

**CHARACTERISATION AND CONTROL OF *XANTHOMONAS CAMPESTRIS* PV.  
*MANGIFERAEINDICAE* CAUSING MANGO (*MANGIFERA INDICA* L.) BACTERIAL  
BLACK SPOT DISEASE IN SOME SELECTED DISTRICTS OF THE EASTERN AND  
VOLTA REGIONS OF GHANA**

**BY**

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

**JULY, 2019**

# DECLARATION

I Samuel Osabutey, hereby affirm that except for other people's work which was duly cited, this work is original research and that this thesis has neither in whole nor part been presented for another degree elsewhere.

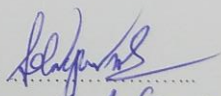
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## **DEDICATION**

I dedicate this research work to Almighty God for His blessings and grace which has brought me this far and to my lovely mother (Patience Osabutey) for her encouragement, support, and prayers which made this work a success.

## **ACKNOWLEDGEMENT**

I thank the Almighty God for His sufficient grace through the provision of strength to complete my study. Throughout this work, I came to understand that I am nothing without Him and I can do nothing without Him.

I would like to express my deepest gratitude to my supervisors Dr. Joseph Okani Honger and Dr. Seloame Tatu Nyaku for their guidance and support during this research work. I am also grateful to Dr. Eric W. Cornelius, Prof. Frank K. Kumagah, and Dr. Christiana Amoatey for their constant guidance and care during my undergraduate and graduate education.

My sincere gratitude to Prof. K. A. Oduro for introducing me to plant pathology and my Head of Department, Dr. Vincent Yaw Eziah for his support and encouragement in tough times. I also appreciate the Leventis Scholarship Foundation for supporting my research work financially.

I am also grateful to all lecturers in the Crop Science Department, University of Ghana. I am indeed very grateful to Azanu Korkor Mercy, James Mckee, Shadrack Coffie, and Mr. Benjamin Otu of the Biotechnology Center Laboratory as well as Mr. Isaac Bedu, Ofoe Raphael, Mr. Richard Otoo and Albertina Naa Adorkor Allotey of the Plant Pathology Laboratory of Crop Science Department.

My gratitude also goes to Miss. Frada Agyapong Ansaah for her support and may God richly bless you, dear.

I am indeed grateful to all my course mates especially Late Miss Larissa Seyrah Ziddah, Camara-Williams I. E. A, Efoo Nutsukpo Bawa, Vigbedor D. Hannah, Muhammed Opuni Nana, and Dompreeh Amofah Daniel.

My heartfelt gratitude also goes to my family for their immense support and guidance.

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## ABSTRACT

Mango bacterial black spot disease is one of the most devastating diseases of mangoes worldwide. The disease was observed in some mango orchards in the Lower Manya and Asuogyaman districts of the Eastern region and North Tongu district of the Volta region of Ghana. The objectives of this study were to assess farmer's perception and knowledge on the prevalence, spread and economic importance of the mango bacterial black spot disease, determine the disease incidence and severity in mango farms, assess the effect of the disease on yield/fruit quality confirm if the causal agent of the current outbreak of the disease in the study area is *Xanthomonas campestris* pv. *mangiferae* and evaluate some selected fungicide/bacteriocides for control of the disease in the field. Questionnaires (60) were administered to randomly selected farmers who were actively engaged in mango farming in the study areas. These were read out and interpreted to farmers when necessary for clarity. The questionnaires covered the general background of the farmers and their perception and knowledge concerning the cause, importance, spread, and control of the disease. Data collected were analyzed using descriptive statistical analysis (means and percentages). Disease incidence in the study area was determined by expressing number of infected trees as a percentage of total number of trees inspected while disease severity was determined by rating the percentage of fruit area diseased on a scale of 0-5 where 0=no disease and 5=more than 50% of fruit area diseased. Data obtained was presented by calculating means and standard errors. The suspected causal agent of the disease was isolated from diseased mango fruits and identified using morphological characteristics, biochemical reactions and molecular methods. Detached mango leaves of kent variety were inoculated with a bacterial suspension of  $1.0 \times 10^6$  CFU/mL using the needle prick method to confirm pathogenicity. The effect of the disease on yield/fruit quality loss of mango was determined on different mango cultivars, namely Keitt, Haden, and Palmer. In vitro

evaluation of the inhibitory effect of some copper based fungicides on the growth of *Xanthomonas campestris* pv. *mangiferaeindicae* was evaluated using the disc fusion method. The treatments were then evaluated on the field using randomised complete block design with three replicates. Farmers in the study areas demonstrated good knowledge of the disease. The disease incidence ranged from 27% in Asuogyaman District to 90% in the North Tongu District, while severities were 0.5 to 3.0 in the same districts. The disease caused a fruit quality/yield loss of 71.5% and the pathogen was confirmed as *Xanthomonas campestris* pv. *mangiferaeindicae* based on cultural, morphological, and sequence analysis of the *cpn60* gene. Copper hydroxide completely inhibited the growth of the bacterium in the in-vitro studies while metallic Copper+Mancozeb (Cuprofix-30 disperse) effectively reduced the disease incidence and severity in the field.

## CHAPTER ONE

### 1.0 INTRODUCTION

Mango (*Mangifera indica*) is one of the most important fruit crops cultivated in Ghana and one of the most significant crops in West Africa. The crop is in the family Anacardiaceae with other crops such as cashew, pistachio, and the mombins. Mango is reported to have originated from Australia, New Guinea, and New Caledonia (Liu *et al.*, 2012) and is now spread in almost all the tropical and sub-tropical regions of the world. Globally, commercial production of mangoes occurs in more than 103 countries and production is increasing each year due to increasing consumer demand as a result of its outstanding sensory, therapeutic and nutritional properties (Bicas *et al.*, 2011).

Production of mango fruits generates income to a large number of people engaged in its cultivation and marketing (Honger *et al.*, 2016). It has been well recognized as an important source of micro-nutrients, vitamins, and other phytochemicals (Fritz, 2017). Mango fruits provide energy, proteins, dietary fibre, carbohydrates, fats, and phenolic compounds which are essential for normal human growth and development (Fritz, 2017). It also has therapeutic properties such as curing of cancer and treatment of heart attack (Chaudhry *et al.*, 1992). Recently, there has been a rise in demand for the peels of mango fruits due to its usage for feeding livestock (Muhammed *et al.*, 2007) further boosting the economic value of the crop.

Good agricultural practices in mango production have been known to yield economic output levels in Ghana since the country is privileged to be among a few countries in the world that have two seasons annually for mango production (Egyir, 2013). Commercial mango cultivation in Ghana is concentrated around the Coastal Savanna (Portions of the Greater Accra, Eastern and Volta

regions), Transitional (Brong and Ahafo Regions) and the Guinea Savanna (Northern region) ecological zones of the country, where the rainfall distribution patterns favour flowering and fruit development of the crop. In these areas growing improved mango cultivars that are exportable is the most lucrative business as it affords employment to about 5000 people in the Eastern Region of Ghana alone which makes the area one of the important regions in Ghana for mango production (MoFA, 2013). The production and marketing of the crop are also becoming important in the coastal areas of the Volta region. The importance of mango production to the economy of these two regions cannot be overemphasized.

Until recently, the major disease affecting the production of mangoes in the Eastern and Volta regions of Ghana was anthracnose caused by the fungus, *Colletotrichum gloeosporioides* (Oduro, 2000; Offei *et al.*, 2008; Honger *et al.*, 2018). The disease caused a reduction in farmer's earnings but control measures were developed to effectively reduce its impact on yield losses (Honger *et al.*, 2018). Currently, a new disease, suspected to be a bacterial black spot (BBS), caused by *Xanthomonas campestris* pv *mangiferae* has been reported on several farms in some of the major mango districts in the two regions. Bacterial black spot is one of the most important diseases of mango and a major challenge in the mango industry worldwide (Gagnevin and Pruvost, 2001). The disease has been reported to reduce yield by 80-100% in susceptible cultivars wherever it was found (Gagnevin and Pruvost, 2001). Its symptoms include black, star-shaped spots on the fruit surface, which ruptures to emit latex and finally fruit drop. A survey in 2015 by the Ministry of Agriculture, in Ghana, indicates average fruit loss from the disease per acre as 5.1 tons resulting in a monetary loss of GHS 19,969,560.00 (MoFA, 2015). There is currently no effective control

for the disease in the country and therefore there is an urgent need to develop methods for control of the disease.

Two of the major mango growing districts in the Eastern region are the Lower Manya Krobo and the Asuogyaman districts while the North Tongu is an important district in the Volta region. Among these three districts, the Lower Manya and North Tongu districts have some of the largest commercial mango farms in Ghana (J. O. Honger, personal communication, March 06, 2019). Mango bacterial black spot was recently reported for the first time in these areas and therefore, information about factors that contribute to the spread of the disease and level of damage is scanty and has to be properly assessed. Farmers' knowledge about the disease has also not been assessed and therefore, problems being attributed to the disease could not be verified. However, to formulate effective control measures against the disease, information such as farmer's knowledge and experience on the disease and factors that promote its' spread could be useful.

Mango bacterial black spot was first reported in Ghana in 2011 as caused by *Xanthomonas campestris* pv *mangiferaeindicae* in some mango orchards in the Northern region (Pruvost *et al.*, 2011). Though the same pathogen is suspected to be the cause of the recent outbreak of the disease in the Eastern and Volta regions, there is the need for work to be done to confirm the identity of the causal agent to aid in formulating control measures against the disease in the area. Key to the confirmation of a causal agent of a disease is the accurate identification of the suspected pathogen using molecular techniques (Tian *et al.*, 2016; Zeigler, 2003; EPPO, 2005). The advantage of these DNA-based methods over the traditional methods (Lelliot and stead, 1987) is that the reliability of the identification assay is not dependent on the environment or the physiological state of the

pathogen in question (Louws and Cuppels, 2001). In this study, the sequence analysis of a diagnostic gene was used to aid in the identification of the causal agent of the mango bacterial black spot disease in the study area.

The sequencing of genes could be very expensive, though it gives very accurate and reliable results. It would therefore, be worthwhile for other DNA based methods, such as polymerase chain reaction (PCR) using species-specific primers for the identification of the causal agent of the disease in these areas. The species-specific primer, MV3/MV4 (Mavrodieva *et al.*, 2004), which was designed based on the pthA gene to identify *Xanthomonas campestris pv citri*, could be able to identify the pathogen on mango as well, though this has not been investigated.

The presence of mango bacterial black spot in Ghana was confirmed in 2011 (Pruvost *et al.*, 2011). This disease was found in a mango orchard established with planting materials imported into Ghana from Burkina Faso. However, after the identification of the disease, it appeared to have been confined in the said orchard in the Northern region, because the disease was never reported in any part of the country. However, recent reports suggest that the disease has now spread to several parts of the country where mangoes are grown (Abeiku-Amissah, 2018). Therefore, when farmers in the Lower Manya Krobo, Asuogyaman and North Tongu Districts of the Eastern and Volta regions reported that the disease was ravaging their farms, it was necessary to conduct this study to confirmed the presence of the disease in the area and to carry out further studies to identify appropriate control strategies to combat the disease and hence this study.

Several reports from farmers suggest that mango bacterial black spot in the Eastern and Volta regions of Ghana is currently uncontrollable. Most farmers reported that the fungicides currently available on the Ghanaian market have not been effective but elsewhere, where the disease has been reported, it has been controlled by these chemicals. Therefore, if these chemicals are not effective in Ghana against the disease, it could be due to factors such as the development of resistant strains or improper use of these chemicals. It is therefore important that a proper field trial is carried out to ascertain the veracity or otherwise of farmers' reports of poor control of the disease and to recommend appropriate methods to be used for the control of the disease. This will increase yield/fruit quality and improve the income and livelihood of farmers and other actors in the mango production value chain.

In view of the limited information concerning the outbreak of the mango bacterial black spot disease in the Eastern and Volta regions of Ghana, this research work was carried out to determine the importance of the disease, confirm the causal agent as *Xanthomonas campestris* pv. *mangiferaeindicae* and evaluate some control measures against the disease in the field.

Specific objectives were to:

1. Assess farmer's perception and knowledge on the prevalence, spread and economic importance of the mango bacterial black spot disease.
2. Study the nature of the disease symptoms and possible factors that exacerbate the disease in the field and determine the disease incidence and severity in selected mango farms
3. Determine the effect of the disease on yield/fruit quality of mango.
4. Confirm if the causal agent of the current outbreak of the disease in the study area is *Xanthomonas campestris* pv. *mangiferaeindicae* using morphological, biochemical and

physiological characteristics and analysis of the cpn60 gene and to evaluate the primer MV3/MV4 for its specificity for the bacterium.

5. Evaluate some selected fungicide and bactericides for control of the disease in the field.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Origin and distribution of mango

Mango (*Mangifera indica*) belongs to the family Anacardiaceae which consists of important fruits such as cashew (*Anacardium occidentale*), pistachio (*Pistacia vera*) and many species of *Spondias*. The crop is believed to have originated from the Indo-Burma region. Vavilov and Freier (1926) reported that there are wild cultivars and many species of mango in India an indication that the crop could have originated from the Indo-Burma region. Numerous allied species of the crop however exist in Malaysia creating another thought that Malaysia is the actual origin of mango (Pavani, 2009).

Mangoes have been cultivated for about 4000 years mostly in Southeastern Asia countries such as the Philippines, Java, Indonesia, Burma, Thailand, Sri Lanka, and Malaysia. The crop is believed to be introduced to East and West Africa through to Brazil in the sixteen centuries. The crop was introduced to Florida in 1833 and to Mexico in the nineteenth century (1998). Presently, mango is cultivated in most tropical and sub-tropical regions of the world.

As of 2010, India was the largest producer of mangoes, with about 70% of the world's total production (Table 1). Some other major grower countries include China, Mexico, Brazil, and Thailand. In Africa, Kenya is the highest producer of mangoes followed by Egypt. In West Africa, Côte d'Ivoire and Ghana are the two countries that are into commercial production of mango (FAOSTAT, 2010a).

**Table 1. Major mango producing countries in the world**

| <b>Producing Country</b> | <b>Production (MT)</b> |
|--------------------------|------------------------|
| 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                  |

Adapted from FAOSTAT (2010a) MT: Metric tonnes.

## **2.2 Economic importance of mango fruit production**

Mango production provides producers, manufactures, and traders with various jobs on a both domestic and international basis as well as generating income for other people involved in the production value chain. There are also health and nutritional benefits of the fruit to consumers (Naz, 2012). The labor involved in mango production around the world per day is 200 million (FAOSTAT 2009). Polyphenolic compounds such as mangiferin,  $\beta$ -glucogallin, ellegic acid, gallic acids, gallotannins, and isoquercetin (Schieber *et al.*, 2000) and antioxidant compounds (Ajila *et al.*, 2007) are present in the mango fruit. These compounds are known to inhibit chemical reactions that lead to degenerative diseases (Sigler and Ruch, 1993; Scalbert and Williamson, 2000). Calatrava (2014) also observed that the plant provides some animals, especially livestock in the tropics with shelter, habitation, and food. Apart from the avocado fruit, mango contains high protein content compared to other fruits and known for the niacin, vitamin A, iron, and calcium it provides for the body upon consumption (Griesbach, 2003).

## 2.3 Important diseases of mango

The mango plant is infected with the diseases caused by several causal agents including fungi and bacteria. Some of the common diseases caused by fungi include stem-end rot caused by *Lasiodiplodia theobromae* and anthracnose caused by *Colletotrichum* species. Others include powdery mildew caused by *Oidium mangiferae* (Berthet) and black rot caused by *Alternaria alternata* (Fr.: Fr.) Keissl. Worldwide, integrated control methods have been developed and are available for managing these fungi diseases leading to a reduction in their destructive effect in mango fields. On the other hand, diseases caused by bacteria on mango are few. In Spain, Florida, and Israel, a bacterial disease caused by *Pseudomonas syringae* pv. *syringae* characterized by apical necrosis but absence of symptoms on fruits was reported. This disease was reported in areas where low temperature and high humidity occur simultaneously (Moll, 1985; Cazorla *et al.*, 1998). Another disease associated with an Erwinia-like bacterium has been reported in Central America (McMillan and Wang, 1992, Guevara *et al.*, 1980; Guevara *et al.*, 1985). These two bacterial diseases have minimal impact on the fruit production and hence are not considered important. However, one of the most destructive diseases of mango is the bacterial black spot caused by *Xanthomonas campestris* pv. *mangiferae* indicae.

## 2.4 The mango bacterial black spot disease

### 2.4.1 Origin, spread and importance of mango bacterial black spot disease

Mango bacterial black spot disease was originally described in South Africa in 1909 (Doidge, 1915). Presently, the disease has been reported in Australia (Moffett *et al.*, 1979). The Comoro Islands (Pruvost *et al.*, 1995), India (Patel *et al.*, 1948), Japan (Fukuda *et al.*, 1990), Kenya (Buruchara *et al.*, 1990), Malaysia (Lim *et al.*, 1991), Mauritius (Pruvost *et al.*, 1995), New

Caledonia (Pruvost *et al.*, 1995), Pakistan (Afzal, 1987), the Philippines (Pruvost *et al.*, 1995), Réunion (Daniel *et al.*, 1975), Taiwan (Ann, 1978), Thailand (Pruvost *et al.*, 1995) and the United Arab Emirates (El-Goorani, 1987). The disease was suspected to be present throughout southern and eastern Africa as well as in most parts of Asia (Gagnevin and Pruvost, 2001). By the 1950s, the disease has been spotted in Brazil. In 2011, the disease was first recorded in Ghana, which also constituted the first report of the disease in West Africa. Currently, the disease has been reported in several West African countries and is suspected to be present in all mango growing areas of the world (Gagnevin and Pruvost, 2001).

Mango bacterial black spot is a devastating disease and yield losses due to the disease could reach up to 100% since the disease could cause heavy dropping of immature fruits and could leave rotten dark spots on fruits that go into maturity. Therefore, in severe infestation, all fruits could be lost (Honger, 2018). Other reports suggest that yield loss due to the disease could range from 50% to 100% (Kishun, 1982; Prakash and Misra, 1992). Moreover, all commercial varieties of mangoes are susceptible to the disease. In 1996, South Africa, 5 million Rands (approximately U.S. \$1 million) due to the disease (Gagnevin and Pruvost, 2001). Apart from fruit destruction, the disease causes canker symptoms on branches that weaken them and therefore easily break with the slightest wind breeze. The disease also has the potential of restricting the movement of mango from producer countries with the disease since the causal agent is a quarantine pest in most countries (J. O. Honger, personal communication, March 06, 2019).

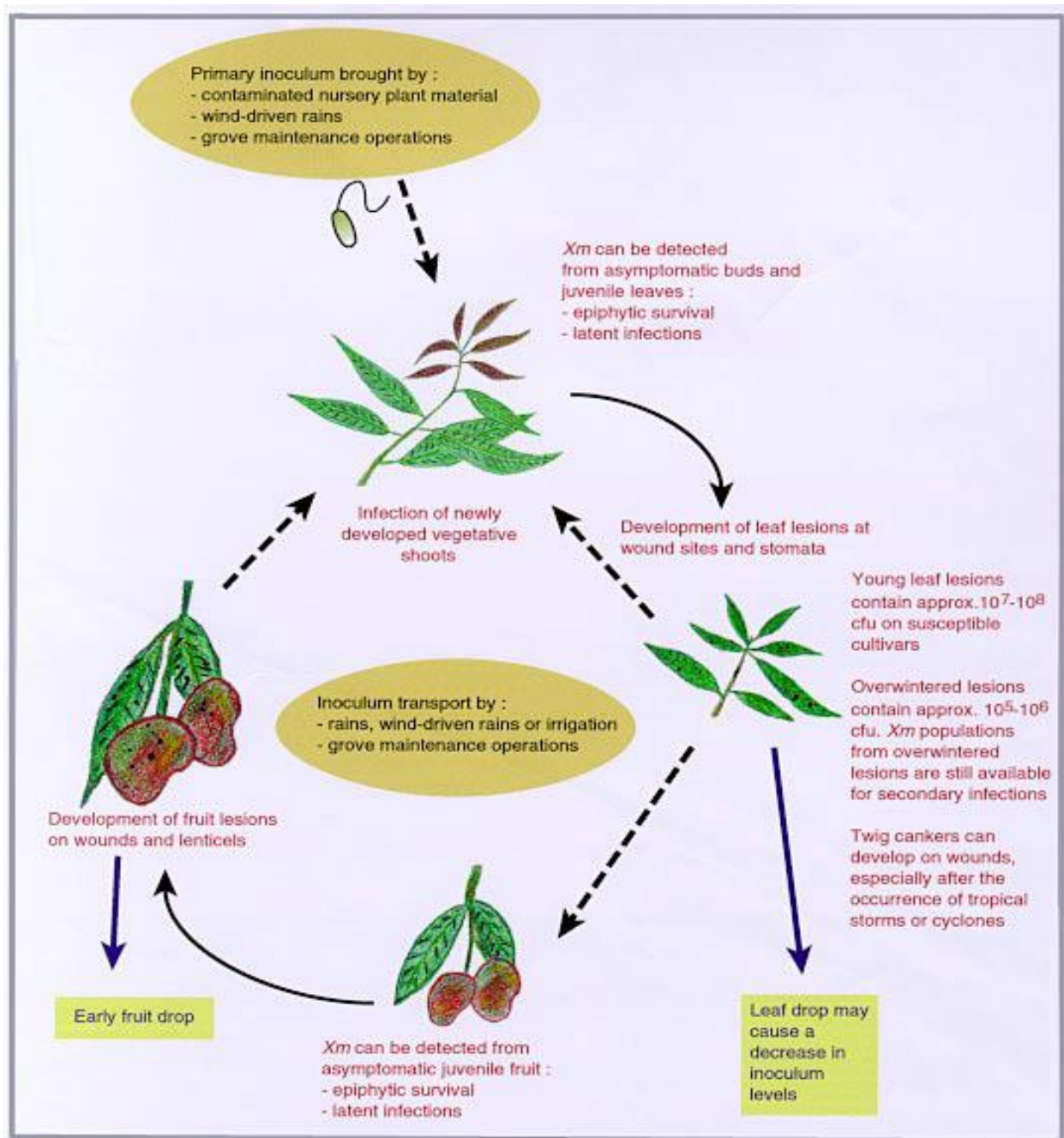
#### **2.4.2 Symptomatology**

Symptoms of mango bacterial black spot disease could be found in all the above-ground parts of the mango tree. On leaves, symptoms begin as small water-soaked spots delineated by veins, becoming raised, black, sometimes with a chlorotic halo (Jackson, 2009). The spots are mostly accompanied by oozing of latex-containing the bacterium. Spots are normally tiny, but several may coalesce to form large chlorotic areas of the leaf. When the spot advances in age, it may dry and turn light brown or ash-gray. Under conditions of severe infection, leaf abscission occurs (Gagnevin and Pruvost, 2001). On the fruits, black oval to irregular raised spots develops, with greasy margins. The spots may be pin-shaped or may join together to form a large crack, and sap oozes out. The spots are only skin deep, but they affect the quality and lead to the fruit being rejected or sold for a low price (Jackson, 2009). Severe fruit infections will cause premature fruit drop. Less severe infections reduce fruit quality and allow entry of other pathogens (Gagnevin and Pruvost, 2001). The disease also causes canker symptoms on twigs and branches. These appear as cracked surfaces of the branches which are mostly accompanied by oozing of latex.

#### **2.4.3 Disease cycle**

The epidemiological cycle of mango bacterial black spot is shown in Fig. 1. Primarily, the causal agent of the mango bacterium black spot disease are disseminated into un-infected mango orchards by contaminated seedlings, which is the major mode of long-distance spread (Jackson, 2009), and wind-driven rains or contaminated tools used for the maintenance of the orchard (Gagnevin and Pruvost, 2001). Another important source of dissemination of bacterial inoculum is aerosols. These are produced during the mid-day and as they move they collect large sources of bacterial inoculum from infected orchard floors and deposit them on the way as they move (Honger, 2018). Insects

have also been implicated in the spread of the bacterium within the tree canopy (Jackson, 2009). On the other hand, dissemination of the inoculum by seed has not been demonstrated (Gagnevin and Pruvost, 2001).



**Fig. 1. The epidemiological cycle of mango bacterial black spot (MBBS).** Dotted lines represent inoculum redistribution. Solid lines represent the evolution and consequences of the disease on mango organs. Adapted from (Gagnevin and Pruvost, 2001).

The secondary sources of inoculum includes infected leaves, cracks on old twigs and branches, and epiphytic populations on healthy leaves. The bacterium infects the hosts passively, requiring open stomata on leaves and lenticels on fruits for inoculation. Once inside the host, the bacteria produce hypertrophy of leaf tissue as a result of the enlargement of intercellular spaces of spongy parenchyma early in the interaction process. This causes the epidermis to rupture allowing bacteria to exude from lesions. These exudates then serve as secondary sources of inoculum and infect fresh tissues on the plant to complete the disease cycle (Gagnevin and Pruvost, 2001). The disease can be severe in both high and low rainfall areas as dew and wounding are important in the development of the disease. Cyclones have been reported as providing perfect condition for the spread of the disease (Jackson, 2009).

Current information suggests that of survival of epiphytic populations of the causal agent on the leaf phyllosphere and subsequent ability to cause the disease are mainly dependent on the availability of juvenile plant tissues. These epiphytes after surviving can gain access to protected sites (e.g., mesophyll) through stomata of the leaves where they eventually become endophytic and causes disease (Pruvost *et al.*, 2009).

#### 2.4.4 Aetiology

Mango bacterial black spot is caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (syn. *Xanthomonas citri* pv. *citri*). The naming of the pathogen has undergone several changes. The pathogen was first identified as *Pseudomonas mangiferaeindicae* and described as species that were pathogenic to both *Mangifera indica* L. and *Anacardium occidentale* L (Pate *et al.*, 1948). The pathogen was then reclassified as *Xanthomonas campestris* subsp. *mangiferaeindicae* (Robbs *et al.*, 1974) while other authors in the same period retained the name *P. mangiferae-indicae* (Steyn

*et al.*, 1974). Later, it was suggested that the original name be maintained but the taxonomic position of the organism must be defined (Daniel *et al.*, 1975). Finally, the name, *Xanthomonas campestris* pv. *mangiferaeindicae* was proposed and backed with neopatho-type strains (Dye *et al.*, 1980). However, as late as the year 2009, the name *citri* pv. *mangiferaeindicae* was preferred by some authors (Pruvost *et al.*, 2009), though in 1984 some authors have supported the name *X. campestris* pv. *mangiferaeindicae* with several chemical and morphological pieces of evidence (Manicom *et al.*, 1984).

Phenotypically, the strains of the bacterium causing the mango bacterial black spot were identified as non-pigmented (Dye *et al.*, 1980). Yellow-pigmented strains of *Xanthomonas*, causing black angular lesions on mango were however reported from Brazil (Robbs *et al.*, 1978), South Africa (Pruvost and Manicom, 1993) and Reunion Island (Pruvost and Luisetti 1989). Reports however suggest that the reported yellow-pigmented strains from Brazil and Reunion were less pathogenic than the non-pigmented isolates (Pruvost and Luisetti, 1989). Morphologically, cells of the bacterium are gram-negative with single, short rods and straight rods (0.4 - 0.7 x 0.7 -1.6 µm) being motile with a single flagellum (Kwee and Chang, 1985). The isolates of the bacterium grow well temperature range of 10-35 °C with growth mostly pronounced at 30 °C (Lakpale *et al.*, 2006). *Xanthomonas campestris* pv. *mangiferaeindicae* on mango could be differentiated into three pathogenic clonal groups. Group I strains reproduced significantly in tissues of mango and cashew leaves and produced the characteristic black lesions associated with the original pathovar, Group II reproduced significantly in the tissue of cashew but not in mango, while Group III produce symptoms only on mombin (*Spondias mombin*) and ambarella (*Spondias dulcis*)(Ah-You *et al.*, 2007).

## **2.5 Control of Mango bacterial black spot**

The mango bacterial black spot disease remains a limiting factor in most mango growing areas because the control measures practiced in most of these areas are not effective. However, it has been suggested that an integrated control measure comprising of preventive, good cultural practices and chemical control can be effective in minimizing the damages caused by the disease (Honger, 2018). One of the important preventive measures is to source planting materials from uncontaminated nurseries (Gagnevin and Pruvost, 2001). This is because infected planting materials are the major source of inoculum introduction into un-infected areas. Another preventive measure is to restrict access to the orchards from potential sources of contamination such as trucks and infected fruit trays (Honger, 2018).

Some of the good cultural practices are the selection of tolerant varieties and maintenance of a clean farm (farm sanitation). Although no variety is completely resistant, some varieties have demonstrated an appreciable level of resistance, and using these can help to greatly reduce the impact of the disease (Gagnevin and Pruvost, 2001). Since the inoculum survives in old branches and defoliated leaves in the farm, prompt removal of these however reduces the inoculum load in the farm (Honger, 2018). Also carrying out good pruning of the trees to reduce foliage density and hence the humidity in the tree canopy is another important cultural practice to manage the disease (Jackson, 2009). This is because the activities of the bacterium are normally reduced during periods of dryness. However, pruning tools must be sterilized before being re-used; else they may serve as a means of spreading the disease in the orchard (Pitkethley, 2006). Other practices such as drip irrigation and windbreaks play a key role in disease control (Gagnevin and Pruvost, 2001). Elsewhere, foam cups are placed by farmers on growing fruits to avoid been infected by inoculi

carried by wind-blown water (Boshoff *et al.*, 1998). Wrapping of young fruits in rain-resistant envelopes to protect them from rain splashed inoculum could be a viable option, but this has not been experimented (J. O. Honger, personal communication, March 06, 2019).

The use of copper-based fungicides in managing plant-bacteria disease is a common practice all over the world. There have been several reports in which copper-based fungicides have proved to be effective in controlling plant-bacteria diseases on tree crops such as mango, citrus and pear (Iliescu *et al.*, 1985; Rajput *et al.*, 2006; Iqbal *et al.*, 2010; Govindappa *et al.*, 2011; Ahmed *et al.*, 2012). It has been recommended that copper-based fungicides should be applied at 2-4 weeks intervals), except during flowering when mancozeb should be used instead (Jackson, 2009) to control the disease. Also, a combination of copper-based fungicide and mancozeb applications is effective in the control of the disease (Honger, 2018).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Survey to assess farmers' knowledge and perception on prevalence, spread and economic importance of bacterial black spots of mango in Eastern and Volta regions of Ghana**

In March 2019 a survey was conducted to assess the perceptions and reactions of mango farmers to the mango bacterial black spot disease in the Lower Manya Krobo and Asuogyaman Districts of the Eastern Region and North Tongu District of the Volta Region of Ghana. Before the survey, focus group discussion was held with the heads of mango farmers Associations in the three districts to aid in the selection of mango farmers for the study. Questionnaires were designed, pre-tested, and distributed to 60 farmers to gather data on their knowledge and perception of the spread and economic importance of the mango bacterial black spot disease. Five mango farming communities were randomly selected from each district in the Eastern Region while the study in the Volta region was restricted to one mango farming community in the North Tongu district, where the disease has been reported by farmers. In each of the farming communities, twenty mango farmers were randomly selected and interviewed. The questionnaires were read out and interpreted to the farmers as their responses were documented in each experimental community. Subject matter considered in the survey questionnaires is shown in Appendix 1.

#### **3.2 Determination of the nature of the mango bacterial black spot disease symptoms, factors exacerbating the disease in the field and disease incidence and severity in the field**

### **3.2.1 Study area**

A field survey for the determination of the nature of the disease and its spread and the disease incidence and severity in the field was conducted in the Lower Manya Krobo and Asuogyaman Districts of the Eastern Region and North Tongu District of the Volta Region of Ghana.

### **3.2.2 Determination of the nature of disease symptoms of mango bacterial black spot disease**

Mango farms reportedly infected with the mango bacterial black spot disease in the study areas were visited to study the nature of the disease symptoms and observe other factors that could be responsible for the spread of the disease. Leaves, twigs, and fruits showing the different stages of the disease were tagged, observed daily, and observations recorded. Farmers were also interviewed to confirm some of the observations made. Samples of diseased plant parts were collected and sent to the laboratory for isolation of causal agents.

### **3.2.3 Determination of disease incidence and severity in farmers' fields**

Field visits to determine the incidence and severity of the mango bacterial black spot disease (MBBS) were carried out between March and May 2019 in commercial mango farms in the three districts selected for the study. In each district, ten mango farms were selected randomly and used for the study. In each farm, 10 mango trees were assessed for the incidence and severity of mango bacterial black spot disease. For better field coverage and reduction of bias in the sampling of the mango trees, a systematic method of sampling (zig-zag method) was used. The number of mango trees that were found exhibiting the MBBS symptoms within a farm was used to determine the disease incidence using the formula:

$$\text{Disease incidence (DI)} = \frac{\text{Number of trees showing disease symptom}}{\text{A total number of trees sampled (infected and uninfected)}} \times 100$$

Ten other trees on the same farm were then selected using the same zig-zag method and used for the determination of disease severity. Each selected tree was circled at walking paces. After every two steps, a stop was made and all fruits that were found at the head level were inspected. The percentage of the fruit surface covered by the disease symptom was rated visually on a scale of 0-5 after Lakshmi and Prasad (2011) (Table 2). The disease severity index for each assessed mango tree 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 2. Disease severity rating scale used to determine the severity of mango bacterial black spot disease in some farms in Eastern and Volta Region**

| Rating | Meaning                                         |
|--------|-------------------------------------------------|
| 0      | No infection                                    |
| 1      | Up to 5% of fruit surface area covered          |
| 2      | 6-10% of the area affected                      |
| 3      | Between 11 and 20% of fruit area covered        |
| 4      | 21-50% of fruit area affected                   |
| 5      | More than 50% of the fruit surface area covered |

Adapted from Lakshmi and Prasad (2011)

The means and standard errors of the disease incidence and severity for each district were computed and recorded.

### **3.3 Determination of the effect of mango bacterial black spot disease on the yield/fruit quality loss of mango**

The method employed for the assessment of the yield/fruit quality loss caused by the disease was modified after Honger (2013). The experiment was carried out using three different mango cultivars, namely Keitt, Haden, and Palmer at the Soil and Irrigation Research Centre at Kpong.

These were the different varieties that had enough trees producing fruits during the period of the experiment. The number of trees per variety used ranged from 6 to 12.

Trees were inspected regularly after fruit set and records were taken on fruits that were prematurely dropped was inspected and the presence of the bacterial black spot symptom was taken as an indication that the fruit was dropped due to the disease. Otherwise, it was considered dropped due to other factors. When fruits were matured, 150 fruits were harvested at 50 fruits per tree from three trees found in the middle of each plot. The fruits were bulked and 50 were selected at random. These were used to determine disease incidence as follows;

$$DI = \frac{\text{Number of fruits expressing symptoms of the disease}}{50} \times 100$$

The percentage of the surface of each of the selected fruits covered by the disease lesion was determined and used to calculate disease severity as carried out in section 3.2.3. After that, all fruits on trees found in the middle of each plot were harvested and separated into those that were free of the disease and those that were not. The following data were collected:

- Number of fruits fallen mainly because of the disease (Nd)
- Number of fruits without disease symptoms after harvest (Nc)
- Total number of fruits diseased but marketable (Nm)
- Quantity of fruits fallen because of other factors (No)
- Total number of fruits that could not be marketable due to the disease (Nu)
- Number of fruits that could not be marketable because of other factors (Nf)

Data collected were used in the calculation of the following:

- Total number of fruits per tree (Nt)=Nd + No+ Nc + Nm+ Nu +Nf
- Percentage of fruits that dropped due to other factors (%No)= $\frac{No}{Nt} \times 100$
- Percentage of fruits that dropped due to the disease (%Nd)= $\frac{Nd}{Nt} \times 100$

$N_t$

- Percentage of diseased but marketable fruits ( $\%N_m$ ) =  $\frac{N_m}{N_t} \times 100$
- Percentage of fruits without the disease ( $\%N_c$ ) =  $\frac{N_c}{N_t} \times 100$
- Percentage of fruits that could not be marketed due to other factors =  $\frac{N_d}{N_t} \times 100$
- Percentage of fruits that were diseased and could not be marketed =  $\frac{N_u}{N_t} \times 100$

### **3.4 Isolation, identification and confirmation of *Xanthomonas campestris* pv *mangiferaeindicae* as the causal agent of the disease.**

#### **3.4.1 Collection of diseased plant parts**

Several diseased plant parts (leaves, twigs and fruits) were collected from infected mango farms in the study area, placed in polythene bags and transported in an ice chest to the Plant Pathology Laboratory, Department of Crop Science, University of Ghana, Legon for isolation of causal organism of the disease. The collected diseased samples were stored in a refrigerator at 4°C in the laboratory until required.

#### **3.4.2 Isolation of the causal bacterium from diseased mango leaves, twigs and fruit samples**

The isolation of the suspected causal agent of the disease was done on nutrient agar (oxoid, England). Nutrient agar (NA) was prepared following the manufacturer's instruction. 39 g powder was dissolved in 1 L of water and autoclaved at 121°C for 15 minutes. The molten medium was allowed to cool and poured into oven sterilized Petri dishes to solidify. Diseased mango fruits and leaves exhibiting BBS symptoms were thoroughly washed under running tap water and blotted dry with sterile tissue paper. Pieces of the leaves and fruit tissues at the advancing edge of the disease

lesions including some portions of the lesions were excised with sharp sterile scalpel and surface sterilized with 5% sodium hypochlorite and the chemical flushed off three times with sterile distilled water. The excised tissues were macerated in the sterile mortar with a pestle and the preparation was allowed to stand for 10 minutes to allow the bacterium to ooze out. A loop full of bacterium suspensions were streaked on the NA in the Petri dish and incubated at 25°C and 70% relative humidity on benches in the laboratory. Three days later single colonies of creamy white bacterium that grew were sub-cultured on separate NA plates to obtain pure cultures. In all, a total of 30 isolates were used in the study.

### **3.4.3 Morphological and biochemical characterisation of the isolated bacterium**

#### **3.4.3.1 Nature of growth on glucose medium**

The preparation of glucose agar medium was done using the method described by Abo-Haded *et al.* (2017). Twenty-three (23 g) of nutrient agar was added to 2.5 g of glucose powder in a litre of distilled water placed in a conical flask. The conical flask was corked with cotton wool and covered with aluminum foil. The mixture was shaken thoroughly for uniform mixture and autoclaved at a temperature of 121 °C for 15 minutes and allowed to cool and set in sterile plates. Disposable plastic sterile inoculation loop was used to streak single colonies of each of the isolated bacterium separately onto the glucose nutrient agar in a sterile conditions. Samples were covered and sealed with cellophane film and packed into white polythene bags. The plates were turned downward to avoid the landing of fungi spores and incubated in the laboratory at 23 °C and 50-60% RH. The nature of the growth characteristics of the bacteria on the medium was recorded after two days to enhance identification.

#### **3.4.3.2 Nature of growth on yeast dextrose carbonate medium (YDC)**

Yeast dextrose carbonate agar was prepared by dissolving 10 g of yeast extract, 20 g of  $\text{CaCO}_3$ , 20 g of D-glucose and 17 g of water agar in 1 litre of distilled water. The mixture was pre-heated and autoclaved at 121 °C for 15 minutes and allowed to cool. Mixing thoroughly by shaking and then poured in 6 mm Petri plates. The creamy white single colony of bacteria was streaked using sterile disposable inoculation loops in a lamina flow chamber on the YDC medium. The plates were then covered, sealed in cellophane plastic bags and incubated at 23 °C and 50-60% RH. Observations of growth characteristics of the bacteria colonies were recorded after 2 days of incubation. The nature of the growth of the bacterium on the YDC was recorded to aid its identification.

#### **3.4.3.3 Gram staining**

The gram staining reaction was done by using a method developed by Bruckner *et al.*, (2007) using freshly prepared reagents. A drop of sterile distilled water was first placed on a clean slide and a single colony of the bacterium was carefully picked onto the slide. Smear was made by robbing the sterile inoculating loop on the surface of the slide and the bacterium was fixed on the slide by heat by flaming the slide through a light flame. The bacterium smear was covered with crystal violet solution for 60 seconds; the specimen was then washed under tap water for 10 seconds after which excess water drained off. This was carefully blotted dry with tissue paper and flooded with iodine solution for 1.0 minute, washed in tap water for 15 seconds, and blotted dry. Ethanol (absolute) was used to decolourised the smear for 30 seconds and quickly rinsed with tap water for 2 seconds. Counterstain was done by spreading Safranin solution on the smear for 10 seconds and it was gently washed with water and blotted dry. Observation of the specimen was done using

immersion oil and viewed with a compound light microscope. Morphological characteristics such as the shape and colour of the cell wall were observed and recorded for further characterization. A culture of *Escherichia coli* obtained from the Noguchi Memorial Institute for Medical Research served as the reference bacterium and was the same treated as the test bacteria isolates.

#### **3.4.3.4 Potassium hydroxide (KOH) solubility test**

Potassium hydroxide (KOH) solubility test was carried out on isolated bacteria to determine if the isolates were planted pathogenic using a method described by Gregerson (1978) and Suslow *et al.* (1982). A drop of 3% freshly potassium hydroxide aqueous solution was placed on a sterile glass slide by using Pasteur pipette. Single colonies were picked with sterile disposable inoculation loops from four-day old culture of YDC and placed on the drop of potassium hydroxide solution on the slide. The suspension was stirred for about 10 and rinsed with an inoculation loop to observe the viscosity of the suspension.

#### **3.4.3.5 Catalase test**

Yeast nutrient agar was prepared by adding 23 g of nutrient agar and 5 g of yeast extract to in a litre of distilled water. The mixture was dispensed into test tubes and autoclaved for 15 minutes at a temperature of 121 °C and was placed gently to set as slants. Slant portions were inoculated with two day old bacterium colonies of some isolates selected at random, while the control treatment was inoculated with water only. The preparations were incubated for 48 hours at 50–60% RH and 23 °C. Three percent (3%) of fresh hydrogen peroxide was placed gently on the walls of the test tube and left for a few seconds and the same was repeated for the control treatment. The presence

of gas bubbles in the test tubes was observed and recorded to aid the identification of the causal organism.

#### **3.4.3.6 Pectolytic activity**

Fresh Irish potato was obtained and washed thoroughly with soap under running tap water. was first sterilized and gently pushed into the tuber. The cork-borer was gently removed and the potato tissue plug in the cork-borer was detached and the peels at both ends were removed using a sterile inoculation pin. The slope of the potato plug was cut using a sterile scalpel. The potato plugs were placed in a test tube with a sloped portion facing upwards and an adequate volume of distilled water was used to cover half of the plug in the test tube and capped. The whole set-up was autoclaved for 121°C for 15 minutes and allowed to cool. The slanted portion of the potato plug was then inoculated with a loopful of each bacteria isolates and the set-up was incubated on a rotary shaker at 23 °C and 50–60% RH for three days. The control treatment was a plug inoculated with water only. The set-up was monitored and data was recorded with three days after the set-up, the nature of the slanted edge of the potato plug was recorded to aid its identification.

### **3.4.4 Molecular characterization**

#### **3.4.4.1 Extraction of DNA**

Total DNA extraction was done from bacteria isolates. Five isolates made up of three from the Eastern region and two from the Volta region, showing the creamy white colouration were sub-cultured onto nutrient agar to obtain single colonies. A two-days old culture was sub-cultured on LB broth by picking single colonies with a sterile inoculation loop. Single colonies were placed into 1.5 µL tubes containing LB broth. This was incubated at 28 °C and 250 rpm overnight on a

rotary shaker. Samples were centrifuged to achieve pelleted bacterium at 8000 g for 5 minutes. A modified protocol developed by Llop *et al.* (1999) was used for the extraction of total genomic DNA. DNA pellets were re-suspended in 500 mL of extraction buffer consisting of 0.25 mM/l Na Cl, 0.2 mM/l Tris HCl pH 7.5, 0.5% PVP, 0.5% SDS and 0.025 mM/l EDTA. This was further incubated at 23°C and 200 revolution per minute in an orbital shaker for 1 hour. Suspension samples were then pinned at 1000 g for 5 min; 450 mL of the supernatant was gently pipetted into a different tubes to which isopropanol of equal volume was added; this was mixed gently and placed at room temperature of 23°C for 45 minutes. Pellets were again centrifuged at 8000 g for ten minutes after which the supernatant was discounted. The final pellets were air-dried at room temperature for 1 hour and it was re-suspended in 100 µL of molecular grade sterile distilled water. One percent agarose gel stained with gel red was used to check the quality and presence of DNA on electrophoresis. Extracted DNA was diluted in 1 µL in 8 µL of PCR water and used for the polymerase chain reaction (PCR).

#### **3.4.4.2 PCR amplification**

The extracted DNA was used as templates in a polymerase chain reaction (PCR). Two primer pairs, H1594/H1595 (Table 3) designed to amplify the *cpn60* gene and VM3/VM4 designed to amplify the *pthA* gene in Xanthomonads, were used in the PCR. The sequences of the M13 primer were attached to the 5' end of the H1594/H1594 primer (Table 2) after (Tian *et al.*, 2016). The reaction volume for the PCR was 50 mL. Each mixture comprised of 25 µL master mix (0.25 mM/l Na Cl, 0.2 mM/l Tris HCl pH 7.5, 0.5% PVP, 0.5% SDS and 0.025 mM/l EDTA), 2 µl each of both reverse primers and forward, 8 µL of target DNA and 13 µL of PCR grade water. The amplification conditions used for the PCR are as follows: the initial denaturation was done at temperature 94 °C for 5 min, 35 cycles of denaturation at 93°C for 30 sec, the annealing temperature of 57 °C for 30

sec and extension at 72 °C for 45 sec. The final extension step was 72 °C for 10 min. A volume of 10 µL of polymerase chain reaction products was run on a two percent (2%) agarose gel matrix which was stained with gel red nucleic acid stain using TAE buffer. Amplified products were viewed with the aid of a UV transilluminator.

### 3.4.4.3 Purification and sequencing of amplified products

The products of the *cpn60* gene obtained were sent to Inqaba Company in South Africa for purification and sequencing. The universal primer M13 was used to sequence the gene region from both forward and reverse positions.

**Table 3. List of primers used in the study**

| Name        | Sequences (5'-3') with attached M13                            | Gene         |
|-------------|----------------------------------------------------------------|--------------|
| H1594       | <u>CGCCAGGGTTTTCCCAGTCACGAC</u> CGACGTCGCCGGTGACGGCAC<br>CACAC | <i>Cpn60</i> |
| H1595       | <u>AGCGGATAACAATTTACACAGGAC</u> CGACGGTCGCCGAAGCCCGG<br>GGCCTT | <i>Cpn60</i> |
| VM3         | GCATTTGATGACGCCATGAC                                           | <i>PthA</i>  |
| VM4         | TCCCTGATGCCTGGAGGATA                                           | <i>PthA</i>  |
| <b>M13F</b> | CGCCAGGGTTTTCCCAGTCACGA                                        | -            |
| <b>M13R</b> | AGCGGATAACAATTTACACAGGA                                        | -            |

NB. Underlined sequences are the M13 portion of each primer sequence.

### 3.4.4.4 Basic local alignment search (BLAST)

The sequences were assembled with the aid of BioEdit software (version 6.0). The assembled sequences were used in a basic local alignment search ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and the most

identical isolate was documented to further enhance in the identification of isolates. When the BLAST search did not turn up any significant similarity between the query strains and the type strain of the suspected *X. campestris* pv. *mangiferaeindicae*, a sequence alignment was carried out with query sequences and those of the type strain, to determine the percent similarity between the two.

#### **3.4.4.5 Sequence analysis of the cpn60 gene region**

The sequences of the cpn60 gene of the isolates obtained in this study were compared to those of other *Xanthomonas* species/pathovars elsewhere that were retrieved from the GenBank. Also, two strains of *Xanthomonas campestris* pv *mangiferaeindicae*, had their sequences retrieved from the stable public repository Figshare (DOI: 10.6084/m9.figshare. 4083378). In all, a total of 32 isolates were used in the study. These included sequences of 5 isolates obtained in this study (Table 4) and those of 25 other isolates that were downloaded from GenBank and of 2 others obtained from Figshare (Table 5). ClustalW was used for the alignment of different sequences and gene regions. The aligned sequences were optimized manually to ensure positional homology and gaps were treated as missing data. The multiple sequence alignment obtained was used to construct a phylogram.

#### **3.4.4.6 Phylogenetic analysis and phylograms construction**

All phylograms were constructed using MEGA6 software. Neighbor-joining (NJ) and Maximum Parsimony (MP) algorithms were used to draw the phylograms (gene trees) for the multiple sequence alignment generated. The Maximum Parsimony (MP) tree was obtained using the Close-Neighbor-Interchange algorithm (Nei and Kumar, 2000) with search level 3 (Felsenstein, 1985,

Nei and Kumar, 2000) in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing missing data and gaps were removed from the data set (complete deletion option). The clade stability of the tree resulting from maximum parsimony analysis was assessed by bootstrap analysis with 1000 replicates (Felsenstein, 1985). The Consistency Index (CI), Retention Index (RI), and Composite Index (CI) were all generated by the software to evaluate the stability of the tree.

The Neighbor-Joining method (Saitou and Nei, 1987) was also used to infer evolutionary history. The percentage of replicate trees in which the associated taxa clustered together was evaluated with a bootstrap analysis with 1000 replicates. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*, 2004) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

**Table 4. Isolates collected and used in this study**

| Species                            | Strain identification | Host                    | Country | *GeneBank accession number (cpn60) |
|------------------------------------|-----------------------|-------------------------|---------|------------------------------------|
| <i>X. c. pv. mangiferaeindicae</i> | XamGH1                | <i>Mangifera indica</i> | Ghana   | -                                  |
| <i>X. c. pv. mangiferaindicae</i>  | XamGH2                | <i>Mangifera indica</i> | Ghana   | -                                  |
| <i>X. c. pv. mangiferaindicae</i>  | XamGH3                | <i>Mangifera indica</i> | Ghana   | -                                  |
| <i>X. c. pv. mangiferaindicae</i>  | XamGH4                | <i>Mangifera indica</i> | Ghana   | -                                  |
| <i>X. c. pv. mangiferaindicae</i>  | XamGH5                | <i>Mangifera indica</i> | Ghana   | -                                  |

X. c. pv.=*Xanthomonas campestris* pathovar.-= sequences are yet to be deposited in GenBank

**Table 5. Isolates whose sequences were downloaded from the GeneBank and used for the study**

| Species                              | Strain identification | Host                                                | Country    | GeneBank accession number (cpn60) |
|--------------------------------------|-----------------------|-----------------------------------------------------|------------|-----------------------------------|
| <i>X. c. pv. mangiferaeindicae</i>   | ATCC11637             | <i>Mangifera indica</i>                             | China      | -                                 |
| <i>X. c. pv. mangiferaeindicae</i>   | ICMP                  | <i>Mangifera indica</i>                             | China      | -                                 |
| <i>X. c. pv. euphorbiae</i>          | NCPB1828              | <i>Euphorbia milii</i>                              | Belgium    | -                                 |
| <i>X. campestris pv. campestris</i>  | NCPB528               | <i>Brassica species</i>                             | China      | -                                 |
| <i>X. axonopodis pv. vignicola</i>   | ATCC11648             | <i>Vinicola guaspari</i>                            | Germany    | -                                 |
| <i>X. axonopodis pv. vesicatoria</i> | NCPB936               | <i>Capsicum spp</i> and <i>Solanum lycopersicum</i> | Korea      | AM039952.1                        |
| <i>X. axonopodis pv. vesicatoria</i> | NCPB2572              | <i>Capsicum spp</i> and <i>Solanum lycopersicum</i> | Korea      | AM039952.1                        |
| <i>X. axonopodis pv. phaseoli</i>    | YFS15-3               | <i>phaseoli var. fuscans Vauterin</i>               | France     | AF426387.1<br>AY922370.1          |
| <i>X. axonopodis pv. phaseoli</i>    | YFS15-4               | <i>phaseoli var. fuscans Vauterin</i>               | France     | -                                 |
| <i>X. axonopodis pv. phaseoli</i>    | YFS15-5               | <i>phaseoli var. fuscans Vauterin</i>               | France     | -                                 |
| <i>X. axonopodis pv. manihotis</i>   | ATCC23380             | <i>Manihot esculenta</i>                            | France     | -                                 |
| <i>X. axonopodis pv. manihotis</i>   | ICMP5741              | <i>Manihot esculenta</i>                            | France     | -                                 |
| <i>X. axonopodis pv. glycines</i>    | ATCC43911             | <i>glycines max</i>                                 | Tokyo      | -                                 |
| <i>X. axonopodis pv. citri</i>       | YG290                 | <i>citrus spp.</i>                                  | Florida    | AE008923.1                        |
| <i>X. axonopodis pv. citri</i>       | YG292                 | <i>citrus spp.</i>                                  | Florida    | CP009031.1                        |
| <i>X. axonopodis pv. citri</i>       | G266                  | <i>citrus spp.</i>                                  | Florida    | CP009028.1                        |
| <i>X. albilineans</i>                | DSM3583               | <i>Saccharum spp.</i>                               | France     | -                                 |
| <i>X. albilineans</i>                | ICMP196               | <i>Saccharum spp.</i>                               | France     | FP565176.1                        |
| <i>X. arboricola pv. celebensis</i>  | ATCC19045             | <i>Strigococcus celebensis</i>                      | New        | -                                 |
| <i>X. arboricola pv. celebensis</i>  | DSM50853              | <i>Strigococcus celebensis</i>                      | New        | -                                 |
| <i>X. axonopodis pv. allii</i>       | LMG576                | <i>Allium cepa</i>                                  | Colorado   | -                                 |
| <i>X. axonopodis pv. allii</i>       | LMG578                | <i>Allium cepa</i>                                  | Colorado   | -                                 |
| <i>X. axonopodis pv. begonia</i>     | DSM50850              | <i>Begonia spp.</i>                                 | California | -                                 |
| <i>X. axonopodis pv. begonia</i>     | LMG550                | <i>Begonia spp.</i>                                 | California | -                                 |
| <i>X. axonopodis pv. betlicola</i>   | ATCC11677             | <i>Piper betle</i>                                  | India      | -                                 |
| <i>X. axonopodis pv. betlicola</i>   | ICMP312               | <i>Piper betle</i>                                  | India      | -                                 |
| <i>X. citri subsp. malvacearum</i>   | ATCC12131             | <i>Gossypium hirsutum</i>                           | China      | -                                 |
| <i>X. citri subsp. malvacearum</i>   | DSM1220               | <i>Gossypium hirsutum</i>                           | China      | -                                 |

- =the accession number could not be obtained

### **3.4.5 Pathogenicity test**

Detached mango leaves of the Keitt variety, harvested from a mango orchard free of the disease were first surface sterilized with 5% sodium hypochlorite and the chemical was flushed off by rinsing three times with sterile distilled water. Micro wounds were created on the surface of the mango leaves using sterile inoculating pins. The inoculum of ten of the bacterial isolates obtained in the study was selected at random and used separately for the study. The inoculum of each bacterium was prepared by scrapping the bacterial growth on the surface of nutrient agar into a test tube containing sterile distilled water. The mixture was agitated until the water became cloudy. The inoculum was poured into a hand sprayer and applied on the surface of the leaves at the points where the wounds were created. Control was leaves inoculated with sterile distilled water only. Inoculated leaves were arranged in transparent polyethylene bags and incubated in the laboratory at 23°C and 65% RH. The set-up was observed daily until symptoms developed. Isolates that were able to elicit the symptoms on the inoculated leaves around the points of inoculation were considered pathogenic to the crop. Pathogens were re-isolated from the artificially inoculated leaves to confirm pathogenicity.

## **3.5 Determination of the efficacy of some selected fungicides against the causal agent of mango bacterial black spot disease**

### **3.5.1 In vitro evaluation of the inhibitory effect of some copper base fungicides on the growth of *Xanthomonas campestris* pv. *mangiferaeindicae***

A laboratory trial was carried in the plant pathology laboratory to determine the effect of some selected copper-based fungicides on the growth of *Xanthomonas campestris* pv *mangiferaeindicae* on media. These copper-based fungicides were amongst those that were mentioned by mango

farmers during the survey at the study, as what they use extensively to control mango bacterial black spot disease. The fungicides and their rates applied for the trial are stated in Table 6 below. 50% Copper hydroxide (5 g/l) was used as a standard fungicide in this study. Kirby Bauer's discs diffusion method was used in this experiment (Bauer *et al.*, 1966). Bacteria isolates were cultured on nutrient agar in 9 cm Petri dishes. Bacterial suspension on concentration  $1.0 \times 10^6$  CFU/mL was streaked uniformly on the growth media using a sterile inoculation loop and allowed to stand for 3 min. Two sterilized filter paper disc each were treated with the treatments (20  $\mu$ L) as replicate and control were treated with sterile distilled water. It was then allowed to dry for three seconds and placed on the media in the plates. The set-up was replicated three times and arranged in a completely randomized design and incubated at  $28^\circ\text{C} \pm 2$  for 48 hrs. Data collected were analyzed using analysis of variance (ANOVA) and means comparisons by LSD ( $P < 0.05$ ) using GENSTAT, 12th edition.

**Table 6. Treatments evaluated in the study**

| Product name         | Active ingredient                         | Use          | Recommended rate of application (g/l) |
|----------------------|-------------------------------------------|--------------|---------------------------------------|
| Funguran             | 50% Copper hydroxide                      | Fungicide    | 5                                     |
| Curenox              | 50% Copper oxychloride                    | Fungicide    | 4                                     |
| Funguran+Agrithane   | 50% Copper hydroxide+<br>80%Mancozeb      | Fungicide    | 5:4                                   |
| Curenox+Agrithane    | 50% Copper oxychloride+80%<br>Mancozeb    | Fungicide    | 4:4                                   |
| Caldo                | Copper sulphate+200 g/kg Hydrated<br>lime | Bacteriocide | 6.7                                   |
| Cuprofix-30 disperse | 30% Metallic copper+80% Mancozeb          | Bacteriocide | 6.7                                   |
| Control              | Sterile distilled water                   |              |                                       |

### **3.5.2 Field evaluation of copper-based fungicides against the mango bacterial black spot disease**

A field trial was conducted at a commercial mango orchard located at Lower Manya Krobo District. The trial was conducted in the peak season of mango cultivation between January to May in 2019. The selected mango orchard had a history of the disease infestation in the previous season. Before the beginning of the trial, excess foliage was pruned from the trees which together with leftover fruits from the previous seasons' production, were removed from the field. All trees in the field were sprayed with the systemic fungicide, Carbendazim, to aid in the rapid healing of wounds. Flowering was then induced on all trees by the application of potassium nitrate and calcium source, according to the farmers' practice. Four weeks after flower induction, trees with enough flowers were selected for the trial. The experiment was carried out with seven treatments (Table 6) using a randomized complete block design (RCBD) with three replicates. Each plot was made up of three flowering trees. In all, a total of 63 trees were used for the trial. The chemicals for the trial were selected based on the recommendation by farmers. The control was trees sprayed with sterile distilled water. The treatments were applied using a motorized mist blower at a volume of application rate of 250 mL/ha. Treatment applications began on the 6<sup>th</sup> of February, 2019 and continued at bi-weekly intervals. The last treatment application was done on 2<sup>nd</sup> May when fruits were nearing maturity. In all, there were a total of 7 applications.

Data on disease incidence and severity were collected at 110 days after fruit set when fruits were matured. To determine the disease incidence, 50 fruits were harvested at random from each treatment and the number of fruits with disease symptoms expressed as a percentage of the total number of fruits inspected. The disease severity was then determined using the method stated in section 3.2.3. All fruits were then harvested and those from trees within the same plot were bulked

and separated into healthy, diseased but marketable and rejected fruits (fruits that could not be marketed in any way). The number of fruits that dropped from the start of the spraying to harvesting was monitored and recorded and was used together with the other parameters to calculate yield/fruit quality loss. The yield/fruit quality loss, disease incidence, and severity were then used as a measure of the efficacy of the treatments. Data were analyzed using analysis of variance (ANOVA) and means comparisons by LSD ( $P < 0.05$ ) using GENSTAT, 12th edition.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Mango farmers' knowledge and perceptions on mango bacterial black spot disease

The result obtained from questionnaires and interviews granted to 60 mango farmers from mango cultivation areas in the Volta and Eastern regions of Ghana to assess information on the prevalence, economic impact, spread, and management of MBBS are shown as follows.

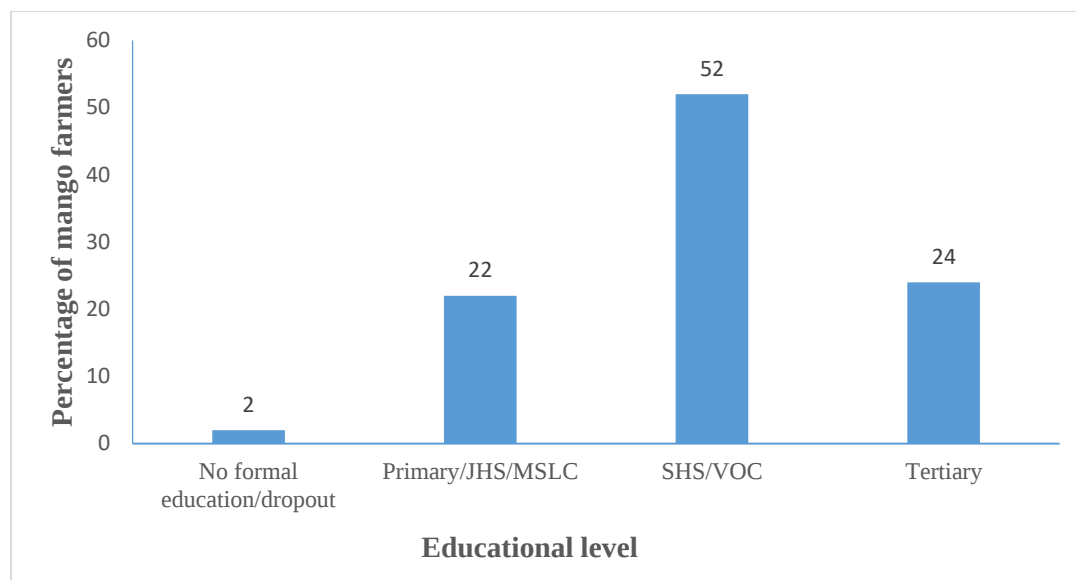
##### 4.1.1 General background of mango farmers

Most of the mango farmers at the study area were males (88%) with females making up 12%. Ages of respondents ranged from 21-60, with 45% (31-40) years, followed by the 41-50 years with 26%, and the least percentage of 6% aged 21-30 years (Table 7).

**Table 7. Ages of farmers in the study area**

| Age of farmers (Years) | Percentage of Mango farmers |
|------------------------|-----------------------------|
| >20                    | 0                           |
| 21-30                  | 6                           |
| 31-40                  | 54                          |
| 41-50                  | 26                          |
| 51-60                  | 14                          |
| 61-70                  | 0                           |
| Total                  | 100                         |

A few farmers had had no formal education (2%) while a larger percentage had either SHS or a vocational school education and about 24% of the farmers had tertiary education (Fig. 2).



**Fig. 2. Level of educational of mango farmers in two agro-ecological zones of Ghana**

#### **4.1.2 Year farmers' detected the disease in their field**

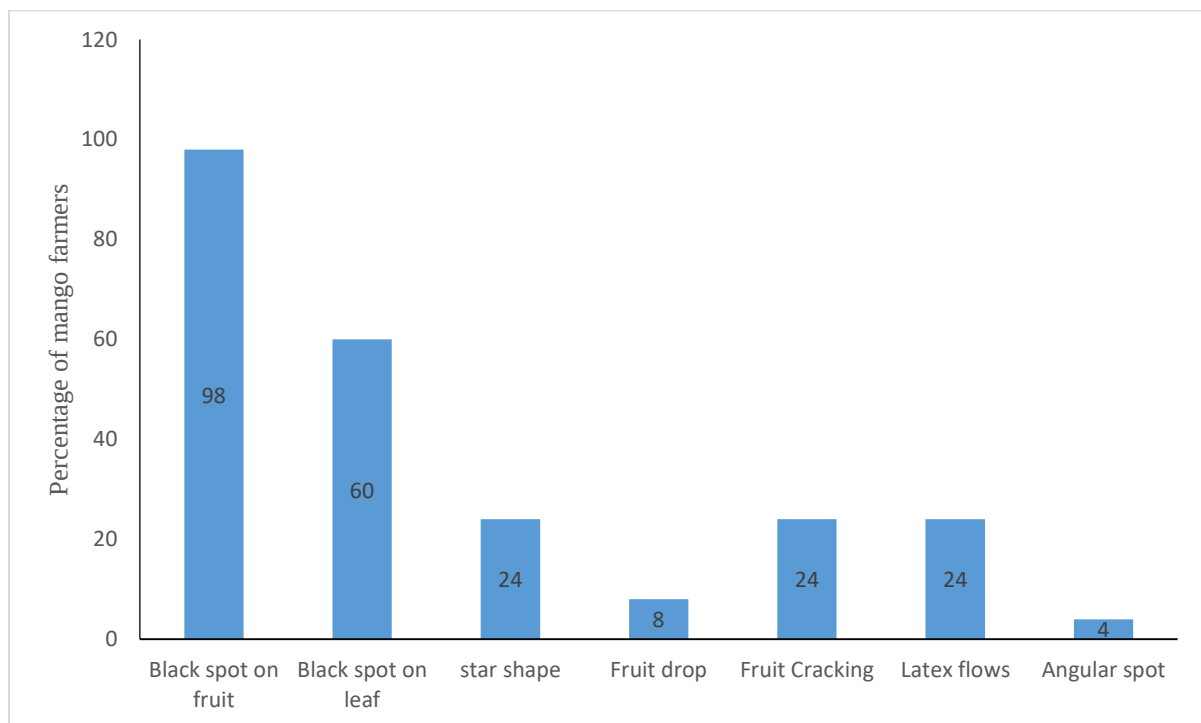
Most of the mango farmers interviewed (66%) first observed the disease between 2011 and 2015 while about 26% of the mango farmers first detected the disease between 2016 and 2019. About 8% of mango farmers in the communities observed the disease between 2006 and 2010 (Table 8).

**Table 8. Period (Years) mango farmers first observed the occurrence of mango bacterial black spot disease in their farms**

| Year      | Percentage of mango farmers |
|-----------|-----------------------------|
| >2000     | 0.0                         |
| 2001-2005 | 0.0                         |
| 2006-2010 | 8.0                         |
| 2011-2015 | 66.0                        |
| 2016-2019 | 26.0                        |
| Total     | 100.0                       |

#### 4.1.3 Farmers' description of symptoms of bacterial black spot disease

All mango farmers interviewed had observed the bacterial black spot disease in their farms. Most farmers (98%) associated the black spot symptoms on the fruit with the disease (Fig. 3). On the other hand, 60% of the farmers also mentioned the black spot on the leaf as an important symptom for identification of mango bacterial black spot disease while 24%, 8%, 24%, 24%, and 4% mentioned the following symptoms; star shape on fruits, fruit drops, fruit cracking, latex coming out from affected fruits and angular leaf spot respectively, as the symptoms associated with the disease.



**Symptoms of mango bacterial black spot disease**

**Fig. 3. Farmers' description of symptoms of the mango bacterial black spot disease**

#### 4.1.4 Farmers' perceptions on the main cause of the disease

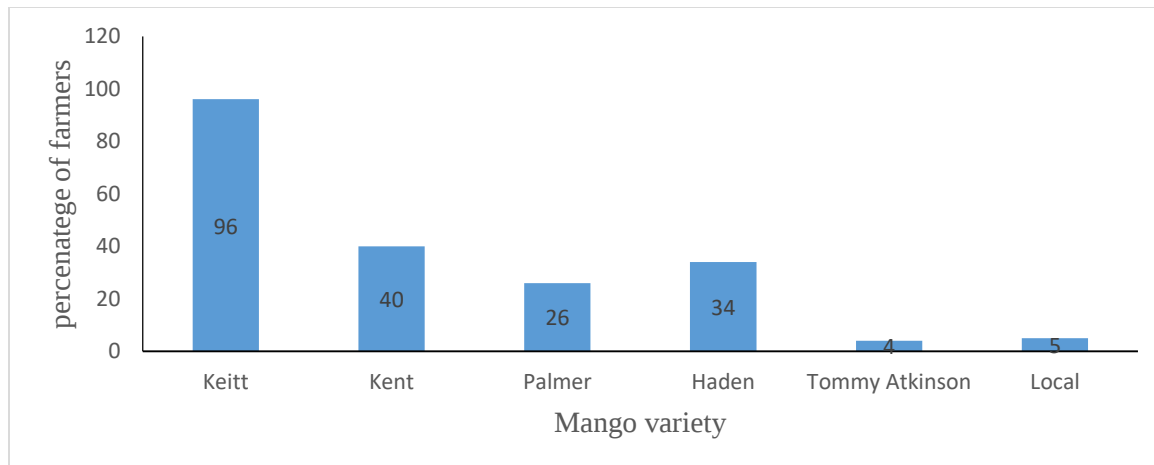
Most farmers (78%) perceived that pathogen was the causal agent of the disease. However, 26% were of the view that the disease was caused by poor management practices. Few farmers believed that the disease was caused by excessive rainfall (6%), planting materials (6%), and wind (6%). Two percent (2%) of the farmers perceived the disease to be caused by poor soil conditions (Table. 9).

**Table 9. Perception on the main cause of the disease**

| <b>The main cause of the disease</b> | <b>Percentage of mango farmers</b> |
|--------------------------------------|------------------------------------|
| Excessive rainfall                   | 6.0                                |
| Infected planting materials          | 6.0                                |
| Poor soil conditions                 | 2.0                                |
| Poor management practices            | 26.0                               |
| Pathogen                             | 76.0                               |
| Wind                                 | 6.0                                |
| Superstitions                        | 0.0                                |
| No idea                              | 0.0                                |
| Others                               | 0.0                                |
| Total                                | 100.0                              |

#### 4.1.5 Varieties of mango grown at the study area

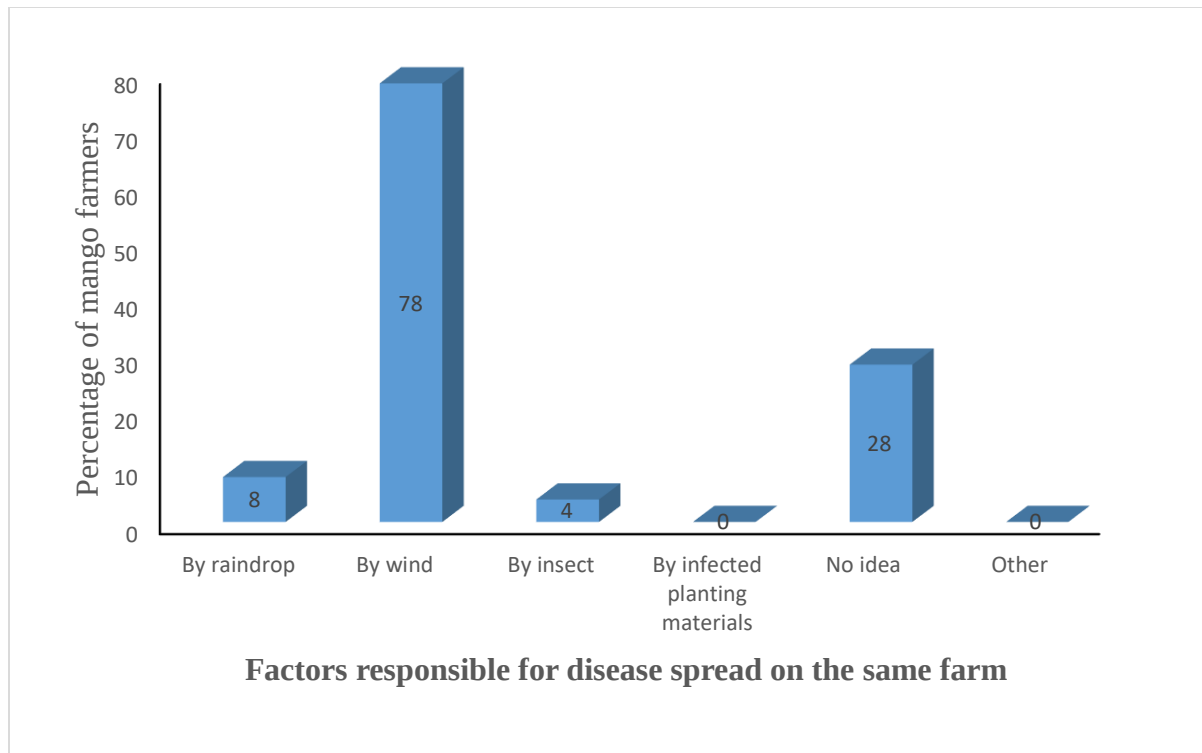
Keitt mango variety (96%) was the most dominant in the study areas, followed by Kent (40%), Haden (34), Palmer (26%), Tommy Atkinson (4) and local variety (5) (Fig. 4).



**Fig. 4. Percentage of mango varieties cultivated at the the three districts**

#### 4.1.6 Perceptions of farmers on means of spread of mango bacterial black spot disease within the same farm

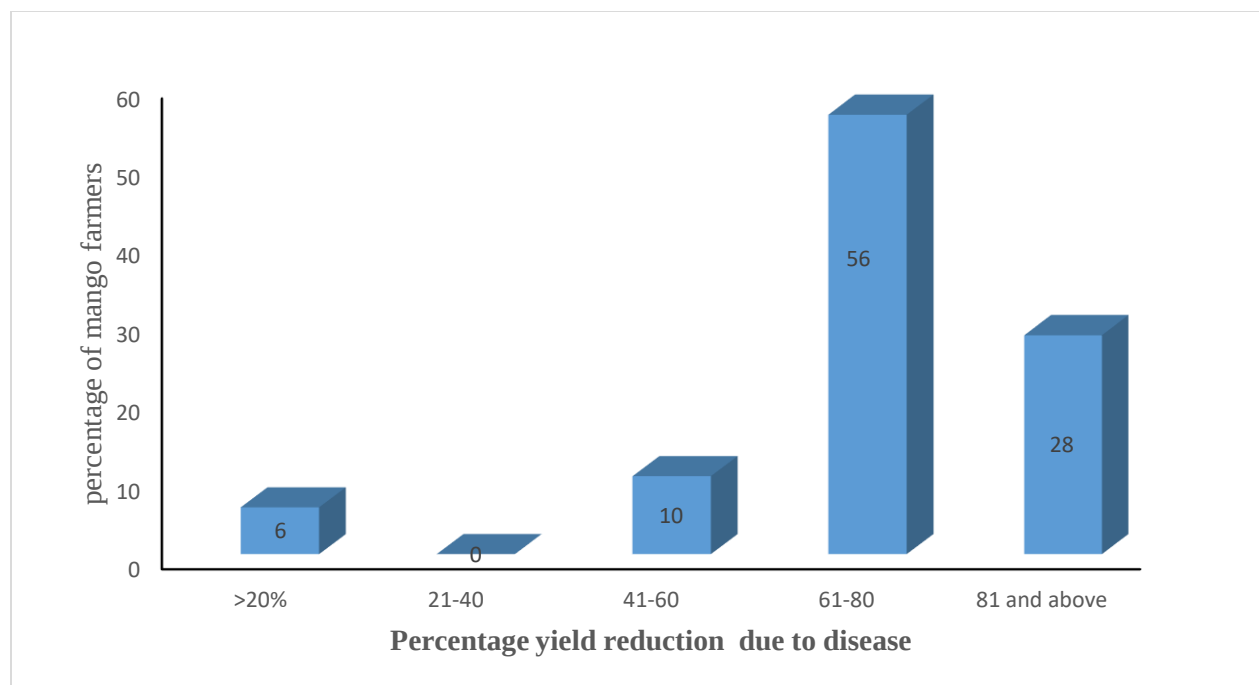
In terms of the mode of spread, 78% of the farmers believed that the disease spreads on the same farm by wind whiles 28% were ignorant about how the disease spread on the same farm. Eight percent (8%) of mango farmers however, associated the spread of the disease on the same farm to a raindrop. Also, 4% of the farmers attributed the spread of the disease to insects. None of the perceived that the disease was spread by infected planting materials (Fig. 5).



**Fig. 5. Perception of farmers' on the spread of the disease within mango**

#### **4.1.7 Percentage reduction of yield as a result of mango bacterial black spot disease experienced on farmers' field**

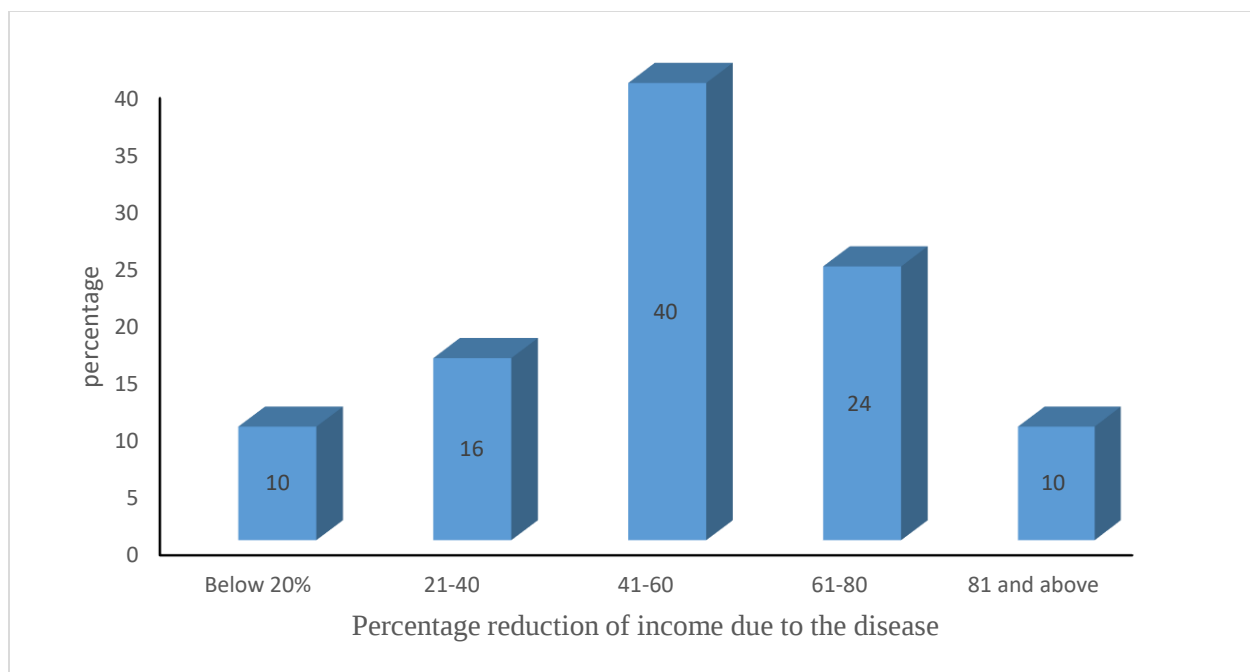
Fifty-six (56%) percent of respondents reported 61-80% yield (fruit) loss while 28% of the farmers experienced 81% and above reduction in yield. Ten percent and six percent of the mango farmers experience the percentage loss in yield was at least 41-60% and >20% respectively (Fig. 6).



**Fig. 6. Percentage reduction of yield experienced by farmers as a result of mango bacterial black spot disease**

#### **4.1.8 Percentage reduction of income as a result of mango bacterial black spot disease**

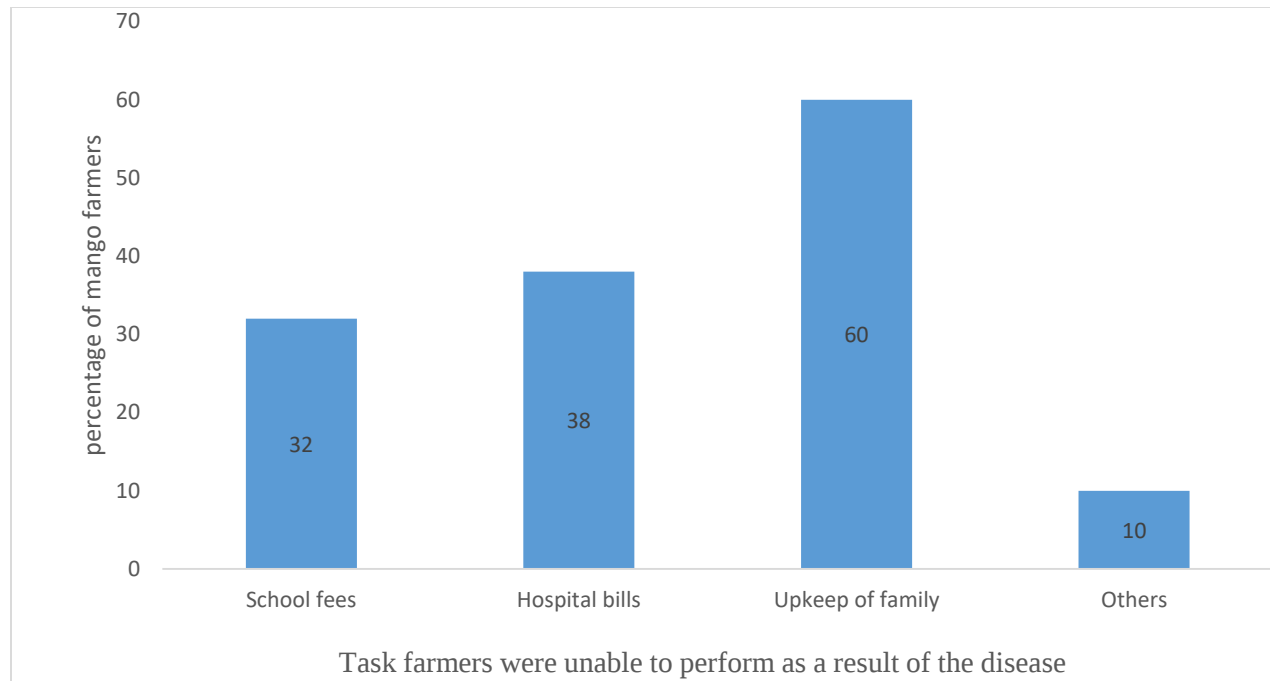
Forty percent (40%) of the farmers experienced between 41-60% reduction in their income per annum, whiles 24% said that they experienced 61-80% income reduction. Sixteen percent (16%) of the farmers had almost 21- 40%. On the other hand, 10 % experienced a below 20% reduction in income while the same percentage experienced income reduction of more than 81% (Fig. 7).



**Fig. 7. Percentage reduction of income as a result of mango bacterial black spot disease**

#### **4.1.9 Effects of the mango bacterial black spot disease on the livelihood of farmers**

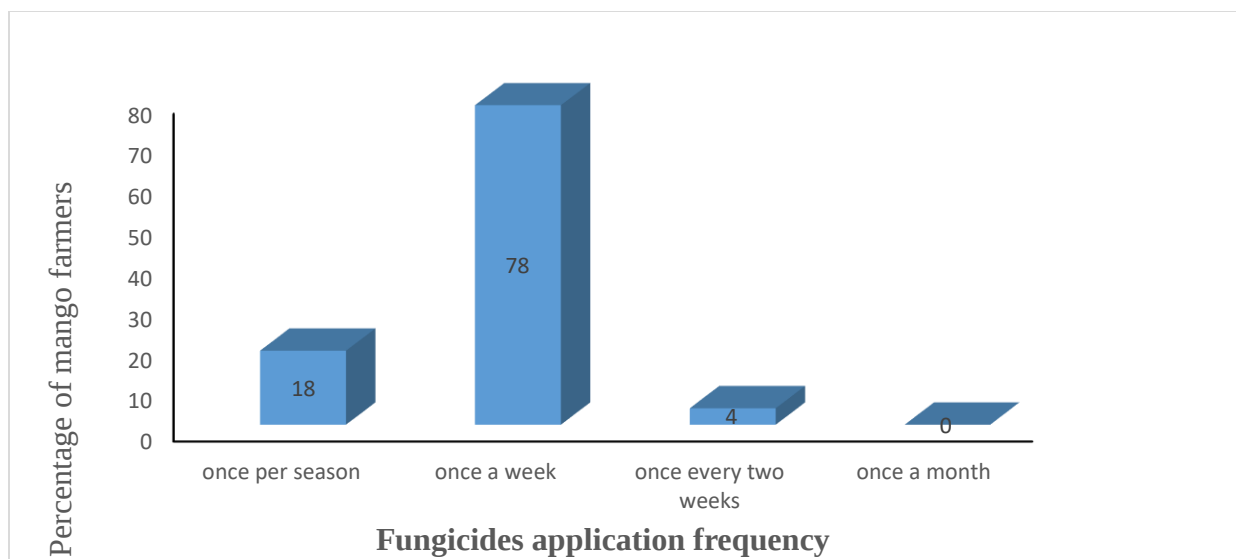
Almost 60% of farmers interviewed said the reduction in their income contributed to their inability to cater for their families. Approximately, 38 % of the farmers had a challenge in paying their hospital bills while 32% were faced with difficulty in financing the education of their wards. However, about 10% of farmers could neither pay their workers as well none purchase farm equipment to expand their farms (Fig. 8).



**Fig. 8. Effects of mango bacterial black spot disease on the livelihood of farmers**

#### **4.1.10 Frequency of fungicide application by farmers and control of mango bacterial black spot disease**

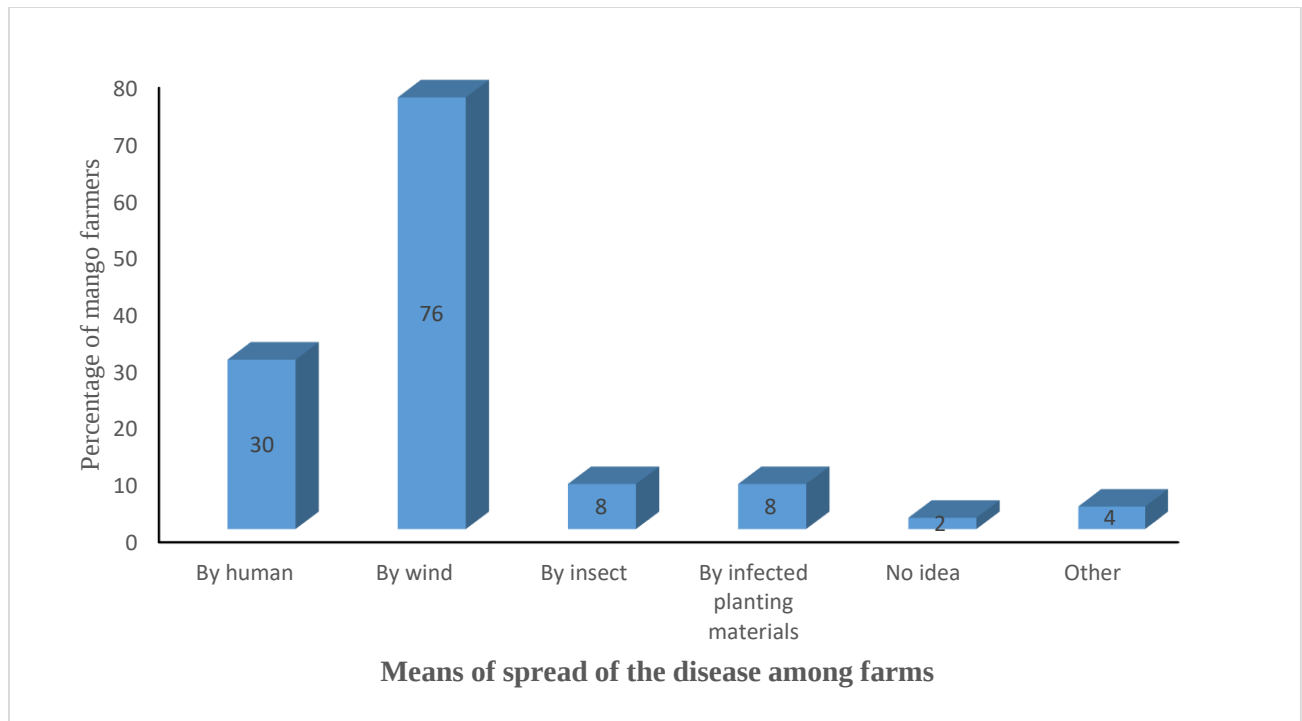
Most farmers (78%) sprayed their farms once a week to control the disease. However, 18% of farmers sprayed their farms once in a season whiles 4% mango farmers sprayed once every two weeks. No farmers sprayed once in a month (Fig. 9).



**Fig. 9. Fungicide application frequency**

#### **4.1.11 Perceptions of mango farmers on agents responsible for the dissemination of mango bacterial black spot disease among farms.**

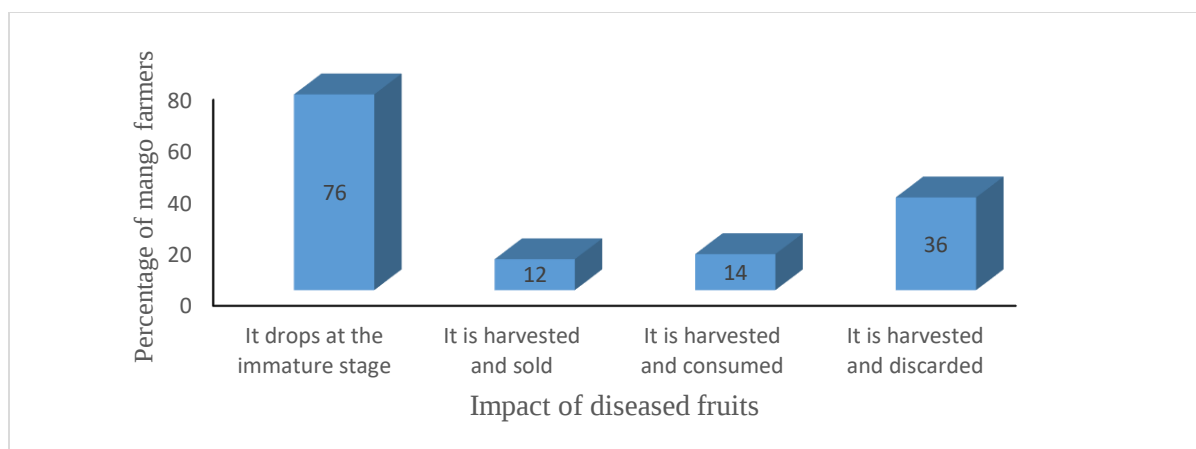
More than half (76%) of the farmers' perceived spread of the disease from farm to farm be caused by wind and 30% by human activities. Other means by which the farmers perceived the disease spread were by an insect (8%) and planting materials (8%). However, 2% of the farmers had no idea of the spread of the disease while 4% attributed the spread to other factors (Fig. 10).



**Fig. 10. Perceptions of mango farmers on agents responsible for the dissemination of mango bacterial black spot disease among farms**

#### 4.1.12 Farmers use of diseased fruits

Most of the farmers (76%) observed mango fruit drop at an immature stage when affected by the disease and 36% harvests and discard infected fruits. However, 14% harvested and consumed diseased fruits while 12% harvested and sold diseased fruits (Fig. 11).

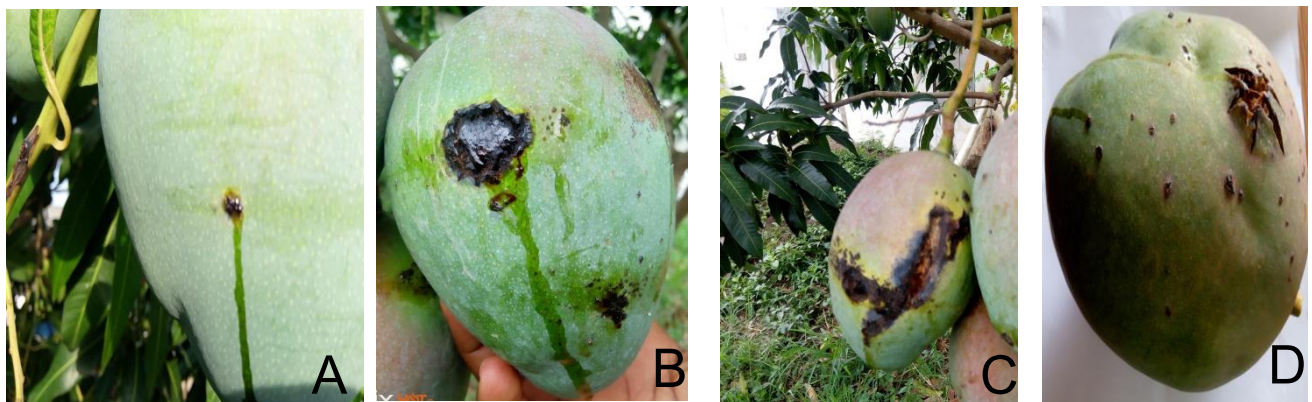


**Fig. 11. Farmers' usage of diseased mango fruits**

## **4.2 Nature of the bacterial black spot disease and disease incidence and severity in farmer fields in the Asuogyaman, Lower Manya Krobo, and North Tongu districts**

### **4.2.1 Disease symptoms, varietal susceptibility and fruit drop due to the disease**

Generally, the symptoms are ‘dirty’ black, and on both fruits and leaves, the spots are slightly raised creating the impression that it could be easily lifted off the surface of the affected plant part. The disease symptoms were found on leaves, stem, and branches of affected trees. On fruits, the spots often crack up in a star-shaped manner. In other instances, the star crack shapes were not evident, with the spots taking on any shape. However, in all instances, there was evidence of latex oozing out of the crack (Fig. 12).



**Fig. 12. Mango fruits showing some of the different shapes of the mango bacterial black spot disease symptoms. (A) Tiny spot showing star crack, (B) Large solitary spot almost round in shape. (C) Large cracked longitudinal spot and (D) Large star crack spot.**

The disease symptoms were found on all the different varieties of the crop found during the survey. These include both exotic and local ones (Fig. 14). The exotic varieties included Julie, Haden, Keitt, Kent, Palmer, and Tommy Atkins. The number of exotic fruits found with the disease symptoms on trees appeared to be far more than those found on trees of local varieties, though the incidence was not determined. One major characteristic of the disease found was premature fruit drop. This appears to occur frequently on fruits that were nearing maturity. On such dropped fruits,

the symptoms of the disease were found on both the fruit pericarp and on the fruit pedicel. Though some premature fruit dropped occur at the tender stage of fruits, the absence of disease symptoms on these fruits meant they were not dropped prematurely because of the disease. During the study, it was found that on some trees, all fruits were dropped prematurely due to the disease.

Another observation from the fields showed that trees that are very large and possess dense canopies have more fruits showing the disease, than smaller trees with sparse foliage. Also, when two or more fruits, hanging on the same peduncle rub against each other, the disease symptoms commonly occur at the point of contact (Fig. 13). Similarly, when peduncle hanging above a fruit, rubs constantly against a portion of the fruit, the disease symptoms were commonly observed to occur at the point of rubbing (Fig. 13).



**Fig. 13. Mango fruits showing the rapid development of disease symptoms at points where the fruits rub against each other on a local variety (Left) or when the fruit surface of an exotic variety is rubbed by a peduncle (Right).**

#### 4.2.2 Incidence and severity of disease on mango farms in the Volta and Eastern regions of Ghana

The disease incidence of mango bacterial black spot in the study area ranged from 27% to 90%. The highest disease incidence in the North Tongu district (90%) while the lowest of 27% was recorded in the Asuogyaman district (Table 10). The incidence of the disease (69.2%) observed in the Lower Manya Krobo District was higher than what was obtained in the Asuogyaman district but lower than what was recorded in the North Tongu district. Disease severity was also highest (3.0) in the North Tongu district and lowest (0.5) was in the Asuogyaman district.

**Table 10. Disease incidence and severity of mango bacterial black spot in the Manya Krobo, Asuogyaman and Juapong districts of Ghana.**

| Name of District<br>(Number of farms) | Disease incidence*<br>(%) | Disease severity index* |
|---------------------------------------|---------------------------|-------------------------|
| Lower Manya (Krobo)                   | 69.2±7.11                 | 2.2±0.32                |
| Asuogyaman                            | 27.0±11.22                | 0.5±0.27                |
| North Tongu                           | 90.0±0.00                 | 3.0±0.55                |

\*=Means+standard errors

Severity index based on a scale of 0-5 where 0= no disease symptoms and 5=more than 50% of fruit surface disease.

#### 4.3 Effect of mango bacterial black spot disease on yield/fruit quality

During the period of the study, direct yield loss due to the effect of the disease was recorded on all the trees used in the study. One week after fruit set, some of the young fruits abscised. However, none of these abscissions could be attributed to the mango bacterial black spot disease, since the disease symptoms were not found on the dropped fruits. Almost eight weeks after fruit set, disease symptoms were found on some of the fruits that had dropped prematurely. In all, the percentage

of fruits solely as a result of the disease ranged from 25.5% on Haden variety to 85% on Palmer (Table 11). This means a direct yield loss of 48.3% of the seasons' total fruit production. At fruit maturity, fruits harvested that were found with the disease symptoms was 10% to 33.3% and none was fit for marketing. In total, the yield/quality fruit loss due to the mango bacterial black spot disease therefore, ranged from 58.5% to 95% with a mean of 71% comprising of the percentage of fruits that formed but were dropped prematurely due to the disease and those that reached maturity but could not be utilized in any way because they were badly affected by the disease symptoms (Table 11).

**Table 11. Effect of the mango bacterial black spot disease on yield/fruit quality of mango fruits at SIREC in the major mango growing season of 2019**

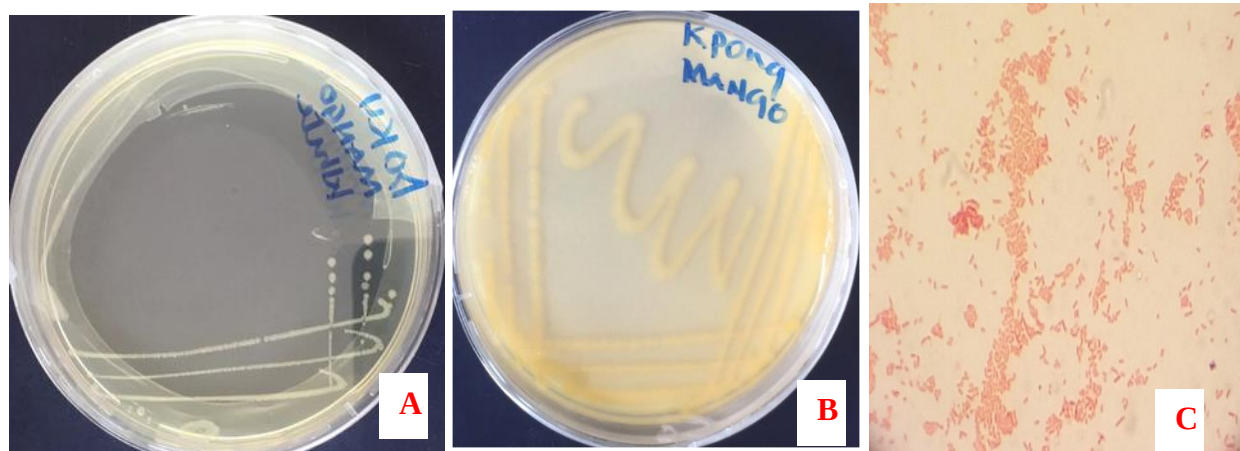
| *Var./<br>no of<br>trees | Factors<br>accounting for<br>fruit drop |                         | Quality of fruits harvesting at maturity |                                           |                                                       |                                               |            |     |                                       |
|--------------------------|-----------------------------------------|-------------------------|------------------------------------------|-------------------------------------------|-------------------------------------------------------|-----------------------------------------------|------------|-----|---------------------------------------|
|                          | <sup>a</sup> Disease<br>only<br>(%)     | Other<br>factors<br>(%) | Clean<br>Fruits<br>(%)                   | Diseased<br>but can be<br>marketed<br>(%) | <sup>b</sup> Rejected<br>due to the<br>disease<br>(%) | Rejected<br>due to<br>other<br>factors<br>(%) | DI.<br>(%) | DSI | <sup>(a+b)</sup> Total<br>loss<br>(%) |
| Keitt<br>(12)            | 34.3                                    | 15.1                    | 13.3                                     | 0.0                                       | 27.0                                                  | 10.3                                          | 90         | 3.6 | 61.3                                  |
| Palmer<br>(6)            | 85.0                                    | 5.0                     | 0.0                                      | 0.0                                       | 10.0                                                  | 0.0                                           | 100        | 3.5 | 95.0                                  |
| Haden<br>(6)             | 25.5                                    | 4.5                     | 29.0                                     | 0.0                                       | 33.0                                                  | 8.0                                           | 98.3       | 1.2 | 58.5                                  |
| Mean                     | 48.3                                    | 8.2                     | 14.1                                     | 0.0                                       | 23.3                                                  | 6.1                                           | 96.1       | 2.8 | 71.6                                  |

\*Variety of mango, DI\*= disease incidence, DSI\*=disease severity index

#### 4.4 Identification and characterisation of the isolated bacteria

##### 4.4.1 Cultural, morphological and biochemical characteristics of isolates

Colonies of the bacterium on the nutrient agar were shallow and convex with complete margins and were mainly creamy white in colour (Fig. 15). On the YDC medium, the isolates produced yellow, shiny, and mucoid colonies after six days of incubation at 25-30 °C. The isolates were gram-negative short rods. In the potassium hydroxide test, the isolates were mucoid and formed a thread when raised with the toothpick (Fig. 14). All isolates used in the pectolytic test were able to macerate the potato plug to indicate that pathogen was plant pathogenic. However, the maceration was not evident in the control. In the catalase test, gas bubbles were found emanating from the growth in the test tube after the few drops of the 3% Hydrogen peroxide has been placed in the tube. However, this was absent in the control test.

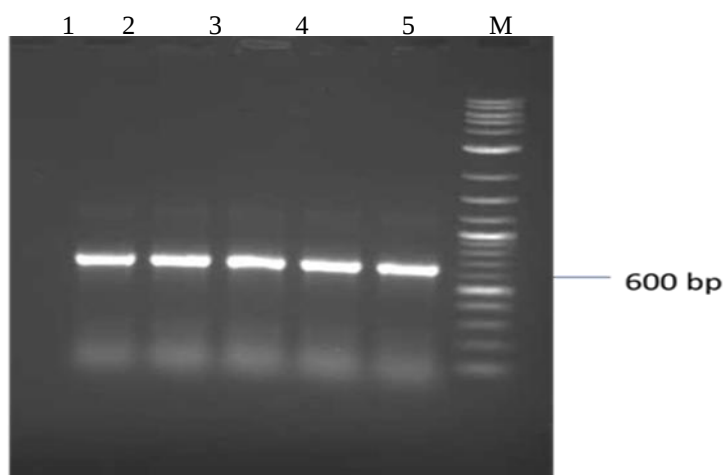


**Fig. 14. Cultural characteristics, Grams reaction, and shape of bacteria isolates obtained from diseased mango fruits. (A) Growth on nutrient agar (B) Growth on YDC. (C) Red and shaped bacteria cells.**

#### 4.4.2 Molecular characterization

##### 4.4.2.1 PCR with universal primer H1594/H1595

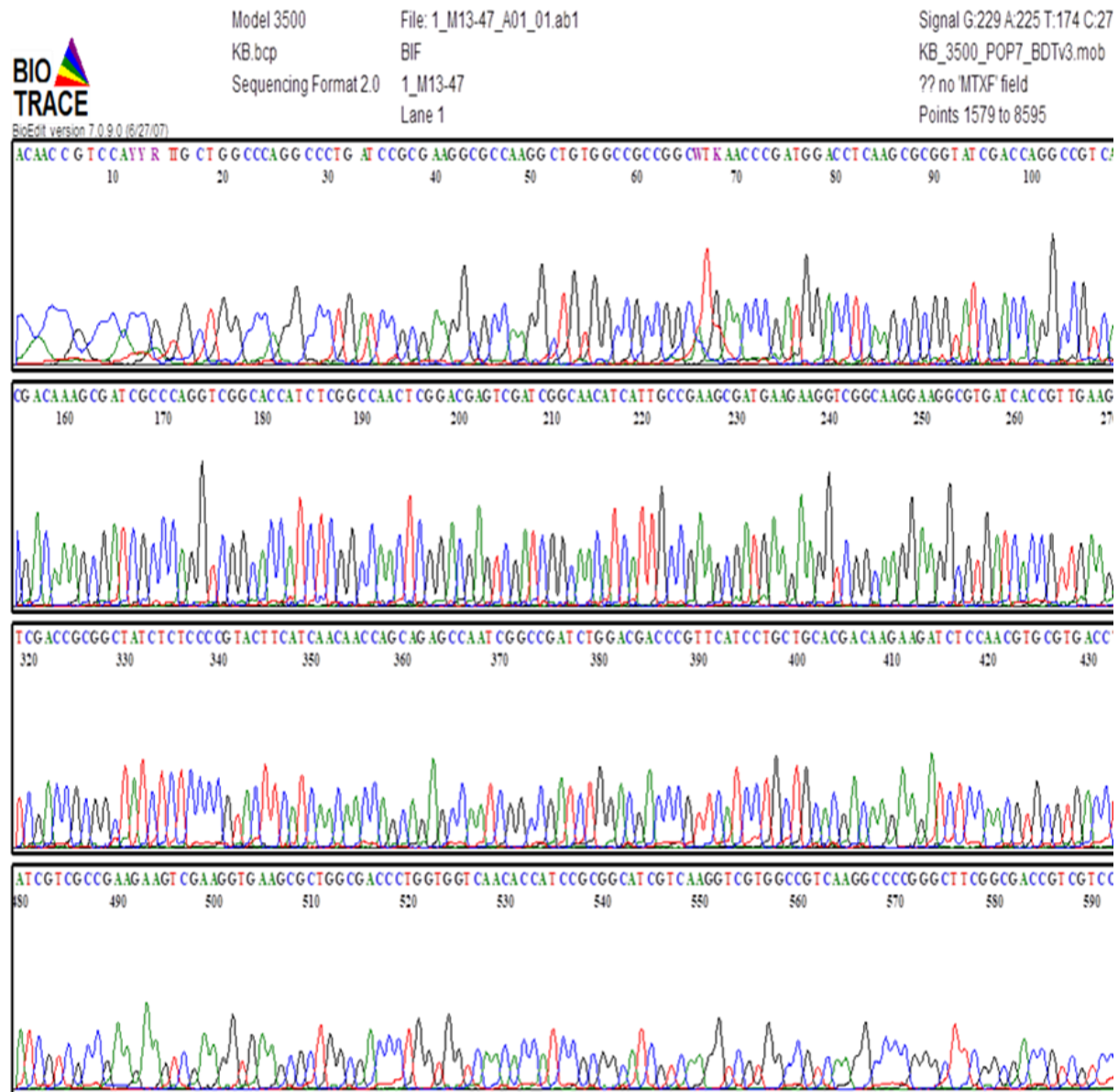
In the PCR using the universal primer pair, H1594/H1595, an approximately 600 bp PCR product was amplified from the DNA of all isolates used in the study but absent in the water control (Fig. 15).



*Fig. 15. A gel image showing amplification of an approximately 600 bp product from DNA extracted from bacteria isolated from mango. Lane 1=water control; Lane 2-6=DNA from isolates; Lane M=100 bp marker. The PCR was carried out with H1594/H1595 primer pair.*

##### 4.4.2.2. Sequence analysis of the cpn60 gene

The cpn60 gene of the selected isolates was successfully sequenced and the chromatographs generated were very clear (Fig. 16). The assembled nucleotide sequences of the cpn60 gene obtained from the isolates from the diseased mango fruits and leaves in Ghana were 555 bp long.



**Fig. 16. Sample of the chromatograph of the nucleotide sequences of the cpn60 gene of isolates used in the study**

#### 4.4.2.3 Comparison of the sequences obtained from isolates from Ghana and the type strain of *Xanthomonas campestris* pv. *mangiferaeindicae*

The BLAST search carried out with the sequences of the isolates obtained from this study did not turn up any significant homology of the isolates with the *X. campestris* pv. *mangiferaeindicae*. However, sequence alignment of the isolates from Ghana and the type strain of *X. campestris* pv. *mangiferaeindicae* downloaded from Figshare, showed a 100% sequence similarity (Table 12)

**Table 12. Sequence alignment of the sequences of the cpn60 gene of a representative isolate from Ghana and that of the type strain of *X. campestris* pv *mangiferaeindicae*. (Alignment was carried out using the clustalw software).**

CLUSTAL 2.1 Multiple Sequence Alignments

Sequence type explicitly set to DNA

Sequence format is Pearson

Sequence 1: XaM 555 bp

Sequence 2: XaM-GH1 554 bp

Start of Pairwise alignments

Sequences (1:2) Aligned. Score: 100

Aligning...

Group 1: Sequences: 2 Score: 10526

Alignment Score 4346

---

clustalw.aln

CLUSTAL 2.1 multiple sequence alignments

```

XaM      GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGCGCATG
XaM-GH1  GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGCGCATG
*****

XaM      AACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCGTCGAGCTG
XaM-GH1  AACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCGTCGAGCTG
*****

XaM      AAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTCGGCACCATCTCG
XaM-GH1  AAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTCGGCACCATCTCG
*****

XaM      GCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGATGAAGAAGGTCGGCAAG
XaM-GH1  GCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGATGAAGAAGGTCGGCAAG
*****

XaM      GAAGGCGTGATCACC GTTGAAGAAGGCTCGGGCCTGGAAAACGAGCTGGACGTGGTCGAG
XaM-GH1  GAAGGCGTGATCACC GTTGAAGAAGGCTCGGGCCTGGAAAACGAGCTGGACGTGGTCGAG
*****

XaM      GGCATGCAGTTCGACCGCGGTATCTCTCCCGTACTTCATCAACAACCAGCAGAGCCAA

```

```

XaM-GH1      GGCATGCAGTTCGACCGCGGCTATCTCTCCCCGTACTTCATCAACAACCAGCAGAGCCAA
*****
XaM          TCGGCCGATCTGGACGACCCGTTTCATCCTGCTGCACGACAAGAAGATCTCCAACGTGCGT
XaM-GH1      TCGGCCGATCTGGACGACCCGTTTCATCCTGCTGCACGACAAGAAGATCTCCAACGTGCGT
*****
XaM          GACCTGCTGCCCCGTGCTGGAAGGCGTGGCCAAGGCCGGCAAGCCGCTGCTGATCGTCGCC
XaM-GH1      GACCTGCTGCCCCGTGCTGGAAGGCGTGGCCAAGGCCGGCAAGCCGCTGCTGATCGTCGCC
*****
XaM          GAAGAAGTCGAAGGTGAAGCGCTGGCGACCCTGGTGGTCAACACCATCCGCGGCATCGTC
XaM-GH1      GAAGAAGTCGAAGGTGAAGCGCTGGCGACCCTGGTGGTCAACACCATCCGCGGCATCGTC
*****
XaM          AAGGTCGTGGCCGTC
XaM-GH1      AAGGTCGTGGCCGT-

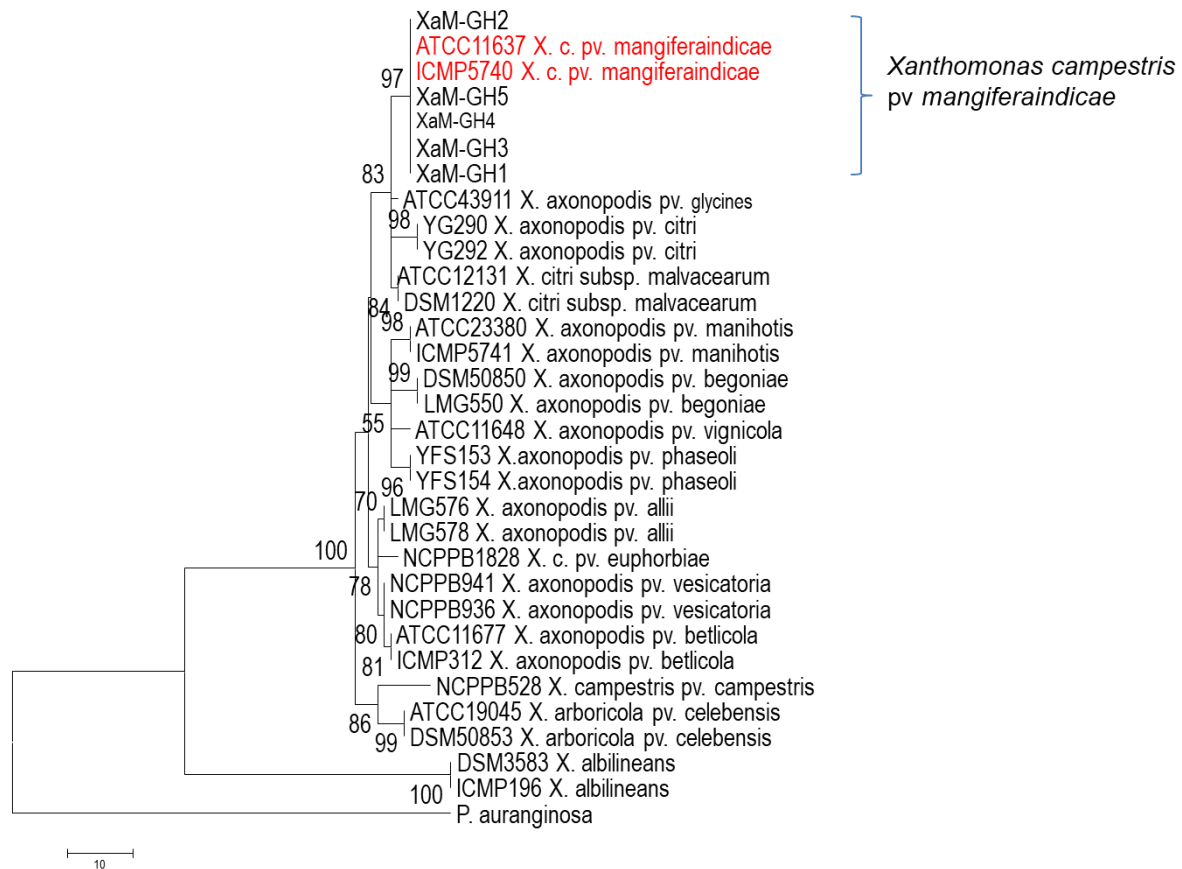
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#### 4.4.2.4 Phylogenetic study of the cpn60 gene

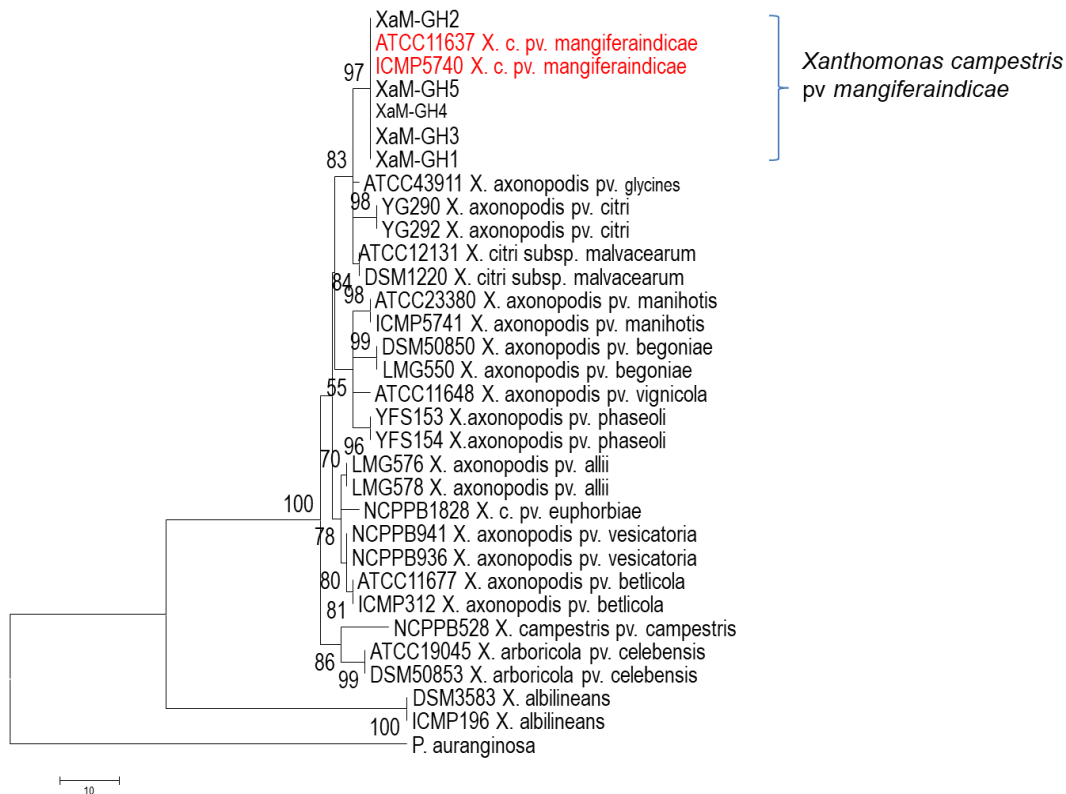
A 560 bp long sequence of the cpn60 gene of the isolates obtained from this study and those retrieved from Figshare were used in the multiple sequence analysis, and for the construction of phylograms. Both the maximum parsimony and neighbor-joining trees drawn had a similar topology. Tree number 1 out of 5 most parsimonious trees with length of 212 obtained with the multiple sequence alignment of the cpn60 of isolates using the maximum parsimony method is shown (Fig. 18). The consistency index was 0.811024, the retention index was 0.893805, and the composite index was 0.792620 (0.724897) for all sites and parsimony-informative sites (in parentheses). The Bootstrap values in percentages are shown next to the branches. The phylogram obtained with the neighbor-joining method (Fig. 17), had the optimal tree with the sum of branch length of 0.70669490, and 554 positions in the final data set.

The phylogram drawn using Maximum parsimony showed all isolates from Ghana clustering with the *Xanthomonas campestris* pv. *mangiferaeindicae* clade. The clade was supported by a high bootstrap value of 97% (Fig. 17). The clade also included strains of *Xanthomonas campestris* pv. *mangiferaeindicae*. All other isolates downloaded from GenBank clustered according to their

species. The phylogram is drawn using the neighbor-joining also gave similar clustering patterns as obtained with the maximum parsimony tree (Fig. 18).



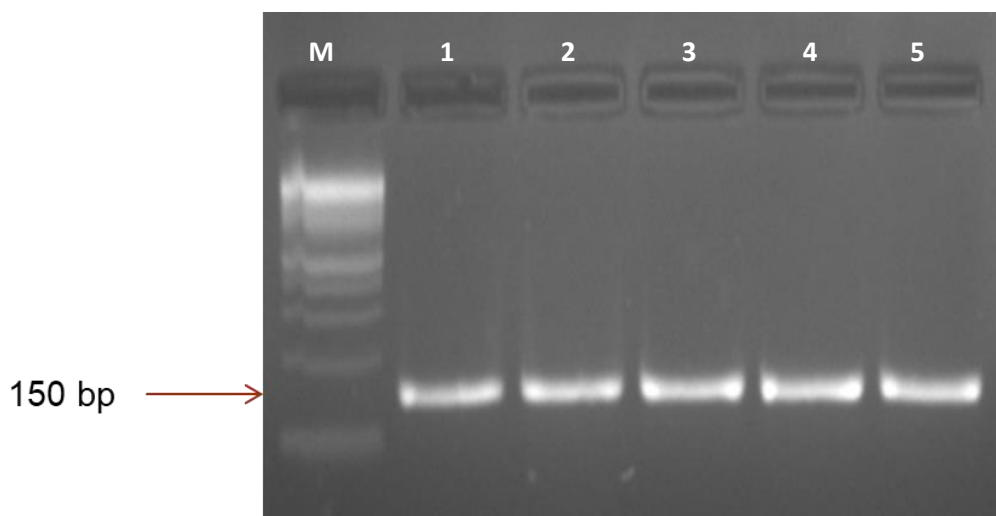
**Fig. 17.** A phylogram is drawn with the multiple sequence alignment generated with the nucleotide sequences of the *cpn60* gene, using Maximum Parsimony analysis. *P. auranginosa* was used as an out-group of the tree. The ex-type strains are in red. All isolates with names beginning with 'XAM-GH' were collected and sequenced by the author. Sequences of strains ATCC11637 and ICMP5740 were downloaded from Figshare database. Sequences for all other strains were downloaded from the GeneBank.



**Fig. 18.** A phylogram is drawn with the multiple sequence alignment generated with the nucleotide sequences of the *cpn60* gene, using the Neighbour Joining method. *P. auranginosa* was used as an out-group of the tree. The ex-type strains are in red. All isolates with names beginning with ‘XAM-GH’ were collected and sequenced by the author. Sequences of strains ATCC11637 and ICMP5740 were downloaded from Figshare database. Sequences for all other strains were downloaded from the GeneBank.

#### 4.4.2.5 Identification of isolates using species-specific primer

An approximately 150 bp PCR product was amplified in all isolates tested when PCR was carried out with the species-specific primer VM3 and VM4 (Fig. 19).



**Fig. 19. PCR product of DNA extracted from *Xanthomonas* isolates electrophoresed on 2% agarose gel stained with ethidium bromide. Lane M = 100 bp DNA marker, lanes 1-5=DNA from isolates identified as *Xanthomonas campestris* pv. *mangiferaeindicae***

#### **4.4.3 Pathogenicity test**

All isolates of the creamy white bacterium obtained from the disease mango parts were able to cause the disease symptoms on the inoculated leaves. The initial symptom of a water-soaked area was first observed three days after inoculation around the point of inoculation. Five days later, the symptoms began to darken and expand on the leaf surface (Fig. 20). The bacterium was successfully re-isolated from the infected leaves to complete Koch's postulates.

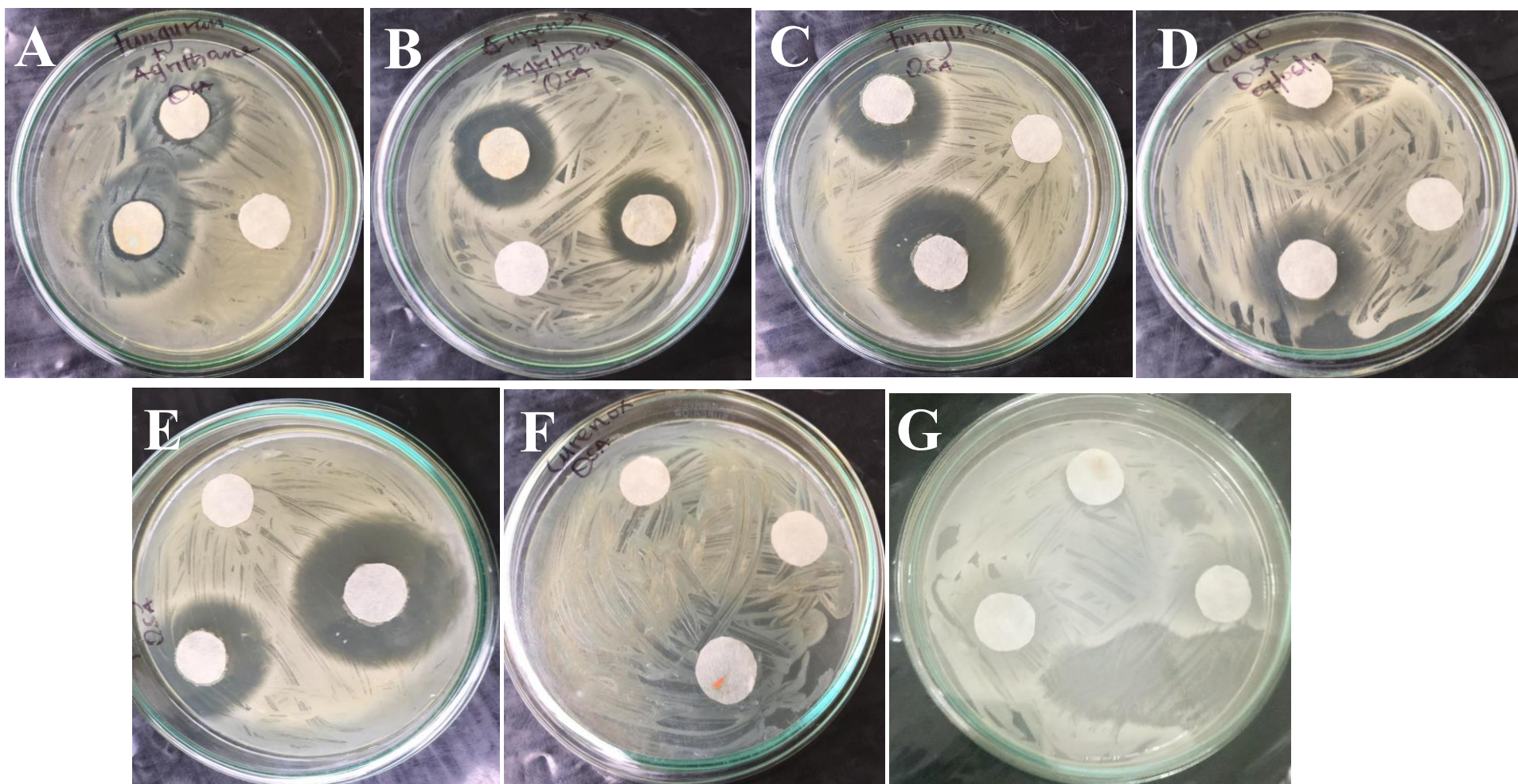


**Fig. 20. Mango leaves showing disease symptoms (dark brown patches) after inoculation with *Xanthomonas campestris* pv. *mangiferaeindicae*. Note the absence of disease symptom on control leaf (far left).**

#### **4.5 Chemical control of mango bacterial black spot disease**

##### **4.5.1 In vitro evaluation of chemicals against *Xanthomonas campestris* pv. *mangiferaeindicae*.**

The diameters of zone of inhibition of the bacterium in media by the selected fungicides are indicated in Table 13 and Fig. 21. There was a significant difference in the zone of inhibition ( $p < 0.05$ ) among treatments. The highest zone of inhibition of 4.93 was observed in the medium amended with 50% Copper hydroxide, while the zone was not observable in the medium amended with 50% Copper oxychloride (Table 13). There was no significant difference in the zones of inhibition on medium amended with 50% Copper oxychloride+80% Mancozeb and 30% Metallic copper+80% Mancozeb. Copper sulphate+Hydrated lime amended medium produced a higher zone of inhibition compared to the mixture of 50% Copper hydroxide+ 80%Mancozeb (Table 13).



**Fig. 21.** Zone of inhibition of *Xanthomonas campestris* pv. *mangiferaeindicae* cultured on nutrient agar amended with 50% Copper hydroxide+ 80%Mancozeb (A), 50% Copper oxychloride+80% Mancozeb (B), 50% Copper hydroxide (C),

**Table 13. Inhibitory effect of some copper based fungicides on the growth of *X. campestris* pv. *mangiferae* isolated from diseased mango fruits**

| Treatment (g/L)                             | The diameter of zone of inhibition (cm) |
|---------------------------------------------|-----------------------------------------|
| 50% Copper hydroxide (5)                    | 4.93                                    |
| 50% Copper oxychloride (4)                  | 0.00                                    |
| 50% Copper hydroxide+ 80%Mancozeb (4 g:4)   | 1.92                                    |
| 50% Copper oxychloride+80% Mancozeb (4 g:4) | 3.93                                    |
| 30% Metallic copper+80% Mancozeb (6.7)      | 3.62                                    |
| Copper sulphate+Hydrated lime (6.7)         | 2.72                                    |
| Control                                     | 0.00                                    |
| LSD                                         | 0.30                                    |

Means followed by the same alphabets are not significant at 5% LSD

#### 4.5.2 Efficacy of various chemicals on the disease incidence, severity and yield loss of mango

There was a significant difference in disease incidence on trees treated with the selected chemicals ( $P < 0.05$ ). The highest and lowest incidences of 99.3% and 55.0% were observed on control and 30% Metallic copper+80% Mancozeb treated trees. The incidence of trees treated with 50% Copper hydroxide was lower than those of the other treatments except 30% Metallic copper+80% Mancozeb. However, there were no significant differences in disease incidence between trees treated with 50% Copper oxychloride+80% Mancozeb and those treated with Copper sulphate+Hydrated lime, and 50% Copper oxychloride (Table 14). Also, there was a significant difference ( $P < 0.05$ ) in disease severity recorded on the trees treated with the various chemicals. The lowest severity of 1.9 was observed on trees treated with 30% Metallic copper+80% Mancozeb while the highest severity of 3.8 was obtained on control trees. On the other hand, there was no significant difference in disease severity on trees treated with 50% Copper hydroxide and 50% Copper hydroxide+ 80%Mancozeb. The

difference in disease severity on trees treated with 50% Copper hydroxide+ 80%Mancozeb and 50% Copper oxychloride+80% Mancozeb was significant. Disease severity on 50% Copper oxychloride and Copper sulphate+Hydrated lime was not significantly different from what was recorded on control trees (Table 14). There was no significant difference in percentage of fruit yield/quality loss among treatments. The lowest yield loss of 38.2% was observed on trees treated with 30% Metallic copper+80% Mancozeb while the highest yield loss of 81.2% was recorded on control plots. The yield/fruit quality loss obtained on trees treated with 50% Copper hydroxide only, 50% Copper hydroxide+ 80%Mancozeb, and 50% Copper oxychloride+80% Mancozeb was not significantly different. Also, the yield loss on trees treated with 50% Copper oxychloride only was not significantly different from what was observed on control treatment (Table 14).

**Table 14. Disease incidence, severity and yield loss of mango (Keitt variety) spray with copper-based fungicides at Lower Manya Krobo district of Ghana.**

| Treatment (g/L)                           | Disease incidence (%) | Severity index (0-5) | *Yield/fruit quality loss (%) |
|-------------------------------------------|-----------------------|----------------------|-------------------------------|
| 50% Copper oxychloride (5)                | 93.3 (75.1)e          | 3.4d                 | 77.3 (61.6)                   |
| Copper sulphate+Hydrated lime (6.7)       | 91.3 (72.9)de         | 3.3cd                | 63.8 (52.9)                   |
| 50% Copper oxychloride+80% Mancozeb (5:4) | 90.0 (71.6)d          | 2.7bc                | 62.8 (52.1)                   |
| Funguran+Mancozeb (4:4)                   | 72.7(58.5)c           | 2.3ab                | 45.9 (42.6)                   |
| 50% Copper hydroxide (4)                  | 66.0 (54.3)b          | 2.0a                 | 49.8 (44.9)                   |
| 30% Metallic copper+80% Mancozeb (6.7)    | 55.0 (47.9)a          | 1.8a                 | 38.2 (38.1)                   |
| Control                                   | 99.0 (88.9)f          | 3.7d                 | 81.2 (64.3)                   |
| LSD (5%)                                  | (3.4)                 | 0.7                  | (5.8)                         |

- Incidence: Percentage of fruits showing the disease symptoms at harvesting.
- Severity index based on a scale of 0-5 where 0= no disease symptoms and 5=more than 50% of fruit surface disease
- Transformed values in parenthesis.

## CHAPTER FIVE

### 5.0 DISCUSSION

#### **5.1 Farmers perception of the causes and control of mango bacterial black spot disease.**

Most farmers at the study areas perceived that the disease occurred mostly in the raining season and suggested that the spread of the disease is by wind-driven rain. These findings are similar to reports by Manicom (1986) and MOAP-GIZ (2015) who suggested that the agent of the spread of the disease in the orchard was by wind-driven rains and windblown rains increase the spread of organism from tree to tree. As a means of reducing the spread of the disease in their orchards, mango farmers should plant windbreaks around their farms to reduce the spread of the disease. This is also because cracks and wounds created by wind enhance penetration into the fruits by bacteria. Most farmers were able to associate the cause of bacterial black spot disease to a pathogen. This could be attributed to the fact that most of the farmers were well educated and thus could read and obtain information about the disease elsewhere. Most of their responses were accurate, in terms of what causes the disease. Estimates they therefore, gave concerning the yield losses and the effect of the disease on their livelihood were considered well informed. It can, therefore, be conjectured that farmers in the study area were not ignorant of the type of disease they were dealing with and its destructive nature.

Another important observation was that most farmers in the study areas abused fungicide in their attempts to control the disease. This could result in the build of resistant strains of the bacterium and residual build up in fruits. It is, therefore, necessary that MoFA extension agents educate and train the farmers on the safe handling and use of fungicides. Pesticide residue levels in mango fruits should be determined intermittently and compared to maximum residual levels to ensure the safety of consumers.

Mango farmers in the study areas resorted to the use of cultural practices such as moderate to heavy pruning to manage the disease. 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 most diseases efficiently (Parkash and Raoof, 1989). Regular pruning of the mango trees facilitates the penetration of sunlight into the canopy to reduce humidity and hence reduced incidence of the mango bacterial black spot disease.

### **5.2 Nature of the mango bacterial black spot disease in the study area.**

In this study, the type of disease symptoms found on lesters, leaves and fruits on infected trees and the premature dropping of fruits confirmed that disease as the much dreaded mango bacterial black spot disease (Manicom and Pruvost, 2001). It was observed that any action that created micro-wounds e.g. fruits rubbing against each other or fruit peduncle rubbing on the surface of nearby fruits, increased the number of fruits that were infected in the field. This nature of the disease has been ascribed to mango bacterial black spot in many mango producing areas of the world (Manicom and Pruvost, 1998; Pruvost *et al.*, 2011). This means one way of controlling the disease is to prune off excess fruits and fruit pedicel to minimize the rubbing of these plant parts against the fruits.

### **5.2 Economic importance of the disease in the study area**

The mango bacterial black spot disease was found to be very devastating in all the districts surveyed. In the Lower Manya and North Tongu Districts, the disease incidence of 69.2% and 90% respectively was an indication that the disease was widespread in these areas. In general, the incidence and severity of plant diseases are influenced by the many factors including cultural

practices, host genotypes, and the growing environment (Neya and Normand, 1998). Both districts in the coastal savanna zone of Ghana, where rainfall frequency higher especially during the major mango growing season between January and July. This kind of climate is known to increase relative humidity (Chala *et al.*, 2010). High relative humidity and rainfall greatly exacerbate plant diseases and therefore the climate in these areas could explain the high incidence and severities of the disease. Secondly, there is a high concentration of mango farms in these areas which increases the probability of farm to farm spread of the disease. Keitt was the major type of mango variety grown in these areas. Several reports have shown that the variety was highly susceptible to the disease (Smith, 1982). On the other hand, disease incidence and severity were lower in the Asuogyaman district. This could be attributed to the widely spaced which limit mango farms in the district, such that there was a reduced incidence of farm to farm spread of the disease. The area was now also being considered as an important mango growing area and mango farms were now springing up. Therefore, the intense activities associated with mango commerce which could aid in the rapid spread of the disease from farm to farm was non-existent.

In this study, it was found out that the disease could potentially cause between 25.5% and 85% of the total number of fruits produced per tree per season to be lost through premature fruit drop. When combined with the percentage of fruits that could not be marketed because they were infected at maturity, the disease could potentially cause yield loss of between 58.5% and 95% of total fruit produced per season, depending on the variety. The fruit loss through premature drop and blemishes on mature fruit has been reported by Gagnevin and Pruvost (2001), giving credence to the findings in this work. Also, the estimates obtained in this study are in agreement with reports elsewhere suggesting that the disease could cause between 50% to 100% loss of the seasons' fruits

production (Kishon, 1982; Prakash and Misra, 1992). This makes the mango bacterial black spot a threat to the mango industry in Ghana.

### **5.3 Confirmation of *Xanthomonas campestris* pv. *mangiferaeindicae* as the causal agent of the mango bacterial black spot disease in the study area**

The bacterium, *Xanthomonas campestris* pv. *mangiferaeindicae* was reported as the causal agent of the mango bacterial black spot in the Northern part of Ghana in 2011 (Pruvost *et al.*, 2011). The pathogen has also been associated with the disease in the transitional zone and parts of the coastal savanna zones of Ghana (Abeiku-Amissah, 2018). Before this study, the pathogen was not confirmed to be present in the Lower Manya, Asuogyaman, and North Tongu districts. This study therefore, confirm earlier reports that the pathogen was responsible for the disease in Ghana.

In this study, the *cpn60* gene was selected for the identification of the bacterium. This gene is one of several that have been touted as possible bar codes for differentiation of strains and species of *Xanthomomas* (Qian *et al.*, 2016). The *cpn60* sequences of the isolates obtained in this study showed a 100% similarity with these sequences of the same gene isolated from *Xanthomonas campestris* pv. *mangiferaeindicae* strains collected from China. The phylogram is drawn separated the *Xanthomonas campestris* pv. *mangiferaeindicae* strains from all other species/pathovars without any ambiguity. This was a confirmation of the report by Tian *et al.* (2016) that the *cpn60* gene was very reliable in delineating the species and pathovars status of members of the *Xanthomonas* genera. It therefore widens the spectrum of choices available for the choice of diagnostic genes for identification of *Xanthomonas campestris* pv. *mangiferaeindicae*.

The nucleotide BLAST search with the sequences of the isolates obtained in this study failed to turn up any significant similarity to the suspected *Xanthomonas campestris* pv. *mangiferaeindicae* strains. This could be because sequences of these strains had not been deposited in the GenBank, where BLAST results are obtained. The sequences of the type strains of the pathogen were only available at Figshare. When the sequences from this study are finally deposited in the GenBank, it will help to correct the problems created in the absence of the sequences from the GenBank.

The species-specific primer MV3/MV4 originally was designed to amplify a 150 bp product in the *Xanthomonas campestris* pv. *citri* on citrus (Mavrodieva *et al.*, 2004). The primer was able to successfully amplify the expected PCR product in the mango isolates also. This could be because the MV3/MV4 primer was designed based on the sequences of the pthA gene, which is required for pathogenicity in some *Xanthomonas* species and pathovars. These primers have been reported as a potential for detecting all strains of any species or pathovar in which a homologue of pthA gene is required for pathogenicity (as appears to be the case for rice blight, cotton blight, and common bean blight) (Mavrodieva *et al.*, 2004) and in mango bacterial black spot disease (Gagnevin and Pruvost, 2001). The identification of the isolates from mango using the MV3/MV4 primer pair in this study is therefore given credence. This finding is very important as the use the primer pair will shorten the time needed to identify the bacterium on mango orchards compared to the use of the sequencing method. The only limitation with these primers is that if the host of an isolate is unknown, it would be very difficult to name it to the pathovar level. However, if the host is known it would be easy to use the primer to identify to the pathovar level because most of *Xanthomonas campestris* species are host specific.

#### **5.4 Chemical control of the mango bacterial black spot disease**

In this laboratory experiment, some of the selected copper-based fungicides, either solely or in combination with mancozeb, demonstrated anti-bacterial activity against the causal agent of the disease. In general, copper is known to be toxic to bacteria. Copper fungicides used in controlling bacterial diseases function in different ways such as altering bacterial protein synthesis, altering membrane integrity, and is detrimental to microbial DNA (Warnes *et al.*, 2010; Grass *et al.*, 2011; Chaturvedi and Henderson, 2014). The findings in this study therefore, buttressed the point that copper was toxic to the bacterium (Warnes *et al.*, 2010).

In the fieldwork, variation in the success of bacterial black spot disease control was recorded with different forms of copper-based fungicides. Some of the treatments, such as 50% Copper hydroxide and 50% Copper oxychloride disperse were able to reduce the disease incidence and severity and the percentage fruit loss as compared to the control. The better performance of these copper chemicals is similar to what was recorded in South Africa where some copper-based formulations were able to control the disease (Frean, 1985; Vermuelen *et al.*, 1992). Due to the problems associated with the use of antibiotics such as the development of resistant varieties in food, copper-based fungicides are currently the most viable option available for the control of the mango bacterial black spot disease. Mancozeb is also reported to interfere with some survival activities of the bacteria in general (Pozo *et al.*, 2008). A combination of the two was therefore expected to give some level of control against the disease. However, despite the better performance of these treatments compared to the non- treatment control, the yield loss experienced with those treatments was very high. This could be one of the reasons why farmers in the study area are of the view that

the application of these copper-based fungicides is not effective. Therefore until a more effective chemical that will reduce the yield loss due to the disease is found, mango farmers in Ghana must consider the use of 50% Copper hydroxide and 50% Cuprofix disperse for the management of the disease.

Mango bacterial black spot disease was confirmed to be prevalent in mango orchards of the selected districts in the Eastern and Volta region. The causative organism was confirmed as *Xanthomonas campestris* pv. *mangiferaeindicae* using both the traditional methods and sequence analysis of the Cpn60 gene. Copper-based fungicides such cuprofix disperse was found to be effective against the disease. It is however recommended that, field trials of the research is repeated to conclusively find the most effective control measure against the disease.

## CHAPTER SIX

### 6.0 CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 CONCLUSIONS

- Bacterial black spot disease of mango was common and widespread in the two agro-ecological zones of Ghana. The majority of farmer groups perceived that the pathogen (*Xanthomonas campestris* pv. *mangiferaeindicae*) was the cause of the disease and that the disease spreads among orchards through rains. The majority of mango farmers experience 61-80% yield reduction. This had negatively affected their income and living standards.
- The disease symptoms, made up of star crack with oozing of sap, were found on all the different varieties of the crop found during the survey. Also, when two or more fruit, hanging on the same peduncle rub against each other, the disease symptoms commonly occur at the point of contact.
- The disease incidence ranged from 27% in Asuogyaman District, 69.2% in Lower Manya Krobo, and 90% in the North Tongu District, while severity were 0.5 to 3.0 on a scale of 0-5 respectively.
- The disease was devastating as it caused as high as 95% fruit quality/yield loss in mango orchards in the study area.
- The causal organism of the mango bacterial black spot disease of mango was confirmed as *Xanthomonas campestris* pv. *mangiferaeindicae* based on cultural, morphological, and sequence analysis of the cpn60 gene.

- Cuprofix disperse (Metallic copper+Mancozeb) fungicide is a potential chemical for the control of the disease as it significantly reduced the fruit quality/yield loss caused by the disease to 38.25 as compared to 81.2% on control trees.

## 6.2 Recommendation

- The bacterium causing the disease in the study area must be compared to those from other parts of the country to determine whether there are different strains in the country.
- Other *Xanthomonas* species such as *Xanthomonas citri* pv. *citri* have been reported on other crops in Ghana. It will be important to determine whether these can cross-infect mangoes in the field.
- The field trial for the control of the disease must be repeated and more copper-based fungicides, such as Nordox can be included in the trial to increase the spectrum of products that can be used to control the disease.

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## 7.0 APPENDICES

### **Appendix 1. Questionnaire: Survey on Perception of Mango Farmers on a mango bacterial black spot disease in the Eastern and Volta regions of Ghana**

Kindly spend about 30 minutes to respond to the following questions on the Mango Bacterial Black spot disease. 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 [ ☐ ] 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).....

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

8. Have you observed a new mango disease on your farm? Yes [ ] No [ ]

9. If yes, describe it

.....

.....

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

a. excessive rainfall [ ] b. infected planting material [ ] c. belief and superstition [ ]

d. poor soil [ ] e. poor management practices [ ] f. pathogen [ ] g. wind [ ] h. no idea [ ]

j. others (specify).....

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

**Knowledge, perception, and experiences concerning spread (for those who have seen the disease on their farms)**

12. 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 [ ]

13. When (what season) do you normally observe this disease on your farm? a. rainy season [ ]

b. dry season [ ] c. both rainy and dry seasons [ ] d. All year round

14. If seasonal, which of these seasons is the disease prevalence high

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

15. 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. others (specify).....

16. 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).....

**Knowledge and perception of farmers concerning the economic impacts of mango bacterial back spot disease on the livelihood of farmers. (for those who have seen the disease in the exotic trees)**

17. What happens to the fruits affected by the disease?

a. it drops at the immature stage [ ] b. it is harvested and sold [ ]

c. it is harvested and consumed [ ] d. it is harvested and discarded [ ]

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

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

19. 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 [ ]

20. How does the disease affect your livelihood?

a. school fees [ ] b. hospital bills [ ] c. upkeep of family [ ] d. others (specify)

.....

**Farmers' knowledge and perception concerning the control of mango bacterial black spot disease.**

21. Which of the following methods do you use to control the Mango bacterial black spot disease?

a. heavy pruning [ ] b. slight pruning [ ] c. no pruning [ ]

d. apply fertilizer [ ] e. spray with pesticide [ ] f. no control

22. How often do you spray (fungicide) your mango plants in a year to control BBS?

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

23. Which type of fungicide?

Specify.....

24. At what stage of fruit development do you start spraying?

a. at flowering [ ] b. at fruit set [ ] c. Near maturity [ ]

25. What is your frequency of application?

a. Once per season b. once a week c. once every two weeks d) once a month

26. For how long.....

**Thank you**

## Appendix 2. Disease assessment key of mango bacterial black spot disease

| Rating | Meaning                                         |
|--------|-------------------------------------------------|
| 0      | No infection                                    |
| 1      | Up to 5% of fruit surface area covered          |
| 2      | 6-10% of the area affected                      |
| 3      | Between 11 and 20% of fruit area covered        |
| 4      | 21-50% of fruit area affected                   |
| 5      | More than 50% of the fruit surface area covered |

Adapted from Lakshmi and Prasad (2011)

### a. Analysis of variance of clean fruits untransformed data

| Source of variation | d.f.  | s.s.    | m.s.   | v.r.  | F pr. |
|---------------------|-------|---------|--------|-------|-------|
| Treatment           | 6     | 3401.20 | 566.87 | 11.75 | <.001 |
| Residual            | 12    | 578.94  | 48.24  |       |       |
| Total               | 20    | 4337.49 |        |       |       |
| l.s.d.              | 12.36 |         |        |       |       |

### b. Analysis of variance of clean fruit transformation data

| Source of variation | d.f. | s.s.    | m.s.   | v.r.  | F pr. |
|---------------------|------|---------|--------|-------|-------|
| Treatment           | 6    | 1393.81 | 232.30 | 12.06 | <.001 |
| Residual            | 12   | 231.21  | 19.27  |       |       |
| Total               | 20   | 1765.35 |        |       |       |
| l.s.d.              | 7.81 |         |        |       |       |

## c. Analysis of variance of fruit drop due to disease

| Source of variation | d.f. | s.s.    | m.s.   | v.r.  | F pr. |
|---------------------|------|---------|--------|-------|-------|
| Treatment           | 6    | 1742.03 | 290.34 | 11.18 | <.001 |
| Residual            | 12   | 311.75  | 25.98  |       |       |
| Total               | 20   | 2093.88 |        |       |       |
| l.s.d.              | 9.07 |         |        |       |       |

## d. Analysis of variance of fruit dropped due to disease

| Source of variation | d.f. | s.s.    | m.s.    | v.r.  | F pr. |
|---------------------|------|---------|---------|-------|-------|
| Treatment           | 6    | 693.035 | 115.506 | 11.89 | <.001 |
| Residual            | 12   | 116.623 | 9.719   |       |       |
| Total               | 20   | 827.895 |         |       |       |
| l.s.d.              | 5.55 |         |         |       |       |

## e. Analysis of variance of diseased but can be marketed

| Source of variation | d.f.  | s.s.   | m.s.  | v.r. | F pr. |
|---------------------|-------|--------|-------|------|-------|
| Treatment           | 6     | 10.383 | 1.730 | 1.11 | 0.413 |
| Residual            | 12    | 18.754 | 1.563 |      |       |
| Total               | 20    | 29.243 |       |      |       |
| l.s.d.              | 2.224 |        |       |      |       |

## f. Analysis of variance of Diseased but can be marketed

| Source of variation | d.f. | s.s.   | m.s.  | v.r. | F pr. |
|---------------------|------|--------|-------|------|-------|
| Treatment           | 6    | 93.50  | 15.58 | 1.52 | 0.253 |
| Residual            | 12   | 123.06 | 10.25 |      |       |
| Total               | 20   | 224.70 |       |      |       |
| l.s.d.              | 5.70 |        |       |      |       |

## g. Analysis of variance of diseased only

| Source of variation | d.f.  | s.s.    | m.s.   | v.r. | F pr. |
|---------------------|-------|---------|--------|------|-------|
| Treatment           | 6     | 62.203  | 10.367 | 2.82 | 0.060 |
| Residual            | 12    | 44.106  | 3.675  |      |       |
| Total               | 20    | 109.763 |        |      |       |
| l.s.d.              | 3.411 |         |        |      |       |

## h. Analysis of variance of dropped due to other factors

| Source of variation | d.f. | s.s.    | m.s.   | v.r. | F pr. |
|---------------------|------|---------|--------|------|-------|
| Treatment           | 6    | 121.919 | 20.320 | 2.92 | 0.054 |
| Residual            | 12   | 83.558  | 6.963  |      |       |
| Total               | 20   | 210.570 |        |      |       |
| l.s.d.              | 4.69 |         |        |      |       |

## i. Analysis of variance Diseased but can be marketed

| Source of variation | d.f.  | s.s.    | m.s.   | v.r. | F pr. |
|---------------------|-------|---------|--------|------|-------|
| Treatment           | 6     | 1441.28 | 240.21 | 3.67 | 0.026 |
| Residual            | 12    | 785.65  | 65.47  |      |       |
| Total               | 20    | 2245.04 |        |      |       |
| l.s.d.              | 14.39 |         |        |      |       |

## k. Analysis of variance due to other factors

| Source of variation | d.f. | s.s.   | m.s.  | v.r. | F pr. |
|---------------------|------|--------|-------|------|-------|
| Treatment           | 6    | 35.423 | 5.904 | 1.25 | 0.348 |
| Residual            | 12   | 56.644 | 4.720 |      |       |
| Total               | 20   | 94.795 |       |      |       |
| l.s.d.              | 3.87 |        |       |      |       |

## l. Analysis of variance of Rejected due to the disease

| Source of variation | d.f.  | s.s.   | m.s.  | v.r. | F pr. |
|---------------------|-------|--------|-------|------|-------|
| Treatment           | 6     | 9.097  | 1.516 | 1.21 | 0.364 |
| Residual            | 12    | 15.010 | 1.251 |      |       |
| Total               | 20    | 24.870 |       |      |       |
| l.s.d.              | 1.990 |        |       |      |       |

## m. Analysis of variance of Disease only

| Source of variation | d.f. | s.s.    | m.s.   | v.r.  | F pr. |
|---------------------|------|---------|--------|-------|-------|
| Treatment           | 6    | 4642.33 | 773.72 | 26.49 | <.001 |
| Residual            | 12   | 350.46  | 29.20  |       |       |
| Total               | 20   | 5104.75 |        |       |       |
| l.s.d.              | 9.61 |         |        |       |       |

## n. Analysis of variance of other factors

| Source of variation | d.f. | s.s.    | m.s.   | v.r.  | F pr. |
|---------------------|------|---------|--------|-------|-------|
| Treatment           | 6    | 1707.96 | 284.66 | 27.00 | <.001 |
| Residual            | 12   | 126.53  | 10.54  |       |       |
| Total               | 20   | 1874.78 |        |       |       |
| l.s.d.              | 5.78 |         |        |       |       |

## o. Analysis of variance rejected due to other factors

| Source of variation | d.f.  | s.s.    | m.s.   | v.r. | F pr. |
|---------------------|-------|---------|--------|------|-------|
| Treatment           | 6     | 746.53  | 124.42 | 2.48 | 0.085 |
| Residual            | 12    | 602.20  | 50.18  |      |       |
| Total               | 20    | 1355.94 |        |      |       |
| l.s.d.              | 12.60 |         |        |      |       |

p. Analysis of variance of disease Incidence transformed data

| Source of variation | d.f.  | s.s.   | m.s.  | v.r. | F pr. |
|---------------------|-------|--------|-------|------|-------|
| Treatment           | 6     | 655.3  | 109.2 | 0.68 | 0.669 |
| Residual            | 12    | 1927.4 | 160.6 |      |       |
| Total               | 20    | 2705.2 |       |      |       |
| l.s.d.              | 22.55 |        |       |      |       |

q. Analysis of variance of disease incidence of raw data

| Source of variation | d.f.  | s.s.    | m.s.   | v.r.  | F pr. |
|---------------------|-------|---------|--------|-------|-------|
| Treatment           | 6     | 2615.24 | 435.87 | 40.26 | <.001 |
| Residual            | 12    | 129.90  | 10.83  |       |       |
| Total               | 20    | 2783.24 |        |       |       |
| l.s.d.              | 5.853 |         |        |       |       |

r. Analysis of variance of disease severity of raw data

| Source of variation | d.f.   | s.s.    | m.s.   | v.r.  | F pr. |
|---------------------|--------|---------|--------|-------|-------|
| Treatment           | 6      | 10.0781 | 1.6797 | 12.72 | <.001 |
| Residual            | 12     | 1.5848  | 0.1321 |       |       |
| Total               | 20     | 11.6714 |        |       |       |
| l.s.d.              | 0.6465 |         |        |       |       |

s. Analysis of variance of diseased severity of transformed data

| Source of variation | d.f.  | s.s.     | m.s.    | v.r.   | F pr. |
|---------------------|-------|----------|---------|--------|-------|
| TREATMENT           | 6     | 51.28476 | 8.54746 | 240.13 | <.001 |
| Residual            | 14    | 0.49833  | 0.03560 |        |       |
| Total               | 20    | 51.78310 |         |        |       |
| l.s.d.              | 0.955 |          |         |        |       |

Appendix 5. The sequence of isolates from Ghana

Xan-GH 1

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTATCTCTCCCCGTAC  
TTCATCAACAACCAGCAGAGCCAATCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCTGCTGGAAGGCGTGGC  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGTGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGT

Xan-GH 2

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTATCTCTCCCCGTAC  
TTCATCAACAACCAGCAGAGCCAATCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCTGCTGGAAGGCGTGGC  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGTGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGT

Xan-GH 3

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTATCTCTCCCCGTAC  
TTCATCAACAACCAGCAGAGCCAATCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCTGCTGGAAGGCGTGGC  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGTGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGT

Xan-GH 4

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG

TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTATCTCTCCCCGTAC  
TTCATCAACAACCAGCAGAGCCAATCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCTGCTGGAAGGCGTGCG  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGTGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCTGCGCCGT

Xan-GH 5

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTATCTCTCCCCGTAC  
TTCATCAACAACCAGCAGAGCCAATCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCTGCTGGAAGGCGTGCG  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGTGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCTGCGCCGT

#### Appendix 6. Sequences obtained from GenBank

> ICMP5740 *X. c. pv. mangiferaeindicae*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTATCTCTCCCCGTAC  
TTCATCAACAACCAGCAGAGCCAATCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCTGCTGGAAGGCGTGCG  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGTGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCTGCGCCGT

> ATCC11637 *X. c. pv. mangiferaeindicae*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAAGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA

AACGAGCTGGACGTGGTCGAGGGGCATGCAGTTCGACCGCGGCTATCTCTCCCCGTAC  
TTCATCAACAACCAGCAGAGCCAATCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGGC  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGTGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> NCPPB1828 *X. c. pv. euphorbiae*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGTGGTATCGACCAGGCCGTCAAGGCTGCGGTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATTGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAGGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGATCTGCTGCCCCGTGCTGGAAGGCGTGG  
CCAAAGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> NCPPB528 *X. campestris pv. campestris*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGATCTGAAGCGCGGTATCGACCAGGCCGTCAAGGCCGCCGTCA  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATTGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAATCGATCGGCAACATCATTGCCGAAGCGAT  
GCAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGGCATGCAGTTCGATCGCGGCTACCTCTCCCCGTAC  
TTCATCAACAACCAGCAGAGCCAGTCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGGC  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCTGAAGAAGTCGAAGGCGAAGCCCTGG  
CGACGCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> ATCC11648 *X. axonopodis pv. vignicola*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAAGCCGTCAAGGCTGCGGTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GCAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGG  
CCAAGGCCGGCAAGCCGCTGCTGATCGTGGCTGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> NCPPB936 *X. axonopodis* pv. *vesicatoria*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGTGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATTGCCCAGGTC  
GGCACCATTTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGG  
CCAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> NCPPB941 *X. axonopodis* pv. *vesicatoria*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGTGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATTGCCCAGGTC  
GGCACCATTTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGG  
CCAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> NCPPB1438 *X. axonopodis* pv. *vesicatoria*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGTGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATTGCCCAGGTC  
GGCACCATTTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGG  
CCAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> NCPPB2572 *X. axonopodis* pv. *vesicatoria*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGTGGTATCGACCAGGCCGTCAAGGCTGCGGTCG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATTGCCCAAGCGAT  
GGCACCATTTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GAAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTG  
CCAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> YFS153 *X.axonopodis* pv. *phaseoli*

GCCACTGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
TATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GCAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTG  
CCAAGGCCGGCAAGCCGCTGCTGATCGTGGCCGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATTTCGCGGCATCGTCAAGGTCGTGGCCGTC

> YFS154 *X.axonopodis* pv. *phaseoli*

GCCACTGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
TATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GCAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA  
AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTG  
CCAAGGCCGGCAAGCCGCTGCTGATCGTGGCCGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATTTCGCGGCATCGTCAAGGTCGTGGCCGTC

YFS155 *X.axonopodis* pv. *phaseoli*

GCCACTGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
TATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GCAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCCTGGAA

AACGAGCTGGACGTGGTCGAGGGCATGCAGTTCGACCGCGGCTACCTCTCCCCGTA  
CTTCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCT  
GCACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGG  
CCAAGGCCGGCAAGCCGCTGCTGATCGTGGCCGAAGAAGTCGAAGGCGAAGCGCTG  
GCGACCCTGGTGGTCAACACCATTTCGCGGCATCGTCAAGGTCGTGGCCGTC

> ATCC23380 *X. axonopodis* pv. *manihotis*

GCCACGGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GCAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCTTGGA  
ACGAGCTGGACGTGGTCGAGGGTATGCAGTTCGACCGCGGCTACCTCTCCCCGTACT  
TCATCAACAACCAGCAGAGCCAGTCGGCCGACCTGGACGACCCGTTTCATCCTGCTGC  
ACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGGCC  
AAGGCCGGCAAGCCGCTGCTGATCGTGGCCGAAGAAGTCGAAGGCGAAGCGCTGGC  
GACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> ICMP5741 *X. axonopodis* pv. *manihotis*

GCCACGGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCCGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
GCAGAAGGTTCGGCAAGGAAGGCGTGATCACCGTTGAAGAAGGCTCGGGCTTGGA  
ACGAGCTGGACGTGGTCGAGGGTATGCAGTTCGACCGCGGCTACCTCTCCCCGTACT  
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ACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGGCC  
AAGGCCGGCAAGCCGCTGCTGATCGTGGCCGAAGAAGTCGAAGGCGAAGCGCTGGC  
GACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> ATCC43911 *X. axonopodis* pv. *glycines*

GCCACCGTGCTGGCCCAGGCCCTGATTCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
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GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
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CACGACAAGAAGATCTCCAACGTGCGTGACCTGCTGCCCCGTGCTGGAAGGCGTGCG  
CAAGGCCGGCAAGCCGCTGCTGATCGTCGCCGAAGAAGTCGAAGGCCGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTCAAGGTCGTGGCCGTC

> YG290 *X. axonopodis* pv. *citri*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
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CACGACAAGAAGATCTCCAACGTGCGCGACCTGCTGCCCCGTGCTGGAAGGCGTGCG  
CAAGGCCGGCAAGCCGTTGCTGATCGTCGCCGAGGAAGTCGAAGGCCGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTGAAGGTCGTGGCCGTC

> YG292 *X. axonopodis* pv. *citri*

GCCACCGTGCTGGCCCAGGCCCTGATCCGCGAAGGCGCCAAGGCTGTGGCCGCCGG  
CATGAACCCGATGGACCTCAAGCGCGGTATCGACCAGGCCGTCAAGGCTGCGGTCTG  
TCGAGCTGAAGAACATCTCCAAGCCCACCACCGACGACAAGGCGATCGCCCAGGTC  
GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
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> YG266 *X. axonopodis* pv. *citri*

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GGCACCATCTCGGCCAACTCGGACGAGTCGATCGGCAACATCATTGCCGAAGCGAT  
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TTCATCAACAACCAGCAGAGCCAGTCGGCCGATCTGGACGACCCGTTTCATCCTGCTG  
CACGACAAGAAGATCTCCAACGTGCGCGACCTGCTGCCCCTGCTGGAAGGCGTGGC  
CAAGGCCGGCAAGCCGTTGCTGATCGTCGCCGAGGAAGTCGAAGGCCGAAGCGCTGG  
CGACCCTGGTGGTCAACACCATCCGCGGCATCGTGAAGGTCGTGGCCGTC

> DSM3583 *X. albilineans*

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> ICMP196 *X. albilineans*

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> ATCC19045 *X. arboricola* pv. *celebensis*

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> DSM50853 *X. arboricola* pv. *celebensis*

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> LMG576 *X. axonopodis* pv. *allii*

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> LMG578 *X. axonopodis* pv. *allii*

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>DSM3585 *X. axonopodis* pv. *axonopodis*

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> LMG539 *X. axonopodis* pv. *axonopodis*

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> DSM50850 *X. axonopodis* pv. *begoniae*

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> LMG550 *X. axonopodis* pv. *begoniae*

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> LMG553 *X. axonopodis* pv. *begoniae*

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> ATCC11677 *X. axonopodis* pv. *betlicola*

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> ICMP312 *X. axonopodis* pv. *betlicola*

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> LMG555 *X. axonopodis* pv. *betlicola*

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> ATCC12131 *X. citri* subsp. *malvacearum*

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> DSM1220 *X. citri* subsp. *malvacearum*

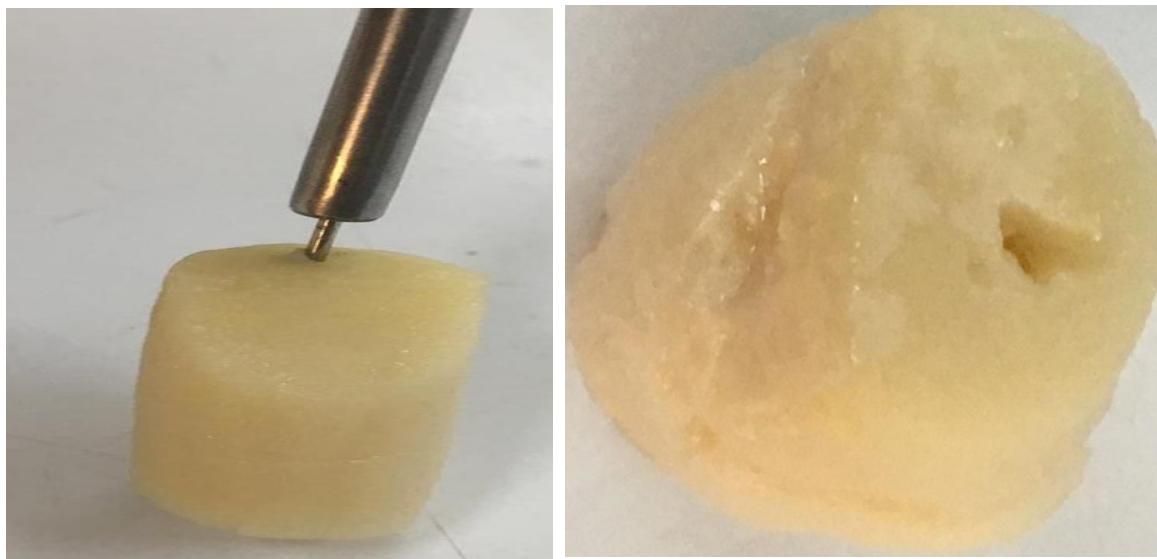
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> DSM3849 *X. citri* subsp. *malvacearum*

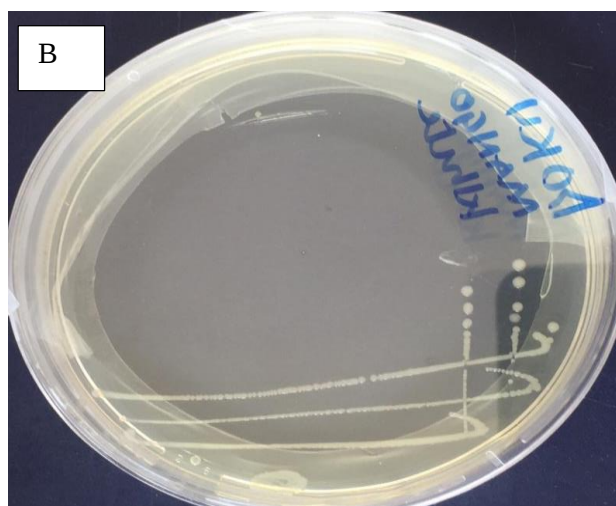
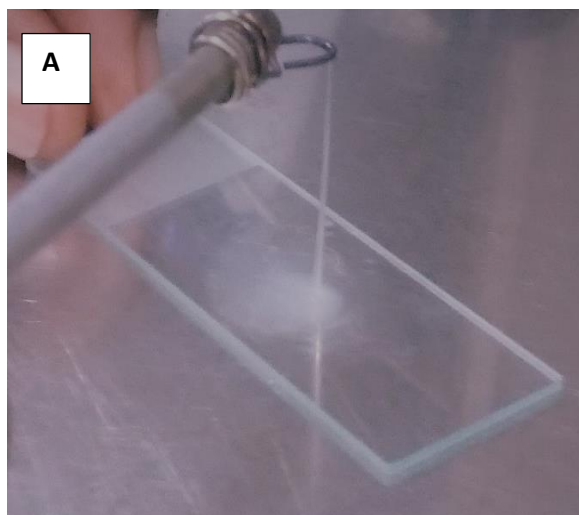
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> *P. auranginosa*

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A=control, B= pectolytic activities,



Cultural characteristics and KOH test of the isolates of the bacterium obtained from diseased mango fruits. A=Viscous mucoid thread of bacterial cells, B=Growth on nutrient agar,