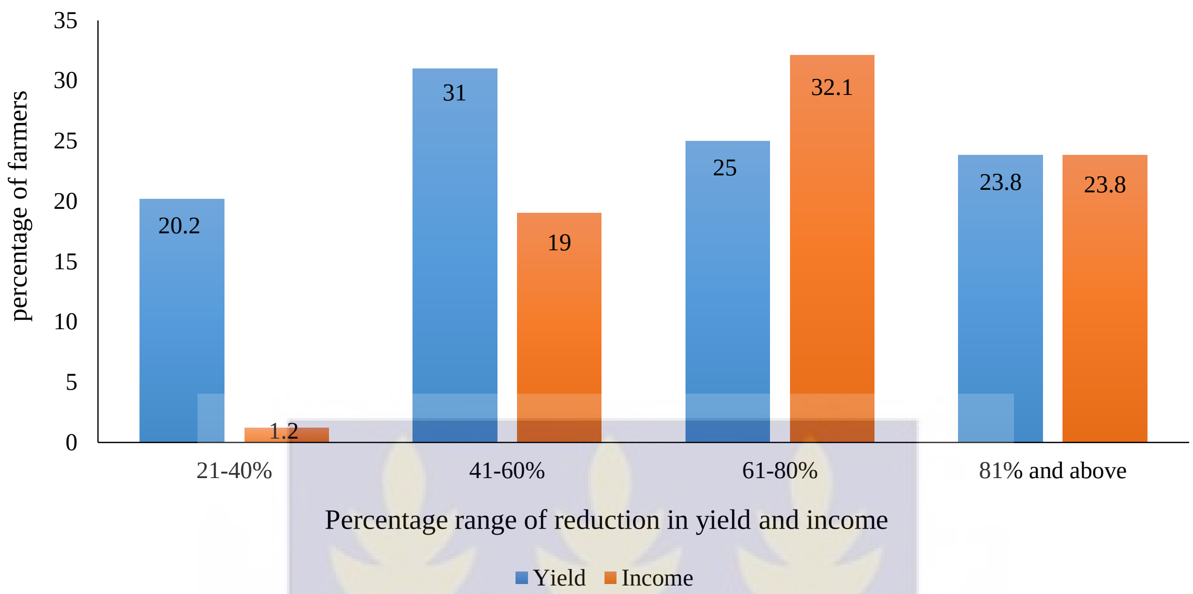


Fig. 4.5 Percentage reduction of yield and income of farmers as a result of mango tree decline disease

Table 4.7 Effects of mango tree decline disease on the livelihood of farmers

Form of the impact on livelihood on farmers	Percentage of mango farmers
Unable to pay fees	16.7
Inability to settle hospital bills	13.1
Upkeep of family becomes a problem	64.3
Others	6.0
Total	100.0

4.1.5 Perception of farmers on control of mango tree decline disease

Most farmers (74%) use pruning as their main control measure with 38% using heavy pruning whiles 36% practice light pruning. On the other hand, 12% use chemicals in controlling the disease whereas 7.1% of the farmers apply fertilizer as means of managing the disease. The rest of the farmers (7.1%) cut and burn affected trees as a control measure (Table 4.8).

Table 4.8 Methods used by mango farmers to control mango tree decline disease

Control Methods	Percentage of mango farmers
Heavy pruning	38.1
Light pruning	35.7
Cut and burn	7.1
Apply fertiliser	7.1
Spray with pesticide	11.9
Total	100.0

Thirty-five percent (35%) of the mango farmers indicated that they spray 3-4 times in a year with 30% of them spraying 5-6 times annually. About 25% of the mango farmers sprayed their farms for at most two times whiles close to 11% of them sprayed more than six times (Fig. 4.6).

Based on suggestions made by the farmers in respect to other means of controlling the disease, 85% of them believe frequent irrigation can help control the disease while 15% of them consider fertilizer application as a possible means of controlling the disease.

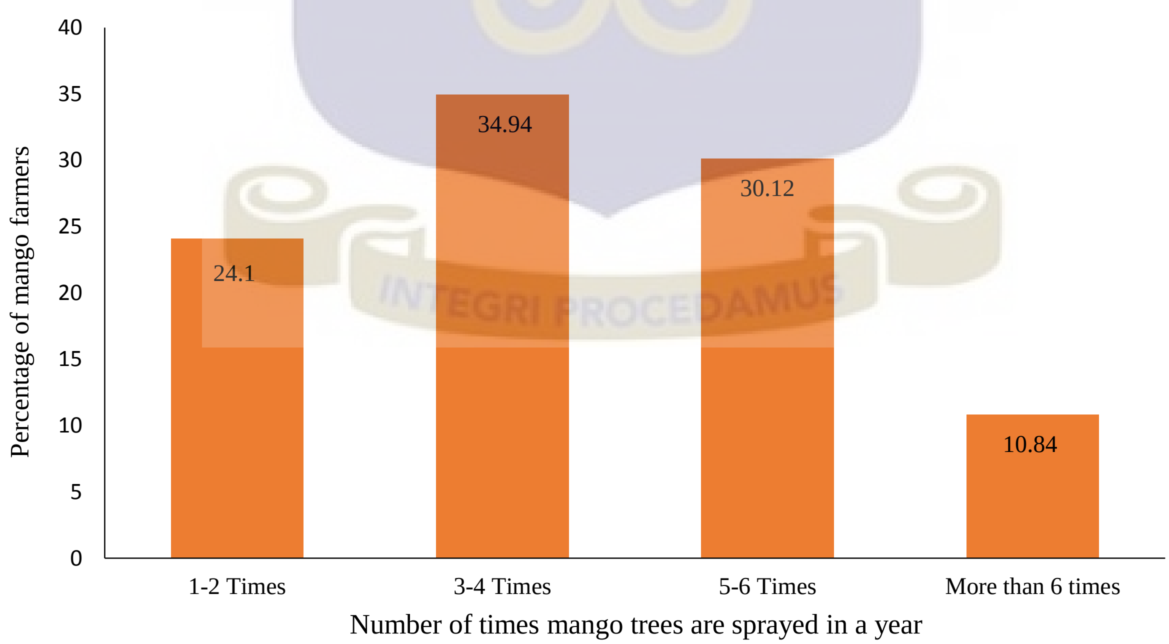


Fig. 4.6 Number of times mango plantation is sprayed by farmers

For farmers who use chemicals as a means of controlling the disease, 41% of them start pesticide spraying on their farms when the trees are 1-2 years old while 32% of them do so when the trees are below one year. Over 14% of the farmers initiate spraying when the mango trees are between 3-4 years old. Thirteen percent (13%) of the farmers, however, sprayed when the mango trees are at least five years (Table 4.9).

Table 4.9 Stages in the development of mango trees at which farmers initiate spraying of pesticides to control mango tree decline disease

Stage at which spraying starts	Percentage of mango farmers
Below 1 yr	32.1
1-2 yrs	40.5
3-4 yrs	14.3
5-6 yrs	7.1
6 yrs and above	6.0
Total	100.0

4.2 Nature of the mango tree decline disease

When mango plants infected by the mango tree decline disease were examined on the field, various symptoms were found to be associated with the disease. Symptoms of the disease at the early stage of the affected tree leaves found on the smaller branches exhibited signs of necrosis but remained attached to the dying trees with a trace of gum from the bark. This is accompanied by the splitting or cracking of the bark with profuse gum exudation. Rotten Canker was observed on severely affected trunks as a result of the gum exudates. A division made on the branches showed a discolouration of the vascular system. At the advanced stage of the disease, wilting of leaves on the smaller branches eventually progressed to the larger branches with total defoliation of leaves. The series of symptoms depicting the disease syndrome is shown in (Fig. 4.7).

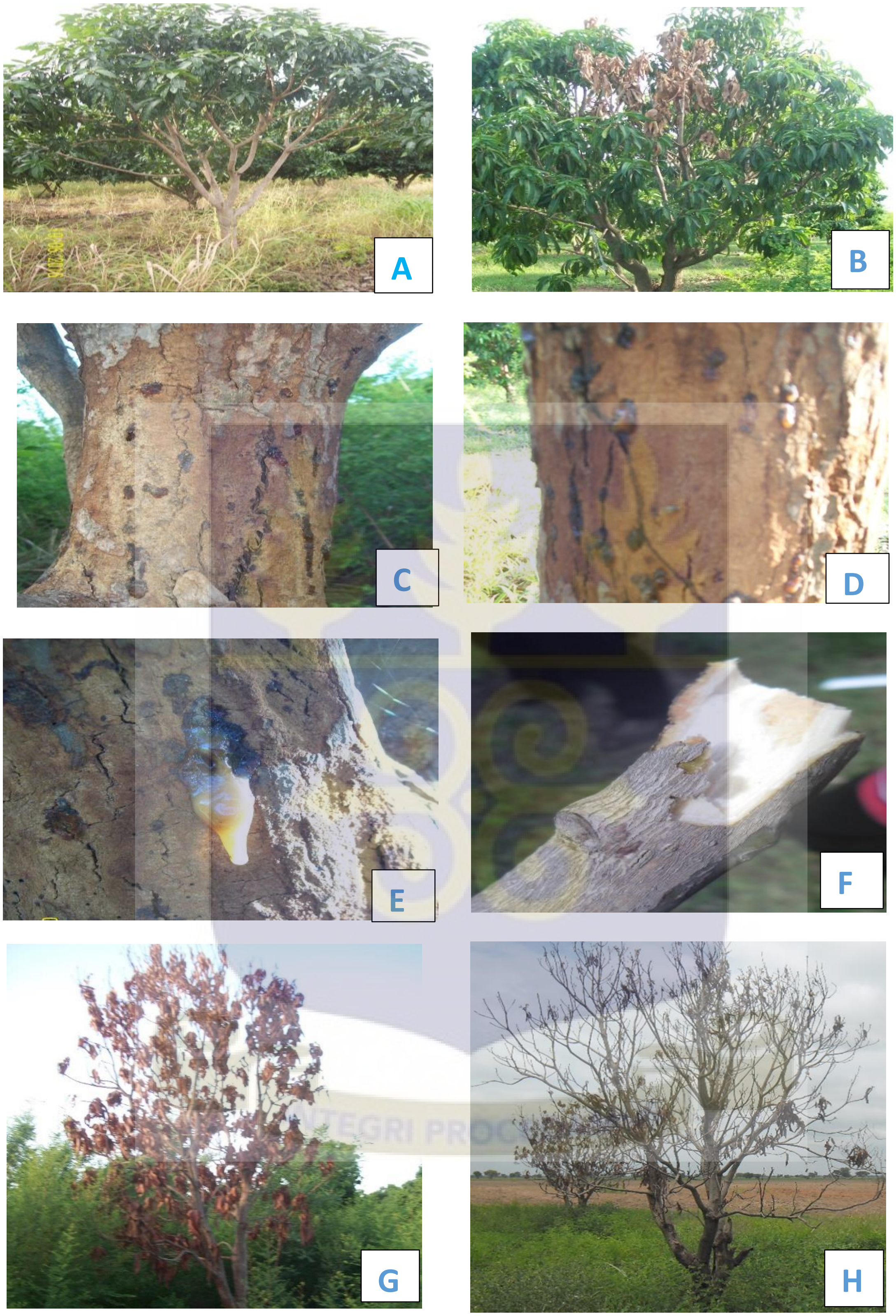


Fig.4.7 Stages of symptom development of mango tree decline. Healthy mango (A), Tip die-back (B), Gum traces (c), Bark cracking/ splitting (D), Heavy gum exudation (E), Vascular discoloration (F), Total wilting of leaves (G), Leaf defoliation (H).

4.3 Impact of the mango tree decline disease

The death and decay of mango trees have been a worry for the farmers over the years. This has resulted in low yield and in some cases no yield being produced by the mango trees.

The desperate mango farmers upon several interventions to curtail the destructive and the devastating nature of the disease has proved futile leaving most farmers with no option than to cut down their diseased trees and quickly replaced them with other arable crops such as cassava, yam, maize and groundnuts (Fig. 4.8). Mango plantations were neglected by the framers resulting to the farms being ravaged by bush fire (Fig. 4.9).

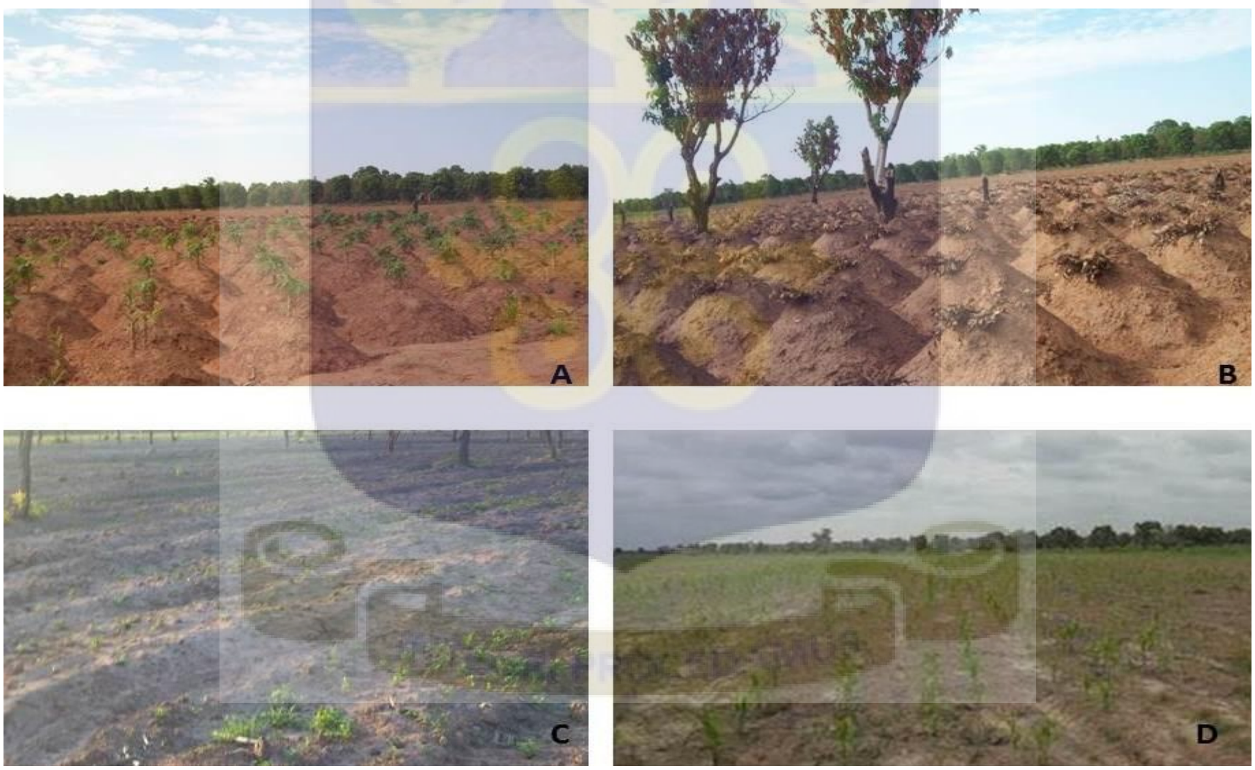


Fig. 4.8 Mango plantation being replaced with arable crops in the Northern Region of Ghana as a result of the mango tree decline disease. Infected mango trees cut down and being replaced with cassava at Kumbungu in the Kumbungu district (A), mango trees being cleared and replaced by yam in Tamaligu in the Karaga district (B), infected mango trees being replaced by groundnut in Gushie in the Savelugu Nanton Municipality (C), infected mango trees being replaced by maize in Tunayili in the Savelugu Nanton Municipality (D)

Fig. 4.9 A neglected mango plantation in Diare in the Savelugu Nanton Municipality as a result of the mango tree decline disease being ravaged by fire (A), An abandoned mango plantation in Zugu in the Kumbungu district as result of the mango tree decline disease (B).

4.4 Incidence and severity of mango tree decline disease in the three mango growing districts

The results obtained in the 12 communities in the three mango growing districts in 2015 are shown in Table 4.10. The highest disease incidence was found in Zankaili (84.3%), Diare (82.9%) and Gushie (80.9%) whereas Zangbalung (55.7%), Zugu (62.9%), and Kumbugu (64.3%) recorded the lowest disease incidence. However, Pong-Temale and Tamaligu recorded the same disease incidence of (75.7%) with Tunayilli and Gbullung (72.9%), Tamaligu (75.7%) and Maligunayili (71.4%). Also, the disease severity was found to be higher in Diare (2.74), Gushie (2.64), Pong-Tamale (2.47), Zankali (2.23) and Bagurugu and Tunayilli (2.43). The disease severity in Zangballung (1.29) and Kumbugu (1.36) were lower than what was recorded in Zugu (1.39) and Tamaligu (1.54). Gbullung and Maligunayili registered 1.66 and 1.76 respectively (Table 4.10).

With respect to disease incidence in the various districts, Karaga district recorded the highest disease incidence (77.9%) followed by Savelugu- Naton municipality (77.1%) with Kumbugu (63.9%). Savelugu Nanton registered the highest disease severity (2.57) followed by Karaga district (2.0) with Kumbugu district (1.42) being the least (Table 4.11).

Table 4.10 Mean incidence and severity of mango tree decline disease in twelve randomly selected communities in the Northern Region of Ghana

Community	Incidence (%)	Severity
Gushie	80	2.64
Diare	82.9	2.74
Tunayilli	72.9	2.43
Pong-Tamale	75.7	2.47
Kumbugu	64.3	1.36
Zugu	62.9	1.39
Zangbalung	55.7	1.29
Gbullung	72.9	1.66
Tamaligu	75.7	1.54
Zankali	84.3	2.24
Maligunayili	71.4	1.79
Bagurugu	77.1	2.43

Survey was conducted in the major season in 2015

Table 4.11 Average incidence and severity of mango tree decline disease in three (3) randomly selected districts in the Northern Region of Ghana in 2015

District/Municipal	Incidence (%)	Severity
Savelegu Nanton	77.1	2.57
Kumbugu	63.9	1.42
Karaga	77.9	2.00

4.5 Fungi associated with the mango tree decline disease in the Northern Region

Three fungi species namely *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides* and an unidentified one were consistently isolated from the diseased mango plant parts. Identification was based on the following outlined characteristics.

4.5.1 *Lasiodiplodia theobromae*

4.5.1.1 Morphological identification

Culture of *L. theobromae* on a PDA was found to be grey to black, fluffy with abundant aerial mycelium. Matured cultures showed black pigmentation. The fungi colonies had profuse aerial mycelia which eventually turned from grey to black with age and finally formed a black pycnidia. Shiny black pynidia were produced on the surface of the media after six to ten days (Fig.4.10).

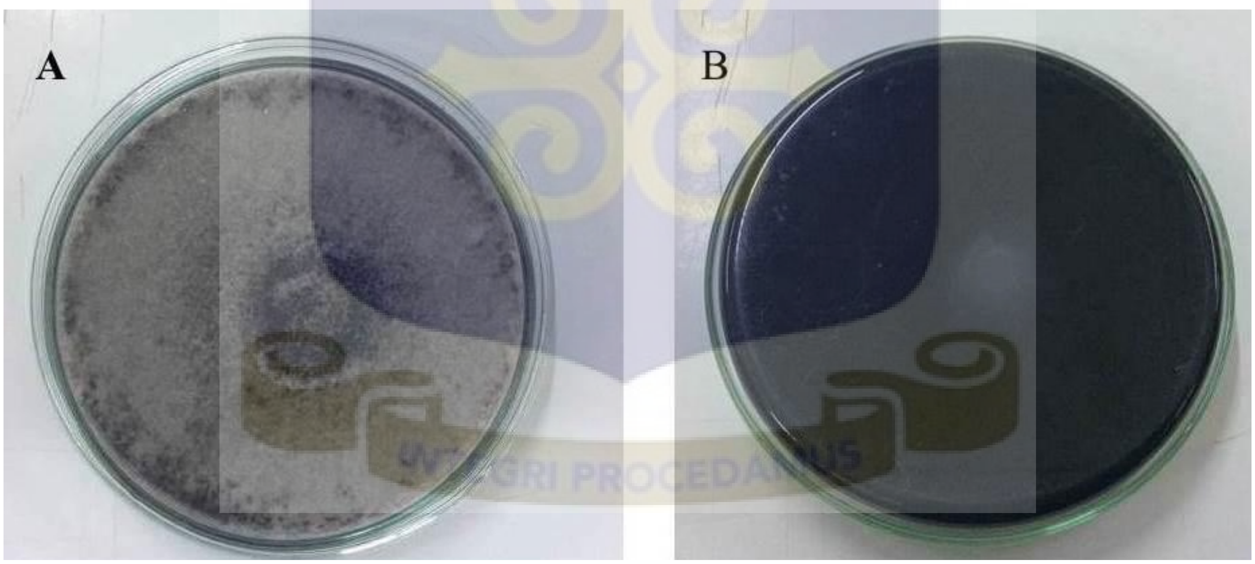


Fig. 4.10 A fifteen-day old culture of *Lasiodiplodia theobromae* on PDA. A sepia of mouse grey black, fluffy with abundance of aerial mycelium (A) and a reverse fuscous to black (B)

Conidia formed were initially observed as unicellular and translucent, sub-ovoid to ellipsoid, oblong, thick-walled, granulose and having truncated base at the immature stage of the spore development. Conidia are two-celled (one-septate) and cinnamon to dark brown, thick-walled, ellipsoidal and longitudinally striated (Fig. 4.11).

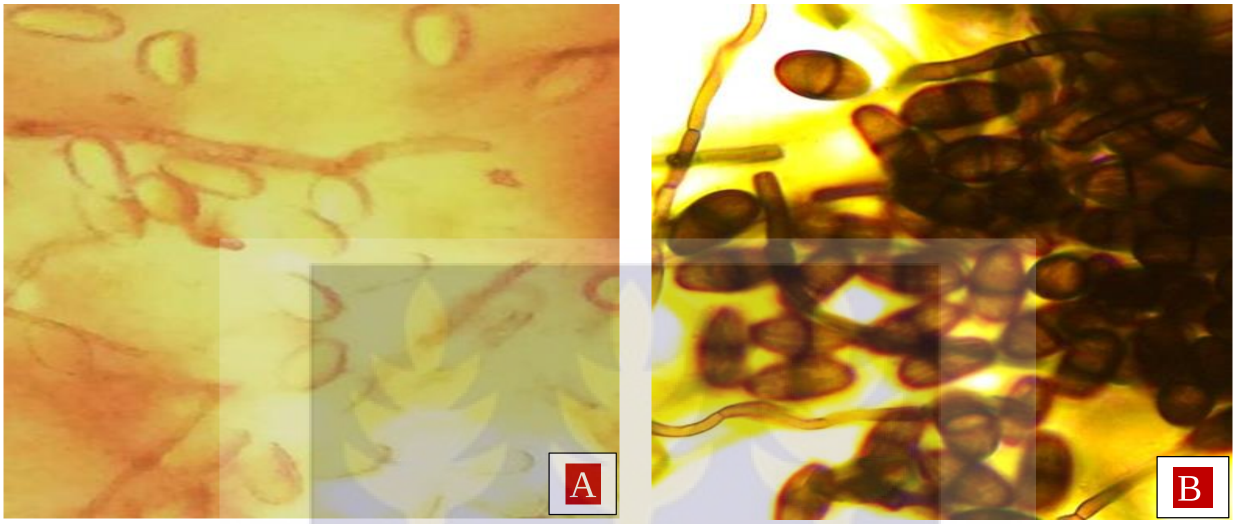


Fig. 4.11 Micrograph of (A) immature, translucent, unicellular conidia (400x) and (B) mature, dark brown, bi-celled, thick walled conidia of *L. theobromae* spores.

4.5.1.2 Molecular identification of *L. theobromae*

The result of the molecular experiment showed that, the species primer in conjunction with ITS primer amplified approximately 347 bp fragment from genomic DNA of mango fungus. Fig. 4.12 shows agarose gel of *Lasiodiplodia theobromae*

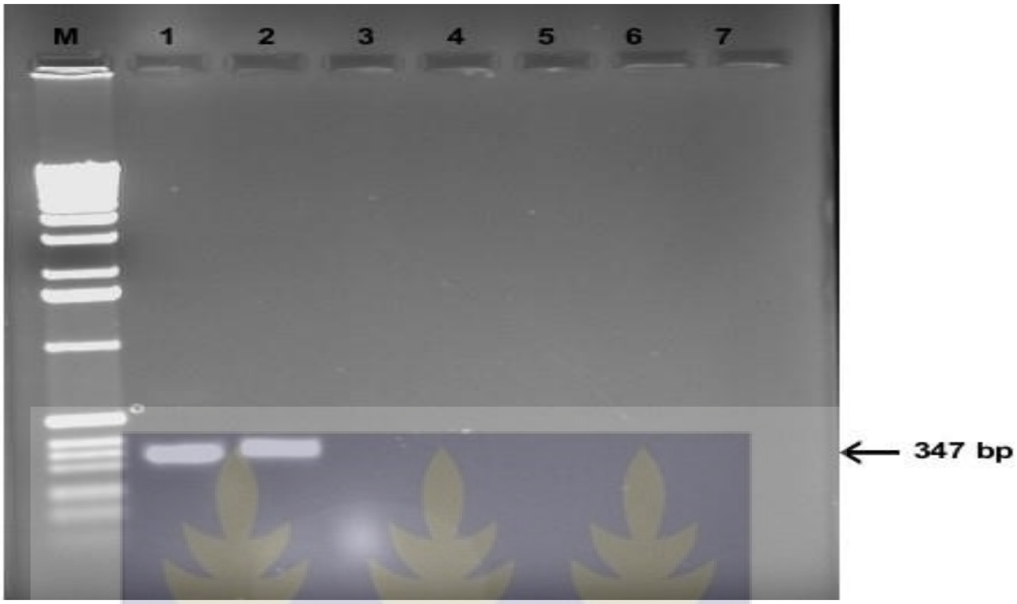


Fig. 4.12 A gel showing amplification of specific fragment of *L. theobromea* isolates from mango. Marker (M), 1 and 2 showing the bands of the isolate.

4.5.2 *Colletotrichum gloeosporioides*

4.5.2.1 Morphological identification

The colonies *C. gloeosporioides* on potato dextrose agar were found to be grayish white to dark grey with aerial mycelia which ranged from a thick mass to sparse tufts associated with fructifications. The conidia were found to be short, hyaline, and unicellular, and were either cylindrical with obtuse ends or ellipsoidal with a rounded apex and a narrow truncated base. The conidia form hyaline to faintly brown conidiophores in acervuli which are irregular in shape.

Setae which are found on the acervuli are one to four septated, brown, slightly swollen at the base and tapered at the apex (Fig. 4.13).

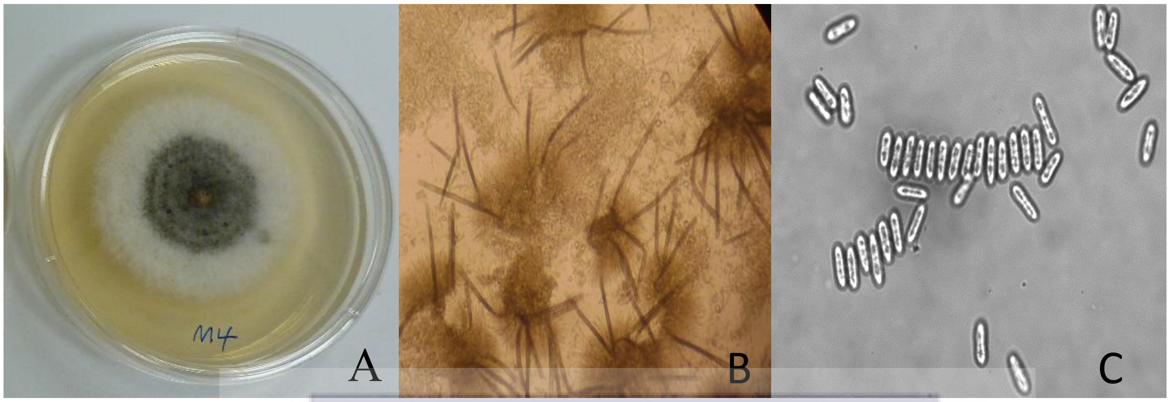


Fig. 4.13 Cultural and Morphological characteristics of *C. gloeosporioides* isolated from mango bark. Mycelium growth on PDA (A), Setae on acervulus (B), Spores (C)

4.5.2.2 Molecular identification of *C. gloeosporioides*

The outcome of the molecular experiment revealed that, the species primer in conjunction with ITS4 primer amplified approximately 480 bp fragment in Fig.4.14 from genomic DNA of mango fungus.

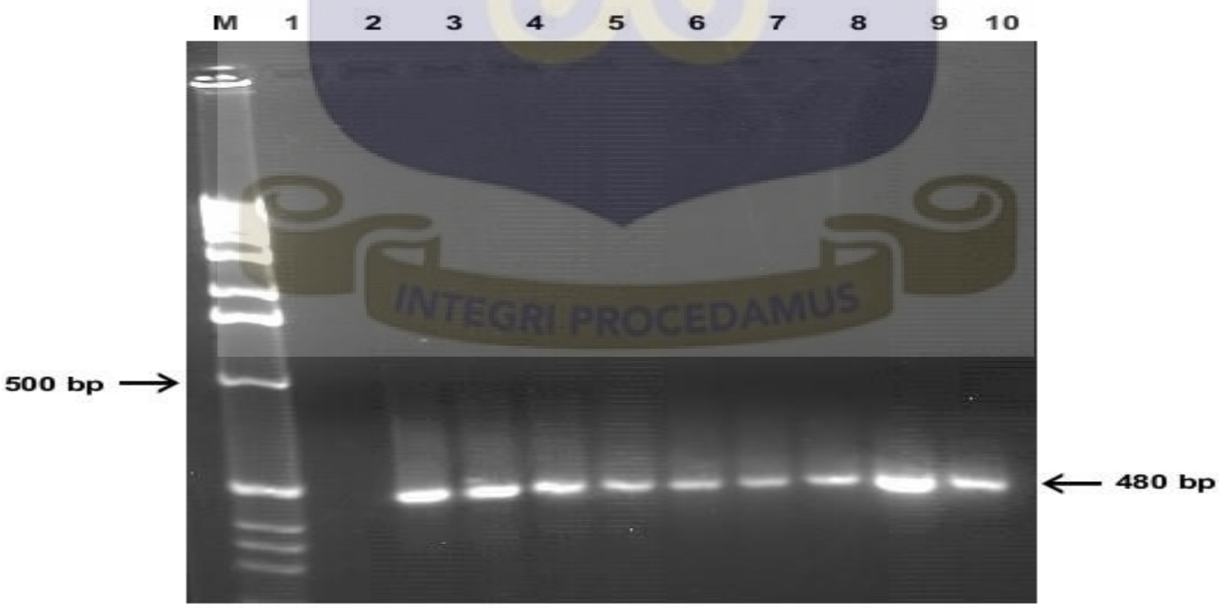


Fig. 4.14. A gel showing amplification of specific fragment of *C. gloeosporioides* isolates from mango. Marker (M)

4.5.3 *Neofusicoccum parvum*

4.5.3.1 Morphological identification

Culture characteristics of *Neofusicoccum parvum* (Fig. 4.15) showed colony mycelium which was fluffy and initially white but turned smoke grey from the middle of the colonies within 4–7 days, with an appressed mycelial mat and had sparse which was moderately dense. Also observed was Cottony aerial mycelium towards the edge of the colony which was characterized by pale olivaceous grey and with time changed to olivaceous grey and finally greenish black

Fig.4.15 A twelve- day old culture of *Neofusicoccum parvum* on PDA

4.5.3.2 Molecular Characterization of *Neofusicoccum parvum*

A PCR product of approximately 750 bp was amplified from the DNA of the fungus using the primer ITS1/ITS4. The assembled sequence was 600 bp. Blast search using the assembled nucleotide sequences of the rDNA-ITS region of the fungus showed the unidentified fungus to be similar to *Neofusicoccum parvum*. The percentage similarity or homology was 100% between isolate obtained from this study and *N. parvum* STRAIN L12 (accession number KR 260793.1). The isolate was therefore identified as *N. parvum*.

4.6 Frequency of occurrence of fungi on diseased mango plants

Lasiodiplodia theobromae was the highest isolated fungus (98%) followed by *C. gloeosporiodes* (50%) and *Neofusicoccum parvum* (10%) (Table 4.12).

Table 4.12 Frequency of occurrence of fungi on diseased mango plants

Isolated fungus	Percentage of occurrence
Lasiodiplodia theobromae	98
Colletotrichum gloeosporioides	50
Neofusicoccum parvum	10

4.7 Pathogenicity test of fungal isolates

The three fungi species isolated from the diseased plant parts were used in the inoculation. Pathogenicity test of the isolates revealed that *L. theobromae* was pathogenic (Fig. 4.16) but *Colletotrichum gloeosporioides* and the *Neofusicoccum parvum* isolates were not. *L. theobromae* was able to express the disease symptoms on each of the five mango seedlings inoculated. Dieback coupled with gum exudation symptoms were first observed 42 days after inoculation. After 70 days the gum exudation disappeared. The inoculated seedlings equally exhibited leaf necrosis which remained attached to the dying stem though some of the leaves defoliated. The entire seedlings died after four months. A vertical section through the affected stems showed vascular discolouration. The roots developed showed root rot but the control plants had no symptoms of the disease. The pathogen was re-isolated from the diseased lesions to confirm the fungus as pathogenic to mango plants

Fig. 4.16 Pathogenicity trial of *L. theobromae*. Wilting of mango seedlings (D) inoculated with *L. theobromae* after four months and control seedlings (C).

4.8 Inhibitory effect of six fungicides on mycelial radial growth of *L. theobromae*

The trial was to assess the effectiveness of some fungicides in the laboratory for application on the field. There was complete inhibition (100%) of mycelial radial growth of *L. theobromae* by five of the fungicides namely carbendazim, funguran, mancozeb, prochloraz, and zamir. Table 4.13 shows percentage inhibition of radial mycelial growth of *L. theobromae* at various concentrations. The fungicides inhibited the growth of pathogen throughout the four-day period of the trial. One fungicide, sulphur 80 was unable to completely inhibit growth of the fungus at various concentrations. There was no significant difference in growth of the fungus on the second day at the various concentrations of sulphur 80 but days three and four saw a significant difference in growth between the organism grown on the standard and the rest of the concentrations.

Table 4.13 Percentage inhibition of mycelial radial growth of *L. theobromae* by potato dextrose agar amended with six fungicides

Fungicide	Concentration	Mean inhibition % of mycelial radial growth		
		Day 2	Day 3	Day 4
Carbendazim	0.09 g/50ml	100 a	100 a	100 a
	0.17 g/50ml	100 a	100 a	100 a
	0.26 g/50ml	100 a	100 a	100 a
Funguran	0.17 g/50ml	100 a	100 a	100 a
	0.33 g/50ml	100 a	100 a	100 a
	0.50 g/50ml	100 a	100 a	100 a
Mancozeb	0.13 g/50ml	100 a	100 a	100 a
	0.25 g/50ml	100 a	100 a	100 a
	0.38 g/50ml	100 a	100 a	100 a
Prochloraz	0.08 ml/50ml	100 a	100 a	100 a
	0.15 ml/50ml	100 a	100 a	100 a
	0.23 ml/50ml	100 a	100 a	100 a
Sulphur 80	0.09 g/50ml	15 b	25 c	28 c
	0.17 g/50ml	22 b	43 b	36 b
	0.26 g/50ml	20 b	25 c	27 c
Zimir	0.08 ml/50ml	100 a	100 a	100 a
	0.15 ml/50ml	100 a	100 a	100 a
	0.26 ml/50ml	100 a	100 a	100 a

Means followed by same letters in a column are not significantly different at LSD (5%)

4.8.1 Field control

4.8.1.1 Incidence and severity of mango tree decline disease treated with some fungicides

The fungicidal spray showed a drastic reduction of fungal infection in treated mango plants. There was a steady reduction in the disease incidence as well as disease severity in the decline trees treated with fungicides (Fig. 4.17 and 4.18). On the other hand, the incidence and the severity increased in control (untreated) trees with time. Two weeks after the second fungicidal spray carbendazim and funguran showed promising results. There was complete disappearance of all the disease symptoms in affected trees treated with carbendazim after the third spray. Nevertheless, trees sprayed with mancozeb still showed traces of gum exudation. The field trial showed carbendazim to be the most highly effective fungicide for the control of the decline disease followed by funguran and mancozeb.

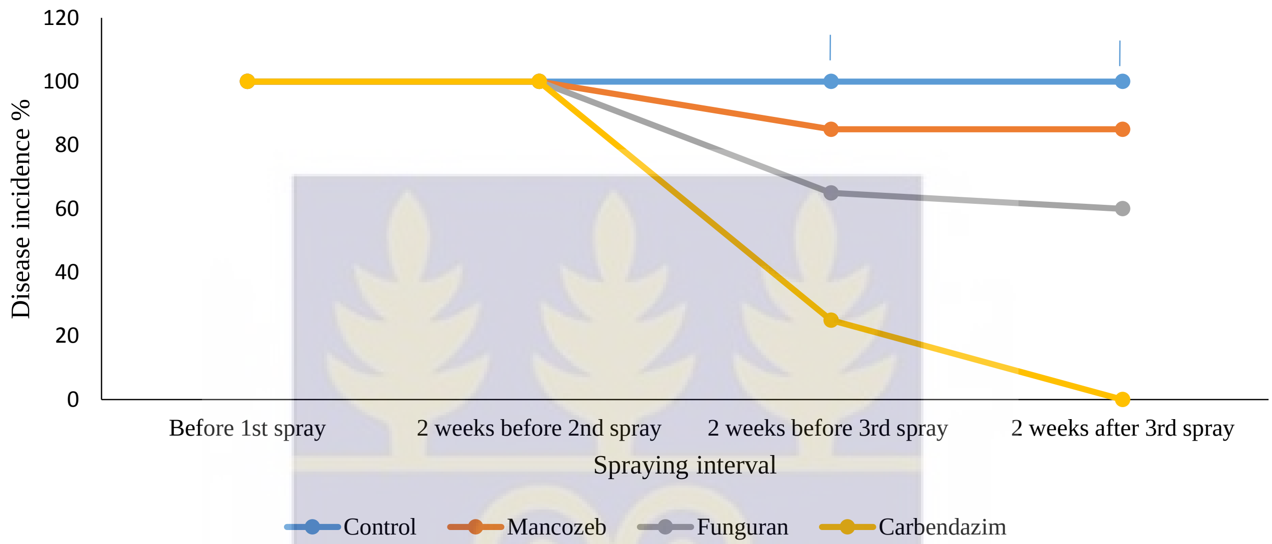


Fig. 4.17 Effect of fungicidal spray on incidence of mango tree decline disease. Vertical bars represent LSD (5%).

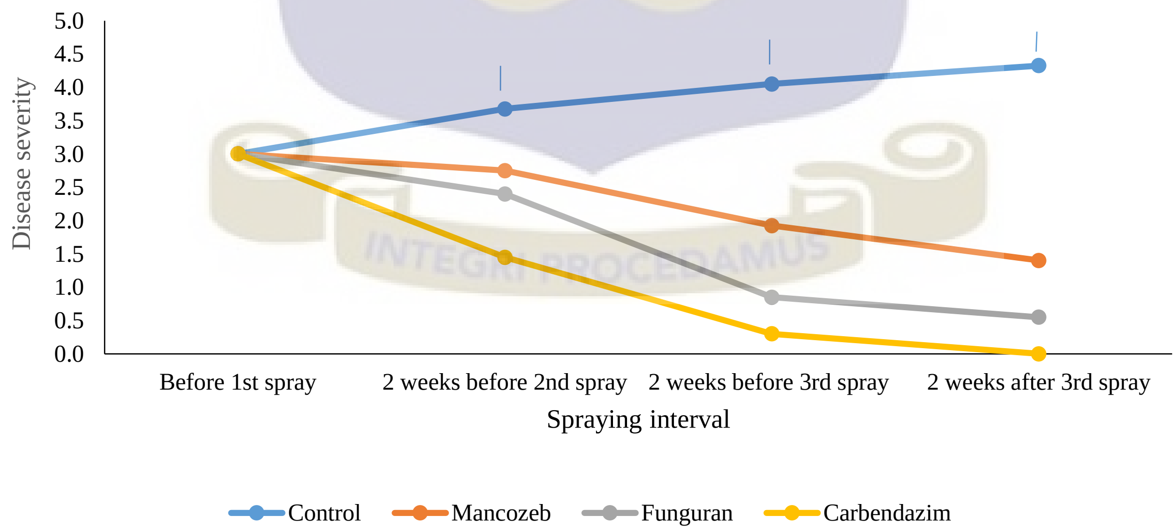


Fig. 4.18 Effect of fungicidal spray on disease severity of mango tree decline disease. Vertical bars represent LSD (5%).

4.8.1.2 Effects of fungicidal spray on shoot and leaf growth of mango tree decline disease.

Average number of shoots and leaves of diseased mango plants tend to increase across all treatments as the fungicides were applied at a two-week interval (Fig. 4.19 and 4.20). Analysis of variance showed a significant difference between the various treatments. Leaves and shoots production consistently increased till the end of the fungicides treatment (Fig.4.21). Mango plants treated with carbendazim produced the highest number of leaves followed by funguran and mancozeb. After the 3rd spray the diseased trees sprayed with carbendazim and funguran showed improvement in vegetative (shoots and leaves) growth. However, trees treated with mancozeb produced less vegetative shoots and leaves. For the untreated control plants, shoot and leaf production decreased with time.

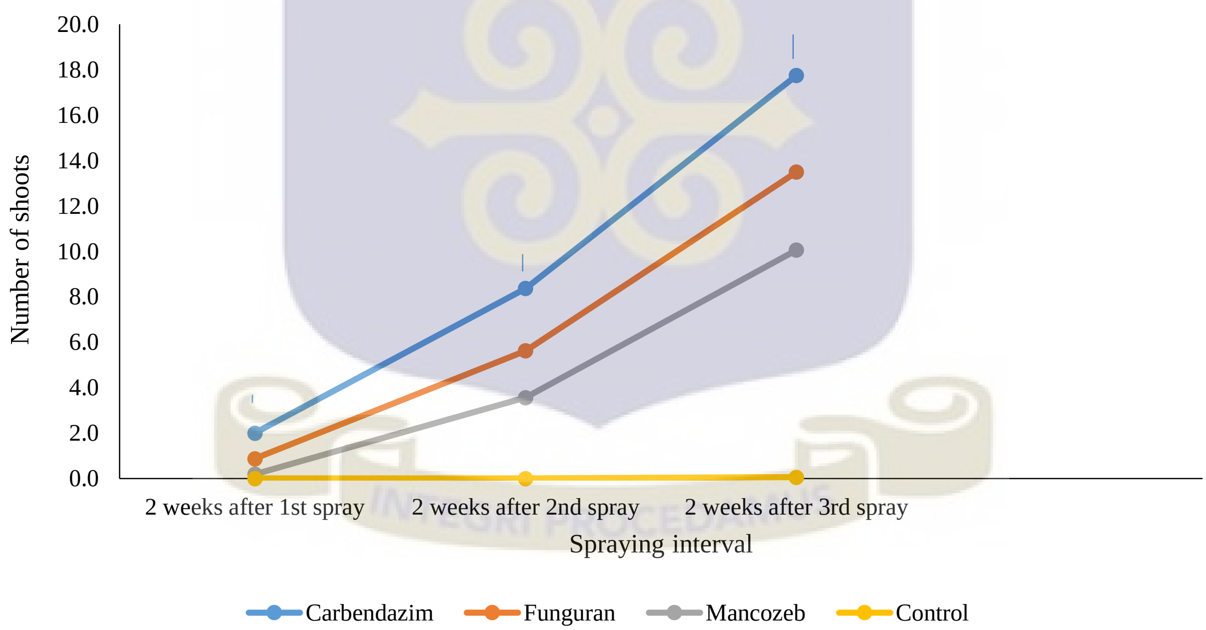


Fig. 4.19 Effect of fungicidal spray on shoot growth of mango tree decline disease. Vertical bars represent LSD (5%).

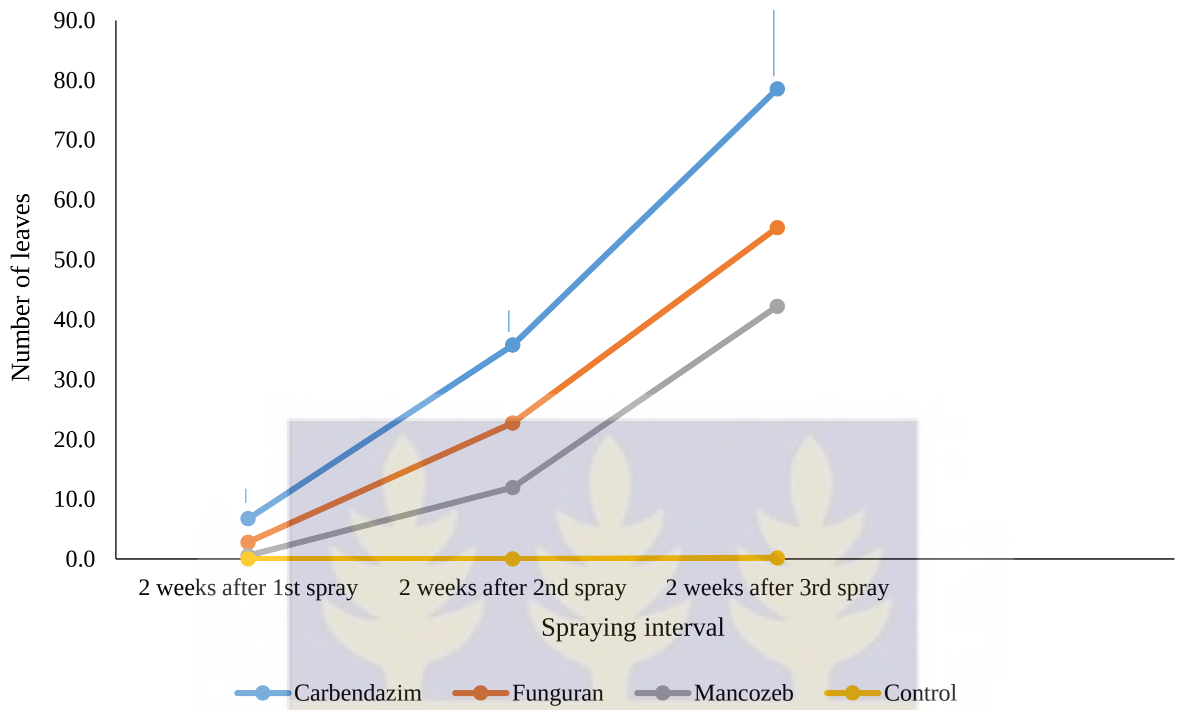


Fig. 4.20 Effect of fungicidal spray on leaf growth of mango tree decline disease. Vertical bars represent LSD (5%).

Fig. 4.21 Effects of fungicidal spray on leaf growth. Carbendazim (**A)**, Funguran **(B)**,Mancozeb (**C)**, Control (**D)**

CHAPTER FIVE

5.0 DISCUSSION

A considerable number of mango farmers in the three districts of the Northern Region of Ghana where mangoes are mostly produced had no formal education but were able to identify the mango disease by its symptoms on the field. Majority of the farmers (82%) were males and this could be attributed to the capital intensive nature of mango production, as well as customs which make it impossible for females to own land titles in the Northern Region. This was supported in a case study conducted by Okorley *et al*. (2014) in Dangme West district where mango farming business is male dominated and predominantly practised as monoculture by small to medium scale holders with Kent and Keitt as the major varieties grown. The present survey revealed that about 84% of mango farmers who were interviewed in this study were above the age of thirty. This implies that the youth are therefore not involved in the mango farming business in the three mango growing districts in the Northern Region of Ghana. With respect to the ages of the mango plantation the results showed that most of the mango trees were planted between 2004 and 2007. This is an indication that mango production in the Northern Region is a new venture being carried out by the farmers. Only a few of the mango farms (4.8%) were found to have been planted over 15 years ago.

Majority of farmers were ignorant of the cause of the mango tree decline disease and so attributed it to factors such as poor management practices, poor soil and drought. However, *L. theobromae* has been identified as the principal pathogen responsible for mango tree decline disease in most countries (Al-Adawi *et al*., 2003; Iqbal *et al.*, 2004; Malik *et al.*, 2005; Al-Adawi *et al*., 2006) among other pathogens. Since most of the farmers did not know the main cause of the disease, they were not able to control it and therefore abandoned their mango plantations. Nutritional deficiency, drought, temperature fluctuations have also been identified as abiotic factors which speed up mango tree decline disease (Schaffer *et al.*, 1988; Ploetz, 2003; Malik *et al.*,2004; Nafees *et al.*, 2010; Masood *et al.*, 2012). In this study, the disease was observed in both rainy and dry seasons but most of the farmers in the study area reported that the highest prevalence of the disease is in the dry season as compared to the rainy season. The observation corroborates to a report by (http: //horticulture world.net/mango-india2.htm) that the disease can be observed at any time of the year but mostly noticed in October and November in most countries including India. Though most of the farmers in the three mango growing districts in Northern Ghana had no idea about the mode of spread of the disease on the same farm, a few attributed the spread of the disease to edaphic (soil) factors, contact with diseased plants and insects. Regarding insects, the farmers were unable to identify the particular insects responsible for the transmission. However, Rawal (1998) identified beetle (*Xeleborus offinis*) as transmitting agent of the disease at a relative humidity above 80% and temperature of 25 – 31^{0}C. Mango bark bettle (*Hypocryphalus mangiferae*) has also been implicated as a vector of the disease in Pakistan (Al Adawi *et al*., 2006; Saeed and Masood 2008; Masood *et al*., 2010). For mode of spread of the disease among farms, 86.9% of the farmers were ignorant of its transmission. Few (10.8%) of the farmers perceived that insects and infected planting materials are responsible for the spread of the disease among farms.

Mango production is a profitable venture hence can bring huge returns to the growers. It was however, realized through this study that mango tree decline disease has a great economic impact on fruit yield especially a reduction in number of fruits per plant. In some cases, the disease may cause mango trees to produce no fruits. Only a negligible number of the farmers (3.6%) perceived that there was no significant negative effect of the disease on fruit yield. Perhaps the disease had not reached its devastating stage on their farms or they are using some cultural practices to manage the disease. Some cultural practices such as frequent pruning, fertiliser application, regular weeding and irrigation could therefore reduce the impact of the disease on yield to improve the livelihood of the farmers in the three mango growing districts of the Northern Region of Ghana.

Almost all respondents in this study recorded a reduction in yield as a result of the disease which consequently decreased their incomes from mango production and resulted in difficulty in affording school fees and hospital bills as well as meeting the basic needs of their families.

In an effort to manage the disease, mango farmers in the experimental communities resorted to the use of phyto-sanitary practices such as moderate to heavy pruning and rouging of diseased trees by burning. Effective pruning of the affected parts of diseased plants followed by spraying of wounded areas with 5:5:50 Bordeaux mixture has been found to control the disease efficiently (Parkash and Raoof, 1989). As a good agronomic practice, it is recommended that regular pruning of the mango trees must be carried out to facilitate the penetration of sunlight into the canopy to reduce humidity and hence the incidence of the mango tree decline disease. Other mango farmers resorted to the use of fertiliser application as well as spraying of pesticides (fungicides) to slow the spread of the disease. Minority (11.9 %) of the mango farmers use pesticides which include Nordox and local plant extracts (neem extract and "Awabili'). Meanwhile Muhammad *et al*. (2005) mentioned carbendazim as effective in inhibiting growth of mycelia under both *in vitro* and *in vivo* conditions suppressing gum exudation, dieback and wilting. This consequently results in significant improvement in the vegetative growth of plants. However, the timing, and the frequency of application and the choice of fungicides by most farmers who used chemical control in the study were inappropriate and may result in the development of fungicide resistance. However, Mahmood *et al*. (2002) have reported that first foliar spray of Topsin-M (Thiophanate-methyl) at 1g/L to manage the disease resulted in 10% reduction in the incidence of infection by

L. theobromae and 2nd spray of the same fungicide completely inhibited the fungus. As a prophylactic measure, majority of the farmers begin to spray their mango plantation with fungicides when the trees are 1-2 years old but about a third of the farmers start spraying fungicides on mango trees when they are below one year.

Symptoms of the mango tree decline disease which was found to be common among all the affected trees across the twelve experimental areas within the three districts where the survey was carried out occurred on different parts of the tree including leaves, bark of the stem, main trunk and root. At the early stage of the disease infection, affected leaves on smaller branches became necrotic and gradually progressed to the main branches where gum exudation from the bark of the main branches was observed. This confirmed the work of earlier researchers (Rasmos *et al*., 1991; Al-Adawi *et al.,* 2006; Saeed and Masood, 2008). Subsequently, profuse gum exudation occurs and this was followed by splitting or cracking of the bark. A related study by Pernezny and Ploetz (2000) also revealed symptoms of mango tree decline disease to include blight, canker, gummosis, twig blight, tip die-back and stem bleeding. On severely affected trees, gum exudates led to rotten canker and in most severe form, the disease caused wilting and defoliation of the entire leaves. The vascular system was observed to have been discoloured upon a longitudinal division of the branches. This observation agrees with findings of Ploetz *et al.* (1996) and Pernezny and Ploetz, (2000) who reported that leaves of trees affected by mango tree decline disease include defoliation, vascular discolouration and marginal chlorosis.

Incidence and severity of the mango tree decline disease was found to vary in the twelve selected communities where the study was conducted. Zankali, Diare and Gushie recorded the highest disease incidence. This could be the result of the neglect of the infected fields by the farmers. The lowest disease incidences were recorded in Zangbalung, Zugu and Kumbugu respectively. The disease was comparatively severe in Diare, Gushe, Pong-Tamale, Tunayilli and Bagurugu respectively. The rest of the communities in the study area recorded a significantly lesser level of severity. Results of the present study revealed a non-significant difference in disease incidence across the three experimental districts. However, similar studies conducted elsewhere revealed incidence of the mango tree decline disease as 20% in Punjab and over 60 % in Sindh Provinces of Pakistan, and 60 percent in Al Batinah region of Oman (Mahmood *et al*., 2002; Al-Adawi *et al*., 2006; Saeed *et al*., 2006)

The mango tree decline disease was found to be significantly different across the study districts with Savelugu Nanton recording the highest disease severity followed by Karaga and finally Kumbungu. Even though Karaga had a relatively higher disease incidence, severity of the disease was rather higher in the Savelugu Nanton munucipality. The disease incidence and severity were low in Kumbungu district as a result of measures adopted by farmers such as watering. As a result of the devastating nature and severity of the disease, most mango farmers within the studied districts reported to have abandoned their farm plantations with some of the farmers opting for replacement of their mango plantations with arable crops such as groundnut, cassava, yam and maize.

In this study *Lasiodiplodia theobromae, Colletotrichum gloeosporioides* and *Neofusicoccum parvum* were consistently isolated from the various parts of the diseased trees (bark, vascular system and roots). *L. theobromae* had the highest frequency of occurrence follow by *C. gloeosporioides* with *Neofusicoccum parvum* being the least isolated fungus. *L. theobromae* was however, identified as the causal organism of the disease through pathogenicity test. This is the first report of the fungus as the causal agent of the disease in Ghana. The finding confirms earlier reports of *L. theobromae* as the pathogen responsible for the disease elsewhere (Al-Adawi *et al*., 2003, Iqbal *et al*., 2004, Malik *et al*., 2005; Al-Adawi *et al*., 2006; Philip, 2007).

The increasing incidence of the disease in the Northern Ghana suggests the need to evaluate fungicides in the Ghanaian market for control of the disease. In this study, Carbendazim, funguran, mancozeb, prochloraz and zamir were able to inhibit the mycelia radial growth of *L. theobromae* at various concentrations in the laboratory. This shows that the active ingredient in these fungicides may contain the right fungicidal properties to manage *L. theobromae* which cause mango tree decline disease. Field application of the fungicides at a two-week interval showed carbendazim to be superior in reducing the disease incidence and severity drastically to promote new vegetative growth (shoots and leaves). This result is in agreement with that of Muhammad *et al.* (2005), who reported carbendazim to be effective in inhibiting the mycelial radial growth *in vitro* and suppressing gum exudation, dieback and wilting thereby promoting vegetative growth of plants in the field. The highly effective nature of carbendazim in controlling the disease may be due to its systemic properties which enables it to be maintained in the system of the tree. Funguran which is a contact fungicide reduced the disease incidence and severity to some extent. Mancozeb, was ineffective in controlling the decline disease with marginal growth of vegetative parts (shoots and leaves) and gumming on the diseased trees. It however, performed better than untreated mango trees. Since mancozeb is a contact fungicide, repeated applications at a shorter interval could help control the disease.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- This study revealed that mango tree decline disease is prevalent in all the three mango growing districts in the Northern Region of Ghana. Almost all the farmers (100%) in the study areas could identify the disease by at least one of its many symptoms but were lacking knowledge or awareness about the causal agent of the disease and therefore, were unable to control the disease. Most of the farmers (61%) observed a higher prevalence of the disease during the dry season as compared to the rainy season. A large number of the mango farmers (87%) were ignorant about the spread of the disease within the same farms and from farm to farm. Few farmers (8% and 5%) attributed the spread to infected planting materials and insects respectively. The devastating nature and severity of the disease, impacted adversely on the livelihood of the farmers hence most farmers had abandoned their farms and others had replaced their mango trees with arable crops such as groundnut, cassava, yam and maize.
- Generally, there was high incidence of the disease in all the three mango growing districts in Northern Ghana. Karaga district recorded the highest disease incidence (77.9%) followed by Savelugu-Nanton municipality (77.1%) and then Kumbugu district (63.9%). However, Savelugu Nanton municipality had the highest disease severity (2.57) followed by Karaga district (2.0) and Kumbugu district (1.42).
- The causal organism of the mango tree decline disease in the three mango growing districts in the Northern Region of Ghana was identified as *Lasiodiplodia theobromae*

- Application of urea fertilizer alongside spraying of carbendazim at a rate of 50g/15 L water with two weeks spraying intervals was effective in reducing the incidence and severity of the disease.

6.2 Recommendations

It is therefore recommended that:

- Further isolation should be carried out to identify other possible organisms that may be associated with the disease.
- Application of fertilizer (urea) alongside two weeks spraying of carbendazim and good farm hygiene should be adopted by farmers to manage mango tree decline disease.
- Similar studies should be conducted in mango growing areas in the coastal Savannah zone of Ghana in order to determine the extent of the disease.
- Studies on bio-control of the disease should be carried out.
- Studies should be carried out on the effects of climate change on the incidence of mango tree decline disease and the epidemiology of the disease.
- Studies should be conducted on the role of nutrient in management of mango tree decline disease.

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APPENDICES

Appendix 1. Questionnaire Survey on Perception of Mango Farmers on a New Mango Tree Decline Disease in the Northern Region of Ghana

Kindly spend about 30 minutes to respond to the following questions on a new tree decline disease of mango. The interviewer assures you of the confidentiality of the information given at all times and that your responses will only be utilized for academic purposes.

Respondent's Phone No…………………………………………

Questionnaire No……………………….

A. ***Background of Respondent and Farm***

1. Name of District………………………………………………………………

2. Name of Village (Community)……………………………………………………

3. Name of farm/farmer(s)……………………………………………………………………

4. Sex: a) male [ ] b) female [ ]

5. Age of farmer

a. 20 and below [ ] b. 21-30 [ ] c.31-40 [ ] d. 41-50 [ ] e. 51-60 [ ] f.60 and above [ ]

6. Level of education of farmer

a. No formal education/dropout [ ] b. Primary/JHS /MSLC [ ]

c. SHS/VOC [ ] d. Tertiary [ ]

7. What variety of mango do you grow?

a. Keitt [ ] b. Kent [ ] c. Palmer [ ] d. Haden [ ] e. Tommy Attins [ ]

f. local [ ] g. others (specify)…………………………………

8. Where is the source of your planting materials? A. Private nursery [ ] b. Certified nursery (FOHCREC Kade/SIREC Kpong) [ ] c. Own nursery [ ] d. Others (specify)……
……………..………..

9. How old is your plantation?

a. 5 yrs and below [ ] b. 6-10yrs [ ] c. 11-15 yrs [ ] d. 15-20 yrs [ ]

e. above 20 yrs [ ]

10. What is the size of your plantation?

a. less than 1 hectare [ ] b. 1- 2 hectares [ ] c. 3- 4hectares [ ]

d. 4-5 hectares [ ] e. more than 5 hectares [ ]

B. *Farmers' knowledge, perception and experiences concerning prevalence.*

11. Have you observed a new mango disease on your farm? Yes [ ] N o [ ]

12. If yes, describe it ……………………………………………………………………

…………………………………………………………………………………………….

13. Have you observed any of the following symptoms on any of your mango trees?

a. Latex oozing form uncut portions of the tree trunk Yes [ ] No [ ]

b. Abnormal bark cracking Yes [ ] No [ ]

c. Abnormal drying up and death of branches Yes [ ] No [ ]

d. Branches drying up and breaking off Yes [ ] No [ ]

e. Stunted growth of tress Yes [ ] No [ ]

f. Gradual death of trees Yes [ ] No [ ]

g. Any other symptom? If yes, specify ………………………………………………………..

14. Do you observe these symptoms together on the same trees? Yes [ ] No [ ]

15. If yes, what is the order in which you observed these diseases in reference to question?

14 above? …………………………………………………………………………………

16. What in your view could be the cause(s) of the disease symptoms observed?

a. excessive rainfall [ ] b. source of planting material [ ] c. belief and superstition [ ]
d. poor soil [ ] e. poor management practices [ ] f. pathogen [ ] g. wind [ ] h. no idea [ ]

j. others (specify)………………………………………………………………..

17. Apart from your farm, have you seen or heard any other farmer complain about similar disease? Yes [ ] No [ ]

C. *Knowledge, perception and experiences concerning spread*

18. In which year was the disease first observed on your farm?

a. Before 2000 [ ] b. 2001-2005 [ ] c.2006 -2010 [ ] d. 2011-2015 [ ] e. no idea [ ]

19. When (what season) do you normally observe this disease on your farm?

a. rainy season [ ] b. dry season [ ] c. both rainy and dry seasons [ ]

20. Which of these seasons is the disease prevalence high?

a. major raining seasons [ ] b. minor raining seasons [ ]

21. How does the disease spread on the same farm?

a. by raindrop [ ] b. by wind [ ] c. by insects [ ] d. by soil [ ]

e. by contact with diseased plant [ ] f. no idea [ ] g. no idea [ ]

h. others (specify)…………………………………………………..

22. How does the disease spread from farm to farm?

a. by human [ ] b. by wind [ ] c. by insect [ ] d. by infected planting materials [ ] e. no idea [ ] f. others (specify)…………………………………………………..

D. *Knowledge and perception of farmers concerning economic impacts of mango tree decline disease on livelihood of farmers.*

23. What happens to the yield (fruits) when any of the trees that shows one or more symptoms of the decline disease?

a. it produces less fruits [ ] b. it does not produce fruits at all [ ]

c. it produces fruits like healthy trees [ ] d. it dies [ ]

24. What is the percentage reduction of yield from the disease?

a..20% and below [ ] b. 21- 40% [ ] c. 41- 60% [ ] d. 61- 80% [ ] e. 81 and above [ ]

25. What is the percentage reduction of your income as a result of the disease?

a. below 20% [ ] b.21 – 40% [ ] c.41 – 60% [ ] d.61 – 80% [ ] e. 81 and above [ ]

26. What do you do to the affected fruits? a. rejected [ ] b. destroyed [ ]

c. sold [ ] d. to feed my animals [ ] e. others (specify)
…………………………………

27. How does the disease affect your livelihood? a. school fees [ ] b. hospital bills [ ]

c. upkeep of family [ ] d. others (specify) ……………………………………………

E. ***Farmers' knowledge and perception concerning control of mango tree decline***.

28. Which of the following methods do you use to control mango tree decline disease?

a. heavy pruning [ ] b. slight pruning [ ] c. cut and burn [ ]

e. apply fertilizer [ ] f. spray with pesticide [ ]

29. How often do you spray (fungicide) your mango plants in a year?

a.1 – 2 times [ ] b. 3 – 4 times [ ] c. 5 – 6 times [ ] d. more than 6 times [ ]

30. Which type of fungicide? Specify……………………………………………………………

31. At what stage of tree development do you start spraying?

a. below 1 yr [ ] b. 1 – 2 yrs [ ] c. 2 – 3 yrs [ ]

d. 3 – 4 yrs [ ] e. 4 yrs and above [ ]

32. Could you suggest any other solution to the disease? …………………………………………
……………………………………………………………………………………………………

Thank you

Appendix 2. Disease assessment key of mango tree decline disease

Key/scale	Qualitative rating
0	No signs of the disease
1	Gum traces oozed out/few smaller branches dried
2	Oozing of gums/bark splitting and few branches dead
3	Up to 35% of the tree is dead
4	More than 35% of the tree is dead
5	Foliage of whole tree wilted. Severity scale of mango tree decline disease

Source : Modified after Panhwar, *et al.*, (2005)

Appendix 3. Analysis of variance of inhibitory effects of mycelia radial growth of *L. theobromae.*

Variate: Day 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	17	0.	0.		
Residual	36	0.	0.		
Total	53	0.			

Variate: Day 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	17	49299.42	2899.97	107.92	<.001
Residual	36	967.36	26.87		
Total	53	50266.78			

Variate: Day 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	17	36405.25	2141.49	59.92	<.001
Residual	36	1286.51	35.74		
Total	53	37691.76			

Variate: Day 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	17	36939.764	2172.927	1919.17	<.001
Residual	36	40.760	1.132		
Total	53	36980.524			

Appendix 4. Analysis of variance of incidence of mango tree decline disease

Variate: Before 1st fungicide spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	0.	0.		
block.*Units* stratum					
TREATMT	3	0.	0.		
Residual	9	0.	0.		
Total	15	0.			

Variate: 2 weeks before_2nd_fungicide spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	0.	0.		
block.*Units* stratum					
TREATMT	3	0.	0.		
Residual	9	0.	0.		
Total	15	0.			

Variate: 2 weeks before_3rd_spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	675.00	225.00	9.00	
block.*Units* stratum					
TREATMT	3	12675.00	4225.00	169.00	<.001
Residual	9	225.00	25.00		
Total	15	13575.00			

Variate: After_3rd_spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	75.00	25.00	1.00	
block.*Units* stratum					
TREATMT	3	23275.00	7758.33	310.33	<.001
Residual	9	225.00	25.00		
Total	15	23575.00			

Appendix 5. Analysis of variance of disease severity of mango tree decline disease

Variate: Before_1st_fungicide spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	0.	0.		
block.*Units* stratum					
Treatmet_s	3	0.	0.		
Residual	9	0.	0.		
Total	15	0.			

Variate: 2 weeks before_2nd fungicide spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	0.26687	0.08896	1.43	
block.*Units* stratum					
Treatmet_s	3	10.14687	3.38229	54.30	<.001
Residual	9	0.56063	0.06229		
Total	15	10.97438			

Variate: 2 weeks before_3rd_spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	0.61688	0.20563	3.03	
block.*Units* stratum					
Treatmet_s	3	32.91687	10.97229	161.72	<.001
Residual	9	0.61062	0.06785		
Total	15	34.14437			

Variate: After_3rd_spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	0.03688	0.01229	0.50	
block.*Units* stratum					
Treatmet_s	3	44.49688	14.83229	605.06	<.001
Residual	9	0.22063	0.02451		
Total	15	44.75437			

Appendix 6. Analysis of variance of shoot count after two weekly spraying of fungicides.

Variate: 2 weeks after 1st spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	0.5117	0.1706	1.26	
REP.*Units* stratum					
TREATMENT	3	9.8242	3.2747	24.11	<.001
Residual	9	1.2227	0.1359		
Total	15	11.5586			

Variate: 2 Weeks after 2nd spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	2.4492	0.8164	1.18	
REP.*Units* stratum					
TREATMENT	3	149.4492	49.8164	72.05	<.001
Residual	9	6.2227	0.6914		
Total	15	158.1211			

Variate: 2 weeks after 3rd spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	2.516	0.839	0.74	
REP.*Units* stratum					
TREATMENT	3	682.391	227.464	200.64	<.001
Residual	9	10.203	1.134		
Total	15	695.109			

Appendix 7. Analysis of variance of leave count after two weekly spraying of fungicides

Variate: 2 weeks after 1st spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	5.797	1.932	1.06	
REP.*Units* stratum					
TREATMENT	3	112.641	37.547	20.58	<.001
Residual	9	16.422	1.825		
Total	15	134.859			

Variate: 2 weeks after 2nd spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	39.043	13.014	1.47	
REP.*Units* stratum					
TREATMENT	3	2791.074	930.358	105.32	<.001
Residual	9	79.504	8.834		
Total	15	2909.621			

Variate: 2 weeks after 3rd spray

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	85.39	28.46	0.43	
REP.*Units* stratum					
TREATMENT	3	12986.08	4328.69	65.95	<.001
Residual	9	590.77	65.64		
Total	15	13662.23			

Appendix 8. Sequence of *Neofusicoccum parvum*

> (*Botryosphaeria parvum* or *Neofusicoccum parvum*).

GAAGGATCATTACCGAGTTGATTCGAGCTCCGGCTCGACTCTCCCACCCTATGTGTACCTACCTCTGTT

GCTTTGGCGGGCCGCGGTCCTCCGCACCGGCGCCCTTCGGGGGGCTGGCCAGCGCCCGCCAGAGGACC

ATAAAACTCCAGTCAGTGAACTTCGCAGTCTGAAAAACAAGTTAATAAACTAAAACTTTCAACAACGG

ATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA

GTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTCGAGCGT

CATTTCAACCCTCAAGCTCTGCTTGGTATTGGGCCCCGTCCTCCACGGACGCGCCTTAAAGACCTCGGC

GGTGGCGTCTTGCCTCAAGCGTAGTAGAAAACACCTCGCTTTGGAGCGCACGGCGTCGCCCGCCGGAC

GAACCTTTGAATTATTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAA