EFFECT OF CASSAVA PRODUCTION PRACTICES ON INTENSITY OF BACTERIAL BLIGHT IN BUSIA COUNTY AND RESISTANCE REACTION OF CULTIVARS GROWN IN KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

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This thesis is my original work and has not been submitted for a degree in another university

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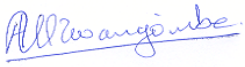

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DEDICATION

I dedicate my thesis to my parents Mr. Martin Nduri Okoth and Mrs. Rose Akinyi Okoth, and my siblings Lynn Barbra, Juliet Adhiambo, and Gabriella Sharon who have been my greatest source of strength and inspiration through God's providence in my academic and life journey.

ACKNOWLEDGMENTS

Firstly, I thank God for his grace and providence that have sustained me all through my academic endeavor. I would like to thank my parents who through their efforts have ensured that I have realized most of the goals I had set throughout the study period. Special thanks to Prof. Agnes W. Mwang'ombe who gave me an opportunity to be part of her project and supported me through the entire period. I would also like to thank her for the professional guidance and utmost concern throughout the project period. I would also like to express my gratitude to Prof. James Muthomi who has also been of immense help in terms of professional guidance through the project period and for his close concern for the progress of the project work. Special thanks to MasterCard Foundation through the Regional University Forum for Capacity Building in Agriculture(RUFORUM) who funded the project and gave me a chance to improve my skills through various trainings under the program. I would also like to appreciate the Department of Plant Science and Crop Protection and Seed Enterprise Management Institute(SEMIs) under Prof. Agnes W. Mwang'ombe for their support in terms of infrastructure within which I conducted my project activities. I would also like to thank Dr. Morag Fergusson and Kenya Plant Health Inspectorate Service (KEPHIS) who aided me in accessing disease free cassava cutting for my greenhouse experiments. I would also like to thank the County Government of Busia and all the cassava farmers for allowing me to conduct a survey and collect samples from Busia. I would also like to thank Mr. Abraham Choti, Mr. Titus Mwangangi, Mrs. Beverlyn Mmera, Mrs. Cecily and Mrs. Nancy Mvungu for the support especially in laboratory access. I would also like to thank all the SEMIs staff for their support during the project period. I also express my gratitude to the Seventh day Adventist Church at Kenyatta National Hospital for their prayers and words of encouragement during the project period. Lastly, I would like to thanks my colleagues Ms. Stacy Odunga, Mr. Patrick Kidasi, Ms. Rachel Wachira, Mr. Simon Vudiya, Mr. Kevin Shitiavai, and Mr. Anthony Livoi for their support and encouragement throughout the project period.

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LIST OF ABBREVIATIONS AND ACRONYMS

AUDPC	Area Under Disease Progress Curve
CBB	Cassava bacterial blight
CBSD	Cassava Brown Streak Disease
CFU/ml	Colony forming units per milliliter
CMD	Cassava Mosaic Disease
FAOSTAT	Food and Agricultural Organization Statistics Database
g	Grams
GPS	Global Positioning System
IITA	International Institute of Tropical Agriculture
KALRO	Kenya Agricultural and Livestock Research Organization
LM1	Lower Midland Zone 1
LM2	Lower Midland Zone 2
mm	Millimeter
ml	Milliliter
NGO	Non-governmental organization
SSA	Sub-Saharan Africa
t	Tons
t/ha	Tons/Hectare
USD	United States Dollars
XAC	*Xanthomonas axonopodis* pv *cassavae*.
XPM	*Xanthomonas phaseoli* pv *manihotis*.
YPGA	Yeast Peptone Glucose Agar

GENERAL ABSTRACT

Cassava (*Manihot esculenta*) is an essential crop to many farming households in Kenya. However, diseases continue to disrupt its huge potential as a food security and economic crop. Among the most devastating diseases is cassava bacterial blight caused by *Xanthomonas phaseoli* pv *manihotis* (syn. *Xanthomonas axonopodis* pv *manihotis*) and *Xanthomonas axonopodis* pv *cassavae*. Therefore, this study was conducted to determine the distribution of cassava bacterial blight in Western Kenya a key cassava belt in Kenya. Multistage sampling was used to select 193 farms from Nambale and Teso south sub counties in Busia county Western Kenya. Information on cassava production practices was obtained through questionnaires. From each of these farms 30 plants were assessed for cassava bacterial blight and symptomatic leaves collected for isolation and conformation of the CBB pathogens. GPS coordinates were also collected from the farms which were used to make distribution maps. Analysis of the questionnaire data was done through descriptive statistics and chi-square tests. The causal agents were isolated aseptically from the diseased leaves using yeast peptone glucose agar media in a laminar hood. The plates were incubated for 24hrs after which biochemical tests and pathogenicity test were done to confirm the causal agents. This aided the generation of distribution maps of the two causal agents across Western Kenya using the previously collected coordinates. A greenhouse experiment was set up to evaluate seven varieties for cassava bacterial blight resistance in a factorial treatment structure within a randomized complete block design. All varieties were inoculated with 10^6CFU/ml of the cassava bacterial blight causal agents on the leaves and stem. Observations were recorded at an interval of six days' post inoculation using a severity scale of 1-5. After 24hrs the cultured plates had white and yellow colonies which are traits of bacterial blight pathogens. Bacteria from both colonies could utilize various sugars, but none could use either lactose or cellobiose. The pathogenicity tests indicated that the white bacterial isolate is *Xanthomonas phaseoli pv manihotis* as it caused both foliar and systemic disease while the yellow one is *Xanthomonas axonopodis pv cassavae* as it only caused foliar diseases. Among the samples collected over 90% of the farms had *Xanthomonas phaseoli pv manihotis* from both sub counties visited while less than 10% had *Xanthomonas axonopodis* pv *cassavae* and a combination of both causal agents. Majority of the farms (89%) had a moderate severity score of 3. There was no association between cassava bacterial blight incidence and training, seed source, and intercropping suggesting that other factors like ignorance could be contributing to the high incidence of the disease. Since 85% of the farmers

interviewed were unaware of the disease thus spreading the disease unknowingly. From the greenhouse experiments *Xanthomonas phaseoli pv manihotis* inoculated varieties had the highest incidences compared to those inoculated with *Xanthomonas axonopodis* pv *cassavae* or both bacteria combined. The varieties also registered high severity score with the highest scores being of *Xanthomonas phaseoli pv manihotis* inoculated varieties. A similar case was registered for the area under disease progress curve values as all of the varieties inoculated with *Xanthomonas phaseoli pv manihotis* had values over 50%. The most affected varieties included mm 96/2480, Naro 56, and mm 96/1871. *Xanthomonas phaseoli pv manihotis* was more severe compared to *Xanthomonas axonopodis* pv *cassavae*. The study concluded that the high prevalence of cassava bacterial blight in western Kenya might be due to ignorance of the disease among farmers, lack of application of existing cassava bacterial blight mitigation measure, and reliance of informal seed systems which are characterized by recycled cuttings. Furthermore, *Xanthomonas phaseoli pv manihotis* was found to be the more severe of the two causal agents and the most prevalent indicating the existence of factors that may be affecting the survival of *Xanthomonas axonopodis* pv *cassavae*. From the greenhouse experiments none of the varieties was resistant to cassava bacterial blight indicating that more varieties currently ought to be assessed for cassava bacterial blight resistance. The following recommendations were made based on the study findings promotion of existing cassava bacterial blight management practices to farmers through elaborate extension services, encourage use of certified cassava cuttings among farmers when establishing their plantations, improve the capacity of community based organizations, nongovernmental organizations and Agricultural institutes through training and infrastructural development to produce enough disease free cuttings for farmers, and study the underlying differences in virulence and pathogenicity by the two cassava bacterial blight causal agents *Xanthomonas phaseoli* pv *manihotis* and *Xanthomona saxonopodis* pv *cassavae*, evaluate more cassava germplasm for resistance against cassava bacterial blight in the field and green house for better deployment of the germplasms.

CHAPTER ONE

INTRODUCTION

1.1 Background

Cassava is an extremely valuable crop socioeconomically to more than 800 million people in the tropics who depend on it for food and income. It is highly diverse and adapted to the production areas of the world where it has been farmed by communities for generations(Adu *et al*., 2021). It is chiefly cultivated for its starch endowed tubers however other communities also consume its protein-laden leaves (Kaluba *et al*., 2021). Sub-Saharan Africa (SSA) is among the foremost cassava producers globally accounting for 177 million metric tons of the total world production (Torkpo *et al*., 2021). Nonetheless, in most cases yields obtained are usually eight tons per hectare which is low in contrast to 90 t/ha which can be realized if good agricultural practices are followed (Adjebeng-Danquah *et al*., 2020). Kenya is among the SSA countries where cassava plays a foundational role in the economic wellbeing of many rural dwellers (Ouma *et al*., 2021). The main cassava production areas include Western, coast, Eastern and Central regions among which western Kenya accounts for 60% of the yearly production (Githunguri and Njiru, 2021). Currently, cassava production in Kenya stands at 970587 tones but it has the potential of attaining higher yields. In Kenya like many other Sub-Saharan countries, cassava production is plagued by a multitude of challenges that prevent the realization of its true potential (Chege *et al*., 2017).

These challenges include pests like green spider mite, whiteflies and cassava mealybug which interfere with cassava growth impacting the revenue of farmers. Moreover, whiteflies act as vectors for viral disease like cassava mosaic disease and cassava brown streak disease which limit cassava production by reducing quality and quantity of yield. However, of the bacterial pathogens that affect cassava, cassava bacterial blight is more severe affecting all parts of the plant and in some instances killing vulnerable varieties causing loss of germplasm (Odongo *et al*., 2019). Two bacterial agents, *Xanthomonas phaseoli pv manihotis* (syn. *Xanthomonas axonopodis* pv *manihotis*) and *Xanthomonas axonopodis pv cassavae*, are normally isolated from plants displaying cassava bacterial blight symptoms(Zárate-Chaves *et al*., 2021). The disease is capable of instigating a loss of 100% depending on the varieties grown, climate, and soil fertility (Toure *et al*., 2020). In Kenya the disease has been documented in all major cassava

growing locales (Odongo *et al.*, 2019), furthermore, recent studies in the coastal regions of Kilifi and Taita taveta have indicated that the disease is present at high incidences of up to 100% on individual farms (Livoi *et al*., 2021). Moreover, presently there is no variety available to farmers that has sufficient levels of resistance against the disease (Mbaringong *et al*., 2017). This is further exacerbated by the fact that most farmers have been reported not to be applying the existing control measures thus contributing to its extensive spread (Njenga *et al*., 2017). Therefore, understanding the extent to which the disease is distributed in major growing areas like Busia will aid in implementing appropriate management strategies. Furthermore, since it has been demonstrated that varieties resistant to the disease are exist in Kenya. Therefore, evaluating more of these diverse cassava varieties within the country increases the prospects of identifying varieties that can be recommended to farmers.

1.2 Problem statement

Cassava bacterial blight (CBB) incidences have been increasing globally and in Kenya new isolations have been made in Coastal Kenya with farms having incidences of over 50% (Chege *et al*.,2017; Mbaringong *et al*., 2017; Odongo *et al*., 2019; Livoi *et al*., 2021). Although cassava bacterial blight has been demonstrated to be prevalent in the country its distribution and that of its causal agents is yet to be determined in Busia County. Furthermore, although many cassava varieties exist in Kenya only a few have been assessed for cassava bacterial blight resistance and these have been found to possess insufficient or no resistance against the disease including some that were intended for breeding (Odongo *et al*., 2019; Mbaringong *et al*., 2017). This leaves farmers in cassava growing regions vulnerable to the disease in case an epidemic occurs. (Mbaringong *et al*., 2017). Furthermore, all evaluated varieties have only been tested for reaction against *Xanthomonas phaseoli* pv *manihoti* with no information on their reaction to *Xanthomonas axonopodis* pv *cassavae* (Kwena *et al*., 1992: Ogunjobi *et al*.,2010). This is despite the fact that cassava varieties have been found to respond differently to both pathogens and each can be disseminated to new areas through asymptomatic stem cuttings (Pereira *et al*., 2000; Ogunjobi *et al*., 2010)

1.3 Justification

Understanding the extent to which cassava bacterial blight is distributed in Busia County Western Kenya will promote stronger surveillance so as to limit its dissemination to disease free

areas. This will safeguard farmers' livelihoods preventing losses that might result from cassava bacterial blight. Assessing some of the locally grown and adapted cultivars grown in Kenya increases the likelihood of identifying varieties that have robust defense against cassava bacterial blight. This is because communities have their preferred cultivars which they have cultivated for years and are adapted to their local conditions. Moreover, these varieties once identified will act as potential sources of resistance in breeding programs (Mbaringong *et al.*, 2017). Although evaluation of some Kenyan varieties has indicated availability of potential resistant sources, reaction of these varieties to both *Xanthomonas axonopodis* pv *cassavae* and *Xanthomonas phaseoli* pv *manihotis* needs to be understood for better varietal selection (Mbaringong *et al.*, 2017). Moreover, majority of the cultivars initially targeted for breeding are extremely vulnerable to cassava bacterial blight creating the need of evaluating more varieties (Odongo *et al*., 2019). Use of resistant cultivars is environmentally friendly, promotes economies of scale, and has been shown to reduce CBB to a minor disease (Pereira *et al*., 2000; Mbayi *et al*., 2014: Sedano *et al*., 2017).

1.4 Objectives

The overall objective of this study is to manage cassava bacterial blight to increase cassava yield through identification of resistant cultivars grown in Kenya.

The specific objectives will be:

i. To determine effect of cassava production practices on intensity of bacterial blight in Busia County.
ii. To determine resistance against cassava bacterial blight among cassava cultivars grown in Kenya.

1.5 Hypothesis

i. Cassava bacterial blight might be widely distributed in Busia County due to lack of knowledge and little or none application of management practices by farmers.
ii. Reaction to cassava bacterial blight varies across different cassava varieties because varieties have different levels of resistance against the disease.

CHAPTER TWO
LITERATURE REVIEW

2.1 Biology of cassava

Cassava (*Manihot esculenta* Crantz) is a perennial crop that is farmed extensively in the tropics. In addition, it is the only domesticated plant within the *Manihot* genus(Wolfe *et al*., 2017).It is immensely diverse showing adaptability to the surrounding conditions within which it is cropped (El-Sharkawy, 2003). The most common method of establishment is via stem cuttings, however, seeds can also be used but these are normally limited to breeding programs (Sonnewald *et al*., 2020). After establishment, it usually takes 8-12 months to harvest but this may extend to 24 months in cooler environments (Cock and Connor, 2021). It is unique in that it can thrive in harsh environments which may prove detrimental to other crops. For example, it can proliferate in nutrient-deprived soils and areas with meager rainfall (Imakumbili *et al*., 2021). This makes it an outstanding food security crop in comparison to many other staples in the wake of changing environmental cycles. Additionally, it is a good food reservoir as its tubers can persist beneath the ground before harvest providing food supplies for longer a periods (Githunguri and Njiru, 2021).

2.2 Socioeconomic importance of cassava

Cassava is a crop of importance to more than 800 million individuals within the tropics of the world (Mugerwa *et al*., 2021). Globally cassava production is projected at 303,568,814t and this earns approximately USD 34,844,500 annually (FAOSTAT, 2021). This is usually through sale of the tubers which are prepared and processed into various products. For example, they can be boiled, fried into chips or crisps, dried and ground to flour, or used in various industrial processes to produce starch as well as plastic bags (Mbanjo *et al*., 2021).The annual cassava production in Sub-Saharan Africa is 177 million metric tons (Torkpo *et al*., 2021). In Kenya as in many Sub-Saharan African countries, cassava production is an important venture to numerous households especially in Western and Coastal regions which account for 90% of the production (Opondo *et al*., 2020). The average yield in Kenya is 7.5 to 10t/ha this results in an annual production of 970587tonnes (FAOSTAT, 2021). Moreover, through the establishment of elaborate value chain systems, cassava production continues to be one of the potentials avenues

of creating employment for the ballooning youthful population hence positively influencing both national and local economic growth.

2.3 Challenges facing cassava production

In as much as cassava has many prospects, its production continues to be negatively affected across its growing belts. The persistent challenges in cassava production include pest and diseases, post-harvest losses, lack of market access, lack of clean seed, and continued cultivation of unimproved varieties (Chavez *et al*., 2021). In Sub Saharan Africa(SSA) these factors have hugely caused production to remain at eight tons per hectare which is low in contrast 90 t/ha which can be realized if good agricultural practices are followed (Adjebeng-Danquah *et al*., 2020). Furthermore, pests and diseases continue to hinder cassava production resulting in annual losses of more than USD 50 million in some SSA countries (Hamza *et al.*, 2020). Among the most rampant pests and diseases are viral diseases: Cassava mosaic diseases and Cassava brown streak disease transmitted via whiteflies and contaminated cuttings. These cause catastrophic losses affecting farmers' income significantly (Chiza *et al*., 2020). However, cassava bacterial blight, which has cemented its place as the most important bacterial disease of cassava has recently been listed among the top 10 most important bacterial diseases and continues to limit cassava production (Bart and Taylor, 2017). This disease can lead to losses of up to 100% when no control measures are applied. This is further aggravated by the fact that most farmers lack access to clean seed yet most of these debilitative diseases are transmitted through seed (Fanou *et al*., 2017). In Kenya pest and disease also continue to pose a serious threat to cassava production. The two major cassava viruses Cassava mosaic diseases and Cassava brown streak are among the major contributors. However, cassava bacterial blight continues to be an ever present threat to cassava production in the country occurring with incidences of up to 100% in major areas of production (Livoi *et al*., 2021). Furthermore, none of the popular varieties currently grown by farmers have been found to have sufficient levels of resistance to the disease (Odongo *et al*., 2019). This has been associated with other production challenges such as lack of clean seed and extensive reliance on neighbors for seed and continued growth of unimproved varieties which promotes the silent proliferation of the disease (Mbaringong *et al*., 2017). Other production challenges in Kenya are post-harvest losses and market access (Githunguri and Njiru, 2021).

2.4 Cassava bacterial blight

Cassava bacterial blight is a devastating disease that affecting cassava. It has been the cause of an historical famine in Zaire where it interfered with the availability of cassava products leading to massive starvation affecting thousands of people (López and Bernal, 2012). Two rod-shaped gram-negative bacterial agents *Xanthomonas phaseoli pv manihotis* (syn. *Xanthomonas axonopodis* pv *manihotis*) (Constantin *et al*., 2016), and *Xanthomonas axonopodis pv cassavae* (Zárate-Chaves *et al*., 2021) are associated with cassava plants presenting symptoms of the disease. *Xanthomonas phaseoli* pv *manihotis* is capable of spreading throughout the plant while *Xanthomonas axonopodis pv cassavae* is usually confined to foliar infections only. One of its causal agent *Xanthomonas phaseoli* pv *manihotis* has been listed among the top ten most important bacterial pathogens globally (Mansfield *et al*., 2012). The disease severity is dictated by soil nutritional profile, prevailing environmental conditions, and types of varieties grown (Toure *et al*., 2020). When all factors for conducive diseases development like favorable climate, nutrients deprived soils, and vulnerable varieties are present yield losses can be as high as 100% (Fanou *et al*., 2017). Moreover, yield loss is not the only effect as the disease is capable of affecting all parts of the cassava plant through contamination of planting materials and destruction of leaves which form an important source of vegetables for some cassava farming communities. In some instances, highly susceptible varieties have been wiped out of existence leading to loss of diversity (Lozano, 1986).

2.5 Distribution of cassava bacterial blight

Globally cassava bacterial blight has presence in almost all major cassava growing regions and continues to be a persistent problem (Veley *et al.*, 2020). Furthermore, it has been reported that the disease incidence continues to gradually increase across the world (López and Bernal, 2012). The greatest avenue that has contributed to its increase is the exchange of contaminated cutting. The disease continues to expand more so in Sub Saharan Africa where new isolations have recently been made in Mali (Kante *et al*., 2020) and Burkina Faso (Wonni *et al*., 2015). In Kenya where the disease was thought to be confined in the Western region (Chege *et al*., 2017), it has been determined that it has pervaded most of the prime cassava growing areas with incidences of up to 100% in areas like Kilifi in the coastal region (Livoi *et al*., 2021). The dissemination has been faulted on the increased cross-country exchange of planting material as well cross border exchange especially for counties like Busia which border Uganda an active

cassava grower. Furthermore, a weak phytosanitary system has been suggested as a key contributor to the rampant spread through uncertified germplasm (Odongo *et al*., 2019).

2.6 Etiology of cassava bacterial blight pathogen

Cassava bacterial blight is caused by *Xanthomonas phaseoli ipv manihotis* (Arrieta *et al*., 2013) formerly known as *Xanthomonas axonopodis* pv *manihotis* (Kante *et al*., 2020). It is gram negative and never produces spores or capsules. Flourishes on media composed of sucrose yielding pigment white colonies, typically possessing single flagellum (Odongo *et al*., 2019). Additionally, most of its biochemical characteristics are similar to those of *Xanthomonads* which include being catalase positive, capability of hydrolyzing milk and aesculin aerobically, they also degrade sodium polypectate, however, they are indole, methyl red and the Voges-Proskauer test negative. However, they differ with other *Xanthomonads* in general in that they lack colony pigmentation (Ongujobi *et al*., 2008).

Morphologically the pathogen forms shiny-mucilaginous, whitish, smooth colonies. Nonetheless, the appearance may differ based on the media used. When it is plated on sucrose peptone agar it gives a mucoid appearance while this is not the case in nutrient agar (Ogunjobi *et al*., 2007). *Xanthomonas axonopodis* pv *cassavae* previously *Xanthomonas campestris* pv *cassavae* is also normally encountered on cassava bacterial blight infected leaves (Ogunjobi *et al*., 2007). It also grows on sucrose based culture media generating yellow colonies which distinguishes it from XPM. Most of its biochemical characteristics are similar to those of *Xanthomonas axonopodis* pv *manihotis*. However, differences in utilization of various sugars has been noted as *Xanthomonas axonopodis* pv *cassavae* has been reported to be capable of producing hydrogen sulphide in sucrose peptone broth (Kwena, 1992). They are also incapable of breaking down starch but capable of acid production on different carbohydrate based media like maltose, galactose and xylose. Nevertheless, variance in acid evolution has been noted on sucrose, lactose, rhamnose and raffinose (Ogunjobi *et al*., 2007).

2.7 Infection process of cassava bacterial blight

The casual agents of cassava bacterial blight *Xanthomonas phaseoli pv manihotis* and *Xanthomonas axonopodis pv cassavae* mainly reside on the exogenous surface of the cassava plant (Verdier *et al*., 2004). Normally the best temperature for infection is 28 ℃ and wet climate. The chief access route is usually the stomata, however, other routes such as wounds

offer entry points for the disease causal agents (Yoodee *et al*., 2018). The pathogen is capable of producing enormous amounts of toxic molecules which induce angular leaf spots which merge to produce blight (Tappiban *et al*., 2018). However, of the two causal agent it has been reported that only *Xanthomonas phaseoli pv manihotis* is capable of causing disease throughout the plant while *Xanthomonas axonopodis pv cassavae* is usually limited only to the foliar regions of the leaf (Azorji *et al*., 2016). To establish systemic infection, *Xanthomonas phaseoli pv manihotis* access the stem via the leaf vascular system through the petiole. The affected petiole will often give the candle stick symptom as disease progresses. Once the pathogen has accessed the stems blockage of the vascular bundles occur as a result of enormous production of exopolysaccharides as well as tyloses produced during resistance reactions (Büttner and Bonas, 2010). This is usually observed as wilting of leaves. Infection is usually very severe on highly susceptible plant compared to resistant plants. In extreme cases severe infections leads to total destruction of susceptible plants however it has been reported that some infected plants are able to recover and grow to maturity (Mbaringong *et al*., 2017). Furthermore, secondary infection also occurs within the field via splash of bacterial ooze from diseased plant to healthy plants within the same field. This in most cases increases the number of infected plants resulting in serious losses at the end of the season. Nonetheless, primary infection usually occurs when infected cuttings are planted at the start of the season. This will mostly result in wilting of leaves as the first symptom or death of the young developing plants (López and Bernal, 2012).

2.8 Cassava bacterial blight symptoms

Leaves become water soaked with greenish to bluish coloration. These develop into angular leaf spots bounded within the veins which are usually non uniformly distributed on the leaf surface. Seepage of bacterial cells may occur beneath the lesion these appear creamy at first but later become yellow. Blighting results when the spots merge (Azorji *et al*., 2016). Seepage of bacterial cells also occurs on petioles as bacteria move to the stem. These leads to a weakened petiole but it is not dislodged from the plant. Bacterial migration discolors conducting vessels turning them brownish. Wilting affects leaves due to blockage of water conducting vessels. Progression of wilting results in dieback at the tips forming the candle stick symptom (Lopez and Bernal, 2012; Thanasomboon *et al*., 2019). Seldom do roots succumb unless cultivar is highly vulnerable (Lozano *et al*., 1986). These symptoms are normally common with *Xanthomonas phaseoli pv manihotis* which is much more severe (Mooter*et al*., 1986).

Xanthomonas axonopodis pv *cassavae* has been reported to induce angular leafspots and defoliation. However, it is incapable of inducing blight and systemic infection (Mooter*et al*., 1986).

2.9 Transmission and survival of cassava bacterial blight causal agents

The main mode of transmission of both causal agents is chiefly through infected stem cuttings. This is because they are capable of surviving latently within the plant tissue. In as much as they can survive in the true seed these are mostly used in breeding programs hence are a less common route of transmission (Sedano *et al.*, 2017). They are capable of surviving in debris and may get splashed onto healthy plants. This transmission occurs mostly within the growing season as the pathogens has a short life cycle in the soil. Transmission in field occurs when bacterial ooze is splashed onto neighboring susceptible plant via raindrops. Insects have also been proposed as a possible means of transmission within the field (Melo *et al*., 2019; Boher and Verdier, 1994).

2.10 Cassava bacterial blight management practices

As of now cultural practices remain to be the primary on-farm management practices. Some of these practices include the use of clean seed (Njenga *et al*., 2017). This is majorly done through positive selections when the farmer is harvesting at the end of the season. However, the downside to this approach is that the pathogen is capable of symptomless survival in cuttings which can be transferred during cutting preparation. Moreover, most farmers obtain cuttings from fellow farmers (Odongo *et al*., 2019). Secondly, crop rotation has been recommended as a method of reducing bacterial load levels although limitations in cropping space limits its application. The addition of fertilizers containing compounds like silica and phosphorus have been proposed as potential methods of cassava bacterial blight control but their availability to farmers remains a challenge as most of them are seriously resource constrained (Njenga *et al*., 2017). Other methods include pruning of leaves from diseased plants although this has been observed to work better when disease incidence is low as it is not effective on extremely vulnerable plants (Fanou and Wydra, 2014). In so far as these methods have been recommended to farmers they have not to be fully implemented as they are considered strenuous. Hence with no control measure being applied cassava bacterial blight continues to spread preventing the realization of cassava's full potential (Bart and Taylor, 2017).

2.11 Management of cassava bacterial blight through Resistance

Resistance has long been proposed as the most efficient mode of combating the disease in a composite management system (Teixeira *et al*., 2021; López *et al*., 2007). The benefits of resistance as a prospective method of cassava bacterial blight control has been seen in instances where the disease has been reduced to levels of minor importance. Resistance to cassava bacterial blight had been described as polygenic with variation across cassava varieties. It has been observed that response to cassava bacterial blight is rapid in highly resistant varieties while slow in extremely vulnerable varieties (Mbayi *et al*., 2014). Evaluation has been done across the cassava growing belt in Sub Saharan Africa where potential sources of resistance have been identified however these have been found to lack farmer preferred traits like high yielding and good cooking qualities which have derailed their deployment to farmers. In Kenya studies done by Mbaringong *et al.* (2017) and Odongo *et al*. (2019) have shown that potential sources of resistance are present within farmers' field. However, none of these varieties have shown sufficient levels of resistance creating the need for continued identification (Bart and Taylor, 2017). Furthermore, the likelihood of identifying a resistant variety increases with the fact that cassava shows high diversity across the various localities within which it is grown.

2.12 Resistance Mechanism to cassava bacterial blight

Resistance to cassava bacterial blight is due to a number of mechanisms employed by the cassava plant. Numerous factors play a central role in preventing the advance of the pathogen within the plant. These factors are mainly induced resistance mechanisms (Kpémoua *et al*.,1996). The mechanisms exhibited by the cassava plant include deposition of callose which blocks the pathogen from accessing the phloem and cortical parenchyma cells. In addition, other mechanisms involved are cell wall fortification, lignification and suberization which is connected to callose deposition, generation of flavonoids, and polysaccharides. Moreover, the deposition of pectin has also been observed, tyloses formation, and production of phenolic compounds (Kpémoua *et al*.,1996).

Lignification, suberization, deposition of pectin polymers and callose deposition have been suggested to prevent the formation of bacterial lysis sites within the phloem cells. Phenolic compounds have also been shown to have an antimicrobial effect on the bacterial cells (Kpémoua *et al*.,1996). The production of these factors are more prominent in resistant than in

susceptible varieties (Sedano *et al*., 2017). Resistance to cassava bacterial blight is thought to be multigene in nature (Restrepo *et al*., 2004).

2.13 Identification of cassava resistant to cassava bacterial blight causal agents

Limited progress has been made in line with identification varieties that exhibit resistance to both causal agents. However differential reactions have been observed when different varieties have been challenged with both causal agents. Pereira *et al* (2000) evaluated two cassava varieties MCOL 22 and CM 523-7 from Centro International de Agricultura Tropical against two isolates *Xanthomonas axonopodis* pv *manihotis* (Isolate 9646) and *Xanthomonas cassavae* (Isolate 9018) obtained from brazil. CM 523-7 and MCOL 22 were initially thought to be resistant to *Xanthomonas phaseoli pv manihotis* with no data on *Xanthomonas axonopodis* pv *cassavae*. However, both cultivars were found to be susceptible to *Xanthomonas phaseoli* pv *manihotis* (syn *Xanthomonas axonopodis* pv *manihotis*) each having a bacterial load of 9×10^6 and 7.8×10^7 CFU/ml respectively. On the other hand, MCOL 22 showed defense aganist *Xanthomonas axonopodis pv cassavae*. It never displayed any symptoms on the inoculated leaves only minute necrotic regions were spotted at the inoculation points after 1-2 weeks. Furthermore, MCOL22 had a bacterial load of 3.7×10^5 in contrast to CM 532-7 which had a bacterial load of 2.8×10^7 CFU/ml of *Xanthomonas axonopodis pv cassavae.*

Interestingly, the disease progress of *Xanthomonas phaseoli pv manihotis* and *Xanthomonas axonopodis pv cassavae* was different. The latter was much slower than the former. In *Xanthomonas phaseoli* pv *manihotis* susceptible reaction the leaves wilted, dried and detached from plant after 2 weeks while in *Xanthomonas axonopodis pv cassavae* large areas of necrosis had formed over the same period but leaves detached from plants after 4 weeks. For both casual agents the factors engaged in resistance are more elevated in resistant plants as compared to susceptible ones (Pereira *et al*., 1999). Nonetheless the evaluation did not account for the stem resistance as *Xanthomonas axonopodis* pv *cassavae* has been repeatedly observed to be incapable of systemic invasion but has been suggested to a have temporary inhibitory effect on *Xanthomonas phaseoli* pv *manihotis* when they are co-inoculated (Kwena, 1992; Vedier *et al*., 1994).

Recently Mbaringong *et al* (2017) evaluated 21 cassava varieties from prime cassava cropping areas in Kenya. From these the following four categories were generated resistant, moderately

resistant, susceptible and highly susceptible having four, four, eleven and two cultivars respectively. In another Kenyan study Odongo *et al* (2019) evaluated 7 cultivars from the Kenya Agricultural Research Institute against five XPM isolates from Western, Nyanza, Central, Eastern and Coast. All had an area under disease progress curve of more than 50% rendering them susceptible. Some were extreme with area under disease progress curve of 90.4 which was the case of the reaction between the Eastern Isolate and the variety MH 95/0183. However, each study worked with *Xanthomonas phaseoli* pv *manihotis*. Therefore, much still needs to be done in evaluating these cultivars with both causal agents as both have been shown to exist within Kenya and also the fact that a differential reaction within cultivars has also been witnessed (Pereira *et al*., 2000; Chege *et al*., 2017).

2.14 Limitations to the management of cassava bacterial blight through resistance

In as much as resistance has been suggested as a crucial factor in cassava bacterial blight management, various factors ought to be considered that have been seen as a hindrance towards varietal evaluation. Firstly, the disease has shown varied reactions against genotypes across different environments indicating environment pathogen interaction (Zinsou *et al*., 2005). It has been proposed that this can be partially solved through the evaluation of varieties in a controlled environment to improve accuracy in selection. Secondly, evaluation regimes ought to consider varietal traits preferred by farmers and give more focus on evaluating varieties possessing these traits and also finding ways to improve these varieties without interfering with their unique qualities (Fanou *et al*., 2017). In addition, although resistance is integral in cassava bacterial blight control, strategies ought to be set in motion to create awareness among farmers regarding the existing control measures which are critical in complementing resistance. This is important as resistance may be overcome quickly if other avenues exploited by the causal agents are left unchecked (Zárate-Chaves *et al*., 2021). This is especially true for Kenya where it has been reported that most farmers have not been keen on implementing the current control measures and also lack of access to clean seed leading to persistent dependence on seed from other farmers (Njenga *et al*., 2017). Furthermore, most of the varieties have been evaluated using only *Xanthomonas phaseoli pv manihotis* at the expense *of Xanthomonas axonopodis pv cassavae*. Hence monitoring varietal reaction against both will give a better chance of obtaining a variety of better genetic makeup against both casual agents (Mbaringong *et al*., 2017). This is because each is capable of inducing serious disease if left unchecked (Pereira *et al*., 2000).

2.15 Interaction between microorganism

The war against microorganism is constantly evolving and newer complications keep on emerging as we seek to preserve our food resources against these assailants. Since time immemorial only single microbes have been implicated in pathogen host relationship that lead to disease establishment (Abdullah *et al*., 2017). However, this is being reconsidered as many microbes are being isolated in groups from diseased plants. Microorganism are known to coexist in two ways conflict or harmony. The presence of two microbes may lead to elevated, repressed or no change at all in disease. The latter is however difficult to prove. Across all groups pathogens have been known to gang up and produce diseases of magnanimous proportions leading to the extensive depletion of crops causing food security crises (Lamichhane and Venturi ,2015).

Worthy example is the maize lethal necrosis disease which is incited when two viruses *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* band together (Mariki, 2017). In tomato eight bacterial species known to instigate tomato pith necrosis individually, have proved to increase their potency when they have been co-inoculated in the same plant (Lamichhane and Venturi ,2015). This arsenal being displayed by pathogenic microorganisms deserves study for institution of proper management strategies. Moreover, some of the underlying factors that contribute to successful colonization of co-infecting pathogens have been established. For example, natriuretic peptide receptor exhibited by *Pseudomonas syringe* facilitates the later attack of Arabidopsis by virulent *Aternaria brassicola* via suppression of a variety of genes associated with protection against the fungi (Spoel *et al*., 2007). Additionally, evolution of fusaric acid by *Fusarium oxysporum* hinders the expression of genes that confer defense against microbes promoting the colonization of wheat by *Pseudomonas fluorescence* (Cooper *et al*., 2008).

On the other hand, plant immunity can be greatly boosted due to coinfection. This has been shown by coinfection of tomato plants with two strains of *Fusarium oxysporum* one virulent and the other nonpathogenic (Aime*et al*.,2013). Suppression of the pathogenic strain was observed when both were co-inoculated on the same plant. This phenomenon has been attributed to the following factors antagonism, detoxification of the pathogen effectors and heightened immune

response from plants among all patho-systems in which it has been observed (Ravensdale *et al*., 2014; Conrath *et al*., 2015).

On cassava two distinct bacterial pathovars *Xanthomonas axonopodis* pv *cassavae* and *Xanthomonas phaseoli* pv *manihotis* are sometimes co-isolated from the cassava bacterial blight diseased leaf lesion. The interaction of both microbes has been reported to be inhibitive as *Xanthomonas axonopodis* pv *cassavae* has been observed to partially hinders the progression of *Xanthomonas phaseoli pv manihotis* systemically when both are co-inoculated on the same stem lesion. However, the latter has been observed to be capable of overcoming the difficulty and go on to establish a systemic infection. Cassava genotypes have also been reported to respond differently to both pathogens as others show susceptibility to either one or both pathogens. Enumeration of this relationship is important for institution of appropriate management strategies (Kwena, 1992: Verdier *et al*., 1994).

CHAPTER THREE

EFFECT OF CASSAVA PRODUCTION PRACTICES ON INTENSITY OF BACTERIAL BLIGHT IN BUSIA COUNTY

3.1 Abstract

Cassava bacterial blight caused by *Xanthomonas phaseoli* pv *manihotis* (syn. *Xanthomonas axonopodis* pv *manihotis*) and *Xanthomonas axonopodis* pv *cassavae* is a devastating disease that infects cassava. It has been increasing in incidence making it a threat to the livelihood of many cassava farmers. Hence this study was conducted to determine the distribution of cassava bacterial blight in Busia County Western Kenya a key cassava belt in the country. Multistage sampling was used to select 193 farms from two purposively selected sub counties Nambale and Teso south in Busia. Questionnaires were used to obtain information on cassava production practices, and cassava bacterial blight knowledge among farmers. In all farms 30 plants were assessed along two diagonals for cassava bacterial blight and diseased leaf samples collected for isolation and confirmation of the pathogens. GPS coordinates for making distribution maps were also collected from all the farms visited. The diseased leaves were sectioned into healthy and diseased portions then crushed and plated on Yeast Peptone Glucose Agar. Descriptive statistics and chi-square test were used to analyze the data on questionnaires. Over 90% of the farms in both sub counties had *Xanthomonas phaseoli* pv *manihotis* while less than 10% had *Xanthomonas axonopodis* pv *cassavae* and a combination of both causal agents. Majority of the farms (89%) had moderate severity score of 3. The association between cassava bacterial blight incidence and the cassava production practices was not significant ($p>0.005$) indicating that other factors like ignorance led to the high incidence as 85% of the interviewed farmers were unaware of the disease. Therefore, they could have been spreading the disease unknowingly through self-recycled cutting, and use of seed from neighbors. The prevalence of the pathogen in Busia necessitates urgent action to curb the disease.

Keywords: *Xanthomonas phaseoli* pv *manihotis, Xanthomonas axonopodis* pv *cassavae* and *Manihot esculenta*

3.2 Introduction

Cassava (*Manihot esculenta* Crantz) is a crop of extreme importance to numerous individuals dwelling in Sub-Saharan Africa (Spencer and Ezedinma, 2017). It contributes to the livelihoods

of more than 800million people in the world's tropic (Mbanjo *et al*., 2021). In Kenya it serves as a core part of the diet of many households who grow it primarily for its starch endowed tubers but in some communities its leaves also form part of the delicacy (Ouma *et al*., 2021). Furthermore, it is a dynamic crop with industrial potential that cuts across various industries like the pharmaceutical, food and feed processing, and manufacturing. Its positive attributes include the ability to grow in nutrient constrained soils and unfavorable climatic conditions as well as resilience to most pest and diseases (Sedano *et al*., 2017). Nonetheless, its potential as a food security as well as an economic crop continues to be threatened by cassava bacterial blight which is instigated by two causal agents *Xanthomonas phaseoli* pv *manihotis* (syn. *Xanthomonas axonopodis* pv *manihotis*) and *Xanthomonas axonopodis* pv *cassavae* which are capable of causing loss of up to 100% (Bart and Taylor, 2017). Although it has been indicated that cassava bacterial blight exists in all cassava growing areas in Kenya at high incidences of up to 100% (Livoi *et al*., 2021; Chege *et al* ., 2017) its distribution and that of its causal agent is yet to be determined in Busia Western Kenya (Odongo *et al.*, 2019). Therefore, we hypothesized that cassava bacterial blight might be widely distributed in Busia County due to lack of knowledge and little or none application of management practices by farmers. Hence, this study was conducted to determine the effect of cassava production practices on intensity of bacterial blight in Busia county.

3.3 Materials and Methods

3.3.1 Study area

The study was carried out in two agro ecological zones within Busia County in the Western Kenya: Lower midland zone 1 (LM1) and Lower midland zone 2 (LM2) the two zones were purposively selected due to the high cassava production in each compared to the other zones. Two sub counties Nambale (LM1) and Teso south (LM2) were selected one sub County from each zone due to the high cassava production in each Sub County. These areas are characterized by a temperature range of 21- 30 ℃, two rainfall seasons with an average precipitation of 760 and 1750mm, the areas are characterized by well drained, deep, brownish and sandy soils, the altitude ranges between 1200 – 1440 meters above sea level (Owiny *et al*., 2019). Most of the farmers practice subsistence farming mainly growing cassava, sorghum and maize in intercropped or monoculture systems. The study was conducted in the month of November 2020.

3.3.2 Survey of cassava farming households

Information on cassava production practices and cassava bacterial blight knowledge among farmers was collected using a semi structured questionnaire. Field assessment was also conducted to determine the distribution of cassava bacterial blight on the farms visited. A multistage approach was used to select a sample of 193 farmers into the study. In the first stage two agro ecological zones (LM1 and LM 2) were purposively selected due to high cassava production. In the second stage two sub counties Nambale(LM1) and Teso south(LM2) were purposively selected one from each agro ecological zone. In the third stage four wards were selected from Teso south and Nambale sub counties. In the fourth stage 20 -30 households were selected within each ward using purposive random sampling with the aid of field guides. The distance from one household to the next was 2km. The questionnaire comprised of two types of questions open and closed ended. Some questions were adjusted to enable ease of understanding by farmers. The interviews were conducted using Kiswahili and Teso languages. Farmers knowledge on pest and diseases was obtained by showing them images containing symptoms and signs of common pests and disease of cassava. Information on disease management practices, sources of cassava stem cuttings, sources of information on cassava production, knowledge on cassava bacterial blight, and cassava production practices employed was also obtained from the farmers. Geographical coordinates were also collected and recorded from each of the visited farms. The sample size was determined using the following formula as described by Anderson *et al*. (2016) where p = 0.5, Z = 1.96, and E = 0.071

$$n = \frac{p(1-p)Z^2}{E^2}$$

3.3.3 Determination of incidence and severity of cassava bacterial blight

In all farms visited 30 plants were randomly assessed along two diagonals with 15 plants from each diagonal (Sseruwagi *et al*., 2004). The incidence was calculated as the total number of plants showing CBB symptoms over total number of plants assessed multiplied by 100% to obtain percentage:

$$\text{Disease incidence} = \frac{No.of\ infected\ plants}{\text{Total no.of plant assessed}} \text{x} 100$$

The severity was assessed using a scale of 1-5 by Wydra *et al* (2007): where 1 = no symptoms, 2 = angular leaf spotting only,3 = wilting, angular leaf spotting, leaf blight, defoliation, gum exudates on stems or petioles, 4 = wilting, blighting, defoliation, gum exudation, shoot tip die back, 5 = wilting and blighting, defoliation and gum exudation, abortive lateral shoot formation, stunting, complete die back. Leaves from infected plants were collected in khaki bags and stored in cooler boxes and transported to plant pathology laboratory, Faculty of Agriculture, University of Nairobi and stored at 4℃.

3.3.4 Isolation and confirmation of cassava bacterial blight causal agents

Yeast peptone glucose agar(YPGA) (Yeast 7g, peptone 7g, glucose 7g, agar15g, ph7 in 1000ml of sterile distilled water) was prepared at 121℃ for 15minutes at 15psi(Azorji *et al*., 2016). The media is capable of facilitating the differentiation of the two CBB causal agents from other bacteria through formation of glossy mucoid colonies (Zárate-Chaves *et al*., 2021). The two causal agents also differ in terms of colony color further aiding their identification (Ogunjobi *et al*., 2007). The media was then dispensed aseptically into petridishes within a laminar hood and allowed to stand for an hour to solidify. Leaves collected from farms were retrieved from storage. These were then sectioned into portions containing healthy and diseased tissue using a sterile scalpel. After which the cut portions were surface sterilized using 0.5% sodium hypochlorite for 3 minutes after which they were rinsed in three changes of sterile distilled water for five minutes at each stage. The portions were then transferred onto sterile blot papers within laminar hood to drain excess water. After the portions were blotted out, the cut leaf portions were transferred into sterile universal bottles containing 9ml of sterile distilled water and pounded using sterile glass rods. The mixture resulting from the pounding was allowed to stand stood for two hours for the bacteria to ooze from the plant cells. Streaking method was then used to plate 1ml of the mixture on YPGA media which was incubated for 24hrs. Colony characteristics were used to confirm the CBB causal agents because they grow on YPGA media forming glossy, convex, mucoid colonies with entire margins (Zárate-Chaves *et al*., 2021). Colony pigmentation was also used to differentiate the two causal agents (Ogunjobi *et al*., 2007) as *Xanthomonas phaseoli* pv *manihotis* forms white colonies while *Xanthomonas axonopodis* pv *cassavae* forms yellow colonies (Livoi *et al*, 2021).

3.3.5 Biochemical tests

The following biochemical tests were performed on the CBB causal agents:

Gram tests

This was done by first taking bacterial colonies from the culture plates which were smeared on sterile slides. The bacterial smears were then heat fixed. Crystal violet was then poured on the glass slides for 1 minute after which iodine was added and rinsed with water after 1 minute. Alcohol was used to decolorize the smear for 20 seconds after which safranin was added as a counter stain for 30 seconds (Reiner, 2010).

Catalase test

This was performed by placing a drop of 3% hydrogen peroxide on a sterile glass slide then using a sterile wire loop a single bacterial colony was transferred from the plate, stirred and observed for effervescence which would indicate presence of catalase enzyme (Reiner, 2010).

U**tilization of sugars and carbohydrates**

This was done through the use of Hugh and Leifson O-F basal medium (Sodium Chloride 5.0g; Pancreatic Digest of Casein 2.0g; Dipotassium Phosphate 0.3; Bromothymol Blue 0.08g; Agar 2.0g; specific carbohydrate 10g). The carbohydrate sources were sucrose, cellobiose, lactose, glucose. The media was dispensed in test tubes left to cool. After which the bacteria were inoculated then incubated at 27 ℃ and observed daily for 14 days. Yellow colour showed bacterium is capable of using the respective sugars (Goszczynska *et al*., 2000).

3.3.6 Inoculum preparation and inoculation during pathogenicity test

The single colonies of the isolated bacteria were cultured on yeast peptone glucose agar (7g yeast, 7g peptone, 7g glucose, 15g Agar, 1000ml) and incubated for 24 hours at 26℃.The cultures were then aseptically flooded with 1ml of sterile distilled water. Then a sterile glass slide was used to aseptically dislodge the bacteria colonies which was then drained into a conical flask to make the stock. The concentration of the stock was then estimated through serial dilutions by picking 1ml of the stock and diluting it severally through a series of universal bottles containing 9ml of sterile distilled water. After adding 1 ml in last bottle in the series 1 ml was picked again and discarded to maintain 9ml of the mixture. Using a sterile pipette 1ml of

the diluents was picked and dispensed into sterile petridishes. Pour plate method was then used to plate the 1ml of the diluent, by pouring the molten media at approximately 40°C into the plates containing the diluents. The plates were then swirled for the media and the diluent to mix evenly. After which the plates were incubated for 24hrs and the number of colonies determined in the countable plate, 30 colonies was considered too few to count while above 300 was considered to many to count. The concentration of the stock was estimated by multiplying the colonies counted on the countable plate by the dilution factor and dividing it with the plated amount of the diluent. The concentration of the stock bacterial suspension was then adjusted to 10^6CFU/ml by determining how many times the stock was concentrated from the desired concentration. The inocula were the put in spraying cans and used to infect the leaves and stems of one-month old cassava plants. (Pereira *et al*., 2000; Pereira *et al* 1999).

3.3.7 Data analysis

The data from the questionnaires were analyzed using IBM® Statistical Package for Social Sciences (SPSS), Version 21. Farmers were grouped into two broad categories based on the incidence data. These groups included those with high and low incidence. High incidence was considered to be incidence above 10% and low anything below 10%. The categories were used to determine the relationship between incidence and other factors such as sociodemographic and cassava production practices. Descriptive statistics was used to determine the frequencies of sociodemographic characteristics, cassava production practices, knowledge on pest and disease identification. These data were presented as percentages in tables and graphs for each the sub-counties visited. Chi-square test was done to determine whether there was an association between the dependent variable (CBB incidence) and independent variable (cassava production practices).

3.4 Results

3.4.1 Sociodemographic characteristics and cassava production practices

There was no significant difference (P>0.005) in the sociodemographic characteristics across the two sub counties surveyed in Busia. Male led households were over 60% in both sub counties and the pooled mean indicated that 73% more households are male dominated in Busia. Nevertheless, cassava production is mostly done by women in both sub counties who were 10% more from both sub counties compared to male farmers. Most of these farmers were middle aged (36-51yrs) being 28% more compared to each of the other age groups combined. The highest level of education for most farmers was primary which was 20% more compared to the other levels combined across Busia County.

Over 80% of the respondents took part in farming as their sole source of income while less than 10% did it as a part time activity. Most of the farmers 88% had over five years' experience growing cassava. Furthermore, over 70% of farmers grew cassava mainly for household consumption while less than 30% grew it for commercial purposes. There was no significant difference(P>0.005) in most of the cassava production practices across Busia County. Over 60% of the famers grow cassava for a single season while less than 30% grow in both seasons and production is on less than two acres of land. However, there was significant difference(P<0.005) in sources of seed by farmers. Over 60% of the farmers obtained seeds from informal sources. But, considerably more farmers from Teso south 23% obtained seeds from KALRO compared to Nambale (Table 3.2).

Table 0.1: Sociodemographic characteristics of farmers in Lower midland zone 1(LM1) and Lower midland zone 2 (LM 2) zones in Busia County

Characteristic	Sub-counties(n=193)			*p*-value
	Nambale LM 1 (%)	Teso south LM 2 (%)	Pooled (%)	
Age(years)				
<35	23	11	16	0.053
Middle age 36-51	51	47	49	
>51-60 upper middle age	16	24	20	
>60 retired	11	18	15	
Gender				
Male	43	45	44	0.884
Female	57	55	56	
Head of Household				
Male	83	89	87	0.291
Female	17	11	14	
Occupation				
Formal employment	2	3	3	0.092
Business person	8	2	5	
Full farmer	89	95	93	
Education				
None	10	5	7	0.338
Primary	57	61	59	
Secondary	24	29	27	
Tertiary	10	6	7	
Experience				
<a year ago	2	2	2	0.934
2years	2	3	3	
3years	1	0	1	
4years	1	1	1	
>5years	93	95	94	
Reasons for growth				
Food Security	20	35	29	0.036
Food Securityand Commercialization	80	65	71	

Table 0.2: Cassava production practices of farmers in Busia County

Characteristic	Sub counties(n=193)			*p*-value
	Teso South (LM2) (%)	Nambale (LM3) (%)	Pooled (%)	
Planting period				
Long rain	47	43	45	0.881
Short rain	21	24	23	
Both seasons	32	33	33	
Seed Source				
Own seed	20	25	23	0.001
Fellow Farmers	37	55	47	
Local Market	7	6	7	
KALRO	31	8	18	
Others	5	6	5	
Land Preparation Method				
Ox plough	77	63	69	0.171
Handheld hoe	13	22	18	
Ox plough and Handheld hoe	8	9	9	
Ox plough and Tractor	1	3	2	
Tractor	0	3	2	
Training Source				
Government	26	22	24	0.124
NGOs	16	27	22	
Government and NGO	19	10	14	
Never Trained	39	41	40	
Soil Conservation				
Mulching	1	4	3	0.018
Terracing	19	6	12	
Cover Cropping	51	49	50	
No conservation	29	41	36	
Area under cassava				
<2	50	52	51	0.478
2-5	37	41	39	
5-15	11	6	8	
15-50	2	1	2	

3.4.2 Common pests and diseases on cassava in Busia County

Up to 35.2% more of the respondents identified whiteflies as the most common pest compared to green spider mites and mealybugs (Figure 3.1). Over 90% of the respondents could be able to identify the most common cassava disease with majority able to identify Cassava mosaic disease(CMD)compared to Cassava brown streak disease(CBSD) (Figure 3.2). Over 60% of the farmers applied some form of management practice against cassava pest and diseases, the most common strategy was uprooting infected plants (Figure 3.3).

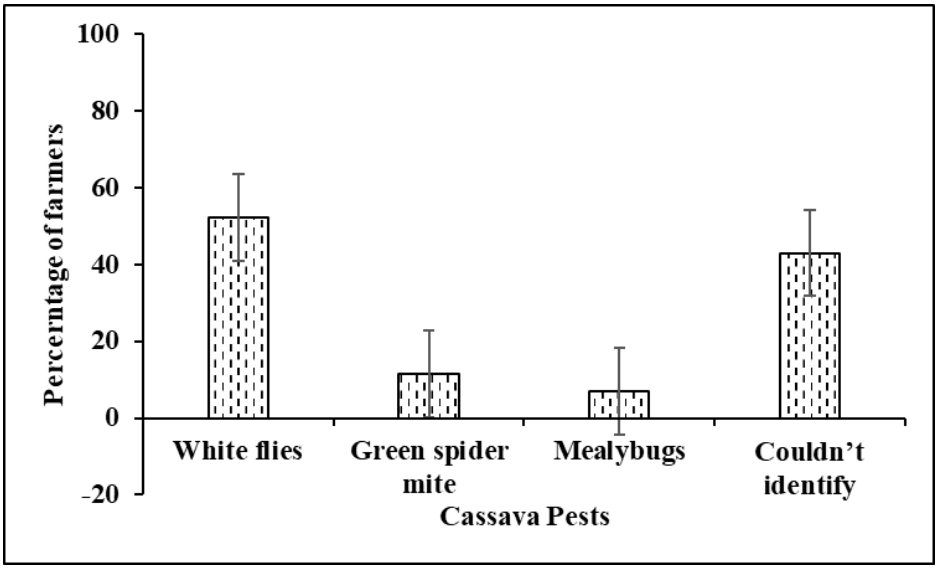

Figure 0.1: Percentage of farmers aware of cassava pest in Busia County.

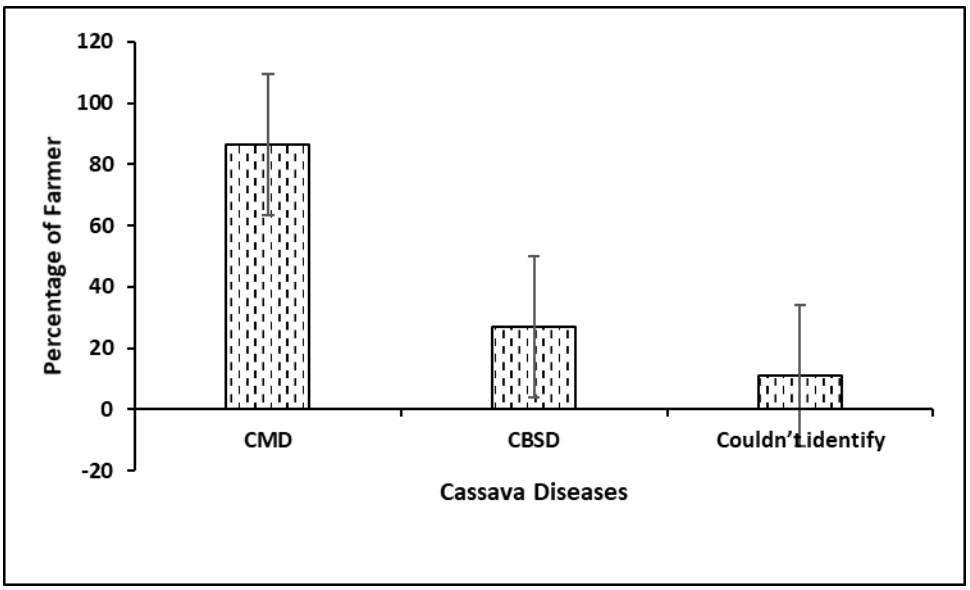

Figure 0.2: Percentage of farmers aware of cassava diseases in Busia County

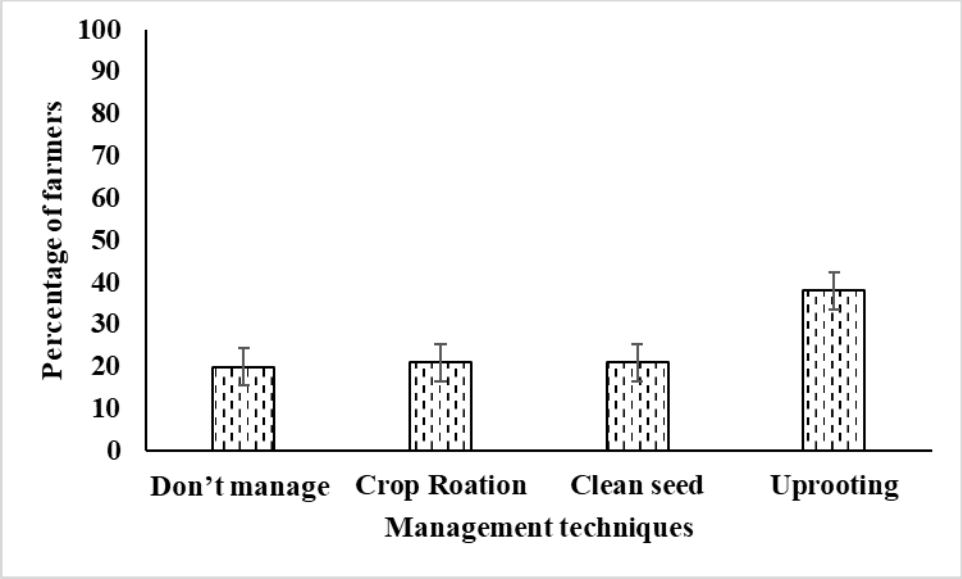

Figure 0.3: Percentage of farmers applying management against cassava pest and diseases

3.4.3 Bacterial growth on culture media

Bacterial growth on yeast peptone glucose agar after 24 hrs was characterized by white, mucoid, shiny convex colonies, and yellow, mucoid, shiny, convex colonies (Figure 3.4).

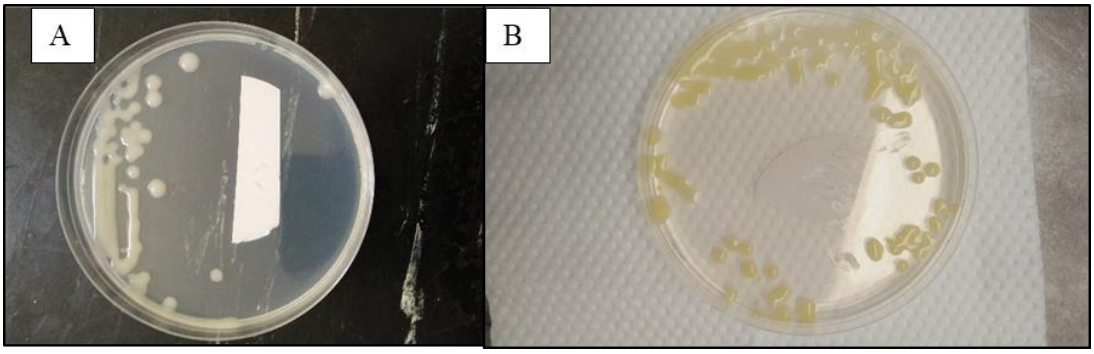

Figure 0.4: Cultural characteristics of cassava bacterial blight causal agents. (A) White colony of *Xanthomonas phaseoli pv manihotis*. (B) Yellow colony of *Xanthomonas axonopodis pv cassavae* after 24 hours on YPGA Media

3.4.4 Biochemical and physiological tests

The cream white and yellow bacteria were rod shaped and gram negative, catalase positive (Figure 3.5) they were capable of utilizing glucose, sucrose and maltose but incapable of breaking down lactose and cellobiose (Figure 3.6).

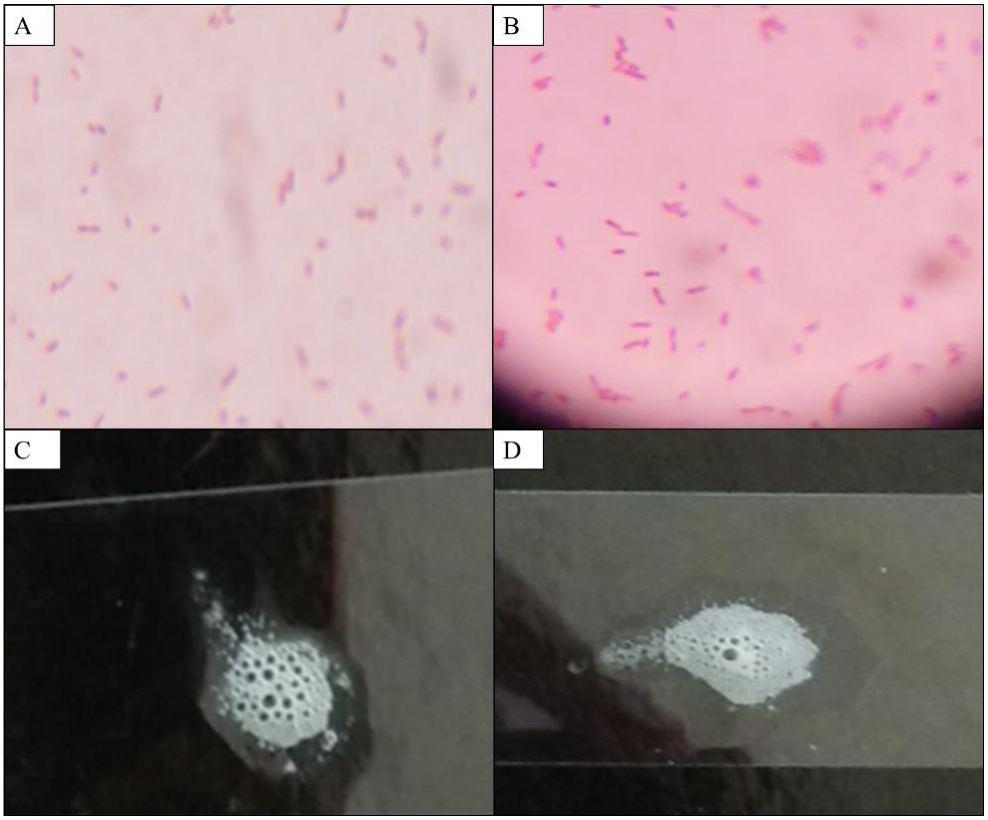

Figure 0.5: Gram stain and Catalase tests of cassava bacterial blight causal agents. (A) Gram negative rods of *Xanthomonas phaseoli pv manihotis*. (B) Gram negative rods of *Xanthomonas axonopodis pv cassavae*. (C) Effervescence reaction of *Xanthomonas phaseoli pv manihotis* during catalase test. (D) Effervescence reaction of *Xanthomonas axonopodis pv cassavae* during catalase test

Table 0.3: Summary of morphological and physiological tests of cassava bacterial blight casual agents *Xanthomonas phaseoli* pv *manihotis* and *Xanthomonas axonopodis* pv *cassavae*

Parameters	**Cassava bacterial blight causal agents**	
	Xanthomonas phaseoli pv *manihotis*	*Xanthomonas axonopodis* pv *cassavae*
Colony traits		
Pigmentation	White	Yellow
Margin	Entire	Entire
Motility	Motile	Motile
Elevation	Convex	Convex
Shape	Rod-shaped	Road shaped
Surface	Mucoid	Mucoid
Physiological characteristics		
Gram stain	Negative	Negative
Sucrose Utilization	Positive	Positive
Lactose utilization	Negative	Negative
Cellobiose utilization	Negative	Negative
Catalase test	Positive	Positive

3.4.5 Pathogenicity reaction

Plants infected with the white bacterial isolate showed systemic infections leading to wilting six days' post inoculation. The angular spots on these plants merged to form blight while plants infected with the yellow bacterial isolate only formed angular leafspots by the 14th day of infection. This confirmed that the white and yellow colonies as *Xanthomonas phaseoli pv manihotis*(XPM) and *Xanthomonas axonopodis pv cassavae* (XAC) respectively (Figure 3.6).

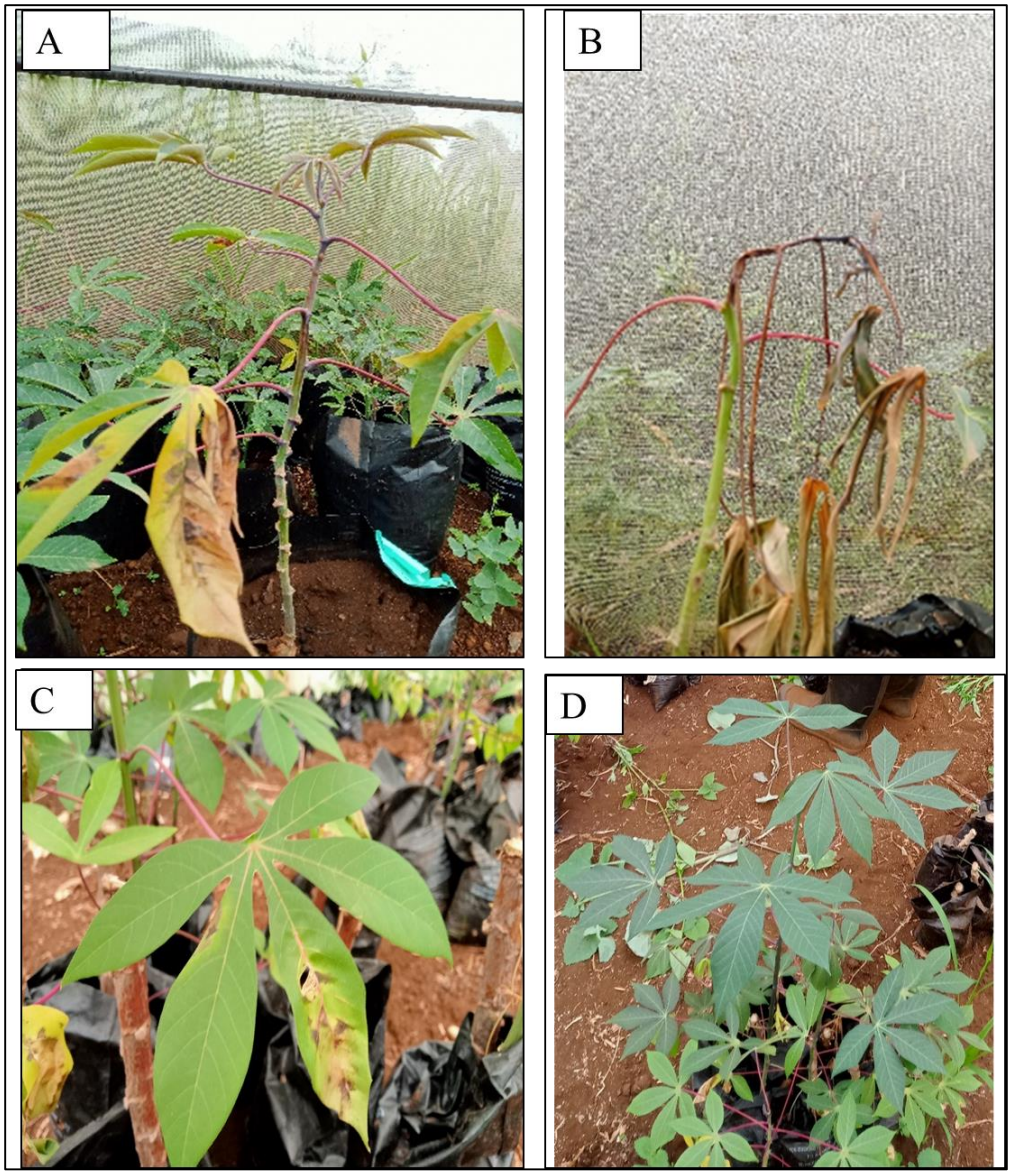

Figure 0.6: Pathogenicity reactions of cassava bacterial blight casual agents. (A&B); Blighted leaves, complete death in plants infected *Xanthomonas phaseoli pv manihotis* (XPM). (C) angular leafspots on plants infected *Xanthomonas axonopodis pv cassavae* (XAC). (D) Control Plant inoculated with sterile distilled water

3.4.6 Incidence, prevalence and distribution of cassava bacterial blight in Busia County

Cassava bacterial blight exhibited high levels of incidences and distribution across the two sub counties visited (Figure 3.9). Moreover, from both sub counties visited over 50% of the farmers had high incidence (Figure 3.10). In Nambale up to 86.2% had high incidence and the lowest and highest incidences observed in Nambale was 3% and 67% respectively. In Teso south up to 81.0% had high incidence, however, one farmer stood out whose farm had apparently healthy crops and hence incidence was 0. The lowest and highest incidence in Teso south were 0 and 73% respectively. Over 80% of the farms visited had moderate severity scores of 3 with more than 70% of the farmers unable to identify cassava bacterial blight in both sub counties surveyed (Table 3.3). The two causal agents were confirmed to be in Busia County. Of the 193 farms visited 94% of the farms had cassava bacterial blight present while the remaining 6% may have been negative probably due to spoilage of sample. Moreover, from each sub County the number of farms from which each causal agent was isolated was as follows Teso south XPM 76 and XAC 2 while Nambale XPM 95, XAC 8 and XPM plus XAC 1 (Figure 3.11). However, of the two *Xanthomonas phaseoli* pv *manihotis* is more widespread as compared to *Xanthomonas axonopodis* pv *cassavae* (Figure 3.9).

Table 0.4: Cassava bacterial blight perception in different farms

Characteristics	**Sub-counties**		
	Teso south (%)	**Nambale (%)**	**pooled(%)**
Famers knowledge			
Aware	23.8	10.1	16.1
Unaware	76.2	89.9	83.9
Severity Score			
2	23.9	0.0	10.8
3	76.1	100.0	89.2

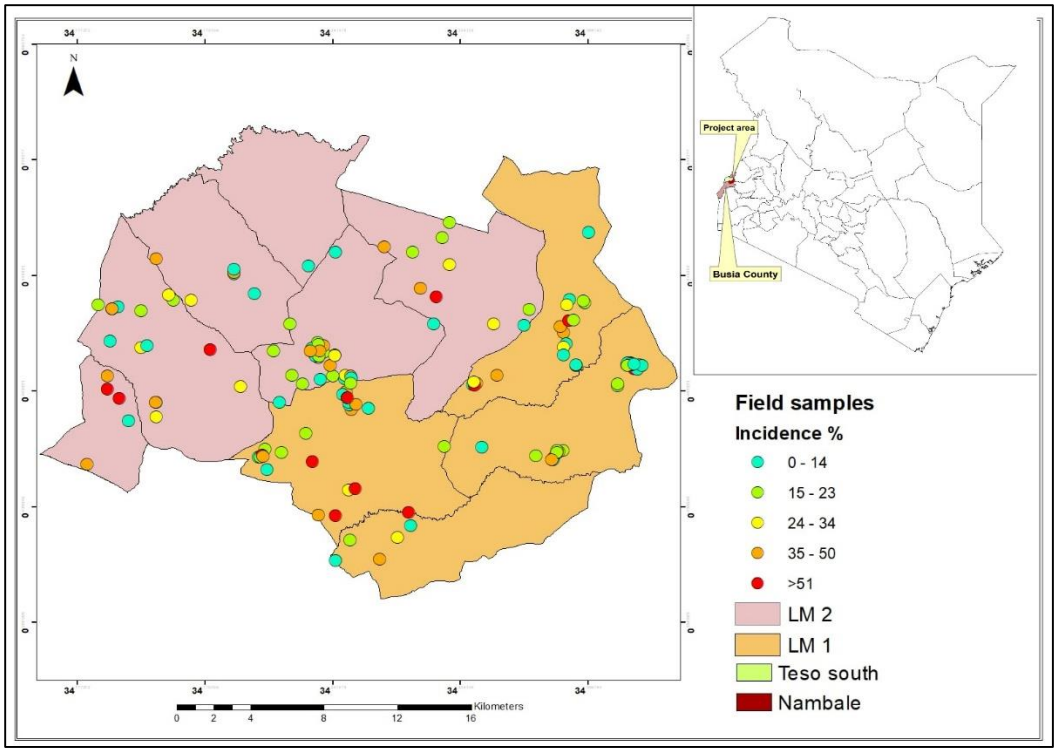

Figure 0.7: Incidence and distribution of cassava bacterial blight across Busia County

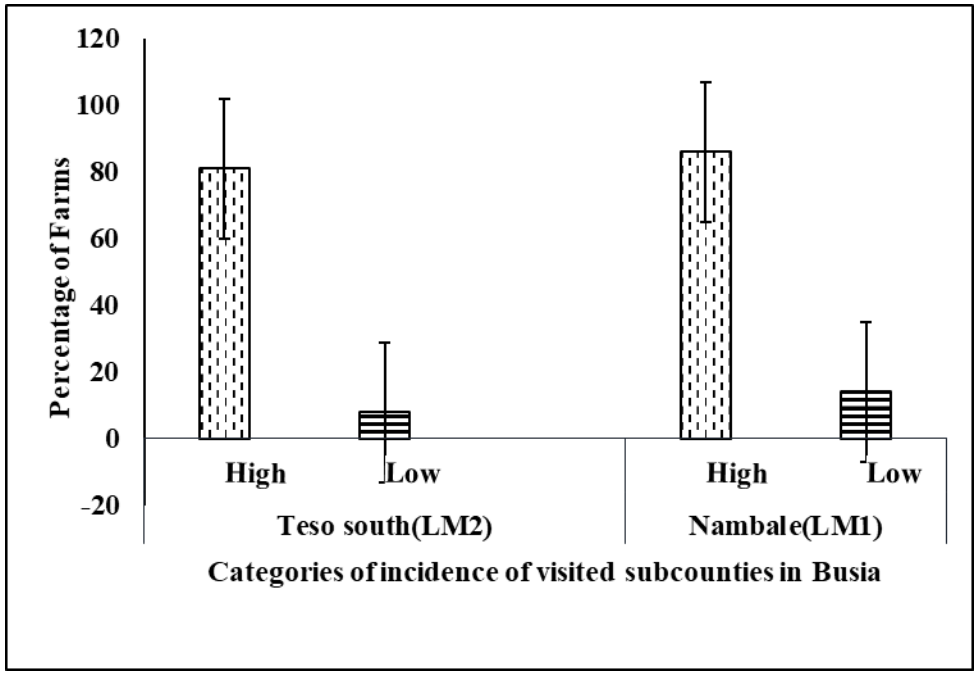

Figure 0.8: Percentage of farms falling into high and low cassava bacterial blight incidences categories in Busia County

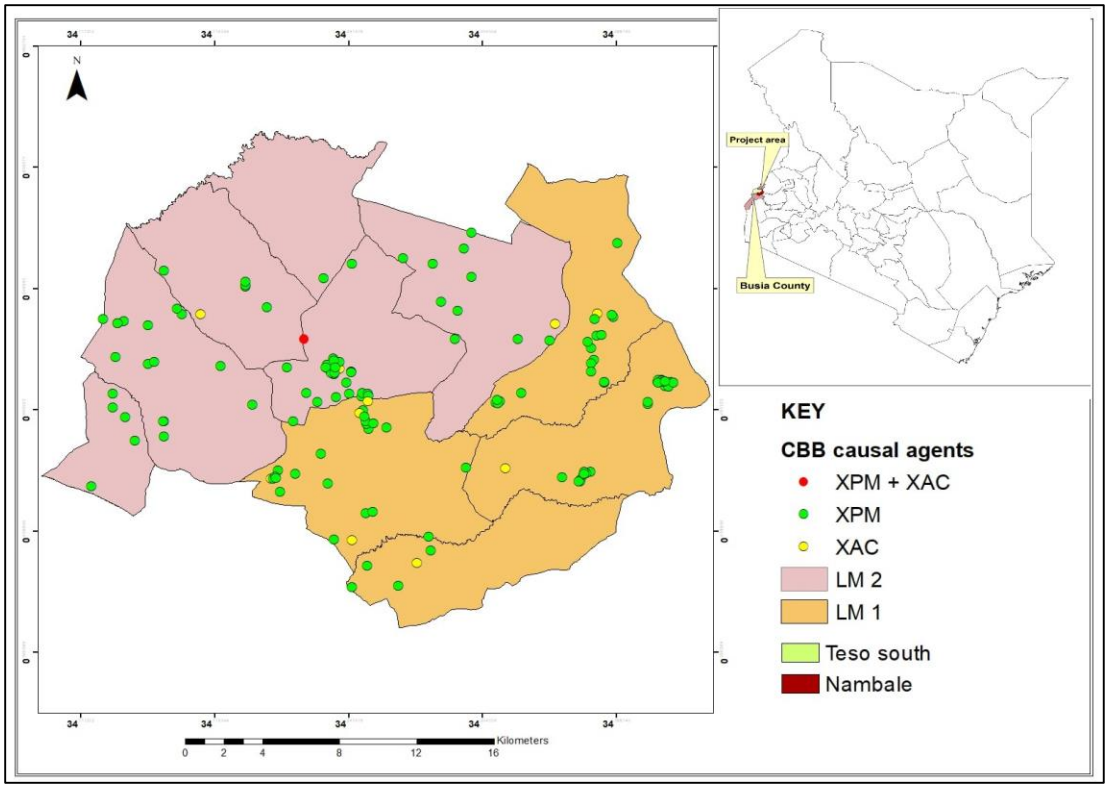

Figure 0.9: Distribution of cassava bacterial blight causal agents *Xanthomonas phaseoli pv manihotis* (XPM) and *Xanthomonas axonopodis pv cassavae* (XAC)in Busia County

3.4.7 Association of cassava bacterial blight incidence and various cassava production practices

The cross tabulation results showed that there was no significant ($p>0.005$) relationship between cassava bacterial blight incidence and training, seed source, and intercrop. Furthermore, all of the odds ratio values had a range which included 1 indicating that no association existed between cassava bacterial blight incidence and the cassava production practices (Table 3.4).

Table 0.5: Association between incidence of cassava bacterial blight and cassava production practices

Factor	Percentage of Respondent with high incidence	P value	odd ratio	95%CI
Trained	86	0.405	0.695	(0.295-1.641)
KALRO cuttings	89	0.461	0.619	(0.171-2.238)
Neighbors cuttings	85	0.671	0.831	(0.353-1.954)
Recycled cuttings	75	0.086	0.453	(0.181-1.136)
Maize intercrop	83	0.594	1.28	(0.515-3.182)
Millet intercrop	80	0.402	1.485	(0.586-3.763)
Beans intercrop	85	0.9	0.945	(0.396-2.260)
Groundnuts intercrop	77	0.14	1.941	(0.797-4.727)
Soya intercrop	78	1.181	1.851	(0.745-4.599)

3.5 Discussion

3.5.1 Distribution of cassava bacterial blight in western Kenya

The study indicated that cassava farming is mostly conducted by women despite most of the households being male headed. Most of these farmers were middle aged and there was no significant difference in the level of education across both sub counties surveyed. This is because for majority of the farmers primary was the highest level of schooling. Additionally, cassava farming was mainly done for substance and majority of the farmers had more than five years of experience. These findings agree with other studies by Tirra *et al* (2019), Kidasi *et al* (2021), and Nyirakanani *et al* (2021) who found similar characteristics in other cassava belts. Furthermore, cassava was mostly planted in less than 2 acres of land in a non-mechanized manner. Coulibaly *et al* (2014) reported that the small sizes of land apportioned to cassava might be due to land fragmentation and preference for other crops deemed more valuable. The study also found that majority of the farmers rely on informal seed systems. However, there were slightly more farmers in Teso south who used certified cutting from Kenya Agricultural and Livestock Research Organization (KALRO) compared to Nambale. This might have been due to the fact that farmers in Teso south are in close proximity to KARLO compared to those from Nambale. This is in agreement with studies by Hounge *et al* (2018), Mdenye *et al* (2018) and El-sharkawy (2003) who found that distance plays a role in farmer access to certified cuttings leaving farmers dependent on informal seed systems which are characterized by

recycled cuttings making them vulnerable to asymptomatic pests and diseases which reside in such cuttings.

Bacterial growth within the laboratory after 24hrs showed two bacterial isolates with white and yellow colonies. These characteristics have been observed in other studies and have been proposed to be a basis for differentiating cassava bacterial blight causal agents (Zárate-Chaves *et* al., 2021). However, (Odongo *et al*., 2019) has reported that colony pigmentation might change after 3-4 days which was not observed in the study as all the colonies retained as similar pigmentation post the period. Both bacteria had convex colonies with entire margins, and a glossy surface which is similar to reports that have been made by Livo *et al*., (2021) when they evaluated isolates from the coastal region. Both bacteria could be able to utilize sugars like sucrose, glucose and maltose but none could breakdown lactose or cellobiose. In other studies, slight variations in the utilization of maltose have been observed as *Xanthomonas axonopodis pv cassavae* has been reported to degrade the sugar at a slower rate compared *Xanthomonas phaseoli pv manihotis* (Ogunjobi 2007; Mooter*et al*., 1986)). Nonetheless, none of these biochemical test has been shown to be reliable in differentiating the two causal agents up to the pathovar level and this was the case in the study (Odongo *et al*., 2019). Therefore, pathogenicity tests were conducted as both causal agents have been reported to differ in their virulence.

The pathogenicity reactions showed that *Xanthomonas phaseoli pv manihotis* is more severe of the two causal agents. This is because *Xanthomas phaseoli pv manihotis* is capable of spreading beyond the infection point through the cassava vascular system resulting in the death of the plant within 6 days' post inoculation. However, *Xanthomonas axonopodis pv manihotis* is incapable of causing systemic infections as it remained limited to the foliar parts of the plant causing angular leaf spots. Its rate of infection was also much slower compared to that of *Xanthomonas phaseoli pv manihotis* as plants infected with it started showing symptoms 14 days' post inoculation in contrast to the latter which started showing infection 6 days' post infection. These results are consistent with observations by Kwena *et al*., (1992) and Pereira *et al*., (2000) who observed a similar trend when they subjected plants to both pathogens. Though pathogenicity was able to distinguish the pathogens on the basis of virulence and symptomatology its didn't differentiating the two causal agents up to the pathovar level.

Moreover, the survey showed that cassava bacterial blight is widespread in Busia at high incidences. Most of the farmers also reported that they have never encountered cassava bacterial blight despite the fact that it had been known to exist in Western as early as the 1980s. This in agreement with studies by Livoi *et al*., (2021), Odongo *et al* (2019) and Chege *et al* (2017) from other cassava belts in Kenya who have reported incidences of over 70% at farm level in these areas. All of the farms surveyed in the study fell into the high incidence category. This might have been associated to the fact that most of them relied on recycled cassava cuttings. Recycled cuttings have been associated with bacterial blight which can survive without notice. Furthermore, other factors such as suitable conditions for the pathogen might have played a role in Busia county which is marked with wet warm weather in the months of November when the survey was conducted. This is in contrast to findings from other cassava regions who observed a low incidence of the disease in areas where the conditions were more dry or cold (Toure *et al*., 2020).

Furthermore, both causal agents of CBB were isolated from Busia of which *Xanthomonas phaseoli pv manihotis* was more prevalent compared to *Xanthomonas axonopodis* pv *cassavae*. This confirms reports by Onyango and Mukunya (1980) and Mooter*et al*., (1986) who indicated that the high prevalence of *Xanthomonas phaseoli* pv *manihotis* might be because its more aggressive compared to *Xanthomonas axonopodis* pv *cassavae*. However, they noted that the later bacterium is also capable of causing severe disease on vulnerable cassava varieties. Interestingly none of the sociodemographic or cassava production practices was associated with the high prevalence of cassava bacterial blight observed in Western Kenya implying that other factors are involved in its spread.

Zárate-Chaves *et al* (2021) observed that lack of cassava bacterial blight awareness among farmers contributes to the spread of the disease eventually leading to its buildup in the long run. This is coherent with the findings of the current study as most farmers reported that they have never encountered CBB. The lack of awareness might be because more attention has been invested in other cassava diseases compared to CBB evidenced by the fact that most farmers in the study could comfortable identify other cassava pests and diseases and even associate them to the respective causes (Chavez *et al*., 2021; Ng'ang'a *et al*., 2019). This was also reported by

Livoi *et al*., (2021) in the Kenyan coast with most farmers 61% being able to recognize the symptoms but none could associate them to CBB. Therefore, because of ignorance most farmers might end up not applying the recommended controls measures against CBB leading to its build up overtime and spread through stem cuttings as most of the farmers reported that they are dependent on cuttings from informal seed systems (Mbaringong *et al*., 2017; Buthelezi and Ngobeni, 2016). Other reasons might be poor soil nutrition reducing the ability of the plant to defend itself. Njenga *et al*., (2017) reported that incorporation of molecules like silica into the soils might enhance the ability of cassava plants to defend themselves against CBB. However, most farmers are resource constrained which might be limiting them from being able to apply these molecules into their soils (Houngue *et al*., 2018; Mdenye *et* al., 2018)). The high prevalence might also be due to the cultivation of susceptible varieties which has been observed by Bart and Taylor (2017) to be the case of most farmers in Sub-Saharan Africa including Kenya leaving farmers vulnerable in the event of an epidemic.

3.6 Conclusion

These findings show that cassava bacterial blight and its causal agents is extensively distributed within Busia County at high levels of incidence. Of the two causal agents *Xanthomonas phaseoli pv manihotis* is more wide spread compared to the *Xanthomonas axonopodis pv cassavae*. Furthermore, most famers are ignorant of the disease indicating that this might be the main factor for the high cassava bacterial incidence. Additionally, most of the farmers surveyed relied on cuttings from neighbors or self for crop establishment. This might have contributed to the high incidence of the disease since the causal agents can survive latently within cuttings. None of the biochemical test use could identify both pathogens to the species or pathovar level. Although pathogenicity test was able to distinguish both pathogens based on symptoms it could not identify them to species or pathovar level. Therefore, more awareness on the disease needs to be done through extension services, molecular techniques should be used to further characterize both pathogens, and disease free cuttings should be produced and distributed to farmers.

CHAPTER FOUR

RESISTANCE AGAINST BACTERIAL BLIGHT AMONG CASSAVA CULTIVARS GROWN IN KENYA.

4.1 Abstract

Cassava bacterial blight associated with *Xanthomonas phaseoli pv manihotis* and *Xanthomonas axonopodis pv cassavae* continues to threaten cassava production and the livelihoods of many farmers. Most of the varieties currently on farmers' fields are susceptible to the disease. Resistance has been suggested as the best Cassava bacterial blight management approach as it is safe for the environment, and can be used in an integrated disease management system. This study was conducted to identify cassava bacterial blight resistant cultivars among the cassava cultivars grown in Kenya. A greenhouse experiment was conducted to evaluate seven varieties for cassava bacterial blight resistance in a factorial treatment structure within a completely randomized block design. All varieties were inoculated with 10^6CFU/ml of the pathogens on the leaves and stems and observations recorded at an interval of six days' post inoculation using a severity scale of 1-5. *Xanthomonas phaseoli pv manihotis* inoculated varieties had the highest incidences compared to those inoculated with or both causal agents. *Xanthomonas phaseoli pv manihotis* inoculated varieties also had higher severity scores compared to those subjected to *Xanthomonas axonopodis pv cassavae*. Furthermore, all varieties had area under disease progress curve values of over 50% and *Xanthomonas phaseoli pv manihotis* inoculated varieties had the highest values. The most affected varieties included mm 96/2480, Naro 56, and mm 96/1871. *Xanthomonas phaseoli pv manihotis* proved to be more severe compared to *Xanthomonas axonopodis pv cassavae*. The experiment also showed that none of the popularly grown varieties is formidable against cassava bacterial blight. This makes it critical for continued identification of cassava bacterial blight resistant varieties.

Keywords: *Manihot esculenta,* Disease intensity, Host resistance, *Xanthomonas axonopodis pv cassavae, Xanthomonas phaseoli pv manihotis*

4.2 Introduction

Cassava occupies an important place in the socioeconomic life of many rural dwelling folks. This is because of its huge potential as a food security, industrial and commercial crop.

Furthermore, it can be processed to provide feeds for both poultry and livestock. The crop is capable of thriving in areas of low rainfall and low soil nutrients (Sangbamrung *et al*., 2020). It's also capable of withstanding many biotic constraints making it a bedrock for many farming households due to of disruptions in environmental climatic cycles (Devi *et al*., 2022). Despite its huge potential cassava productions is still limited by both viral and bacterial diseases in many parts of the world including Kenya. Cassava bacterial blight is among the most severe and causes huge losses to farmers and also has the potential of obliterating some cassava species (Elliott *et al.,* 2022). CBB infected plants are normally associated with two bacteria *Xanthomonas phaseoli* pv *manihotis* and *Xanthomonas axonopodis* pv *cassavae* (Zárate-Chaves *et al*., 2021; Livoi *et* al., 2021). Of the two *Xanthomonas phaseoli* pv *manihotis* is the more severe but *Xanthomonas axonopodis* pv *cassavae* has been observed to cause severe disease in certain environmental conditions (Pereira *et al*., 1999; Verdier *et al*., 1993; Zárate-Chaves *et al.*, 2021). Although, cultural disease management strategies have been suggested farmers have been slow to adopt them leading to CBB's continued persistence (Njenga *et al*., 2017). Nonetheless, resistance has been recommended as the best alternative for farmers in an integrated disease management regime and recent studies by Mbaringong *et al.* (2017) has indicated the existence of resistance among the cassava cultivars grown in Kenya as four of the evaluated varieties displayed some level of resistance. However, the resistance in these varieties was reported to be insufficient, hence more varieties within Kenya need to be evaluated for resistance against cassava bacterial blight (Bart and Taylor., 2017; Odongo *et al*., 2019). This is important as the disease is increasing in incidence worldwide (Lopez and Bernal, 2012). Furthermore, most of the varieties have been evaluated against *Xanthomonas phaseoli* pv *manihotis* only yet varieties vary in their susceptibility to *Xanthomonas phaseoli* pv *manihotis* and *Xanthomonas axonopodis pv cassavae*. Thus, we hypothesized that reaction to cassava bacterial blight varies across different cassava varieties because they have different levels of resistance against the disease. Therefore, a study was conducted to evaluate the popularly grown cassava varieties within Kenya to evaluate their response when subjected to both pathogens either singularly or combined and identify varieties best suited for farmers in cassava growing areas against cassava bacterial blight.

4.3 Materials and Methods

4.3.1 Study site

The experiment was done within a greenhouse at Upper Kabete Campus, University of Nairobi. It is situated in the agroecological zone of upper midland zone three (UM3), on latitude 1º 15' South and longitude 36º 44' East at an altitude of about 1800m above sea level (Jaeztold, 2007).

4.3.2 Collection and preparation of experimental material

Cassava plants used in the study were obtained through from Kenya Plant Health Inspectorate service through the International Institute of Tropical Agriculture. They comprised of cassava varieties MM 96/3567, Fumbachai, MM 96/0067, Serere, MM96/1871, MM96/2480, Naro 56 which had been previously collected from farmer's field, freed from pest and disease contamination. The materials were chosen because of their farmer preferred traits such as good cooking qualities, fast maturity and high yield. The plants were planted in pots measuring 6 by 9 inches containing sterilized potting media composed of forest soil, sand and manure in the ratio of 3:2:1 respectively for one month before infection.

4.3.3 Experimental design

The experimental design was factorial treatment structure with control in a completely randomized block design. The experiment had three blocks with each block having 28 treatments. The blocking was due to light variations within the greenhouse. Randomization of treatments within each block was done independently. Each variety had eight plants per treatment. The experiment was composed of two factors as follows:

1. Bacterial Isolates at four levels:
    i. *Xanthomonas phaseoli* pv. *manihotis* (XPM)
    ii. *Xanthomonas axonopodis* pv. *cassavae* (XAC)
    iii. Combined (XPM + XAC)
    iv. Sterile Distilled water
2. Seven varieties:

MM 96/3567, Fumbachai, MM 96/0067, Serere, MM96/1871, MM96/2480, Naro 56

4.3.4 Inoculum preparation

Stored bacteria of *Xanthomonas phaseoli* pv *manihotis* and *Xanthomonas axonopodis* pv *cassavae* (XAC) which had been isolated from diseased samples from Busia were retrieved and plated using flame sterilized wire loops on yeast peptone glucose agar (7g yeast, 7g peptone, 7g glucose, 15g Agar, 1000ml) and incubated for 24 hrs at 26℃.The cultures were then aseptically flooded with 1ml of sterile distilled water. Then a sterile glass slide was used to aseptically dislodge the bacteria colonies which was then drained into a conical flask to make the stock. The concentration of the stock was then estimated through serial dilutions by picking 1ml of the stock and diluting it severally through a series of universal bottles containing 9ml of sterile distilled water. After adding 1 ml in last bottle in the series 1 ml was picked and discarded to maintain 9ml of the mixture. Using a sterile pipette 1ml of the diluents was picked and dispensed into sterile petridishes. Pour plate method was then used to plate the 1ml of the diluent, by pouring the molten media at approximately 40℃ into the plates containing the diluents. The plates were then swirled for the media and the diluent to mix evenly. After which the plates were incubated for 24 hrs and the number of colonies determined in the countable plate, 30 colonies was considered too few to count while above 300 was considered to many to count. The concentration of the stock was estimated by multiplying the colonies counted on the countable plate by the dilution factor and dividing it with the plated amount. The concentration of the stock suspension was then adjusted to 10^6CFU/ml by determining how many times the stock was concentrated from the desired concentration. The inoculums were the put in spraying cans for application (Pereira *et al*., 2000; Pereira *et al* 1999).

4.3.5 Plant inoculation

The pathogens were inoculated into the plants through two routes the stem and leaves. They inoculation was done either of each individual pathogen alone, and another one was done with the two combined, a control was also included. For the leaf inoculation leaves were injured using carborundum powder and the 10^6 CFU/ml inoculum sprayed on the bruised leaf surface. Stem inoculations were done by picking the bacterial suspension using sterile syringes and puncturing them gently into the cassava stems. After inoculations were done all the plants were covered using humidity bags which were then removed after 24 hrs (Livoi *et al*., 2021).

4.3.6 Determination of incidence and severity of cassava bacterial blight

Incidence was calculated as percentage of total number of plants infected divided by total number of plants assessed. Determination of disease severity was done using a scale of 1 – 5 by Wydra *et al* (2007), 1 = no symptoms, 2 = angular leaf spotting only, 3 = wilting, angular leaf spotting leaf blight, defoliation, gum exudates on stems or petioles, 4 = wilting, blighting, defoliation, gum exudation, shoot tip die back, 5 = wilting and blighting, defoliation and gum exudation, abortive lateral shoot formation, stunting, complete die back. The severity will be evaluated at an interval of 6 days' post inoculation for 6 weeks by assigning scores to the plants based on their symptoms.

4.3.7 Determination of area under disease progress curve

The area under disease progress was obtained on a single plant basis using the trapezoidal integration for the whole duration of assessment by applying the following formula (Jorge *et al*., 2000): $\sum \frac{i[(DSI+DSI-1)\times(ti-ti-1)]}{2}$

Where "i" = (7, 14, 21, 28, 35) periods of assessment, "DS" represents value on severity scale for disease and "t". The disease reaction was determined using a scale described described by Banito *et al*., (2010):

Table 0.1 Cassava bacterial blight disease reaction scale

% AUDPC CATEGORY	Disease Reaction
0 - 33.2	Resistant(R)
33.3 - 49.9	Moderately Resistant (MR)
50 - 100	Susceptible (S)

4.3.8 Determination of cassava fresh biomass

The fresh cassava biomass was determined by taking the weight of the infected cassava plant on 36th day post inoculation using a mini crane scale. This was done by uprooting two month 2 weeks old plants and weight each plant per treatment and recording the weight in grams (Kidasi *et al*., 2021).

4.3.9 Determination of cassava plant height

The cassava height was determined by measuring cassava plant every sixth day post inoculation using a tape measure. The measurements were done from the base of the stem at the soil surface to the tip of the plant. The height was recorded in centimeters (Sim *et al*., 2020).

4.3.10 Data analysis

The means severity score and incidence was calculated for each genotype and analysis of variance done on in Genstat 15th edition. The LSD was calculated at significance difference of P=0.005

4.4Results

4.4.1 Incidence of cassava bacterial blight disease in different varieties

There was significance($P<0.005$) difference in cassava bacterial blight incidence in experiment 1 and 2 across the different varieties. In both experiments varieties inoculated with *Xanthomonas phaseoli pv manihotis* (XPM) had the highest incidences compared to those inoculated with *Xathomonas axonopodis pv cassavae* (XAC) and both bacteria combined. In experiment one 71% of XPM inoculated varieties had incidences of up to 70%, only two varieties (mm96/0067 and Serere) had incidences less than 70%. There was a 16.5% difference between the highest and lowest incidences for XPM inoculated varieties. There was significance difference($p<0.005$) in incidence levels among XAC inoculated varieties. Most of the varieties 57% had an incidence of over 50% while some of the remaining had incidence less than 30%. The difference between the highest and lowest incidence was 33.4%. For varieties inoculated with both bacteria only two had incidences of over 50% while most of remaining had incidences of less than 49%. XPM had the highest incidence in experiment 1 as the difference between its overall mean and the lowest was 26%. In experiment 2 the same trend as experiment 1 was observed however, there was increased disease incidence across all varieties of over 4%. Varieties inoculated with XPM still had the highest incidence in comparison to those inoculated with XAC and a combination of both bacteria Table 4.1.

Table 0.2: Percent incidence of different varieties inoculated with *Xanthomonas phaseoli pv manihotis*(XPM) and *Xanthomonas axonopodis pv cassavae*(XAC)

	Experiment 1			Experiment 2		
Varieties	**XPM**	**XAC**	**XPM+XAC**	**XPM**	**XAC**	**XPM+XAC**
Fumbachai	70.5b	68.8c	49.3ab	73.6b	59.7ab	49.3a
mm96/0067	62.5a	41.7a	42.4ab	75.0b	54.7a	65.3bc
Serere	62.5a	35.4a	33.3a	79.2bc	66.7bc	56.3ab
mm96/3567	79.2c	44.4a	52.1ab	83.3c	57.6ab	65.3bc
mm96/1871	77.8c	50.0ab	48.6ab	75.0b	72.2c	60.4abc
mm96/2480	75.0bc	62.5bc	56.9b	62.5a	61.8abc	74.3c
Naro 56	75.7bc	62.5bc	45.1ab	79.2bc	59.0ab	66.0bc
Mean	73	52	47	75	62	62
P<0.005	0.001	0.001	0.3	0.001	0.087	0.1
LSD	7.0	15.6	19.3	5.7	12.3	15.7
CV(%)	6.2	11.7	11.9	6.3	5	5.5

4.4.2 Severity Scores of cassava bacterial blight disease in different varieties

The severity scores differed significantly ($p<0.005$) across all varieties in both experiment 1 and 2. In both experiments varieties inoculated with XPM showed the highest severity scores compared to those inoculated with XAC and a combination of both bacterial pathogens. In both experiments varieties mm 96/2480 and Naro 56 had relatively higher severity scores compared to the rest while Fumbachai and mm96/3567 showed relatively low severity scores. In experiment 1 for XPM inoculated varieties most of the varieties had a severity score of over 2.5 and the difference between the highest and lowest severity score was 0.5. Fumbachai had the lowest severity score while Naro 56 had the highest. For XAC inoculated varieties majority had a severity score of less than 2, however variety mm 96/2480 had the highest severity score compared to Fumbachai which had the least score. The difference between the highest and lowest severity score was 1.2. for varieties inoculated with a combination of both bacteria majority of had a score of over two. Variety mm 96/3567 had the lowest severity score while mm 96/2480 had the highest. In each case there was significant difference in severity scores among the different varieties with those inoculated with XPM having the highest scores. The trend was similar in experiment 2 as experiment 1 however there was a slight increase in severity score in some varieties. XPM had the highest severity score and variety mm96/2480 and Naro 56 still showed high severity scores across all the inoculations Table 4.2.

Table 0.3: Severity scores of different varieties inoculated with *Xanthomonas phaseoli pv manihotis*(XPM) and *Xanthomonas axonopodis pv cassavae*(XAC)

	Experiment 1			Experiment 2		
Varieties	**XPM**	**XAC**	**XPM+XAC**	**XPM**	**XAC**	**XPM+XAC**
Fumbachai	2.3a	1.4a	2.1bc	2.2a	1.5a	2.0b
mm96/0067	2.4a	1.8b	2.0b	2.3a	1.8b	2.0b
mm96/1871	2.7bc	2.1c	2.2c	2.6b	2.2c	2.0c
mm96/2480	2.7bc	2.6d	2.4d	2.7bc	2.5d	2.4c
mm96/3567	2.5b	1.5a	1.7a	2.6bc	1.6a	1.7a
Naro56	2.8c	2.2c	2.3cd	2.7c	2.2c	2.0c
Serere	2.4a	1.9b	2.0b	2.4a	2.0b	2.1b
Mean	2.5	2	2.1	2.5	2	2
P(<0.005)	0.001	0.001	0.001	0.001	0.001	0.001
LSD	0.154	0.16	0.176	0.177	0.17	0.18
CV(%)	26.2	35.5	36.6	30.6	37.4	36.1

4.4.3 Area under disease progress curve (AUDPC) for different cassava varieties infected with cassava bacterial blight

There was a significant difference in AUDPC values in experiment 1 and 2 across all the varieties. Varieties inoculated with *Xanthomonas phaseoli pv manihotis*(XPM) had the highest AUDPC values compared to those inoculated with *Xanthomonas axonopodis pv cassavae* (XAC) and a combination of both pathogens. Three varieties mm96/1871, mm96/2480 and Naro 56 had relatively higher AUDPC values compared to the other varieties in both experiments. In experiment 1 all varieties had AUDPC values of over 70% while three had AUDPC values of over 80%. Naro 56 had the highest AUDPC value among varieties inoculated with XPM. Varieties inoculated with XAC had AUDPC values of up to 78%. MM96/2480 had the highest AUDPC value of 78%. Varieties inoculated with both XPM and XAC all had a severity value of above 50%. The values ranged between 53 -73%. Variety mm96/2480 had the highest AUDPC value of those inoculated with both bacteria. The XPM had the highest AUDPC mean in experiment 1 having a difference of 18.6% between it and the lowest value. The same trend was observed in experiment 2 as there was a significant difference in the AUDPC values across all the varieties. XPM had the highest AUDPC values and XAC had relatively lower AUDPC values across the varieties compared to the other treatments Table 4.4.

Table 0.4: Percent area under disease progress curve for different varieties inoculated with *Xanthomonas phaseoli pv manihotis*(XPM) and *Xanthomonas axonopodis pv cassavae*(XAC)

	Experiment 1			Experiment 2		
Varieties	**XPM**	**XAC**	**XPM+XAC**	**XPM**	**XAC**	**XPM+XAC**
Fumbachai	71.4a	43.9a	65.0bcd	67.2a	44.4a	61.6bc
mm96/0067	72.1a	55.3b	58.9ab	70.2ab	55.8bc	60.1ab
mm96/1871	81.0bc	64.9c	66.9cde	76.5bcd	68.0d	70.8d
mm96/2480	83.8bc	78.3d	73.0e	81.5d	74.5d	71.6d
mm96/3567	78.0ab	48.0ab	53.0a	77.5cd	48.0ab	53.1a
Naro56	85.9bc	66.8c	68.4de	83.0d	66.9d	68.3cd
Serere	71.6a	56.1b	59.9abc	71.2abc	58.4c	62.9bc
Mean	77.6	59.0	64.0	75.0	59.0	64.0
P (<0.005)	0.001	0.001	0.001	0.001	0.001	0.001
LSD	7.2	8.5	8	6.9	8.5	7.5
CV(%)	16.1	25	21	16.1	25	20.5

4.4.4 Fresh biomass of cassava varieties by the 36th day of severity score evaluation

There was significant difference p (<0.005) in the varietal weights at the 36th day of evaluation in both experiment 1 and 2. Varieties inoculated with *Xanthomonas phaseoli pv manihotis* (XPM)had the lowest overall wet weight compared to those inoculated with *Xanthomonas axonopodis pv cassavae*(XAC) and both pathogens. In experiment 1 Naro 56 had the least weight in comparison to the rest across all bacterial treatments while Fumbachai had the highest wet weight. For Varieties inoculated with XAC most had a wet weight of over 50 g while most of the varieties inoculated with both pathogens had a wet weight below 50g. The same trend wasobserved in experiment 2 where there was a significant difference in wet weight across the different varieties Table 4.5.

Table 0.5: Fresh biomass in grams of different varieties inoculated with *Xanthomonas phaseoli pv manihotis*(XPM) and *Xanthomonas axonopodis pv cassavae*(XAC)

	Experiment 1			Experiment 2		
Varieties	**XPM**	**XAC**	**XPM+XAC**	**XPM**	**XAC**	**XPM+XAC**
Fumbachai	53.1c	57.3b	68.3d	53.3c	61.0c	68.5d
mm96/0067	47.2b	56.7b	45.3b	47.3b	56.9b	45.5b
mm96/1871	45.4ab	54.0b	55.0c	42.5a	55.3b	55.1c
mm96/2480	41.7a	45.5a	42.3a	41.8a	45.6a	42.4a
mm96/3567	47.2b	56.7b	45.3b	47.3b	56.9b	45.5b
Naro56	37.5a	41.6a	43.5ab	37.6a	41.7a	43.6ab
Serere	39.1a	58.1b	56.7c	39.2a	58.2b	56.8c
Mean	46.0	54.0	51.0	46.2	54.7	51.0
P(<0.005)	0.001	0.001	0.001	0.001	0.001	0.001
LSD	4.3	4.0	3.0	4.4	4.1	2.6
CV(%)	16.3	13.6	8.6	16.6	13.7	8.8

4.4.5 Height of cassava varieties during severity score evaluation period

There was no significant difference (P>0.005) in the height of cassava varieties for severity scores in both experiment 1 and 2 at the 36th day of evaluation. The heights were ranging between 28-30 in both experiment 1 and 2 Table 4.6.

Table 0.6: Height in centimeters of different varieties inoculated with *Xanthomonas phaseoli pv manihotis*(XPM) and *Xanthomonas axonopodis pv cassavae* (XAC)

	Experiment 1			Experiment 2		
Varieties	**XPM**	**XAC**	**XPM+XAC**	**XPM**	**XAC**	**XPM+XAC**
Fumbachai	30.0b	29.0a	30.0b	29.8bc	29.6bc	30.2c
mm96/0067	29.2a	28.9a	29.5ba	30.0c	28.4a	28.8ab
mm96/1871	30.1b	30.0b	30.0b	30.2c	30.0c	30.0c
mm96/2480	28.5a	30.0b	28.9a	28.9a	28.9a	29.5bc
mm96/3567	29.8ab	29.5ba	30.4b	28.0a	28.5a	28.7ab
Naro56	30.2ab	29.1ba	28.8a	28.5ab	27.9a	28.3a
Serere	29.2a	28.5a	29.1a	29.0b	30.0c	29.9bc
Mean	29.6	29.2	29.5	29.2	29.0	29.3
P(<0.005)	0.99	0.899	0.999	0.990	0.991	0.999
LSD	0.93	0.95	0.92	0.9	1.0	0.9
CV(%)	13.5	13.7	13.3	13.5	13.7	13.3

4.5 Discussion

4.5.1 Resistance of the popularly grown cassava cultivars in Kenya to cassava bacterial blight causal agents

The study showed that there was significance difference ($p<0.005$) in overall disease incidence across all treatments by the end of the experiment. Varieties inoculated with *Xanthomonas phaseoli pv manihotis* had the highest disease incidence in contrast to those inoculated with *Xanthomonas axonopodis pv cassavae,* and a combination of both bacteria. In a study by Mbaringong *et al* (2017) on selected cassava cultivars grown in Kenya vulnerable varieties to cassava bacterial blight (CBB) were observed to have high incidences compared to those that were resistant. The difference might be due to the fact that *Xanthomonas phaseoli pv manihotis* has been reported to be more aggressive compared to *Xanthomonas axonopodis pv cassavae* hence capable of colonizing susceptible plants faster (Pereira *et al*, 1999). Furthermore, *Xanthomonas axonopodis pv cassavae* has been reported to be able to produce effector molecules capable of leading to the suppression of cassava defense mechanism leading to rapid establishment (Zárate-Chaves *et al*., 2021). There was also significant difference in severity scores across all the different treatments. Varieties inoculated with *Xanthomonas phaseoli pv manihotis* had the highest severity score compared to those inoculated with *Xanthomonas axonopodis pv cassavae* or a combination of both pathogens.

The severity scores recorded were moderate as none of the treatments registered an average score of more than three in the overall experiment. Which is in agreement with what has been reported by Simiyu *et al* (2022). Varieties mm 96/2480, Naro 56, and mm 96/1871 had high severity score for *Xanthomonas phaseoli pv manihotis* while mm 96/2480 had the highest score for *Xanthomonas axonopodis pv cassavae*. Verdier *et al* (1994) also observed varieties inoculated with *Xanthomonas phaseoli pv manihotis* were affected more devastatingly compared to those inoculated with *Xanthomonas axonopodis pv cassavae* and the defense response in susceptible varieties was much slower compared to the cassava bacterial blight resistant varieties. This is also consistent with observations by Pereira *et al* (1999) and Onyango and Mukunya (1980).

In another study by Simiyu *et al* (2022) vulnerable varieties were also found to have high severity scores when evaluated for cassava bacterial blight pathogen *Xanthomonas phaseoli pv*

manihotis and variety mm 96/2480 was found to be one of the most susceptible varieties in that study. Additionally, we also observed in the study that *Xanthomonas phaseoli pv manihotis* is capable of invading the vascular system of cassava while *Xanthomonas axonopodis pv cassavae* remains confined to the foliar parts. This is consistent with other studies that have observed that *Xanthomonas phaseoli pv manihotis* is capable of causing systemic infections while *Xanthomonas axonopodis pv cassava*e remains domiciled on the foliar parts of the plant refraining from invading the vascular tissues of the plant (Zareta *et al*., 2021). However, when co-inoculations were done with both pathogens symptoms developed much slower but the disease became severe by the end of the evaluation period. This might be because of the ability of *Xanthomonas phaseoli pv manihotis* to overcome the temporary inhibitory effect of *Xanthomonas axonopodis pv cassavae* as observed by Kwena *et al* (1992) and Verdier *et al* (1994). Susceptible plants have been shown to respond slowly to CBB infection compared to resistant plants (Kpémoua *et al.* 1996; Lopez *et al*., 2007, Wydra *et al*., 2004, Zinsou, 2001) and this has been associated with the presence or absence of certain factors that make the plant either susceptible or resistant in the presence of the CBB pathogens (Zeng *et al*., 2018). For example, *Xanthomonas phaseoli pv manihotis* has been reported to induce the *MeSweet*10a gene which has been shown to have suppressive effect on plant immunity (Cohn *et al*., 2014). In other studies, Wei *et al* (2017), Li *et al* (2017), and Liu *et al.*, 2018, *MeRAV1* and *MeRAV2*-melatonin biosynthesis genes, *MebZIP3* and *MebZIP5, and MeWRKY75–MeWHY3* have been reported to play a crucial part in cassava bacterial blight resistance response as plants that had these genes silenced showed increased susceptibility to the disease indicating that susceptible plants may be expressing these genes at low levels compared to resistant plants. The difference in severity between varieties inoculated with *Xanthomonas phaseoli pv manihotis* and XAC was also observed in an experiment by Pereira *et al* (1999) where varieties inoculated with *Xanthomonas phaseoli pv manihotis* started to defoliate by the second week after inoculation compared to those inoculated with *Xanthomonas axonopodis pv cassava*e which had most of their infected leaves still on the plants four weeks after the inoculation.

The high severity scores observed in susceptible plants may also be due to slow or lack of deposition of lignin and other phenolic compounds, lack of formation of suberin and tyloses, lack of production of latex with high content or PR-proteins, lack of leaf level resistance such as cell wall pectin which are crucial in checking disease development in cassava (Kpémoua *et al*.,

1996; Pereira *et al*., 2000; Cooper *et al*., 2001; Wydra *et al*., 2004; Mabringon *et al*., 2017). Furthermore, there was significant difference in the area under disease progress curve values by the end of the experiments. Most of the varieties across the different treatments had values of above 50%. All of the varieties inoculated with *Xanthomonas phaseoli pv manihotis* had area under disease progress curve values of over 50% with the seriously affected ones being mm96/2480, Naro 56 and mm96/1871 which had values of over 80. Among the varieties inoculated with *Xanthomonas axonopodis pv cassava*e only varieties Fumbachai and mm 96/3567 had area under disease progress curve values slightly below 50%.

Lastly, varieties inoculated with both *Xanthomonas axonopodis pv cassava*e and *Xanthomonas phaseoli pv manihotis* all had values above 50%. A similar observation was made by Pereira *et al* (1999) where a difference in area under disease progress curve values was observed between varieties inoculated with *Xanthomonas phaseoli pv manihotis* and *Xanthomonas axonopodis pv cassava*e with varieties inoculated by *Xanthomonas phaseoli pv manihotis* having the highest values. A similar observation was also made by Odongo *et al* (2019) who found that most of the Kenyan varieties they evaluated had area under disease progress curve value of above 50% which they reported as susceptible to cassava bacterial blight. This indicated that most of the varieties cultivated in Kenya had low levels of resistance to cassava bacterial blight which is similar to we observed in this study and other observations made in other studies (Bart and Taylor., 2017; Teixeira *et al*., 2021). Mbaringong *et al* (2017) also found that most of the varieties grown in Kenya had high levels of area under disease progress curve showing that most were highly susceptible with the majority of those recognized as resistant having moderate levels of resistance. Furthermore, the results show that *Xanthomonas phaseoli pv manihotis* is the more severe of the two causal agents. This is consistent with findings by Onyango and Mukunya (1980)

4.6 Conclusion

All of the seven varieties evaluated for cassava bacterial blight resistance in this study were susceptible to cassava bacterial blight as all had an area under disease progress curve of 50% or more. All varieties showed susceptibility to both pathogens and *Xanthomonas phaseoli pv manihotis* was more severe of the two. This is because varieties infected with *Xanthomonas phaseoli pv manihotis* had the highest severity scores compared to varieties inoculated with *Xanthomonas axonopodis pv cassavae*. The most affected varieties were mm 96/2480, Naro 56, and mm 96/1871. The fresh biomass was lowest on varieties inoculated with *Xanthomonas phaseoli pv manihotis*. Therefore, these findings highlight the need of continued evaluation of the cassava germplasm within Kenya to find one that can be recommended to farmers.

CHAPTER FIVE

GENERAL DISCUSSION

The results from the study indicated that cassava bacterial blight is widely spread within Western Kenya with incidences of up to 100% on some of the farms visited. This is in agreement with recent studies by (Livoi *et al*., 2021; Odongo *et al*., 2019; Chege *et al*., 2017) who have shown that cassava bacterial blight is prevalent in the cassava belts across. This might be due to the fact that many farmers still rely on informal seed systems to obtain their cuttings and these cuttings are usually from previous crops which has been shown to be a major factor in disease spread **(**Soro *et al*., 2022; Mwango'mbe *et al*., 2013**)** especially since cassava bacterial blight pathogens can reside latently in cuttings (Mbaringong *et al*., 2017**)**. Other studies have also reported that distance from seed multiplying institutions might be a factor in access to certified cuttings (Kidasi *et al*., 2021). This is evident in the study as more farmers in Teso south reported to get their cuttings from Kenya Agricultural and Livestock Research Organization(KARLO) compared to Nambale as KALRO is located in Teso south. Furthermore, most of the cassava that is presently being grown by farmers have been reported to be extremely vulnerable to the disease which might be the cause of the high prevalence (Bart and Taylor., 2017; Odongo *et al*., 2019).

The results from the isolation, biochemical and pathogenicity tests done most of the cultural and biochemical characteristics were similar to those observed by (Livoi *et al.,* 2021; Ogunjobi *et al*., 2007). Bacterial growth within the laboratory after 24hrs showed two bacterial isolates with white and yellow colonies. These characteristics have been observed in other studies and have been proposed to be a basis for differentiating cassava bacterial blight causal agents (Zárate-Chaves *et* al., 2021). However, Odongo *et al*., (2019) reported that colony pigmentation may change after 3-4 days which was not observed in the study as all the colonies retained as similar pigmentation post the period. Both bacteria had convex colonies with entire margins, and a glossy surface which is similar to reports that have been made by Livoi *et al*., (2021) when they evaluated isolates from the coastal region. Both bacteria could be able to utilize sugars like sucrose, glucose and maltose but none could breakdown lactose or cellobiose. In other studies, slight variations in the utilization of maltose have been observed as *Xanthomonas axonopodis pv cassavae* has been reported to degrade the sugar at a slower rate compared *Xanthomonas phaseoli pv manihotis* (Ogunjobi 2007; Mooter*et al*., 1986)). Nonetheless, none

of these biochemical test has been shown to be reliable in differentiating the two causal agents up to the pathovar level and this was the case in the study (Odongo *et al*., 2019). Therefore, pathogenicity tests were conducted as both causal agents have been reported to differ in their virulence.

The pathogenicity test showed that *Xanthomonas phaseoli pv manihotis*is more severe than *Xanthomonas axonopodis pv cassava*e as it is capable of causing systemic infections while the latter only induced foliar infections. This is similar to observations by (Pereira *et* al., 1999). The results also indicated that of the two pathogens *Xanthomonas phaseoli pv manihotis* is the most prevalent compared to *Xanthomonas axonopodis pv cassava*e. This is similar to reports by (Livoi *et al*., 2021; Onyango and Mukunya 1980) who showed that due to some environmental factors yet to be understood *Xanthomonas axonopodis pv cassava*e was less prevalent compared to *Xanthomonas phaseoli pv manihotis*. The study also showed that most farmers were unaware of the disease, and most of them were not doing anything to mitigate it. This is similar to findings by (Zárate-Chaves *et al*., 2021) also indicated that most farmers are unaware of the disease. In another study by Livoi *et al*., (2021) in Kenyan coast it was reported that though most farmers had interacted with the symptoms of the disease majority did not associate it to any cassava disease. This might be due to the fact that much attention had been focused on cassava viral diseases compared to other diseases like cassava bacterial blight (Ng'ang'a *et al*., 2019). Hence, most farmers have been slow to take up any mitigation measures due to ignorance from lack of awareness leading to the increased prevalence of the disease.

The greenhouse evaluation of cassava plants also showed that *Xanthomonas phaseoli pv manihotis* infected plants were the most afflicted by cassava bacterial blight compared to *Xanthomonas axonopodis pv cassava*e inoculated. The most affected plants were mm96/2480, Naro 56 and mm96/1871.The area under disease progress curve (AUDPC) values across most of the bacterial treatments was above 50%. This is in agreement with studies by Odongo *et al*., (2019) and Mbaringong *et al*., (2017) who observed that the AUDPC values of inoculated plants was above 50%. The average severity score for most of the cassava plants was moderate at the end of the evaluation period similar to observations by Simiyu *et al*., (2022)**.** The reason for the high AUDPC values might be due to the in ability of the plants to activate their defense mechanisms against cassava bacterial blight fast as it has been reported that susceptible plants

respond much slower compared to resistant plants when affected by cassava bacterial blight. *Xanthomonas phaseoli pv manihotis* has also been shown to induce factors that increase susceptibility (Cohn *et al*., 2014; Wei *et al*., 2017; Li *et al*.,2017; Liu *et al.*, 2018) in vulnerable plants which might explain its increased severity compared to *Xanthomonas axonopodis pv cassava*e. Cassava plants inoculated with both causal agents also showed slow growth at first but increased in cassava bacterial blight severity afterwards. Similar findings were reported by Kwena *et al* (1992) and Verdier *et al* (1994) who showed that *Xanthomonas axonopodis pv cassava*e might have some temporary inhibitory effects on *Xanthomonas phaseoli pv manihotis* but it can overcome it later and induce a severe systemic infection.

5.1 Conclusion

These results reveal that cassava bacterial blight and its causal agents is extensively distributed within Busia at high incidence levels. Of the two causal agents *Xanthomonas phaseoli pv manihotis* is more wide spread compared to the *Xanthomonas axonopodis pv cassavae*. Furthermore, most famers are unaware of the disease indicating that this might be the main factor for the high cassava bacterial incidence. Additionally, most of the farmers surveyed relied on cuttings from neighbors or self for crop establishment. This might have contributed to the high incidence of the disease since the causal agents can survive latently within cuttings. None of the biochemical test used could identify both pathogens to the species or pathovar level. Although pathogenicity test was able to distinguish both pathogens based on symptoms it could not identify them to species or pathovar level. Therefore, more awareness on the disease needs to be done through extension services, molecular techniques should be used to further characterize both pathogens, and disease free cuttings should be produced and distributed to farmers. Moreover, all of the seven varieties evaluated for cassava bacterial blight resistance in this study were susceptible to cassava bacterial blight as all had an area under disease progress curve of 50% or more. All varieties showed susceptibility to both pathogens with *Xanthomonas phaseoli pv manihotis* being the more severe of the two. This is because varieties infected with *Xanthomonas phaseoli pv manihotis* had the highest severity scores compared to varieties inoculated with *Xanthomonas axonopodis pv cassavae*. The most affected varieties were mm 96/2480, Naro 56, and mm 96/1871. The fresh biomass was lowest on varieties inoculated with *Xanthomonas phaseoli pv manihotis*. Therefore, these findings highlight the need of continued

evaluation of the cassava germplasm within Kenya to find one that can be recommended to farmers

5.2 Recommendations

Based on the study findings, the following actions are recommended:

i. Continued surveillance of cassava bacterial blight prevalence to inform adjustments or change in how management is approached.
ii. Encourage use of certified cassava cuttings by farmers when establishing their plantations
iii. Improve the capacity of Community based organizations, Nongovernmental organizations, and Agricultural institutes through training and infrastructural development to produce enough disease free cuttings for farmers.
iv. Apply molecular methods to study the underlying differences in virulence and pathogenicity by the two cassava bacterial blight causal agents *Xanthomonas phaseoli* pv *manihotis* and *Xanthomonas axonopodis* pv *cassavae*.
v. Use molecular techniques to better understand the resistance reaction against cassava bacterial blight pathogens.
vi. Evaluate more cassava germplasm for resistance against CBB in the field and green house for better deployment of the germplasms.

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APPENDIX

Appendix I. Questionnaire used during baseline survey

SECTION 1

Data Collection Guidelines

Sampling Date: …… Serial Number…….

	County……. Sub-County……..… Location ……. Village …….	Ward…….…… ……. GPS coordinates…… ….. Longitude _____
	Name of Farmer …….	Sex: A) Male () B)Female ( ) Age: <35 (youth) Middle age 36-51 >51-60 upper middle age >60 retirees
	Head of household	Farm size in acres < 2 ( ) 2-5 ( ) 5- 15 ( ) 4)15-50 ( )

		50 + ( )
	Occupation	1) Formal Employment [ ] 2)Casual Employment Time [ ] 3)Business Man [ ] 4) Full Farmer [ ] 5)Other (Specify)
	Academic Qualification	None ( ) Primary ( ) Secondary() Tertiary ( )
	Annual rainfall (mm) Long rains..... Short Rains.....	Temperatures (^{0}c) Long season ..... Short season.....

SECTION 2

Land preparation

When do you expect your rain and when do they end?

Short rains …….

Long rains …….

a) Have you ever experienced non formal agricultural training?

1. yes ( )

2. No ( )

b) If yes, who did the training?

1. Government ( )

2. Non-Government ( )

When do you do your land preparation

……
……
……
…….

How do you prepare your land for cassava production?

……
……
……
……
…….

Do you practice any soil conservation management?

Yes

No

5b) if yes, which soil conservation measures?

……
……
……
……

……
……

What is the total current area under crops?(categorize based on top categories

……..

a) What is the total current area under cassava? categorize as above

……
……
……
……

7. b) On the farm where you have cassava, which was the previous crops?

……
……
……
……

Why do you grow cassava?

……
……
……
……
……
……
……

8b) Which varieties do you grow

Variety	priority
….....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....

8c) which of those varieties do you prefer

8d) Why prefer that variety (list)

1……..

2……..

3……..

9.a) When did you start growing cassava

……

a) When did you start growing the improved cassava variety? (code in terms of years) recall of 10 years

Variety	Year
…… …	……
…….	……
…….	……
…….	……
…….	……
……...	……

What is the spacing for your cassava crop?

……..

a)Do you intercrop or plant cassava alone?

1. Yes ( ) 2. No ( )

11b) Which crops do you use for intercrop with cassava field? (list)

Crops intercropped	order of priority
.....	.....
.....	.....
.....	.....

....

....

11c) Why do you intercrop? (list reasons)

1……..

2……..

3……..

4……..

12.a) Where did you source your planting material? (tick appropriately)

1)Local market ( )

2) Own seed ( )

3) Neighbors ( )

4) KALRO ( )

5) Other, specify …….

12b) What is the reliability of the source of the planting materials?

1) Extremely reliable( )

2) Moderately reliable ( )

3) Low ( )

12c) How frequently do you source your planting materials?

……..

13.a) Do you use farm inputs?

1. Yes ( ) 2. No ( )

13b) If yes which one? (list) Fertilizer/manure?

……

……..……

….

……

……..……

….

13.c)When do you apply the input?(fertilizer/manure)

…….

…….

…….

14.a)When do you plant?

1) Long rains ( ) 2) Short rains ( ) 3). Both ( )

14. b) Specify the month

…….

15. Do you experience pest and disease infestations?

1) Yes ( ) 2) No ( )

15.b) If yes, name the common pests and diseases

Pests Diseases

....

....

....

....

....

....

15.c) How do you identify different pests and diseases?

Signs

……

……

……

……

Symptoms

……

……

……

……

15.d)Do you practice scouting for pests and diseases?

Yes ( ) 2. No ( )

15.e)If yes, how often do you scout?

A) regularly ( )B) Rarely ( )C)Never ( )

15.f)When are the stated pests and diseases most prevalent?

Pests when

_____ _____

_____ _____

_____ _____

_____ _____

Diseases When

_____ _____

_____ _____

_____ _____

_____ _____

15.g) Which management practices do you use? on pests and diseases. (list)

1……

2……

3……..

4……..

5……..

6……..

15.h) If chemicals, which type of chemicals do you use?

……

……

……

16.a) Do you own a sprayer?

Yes ( ) 2 NO ( )

16. b)If no, Where do you borrow the sprayer from?

……
…

……
…

16.c)How often do you spray

……
…

17.a). Have you ever received any information on cassava production?

1) Yes ( ) 2) No ( )

17.b))If yes, what is/are the source of the information

1) Extension staff ( )

2) Media -Radio/T. V/Newspaper ( )

3) Agro input dealer ( )

4) From other farmers ( )

5) Research ( )

17.c)What kind of technical information do you receive ?

1)Extension staff

……

……..

Media -Radio/T. V/Newspaper

……

……..

Agro input dealer

……

……..

from other farmers

……

……..

Research

……

……..

17.d) How often do you receive the information on cassava

1. Weekly ( ) 2. monthly ( ) 3.quarterly ( )

4. Semiannually ( ) 5. annually ( )

18. Where do you get information for new variety?

……

……..

20. What challenges do you face in cassava production?

……

……..

……

……..

……

……..

SECTION 3

Do you know cassava bacterial blight?

1.Yes ( ) 2) NO ( )

If yes, when is the first you encountered cassava bacterial blight in your farm?

2020 ( ) 2) 2019 ( ) 3) 2 018 ( ) 4) > 2018( )

When is the cassava bacterial blight highly prevalent

Long rains ( ) 2. Short rains ( )

How do you identify cassava bacterial blight?

in the leaves

……

……

……

……

in the stem

……

……

……

……

5.a)On which cassava varieties have you seen the cassava bacterial blight?

…….

……

……

……

……

……

6.When do most cassava plants show the symptoms of infection.

Young crops <3 months ( )

Mature crops 3-6 months ( )

Older crops > 6 months ( )

7.Any management practice you apply to manage the cassava bacterial blight ?

Roughing ( )

None ( )

Biological ( )

Chemical ( )

Crop rotation ( )

SECTION 4

CASSAVA PLANTING MATERIALS

1.Prices of the cassava cuttings

How many cassava cutting do you plant?

0- 150 stems [ ] 2. 150-200 stems [ ] 3. 200-250 stems [ ] 4. 250-300 stems [ ] 5. >300 stems [ ]

Of the above, how much do you buy?

…….

what is the cost of the cassava cuttings per stem (1-2 ft)

Kshs 1.00 [ ]

Kshs. 2.00 [ ]

Kshs. 2.50 [ ]

Kshs. 3.00 [ ]

>Kshs. 3.00 [ ]

2.Planting and sprouting of cassava

a. Is there any treatment done on the cuttings before planting?

1.) Yes [ ] 2) No [ ]

b. If yes, which treatment do you do before planting of the cuttings?

Adding water [ ]

Adding bioactivators [ ]

Others [ ]

c.What is the time taken for the cuttings to sprout after planting?

1 week [ ]

2 weeks [ ]

3 weeks [ ]

> 3 weeks [ ]

d. What is the uniformity of the sprouting?

Uniform [ ]

Not uniform [ ]

e. For the sprouting , what is your source of water

Rains [ ]

Irrigation [ ]

Both [ ]

3.Tissue culture cassava

a. Do you plant tissue culture cassava?

1) Yes [ ] 2) No [ ]

b.If yes, where do you obtain them from ?

KARI [ ]

NGOs [ ]

Private sector individuals [ ]

Others [ ]

c.How frequently do you source them ?

Only once during planting then reuse its cutting continuously [ ]

I source the cuttings every season [ ]

Others [ ]

d.Which part of the planting material do you source?

Whole tissue culture plant [ ]

The tissue culture cuttings [ ]

e.What is the price of the tissue culture materials?

Whole tissue culture plant

Kshs. 5.00 [ ] 2. Kshs10.00 [ ] 3. Kshs. 15.00 [ ] 4. >Kshs. 15.00 [ ]

The tissue culture cuttings

Kshs. 1.00 [ ] 2. Kshs. 2.00 [ ] 3. Kshs. 3.00 [ ] 4. Kshs. 4.00 [ ] 5. >Kshs. 4.00[ ]

f. How many bags of cassava roots do you harvest from tissue culture planting materials ?

Whole tissue culture plant

…….

The tissue culture cuttings

…….

g) What are the preferred traits of the of the tissue culture cassava?

Taste [ ]

Can be intercropped [ ]

High yields [ ]

Early maturity [ ]

Others [] Specify

……

……

Section5:

Utilization of cassava roots:

List in Oder of Importance:

1.

2.

3.

4.

5.

6.

7.