PREVALENCE OF CASSAVA BACTERIAL BLIGHT IN KENYAN COAST, ITS CHARACTERIZATION AND EARLY MANAGEMENT IN PLANTING MATERIALS

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This thesis is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

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LIST OF ABBREVIATIONS AND ACRONYMS

ACMV African Cassava Mosaic Virus

CABI Centre for Agriculture and Bioscience International

CBB Cassava Bacterial Blight

CBSD Cassava brown streak disease

CIAT International Centre for Tropical Agriculture

CMD Cassava mosaic Disease

EPAR- Evans School of policy analysis and Research

FAO Food and Agriculture Organization

FAOSTAT Food and Agriculture Organization Statistic Database

FGD Focused group discussion

IITA International Institute of Tropical Agriculture

KALRO Kenya Agriculture Livestock and Research Organization

KEPHIS Kenya Plant health inspectorate service

Mcg- Micrograms

MOA Ministry of Agriculture

MT Metric Tones

NRTS National root and Tuber Strategy

SPSS Statistical Package for Social Sciences

T.C Tissue Culture

Xac *Xanthomonas axonopodis* pv. *cassavae*

Xam *Xanthomonas axonopodis* pv. *manihotis*

YPG Yeast Peptone Glucose

YPGA Yeast Peptone Glucose Agar.

GENERAL ABSTRACT

Cassava (*Manihot esculenta*) is one of the major crops among the roots and tubers consumed in Kenya and Africa at large. It is one of the strategic food security crops in rural livelihoods. The crop is faced by biotic constraints that hinders its maximum productivity. These include Cassava bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *cassavae* and *Xanthomonas axonopodis* pv. *manihotis*, as one of the major cassava diseases after cassava mosaic disease and cassava brown streak disease. Cassava bacterial blight is distributed in 24 countries in Africa and this is due to sharing of infected cuttings among farmers. In Kenya, cassava bacterial blight was first reported in 1980 in the Western region. Lack of healthy clean planting cassava materials, lack of resistant cassava cultivars and poor seed system for cassava are contributing to the spread of the disease nationwide.

This study was conducted to determine the prevalence of cassava bacterial blight in Kilifi and Taita Taveta counties, the existence of the two bacterial pathovars causing CBB, characterizing them and using tissue culture technique to manage the disease. The study involved a focused group discussion and a baseline survey in the two counties. This involved purposive random sampling where 250 cassava farmers were selected and interviewed using a semi- structured questionnaire. Disease assessment was done to establish prevalence as well as sample collection from cassava fields. The collected samples were isolated in the lab and the pathogens were characterized using cultural methods. Tissue culture experiment was set in the laboratory in which raised plants were subjected to a treatment of three antibiotics at optimized concentrations of 5mg/l, 10mg/l, 15mg/l, and 20mg. /l to eradicate CBB causing bacteria and raise healthy cassava seedlings.

From the survey, most farmers were not informed about Cassava bacterial blight. Most of the interviewed farmers (80%) did not practice any management of CBB. Cassava bacterial blight pathogens; *Xanthomonas axonopodis* pv. *cassavae* (Xac) and *Xanthomonas axonopodis* pv. *manihotis* (Xam) were present in both counties with *pv. manihotis* (Xam) as the dominant pathovar. All the varieties (Tajirika, Kibandameno, Kaleso, Shibe) grown in the region were found susceptible to CBB. Among the 70 samples collected 40 samples (11 from Taita Taveta and 29 from Kilifi County) were positive of CBB. Kilifi County had an incidence of 22% while Taita Taveta County had 13% incidence. The two pathovars *Xanthomonas axonopodis pv. cassavae* and *Xanthomonas axonopodis* pv *manihotis* were present in both counties.

Xanthomonas axonopodis pv. manihotis was white to creamish while *Xanthomonas axonopodis pv. cassavae* was yellow-pigmented. Both the pathogens were gram- negative, motile, hydrolyzed gelatin, hydrolyzed starch, and were catalase positive. Both pathovars did not grow on pH below 4.5 neither in salt concentration above 4%. *Xanthomonas axonopodis pv. cassavae* utilized cellobiose while *Xanthomonas axonopodis pv. manihotis* did not. *Xanthomonas axonopodis pv. manihotis* utilized maltose while *Xanthomonas axonopodis pv. cassavae* did not utilize maltose.

Incorporating antibiotics in tissue culture media significantly inhibited bacteria. All the antibiotics suppressed the growth of the bacteria in the media by reducing the colony growth rate. The concentration rate of 20mg/l inhibited bacterial growth but did not allow for optimal plant growth hence not preferred for mass propagation of TC plants. Tetracycline and Streptomycin were found to be the best in suppressing CBB bacteria in tissue culture media at a concentration of 15 mg/l and 20mg/l. In conclusion, CBB is prevalent in Kilifi and Taita Taveta counties, most farmers lack awareness and don’t manage the disease. The tissue culture technique is a better way to manage the disease and increase access to healthy cassava planting materials.

CHAPTER ONE: INTRODUCTION

1.1 Cassava production in Kenya

In the world, cassava provides about 500 million people with food and is important for livelihood improvement in rural communities. It has been ranked fifth after wheat, maize, rice, and Irish potato although its enormous potential has not been fully utilized. In Africa, it is one of the important food crops which provide more than half of the dietary calories to both urban and rural populations in Sub-Saharan Africa (Hillocks, 2002). Cassava adjusts well in difficult conditions such as those of acidic infertile soils, low and unreliable rainfall, and poor agronomic management. Therefore an ideal food security crop in times of extended drought and famine (Burns *et al*., 2010). These characteristics make it a preferred alternative crop for providing food for a growing population in the future (Jarvis *et al.,* 2012).

Cassava is widely adopted in most regions in Kenya majorly western Kenya, Eastern Kenya, and coastal Kenya and across various agro-ecological zones. (Karuri *et al.,* 2001; Abong' *et al*.,2016).The crop has been regarded by many as a crop for the arid and semi-arid lands and even termed as "poor man's food"(G. M. Githunguri, 1995).

Its production is constrained by insect pests and diseases, lack of mechanization, poorly developed value chains, decreasing soil fertility, inadequate quality, healthy planting material and poor agronomic practices (Bull *et al.,* 2011). Pests and diseases, cause economic losses that are valued at $ 100million and are projected to increase if no adequate management strategies are put in place (Mohamed *et al*., 2012). Besides, pests and diseases mainly viruses, bacteria and arthropod pests are easily transmitted through the planting materials (Lozano, 1986). The pathogens and pests are not easily detected with naked eyes and for this reason, CMD, CBSD and bacterial blight of cassava are widely spread as they are embedded within the planting materials. Furthermore, during

prolonged periods of drought and famine, the stakes are not available for planting hence limiting further production of the crop.

The bacterial blight which has remained to be a great threat to cassava requires an integrated approach strategy in management. This includes the use of clean healthy planting materials, crop rotation, uprooting the infected crop and controlling vectors in already infected farms. There is a need to develop a seed system based on micro propagation to avail planting materials all the time and to develop management strategies that will lessen the impact of disease problems in the farmers' cassava fields. According to Munyi and De Jonge (2015), there is renewed attention on seed and food security in Africa causing new thinking on the role of seed sector development in the face of climate change and food price volatility threats. The bulk of seed and planting material for vegetatively propagated crops such as sweet potato (96%), cassava (93%), bananas (80%), and Irish potato (96%) is mostly got from farm-saved seed sources. Farm-saved seeds do contribute to agricultural productivity and need to be backed up by phytosanitary measures.

1.2 Problem statement

Production of cassava is facing challenges, both biotic and abiotic. Among the biotic challenges includes pests and diseases. Diseases remain a great limiting factor in the production of cassava since they lead to crop yield reduction, cause barriers to trade due to phytosanitary issues, reduced market value by lowering quality on infected produce, farm losses which bring food insecurity and loss of planting material required for continuation of the next crop. Cassava Bacterial blight exists as one of the major diseases infecting cassava. Cassava bacterial blight is reported to have caused big losses in cassava production in Sub-Saharan Africa in the 1970s in an outbreak that occurred and caused yield losses of up to 75% (Lozano, 1986).

In Kenya, the disease was reported in Western Kenya after the surveys done by Onyango and Mukunya in 1982. Documentation on the losses is yet to be done though the disease continues to spread and cause economic losses in cassava growing regions in the country across western Kenya, rift valley, coastal Kenya, and Eastern regions. (Odongo, Miano, Muiru, Mwang, & Kimenju, 2019) .The disease exists in latent form in stems which are used by farmers as cuttings for planting, most farmers remain ignorant about the disease and continue sharing cuttings and therefore contributing towards a wide distribution of the disease countrywide. Cassava propagation is done through tissue-based plantings but mainly cuttings and these cuttings are the major ways in which the pathogen *Xanthomonas axonopodis* pv. *manihotis* and *Xanthomonas axonopodis* pv. *cassavae* spread. The pathogen can survive in plant tissues without causing symptoms. (Boher and Vardier, 1994).The gaps in the knowledge about cassava bacterial blight include: detection of the diseaes in cuttings, characterization of the two pathovers responsible for the disease, proper management practices by farmers, to what extent the diseaes is distributed in the country and if both pathovers responsible for cassava bacterial are in the country. There is so far no certification done for cassava planting materials and still, there are no certified sources that can guarantee farmers clean healthy planting materials. The seed system is not well established in order to be used as a start point in managing cassava bacterial blight.

1.3 Justification

Cassava production in Kenya is on the increase an indication that many people are involved in the production. The importance of the crop as a food security crop among the rural livelihoods has called for more efforts to protect the crop from diseases which are the major constraint to its production. To minimize the spread of cassava diseases among farmers in Kenya requires an understanding of the diseases and knowing the farmers' awareness of the diseaes in the regions.

Cassava diseases are spread through planting materials hence this research surveyed to establish the distribution of CBB in the farmers' fields and be able to know disease-free farms which can be used to access clean planting materials.

Cassava bacterial blight is caused by two pathovers, Xam and Xac, and this study establishes the existence of the two pathovers in the country which will help in the management and better understanding of the pathogen in the CBB affected regions.

One way of managing the diseaes is using tissue culture to produce diseaes free planting materials. The study purposed to generate information about the distribution of the disease in Kilifi and Taita Taveta Counties, identify the causal pathogens, and establish ways of disinfecting materials in tissues culture as a contribution towards developing a better seed production system.

1.4 General objective

To increase cassava production through the accessibility of Cassava Bacterial Blight free cassava planting materials.

1.5 Specific objectives.

1) To determine the prevalence, distribution, and incidence of cassava bacterial blight in the coastal region (Kilifi county and Taita Taveta county) of Kenya
2) To characterize *Xanthomonas axonopodis pv. manihotis* and *Xanthomonas axonopodis pv cassavae* from infected cassava samples using cultural methods.
3) Recovery of CBB infected plant materials by eradication of the pathogens using antibiotics in tissue culture propagation medium

1.6 Hypothesis

1) Establishing the distribution of cassava bacterial blight in the Kenyan coast will help in establishing areas free from the pathogen

2) Characterizing the *Xanthomonas axonopodis pv manihotis and Xanthomonas axonopodis pv cassavae* will establish the widespread species in the region.

3). Eradication of cassava bacterial blight causal pathogens using antibiotics in tissue culture propagation medium is feasible.

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin of cassava

Cassava (*Manihot esculent Crantz*), is a tropical root crop grown majorly for its roots. (Nweke, 2005) The crop is reported to be native to South America and introduced in Africa by Portuguese explorers during the 16th and 17th centuries through their trade within the African coastal regions (Nweke, 2005). The crop is grown mostly by low-income and smallholder farmers though its spread is limited due to its nature of propagation which involves the use of stem cuttings(Olsen & Schaal, 2014). In 1972, IITA based in Nigeria was formed with the mandate to promote the cassava and other root and tuber crops(Nweke, 2005). It is one of the few staple crops that do well on a small scale with fewer labor requirements as compared to cereal crops(Hillocks, 2002). The crop can perform well in less fertile soils and can withstand fewer rainfall conditions hence preferred by resource-limited farmers in sub-Saharan Africa for food security and income generation(El-Sharkawy, 2004). Currently, in Africa, more than 40 countries grow cassava with Nigeria and DRC Congo being the largest producers of cassava (FAOSTAT 2013). Predictions by FAO in 2005 show that Africa will produce 60%of the global cassava by 2020. In Kenya, cassava is widely grown in western , coastal regions of and the eastern parts.

2.2 Botany of cassava

Cassava is a woody perennial shrub, which has the potential to grow from 1metre to 5 meters. It is a monoecious crop having both the male part and the female part on the same plant. The crop is propagated from stems but can also be propagated from the seeds. The cuttings are most preferred by farmers since when placed in soil under favorable conditions they sprout and produce adventitious roots within one week and form strong plants compared to those propagated by seed. (Hillocks *et al.*, 2001). The cassava is an evergreen crop and does well in arid and semi-arid

regions and is hence preferred as a climate change mitigation crop. Cassava roots are made of 60% water. Their dry matter is very rich in carbohydrates, of about 250 to 300 kg for every tone of fresh roots. When grown for food the root is used as food and the best time to harvest is from 8 to 10 months after planting although some varieties take longer to mature and the longer the growing period the higher the starch yield is produced (Hillocks *et al*., 2001).

2.3 Cassava production in the world

Cassava is widely used across the world as one of the major sources of daily caloric source to millions of people in tropical America Africa and Asia. It is and also one of the oldest root and tuber crops utilized by humans to produce food, feed, and beverages. Currently, the crop is produced in approximately 100 countries across the world. According to FAO statistics 2018, the current world production of Cassava stands at 277, 808, 759 metric tons. Nigeria is the leading world cassava producer with the main utilization being in form of starch. Its production stands at 60 million metric tons annually FAOSTAT 2019, is followed by Thailand Indonesia and Brazil.

2.4: Cassava production in Africa

Cassava is the third main source of carbohydrates in Africa. It is consumed by 700 million people in Africa hence a vital food security crop. It is a source of income for subsistence farmers, therefore, contributing towards economic development in rural Africa. It is widely adopted in Western, central and eastern Africa with many considering it due to the low cost of production as compared to cereals. Its ability to be grown as a famine reserve crop in sub-Saharan Africa has led to almost every household having a few stems of cassava within the farm yard.

Its status is now evolving with the uptake of industrial activity to produce starch, biofuel, and livestock feed. This has led to cassava being grown as a commercial crop in major cassava-

producing countries in Africa. Nigeria is the largest exporter of cassava in Africa and it contributes to 20.4% world share followed by DR.C Congo at 14.72 % (Olutosin *et al.,* 2019)

2.5 Cassava production in Kenya

Cassava is one of the major staple foods after maize in Kenya. It is widely grown throughout Kenya. This includes the western, coastal region and eastern regions which account for 60%, 30%, and 10% cassava production, respectively (Githunguri & Gatheru, 2017). It enhances household food security and is a source of income to the Kenyan population. In Kenya, cassava is consumed either roasted; boiled or made into chips or dried and milled to produce flour (Abong et al., 2016). In Kilifi county, among the Giriama community cassava is regarded as one of the main staple food. The roots can be boiled, fried, the leaves are used as vegetables and even other special foods like *Kimanga* are prepared from a combination of cassava boiled roots and legumes like beans (Githunguri *et al*., 1995).

Cassava propagation is done through stem cuttings and most farmers depend on sharing of the materials from farmer to farmer. There are few institutions involved in cassava seed multiplication and distribution such as research institutions and special non-governmental organizations interested in the crop. Among the institutions supporting cassava in Kenya include Kenya Agricultural Livestock and Research Organization (KALRO) which is mandated to research development in agriculture in the country and the universities. International Institute for Tropical Agriculture (IITA) is mandated to work with roots and tubers in Africa has its presence in Kenya.

In 2006 the annual production of cassava in the coastal region was estimated at 107,410 t (MoA, 2007). According to FAOSTAT (2017) data, Kenya's annual production of fresh cassava is at 1,112,000 MT with the demand for the crop being high and estimated to be at 301,200 tones. The

current data is expected to be high due to some varieties that have been developed by the research institutions KALRO and IITA which include Karembo, Karibuni, Tajirika, Nzalauka, Shibe, Siri, and mijera (Obiero *et al.*, 2007*).* The National root and tuber crops development strategy 2019 - 2022 developed by the Ministry of Agriculture to promote roots and tuber production in Kenya with cassava being one of the crops is likely to increase farming and utilization of Cassava in Kenya.

2.6 Economic value of cassava in Kenya

Cassava is regarded among the major sources of carbohydrates in cassava-growing regions in Kenya. (Abong' *et al.*, 2016) This includes western Kenya which is the highest cassava producer, followed by coastal Kenya and eastern Kenya respectively. Cassava is mostly peeled and dried to make flour or it is peeled, cut into small pieces and cooked while it is still fresh. (Abong' *et al.*, 2016) It has attracted a good number of value chain actors who are involved in the milling of cassava flour-like MUHOGO FOODS LTD in Kenya, therefore, creating jobs and earning incomes for the farmers. Several products developed in the cassava value chain include Cassava flour, starch and animal feed. At the domestic level, cassava processed products include deep-fried cassava chips, Cassava meal made of cassava fresh cooked roots mixed with beans and other legumes, boiled cassava tubers and cassava crisps. (Githunguri, 1995)

Africa produces about 55% of world cassava and contributes immensely to food security in Africa as a staple crop. Kenya is still lagging in terms of production, marketing and consumption of cassava and processing into cassava-based products despite the enormous potential of such products. Whereas Nigeria is producing over 47 million tons and Uganda 4 million tons, Kenya is currently producing 1.2million tons annually. There is a great potential for cassava in Kenya at the moment as it is one of the target crops under agenda four-under food and nutrition under MTP

2017-2022. Also, a new policy now allows cassava flour to be mixed with maize and millet flour as the country grapples with lowing food insecurity amongst its population. Further to this, cassava has been recognized as a climate-smart crop especially as the country tries to find some long-lasting solutions to climate change challenges. Thus, the establishment of a facility for producing disease-free Tissue culture seedlings for farmers would ideal.

2.7 Constraints limiting cassava production in the world

Like the rest of the world, Kenya's cassava farming is faced with several challenges. These range from biotic factors and abiotic factors. Cassava diseaes remain a great threat to cassava production across cassava growing regions. These diseaes include Cassava mosaic, cassava brown streak and cassava bacterial blight which are the main diseaes of cassava (Kathurima *et al.,* 2016; Odongo *et al.,* 2019).

Other challenges include pests this are scales, aphids, and mites (Njoroge *et al.,* 2016). Most of the pests attack the crop and hinder the maximum productivity of the crops. Availability and access to clean planting materials remain also a great challenge to the successful production of cassava in Kenya (Mwang'ombe *et al.,* 2013). This affects new farmers who are willing to engage in cassava farming.

The attitude toward the crop being poor man's food, high perishability, lack of processing tools and inadequate capacity building (Abong *et al.,* 2016) has reduced the commercial viability within cassava farming therefore make it not an attractive venture to many farmers.

2.7.1 Pests and diseases affecting cassava

Cassava is affected by several diseases and pests which are hindering successful farming and posing a great challenge to farmers in Kenya, Africa and the world at large. The diseases include

viral diseases, bacterial diseases, fungal diseases and nematodes((Valerie *et al*.,2007) The pathogens are classified depending on the part of the plant they attack. This covers those that attack vegetative propagating material, those that attack foliage and green stem portions, and those that cause root rot inducing pre-harvest and post-harvest deterioration(Lozano & Terryl, 1976)

Cassava pests include Cassava mealy bug (*PhenacoccusManihot*), Cassava green spider mite complex (*Mononychellus tanajoa*), variegated grasshoppers (*Zonocerus variegates*) and Whiteflies (*Bemisia tabaci*).These pests damage both the vegetative parts and eventually the tubers hence depriving the required yield of the farmers. These pests also act as vectors of most cassava diseases and cause wounds on plants that act as secondary avenues for entry of disease-causing pathogens on plants (Night *et al*., 2011)

Diseases of cassava include:

i. Bacterial diseases: Cassava bacterial blight (CBB) caused by two species of Xanthomonads namely *Xanthomonas axonopodis pv. manihotis* and *Xanthomonas axonopodis pv. cassavae* (Maraite et al., 1987), bacterial stem gall caused by *Agrobacterium tumefaciens biovar 1*, bacterial stem rot caused by *Erwinia carotovora* and bacterial wilt caused by *Erwinia herbicola* (Álvarez, Llano, & Mejía, 2010)

ii. Fungal Diseases of cassava include: Anthracnose, Armillaria root rot, shoestring root rot, black root and stem rot, blight Leaf spot caused by *Cercospora vicosae*, brown leaf spot caused by *Cercosporidium henningsii*, Cassava ash caused *Oidium manihotis*, Dematophora root rot, Diplodia root and stem rot caused by *Diplodia manihoti*, Fusarium root rot caused by *Fusarium oxysporum*, Phytophthora root rot caused by *Phytophthora cryogen*, Pythium root rot caused *Pythium spp*, Rust caused by *Uromyces spp*, Sclerotium root rot caused by *Sclerotium rolfsii*, Super

elongation caused by *Sphaceloma manihoticola*, Verticillium root and stem rot caused by *Verticillium dahlia* and White leaf spot caused by *Phaeoramularia manihotis* (Álvarez, Llano, & Mejía, 2010)

iii) Cassava viral diseases include: African cassava mosaic caused by African cassava mosaic virus, Cassava brown streak disease caused by Cassava brown streak virus, Cassava common mosaic caused by Cassava common mosaic virus Cassava frog skin caused by Cassava frog skin, Cassava green mottle caused by Cassava green mottle virus and Cassava vein mosaic caused by Cassava vein mosaic virus (Valerie Verdier *et al., 2007*)

The major diseases of cassava include Cassava mosaic disease, Cassava brown streak disease, Cassava bacterial disease, Cassava brown leaf spot, Common cassava mosaic disease and African cassava mosaic disease(Muhungu *et al.*, 1994; Banito *et al* ., 2007)

Among the named cassava diseases, cassava bacterial blight is caused by Xanthomonas *axonopodis pv. manihotis* formerly known as *Xanthomonas campestris pv manihotis* remains a major cassava bacterial disease hindering successful cassava production. The disease has been reported to cause great losses in Nigeria, Ghana, Uganda, Benin and the Democratic Republic of Congo (Nkongolo *et al*., 2014). Cassava bacterial disease has been reported in Kenya but not much work has been done (Odongo *et al*., 2019).

2.8 Cassava bacterial blight

Cassava bacterial blight, caused by the pathogen *Xanthomonas axonopodis PV. manihotis* and *Xanthomonas axonopodis pv cassavae* is the widest spread disease of cassava. The disease was first reported in Brazil in 1912 and from that time it has spread to all cassava growing areas in the

world namely countries in Asia, Africa and Latin America (Lozano, 1986). In Africa, the disease was first reported in Madagascar in 1946. The pathogen only affects members of the genus manihotis (Lozano, 1986). Currently, the disease is extensively spread in Asia, Africa, and South America. Under suitable conditions for disease development and without any measures to control the disease, it can lead to 100% crop loss (Lozano, 1986). The disease spreads from one area to another via infected planting material. Dissemination can occur over small areas through tools, insects especially the grasshoppers and rain splash (Lozano & Terryl, 1976)

Classification of the pathogen

Xanthomonas axonopodis* pv. *manihotis (Xam)

Domain: Bacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Xanthomonadales, Family: Xanthomonadaceae, Genus: Xanthomonas, Species: Xanthomonas *axonopodis pv. manihotis*

Xanthomonas axonopodis* pv. *cassavae (Xac)

Domain: Bacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Xanthomonadales, Family: Xanthomonadaceae, Genus: Xanthomonas, Species: *Xanthomonas axonopodis pv. Cassavae.*

2.8.1 Symptoms

This pathogen *Xam,* is a systematic pathogen and an epiphyte. The pathogen causes a combination of a wide range of symptoms which makes the pathogen unique among other plant pathogenic bacteria. (Lozano and Sequira, 1974) The combination of the symptoms varies from angular spots in leaves, blight, wilt, exudates and lesions on stems (Valitrie Verdier *et al.*, 1994). Symptoms are

expressed on the leaves, the stem, fruits and petioles as follows: the appearance of the water-soaked lesions on infected leaves starting along the veins, margin, and tips of leaf blades (Ogunjobi & Fagade, 2008). In advancing the diseaes, neighboring spots join together to form large brown patches hence killing the leaf blade as it expands, the leaf dries and later falls. Creamish or yellowish gummy exudates are discharged both on leaves and stems and are more distinctive on leaf petioles of infected plants. Petioles of blighted leaves are often horizontally orientated to the main stem axis (A. A. Fanou, Zinsou, & Wydra, 2018). In a critical stage of the disease, dieback of stems is common and new shoots are often seen developing from dead ends of stems of severely infected plants (A. A. Fanou et al., 2018).

The disease is characterized by the presence of water-soaked, angular spots on leaf lobes where small gummy drops of exudate may be observed (Lozano & Terryl, 1976). The pathogen can sometimes invade stem buds and young branches via the phloem (Lozano & Terryl, 1976). Infected leaves show initial lesions surrounded with yellowish halos that coalesce, inducing yellowing of the whole leaf. Leaves fall prematurely leading to plant defoliation, which produces yellow pigmentation in medium containing sugars (Vandeen Mooter, 1987).

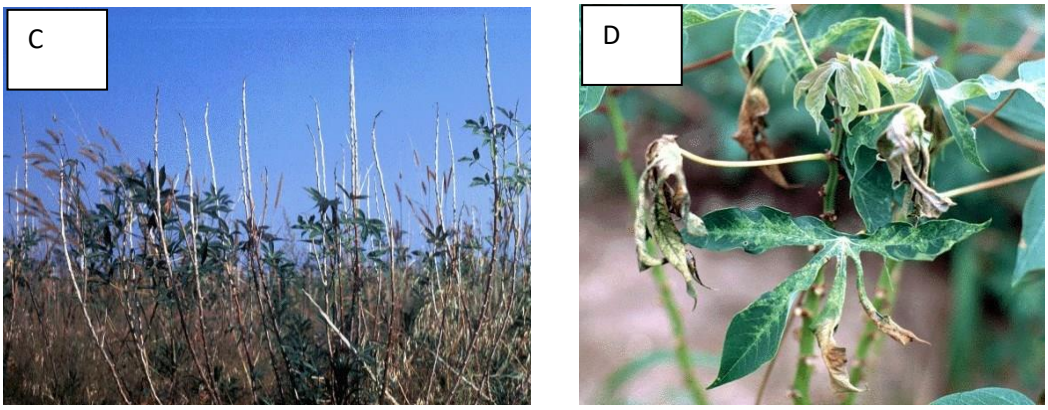


Figure 2. 1; Cassava bacterial blight Symptoms ranging from: **A; water**-soaked lesions, B; **B**lighting, C; **D**ieback and D; **W**ilting (CABI website, 2020)

2.8.2 Etiology

Xanthomonas axonopodis pv. manihotis (Xam) has been renamed severally between 1912 and 1915. From *Bacillus manihotis*, *Phytomonas manihotis*, to *Xanthomonas campestris pv. Manihotis*. In 1995 Vauterin and others proposed the name *Xanthomonas axonopodis pv. manihotis* or Xam. The bacterium grows on sucrose-containing media, producing white to creamish colonies. It is a gram-negative rod-shaped bacterium with a single polar flagellum. It measures 0.5 x 1.0 mm. Most of its physiological and biochemical characteristics are like those of Xanthomonads (Ongujobi *et al*., 2010).

2.8.3 Epidemiology

Infection begins when the pathogen multiplies near the stomata. It enters the leaves through the stomata and wounds. (Lozano, 1986). Twelve hours of humidity is enough for the bacteria to multiply and establish. The most appropriate temperature for infection to occur is around 23^{o}C. The pathogen establishes itself inside the vessel after a preliminary phase of intercellular development in the mesophyll. If the pathogen invades lignified stems, it remains within the vascular tissues where it can survive for up to 30 months (Lozano, 1986). *Xam* degrades both the

middle lamella and the cell wall of plant tissues (Boher *et. al*., 1994). Its lytic activity gives way for intercellular penetration within vascular bundles

Infected propagation material is responsible for the carryover of the pathogen from one planting season to the next (Lopez and Bernal, 2012). The pathogen can also be dispersed through rain splash and contaminated tools used in harvesting or pruning of the crop. The movement of people and animals in cassava fields, especially during or after rains can help spread the pathogen (Lozano 1976). The pathogen can survive as an epiphyte on many weed hosts which may serve as an inoculum source (Lozano 1976).

During drought, the disease development is low although the bacterium is still viable in plant tissues hence providing sources of inoculum when the rainy season arrives ((Álvarez *et al.*, 2010). The disease begins during the rainy season with the establishment of the bacteria on the leaves of the cassava plant, bacteria from contaminated plants and plant debris in the soil get to the leaves by the splashing of rainwater and insects(Álvarez *et al.*, 2010). The bacterium grows on the underside of the leaves forming micro colonies that are protected by mucus (Daniel and Boher, 1985). This epiphytic multiplication leads to the buildup of inoculum that contaminates lamina tissue through the stomatal openings and further cause infections (Harris *et al.*, 2015). Insects may account for up to 10% of within plot spread but are probably important only over short distances (Lozano 1986).

2.8.4 Management strategies

Cassava bacterial blight can lead to yield losses of up to 100% in a conducive environment if no management practices are put in place Cuttings obtained from infected fields and used to establish a plantation are likely to lead to a yield loss of 80% by the third season of production (Rastrepo *et*

al., 2000). Thus, there is a great need for managing the disease to avoid losses that are likely to occur if a crop is started using infected panting materials.

Cassava bacterial blight can be managed using several strategies and this works well when the strategies are combined. This includes the use of cultural practices, varietal resistance, biological control methods, and sanitation measures(A. Fanou & Wydra, 2010). The exchange of cassava cuttings is the major means of disseminating cassava pathogens and pests (Lozano *et al*., 1976) therefore sanitary measures are important in the exchange of materials. This can be achieved through quarantine regulations to ensure that the risk of disseminating the pathogen through propagative material is eliminated (A. Fanou & Wydra, 2010). Certification for clean planting materials can be done and these can be further propagated under controlled environments.

Crop rotation, careful disposal of infected plant residues by burning or burying in designated places, and pruning are some of the practices recommended by (Lozano *et al.*, 1976). Weeding to keep the plantation clean can control vectors and other weeds that can host the pathogen. Cultural practices can be implemented to delay the spread of the pathogen or even to eradicate the pathogens. This can be achieved through crop rotation, fallowing, incorporating infected plant debris into the soil where survival is poor or burned (Lozano *et al.*, 1976). Planting time may also be manipulated to reduce losses. This involves planting towards the end of the rainy season so that the cassava crop can establish and be able to able to escape disease in the drier periods when the weather is unfavorable to the pathogen development. Pruning of the above-ground portion of the infected plant to delay the spread of the disease and secondary infections has proved to be effective (Lozano, 1976).

Farmers should be assisted in the selection of healthy stems when making cuttings. Disease-free areas are suitable for nurseries involved in the propagation and supply of planting materials. This has been reported to be very promising for the production and distribution of high-quality cuttings in South America (Lozano and Wholey, 1974).

2.9 Use of antibiotics in plant tissue culture

Antibiotics can be used to control contamination in tissue culture, both surface contamination, and endophytic contamination. Culture contamination is a major problem in tissue culture (Murashige and Skoog 1974). Contamination can occur at any time during tissue culture work. Most contaminations result from poor unhygienic practices in the laboratories while endophytic contamination results from the explant. Endogenous bacteria multiply within the plant and affect growth, therefore, leading to great losses in tissue culture work. Endophytic bacteria contamination cannot be eliminated by surface sterilization techniques alone and therefore need for antibiotic therapy (Mathias *et., al* 1987). Several attempts have been made to suppress endogenous bacteria from cultures with antibiotics. Most of these bacteria can lose cell walls following antibiotic treatment but remain viable as spheroplasts persisting as cryptic contaminates (Falkiner *et., al* 1999).

The use of antibiotics in the elimination of contamination has been applied in *Pelargonium* tissue cultures whereby carbenicillin and cefotaxime incorporation in tissues culture media has suppressed *Paenibacillus glycanilyticus* and *Lactobacillus pracasei* contaminants. Vancomycine and Cefatoxime both combined at 250mgL^{-1} have been used to suppress *Agrobacterium tumefaciens* in soybeans. They have been recommended for the soybean *Agrobacterium*-mediated transformation procedure. Streptomycine has been used to control microbial contaminants on potatoes growing in MS media in India (Buckseth *et al*., 2017). A combination of antibiotics

including Tetracycline, Rifampicin, Streptomycin, gentamycin have been used in a study to control clover bacteria in *invitro* yam tissue cultures.

Antibiotics used in plant tissue cultures should be soluble, stable, unaffected by the components of the pH of the medium. They should not have any side effects, be broadly active nonresistance, cost-effective and with no toxic effects to humans (Falkiner *et., al* 1997). Effects of antibiotics resulting from resistance and phytotoxicity can be minimized by using antibiotics at relatively lower concentrations (Leifert *et., al* 1992).

Antibiotics were discovered in 1950 and streptomycin has played a major role in managing bacterial diseases (Stockwell *et al.,* 2012). The use of antibiotics has been practically applied in controlling diseases like fire blight of pear and apple and bacterial spots of peach in the USA(Stockwell *et al .,* 2012). Antibiotics that have been put into use include streptomycin, Oxytetracycline, oxolinic acid and gentamicin. Registration of antibiotics has been done in the USA for managing *Erwinia amylovora* on apples (Stockwell *et al*., 2012) while Streptomycin has been used in eradicating *Xanthomonas pruni* from bud wood (Brian and Hemming, 1946).

Antibiotics have a different mode of action although most of them work well if they are absorbed and translocated by plants (Pramer *et al.,* 1959). Antibiotics uptake is greater in cuttings than in rooted plants. Antibiotics can act directly on the pathogen, neutralize toxins secreted by pathogens, act directly on the host, transform within the plant to a substance having greater or different activity and also a combination of any of the above actions. Some antibiotics can penetrate foliage while those that can't penetrate are translocated from the roots.

To have access to clean planting materials, seed producers must ensure clean seed production measures are put in place for elimination of the pathogen by selecting healthy mother plants,

eradicating the bacteria in media in the process of generating healthy tissue culture plantlets in instances where infected materials are used for seed propagation. Tissue culture techniques are instrumental in the rapid production of cassava planting materials and can also be useful in producing healthy planting materials.

CHAPTER THREE: PREVALENCE OF CASSAVA BACTERIAL BLIGHT IN KILIFI AND TAITA TAVETA COUNTIES

3.1 Abstract

Cassava farming is constrained by many challenges and its productivity can only be enhanced if strategies are put in place to lower their impact. Among the many challenges, biotic factors remain a great threat to cassava farming hindering the maximum output in yield as expected. Apart from the major viral diseases, cassava bacterial blight is caused by two bacterial pathovars namely *Xanthomonas axonopodis pv manihotis* (Xam) and *Xanthomonas axonopodis pv cassavae* (Xac) is one of the major bacteria diseases affecting cassava and spreading fast in cassava growing regions in Kenya. A study was carried out in coastal Kenya to establish farmers' knowledge on cassava bacterial blight and distribution of the diseases on the coast with a focus on cassava growing areas of Kilifi county and Taita Taveta County. The study involved focused group discussions with cassava farmers and a baseline survey done in the two counties. Using randomly purposive sampling, 250 cassava farmers were selected and a semi-structured questionnaire was administered. Among the 250 farmers, 61.6% had observed cassava bacterial blight symptoms in their farms. The study sites cultivated several cassava varieties and these included Tajirika, Karembo, Kibandameno Kaleso, Shibe and other land races (local cultivars). The field visits during the survey showed that all these varieties/landraces were found susceptible to cassava bacterial blight according to the farmer's response in the baseline survey. During the survey, plant samples were randomly collected from different cassava farms in the field and taken to the laboratory for isolation. There was no sample found with both pv. *cassavae* and pv.*manihotis*. From the samples, 60% of the samples tested positive for CBB while 43% of the samples were confirmed positive with *X.axonopodis pv. manihotis* and 17% of the samples with *X.axonopodis pv. cassavae* which cause cassava bacterial blight. Both the counties were found to have a

distribution of each of the pathogen with pv. *manihotis* having a higher distribution of 18.4% in Kilifi and 5.6% in Taita-Taveta County. The diseaes incidence was higher in Kilifi county ranging to 22% as compared to Taita Taveta County which had 13% disease incidence.

Farmer knowledge of cassava bacterial blight management is still low, 51% of the farmers in Taita taveta don't manage CBB while 81% of the farmers in Kilifi don't manage CBB. From all the interviewed farmers generally, 80% do not employ any disease management practices. Therefore, there is a need for a proper disease management program to be deployed in managing cassava bacterial blight to reduce its spread and impact on cassava productivity.

There was a high prevalence of cassava bacterial blight on Kenyan coast, a wide distribution in the two counties. This study has proved the presence of CBB in the Kenyan coast, therefore, giving an avenue for intervention in employing management strategies to prevent pandemics that may occur.

3.2 Introduction

Cassava bacterial blight caused by *Xanthomonas axonopodis pv. manihotis* and *Xanthomonas axonopodis pv. cassavae* is one of the major diseases of cassava throughout the world (Verdier *et al.*, 1994). The disease is commonly spread through infected planting materials (Lozano *et al*., 1980). Since the pathogen exists in the planting stems in latent form, it is not easy to detect the disease. Due to ignorance of the disease farmers have played a major role in disseminating the disease unknowingly by sharing the planting materials over a long distance. This is because the disease symptoms cannot be easily recognized by farmers.

In Kenya, the disease was first reported in the western region (Mukunya *et al.,* 1980). A recent survey done across the country in cassava growing regions showed that cassava bacterial blight is

present in all the cassava growing regions (Odongo *et al.,* 2019). This is likely to cause a great pandemic in the future and hinder cassava farming in the country. It is critical to understand the current status of the disease in the cassava growing regions and be able to know the most prevalent areas for abettor future management. Coastal Kenya is the second leading in cassava production in Kenya (Mulu-mutuku *et al.,* 2013; Abong' *et al.,* 2016). This calls for interventions on understanding the disease in the region and how it is distributed for better management. This study was conducted to determine the prevalence of cassava bacterial blight in two cassava growing regions Kilifi and Taita Taveta counties in coastal Kenya.

3.2 Description of the study area

3.2.1Taita Taveta County

It is one of the counties in the coastal region known for its tourist attraction sites and wildlife. The county has some neighboring counties including Makueni, Tana River, and kitui in its North direction. It borders Kilifi and Kwale in its East direction and the south the county borders the Republic of Tanzania. As per the census done in 2019, the county is home to a population of 340,671 people with an area of 17,084 km2. The county has its capital in Mwatate with four sub-counties including Voi, Taveta Wundanyi and Mwatate. (*Kenya county fact sheet, 2019*).

Topographically, the County has three major zones. The upper zone comprises Taita, Mwambirwa, and Sagalla hills regions with altitudes ranging between 304 and 2,208 m. The lower zone has plains while the third zone is the volcanic foothills zone covering the Taveta region. The region has two lakes, Jipe and Challa, both found in the Taveta area and served by springs emanating from Mt. Kilimanjaro. The main rivers are the Tsavo, Lumi, and Voi. The County is largely dry although Taita hills are wetter. Two rainy seasons are experienced, long rains between March and July while the Short rains occur between October and December. In the Taita Hills on January and

August are dry. The annual mean rainfall is estimated at 650 mm. But it ranges from 350 mm to 2200mm per year depending on the agro Eco zone. The County is divided into eight Agro-ecological Zones (AEZs) (Jaetzold *et al.*, 2010).

I. The lower highland zone (LH2), found in Wundanyi at altitudes above 1680 m and receives more than 1,200 mm of mean annual rainfall.
II. The upper midland zone 3 (UM3), found in Wundanyi at altitudes between 1,370 and 1,680 m, receives around 900 – 1,200 mm of mean annual rainfall.
III. The upper midland zone 4 (UM4), found in Wundanyi at altitudes between 1,220 and 1,520 m, receives 700 - 900 mm of mean annual rainfall.
IV. The low midland zone 4 (LM4), including Wundanyi, Mwatate and Taveta at altitudes between 910 and 1220 m, receives 600 - 800 mm of mean annual rainfall.
V. The low midland zone 5 (LM5), including Wundanyi, Mwatate, Taveta and Voi, is situated at altitudes between 790 and 980 m and receives 480 - 700 mm of mean annual rainfall.
VI. The low midland zone 6 (LM6), is located in Taveta National Park, Mwatate, and Voi at altitudes below 790 m; it receives bimodal rainfall
VII. The lowland zone 5 (L5) found in Mwatate, Taveta and Voi at altitudes between 610 and 790 m, receives 480 - 680 mm of mean annual rainfall.
VIII. The lowland zone 6 (L6) found in Tsavo National Park and Voi at altitudes below 610 m; receives bimodal rainfall.

The primary economic activities of the households are crop and livestock production. Over 90% of the total households grow maize; 46% grow beans and 31% grow cowpeas. Drought-tolerant crops exist and these include sorghum, millet, pigeon peas, green grams, and cowpeas which are grown in the lowland areas. In Taita Hills, French beans, snow peas, indigenous vegetables tomatoes and cabbages are the major horticultural crops while in Taveta, the cultivation of tomatoes, onions and bananas is widely practiced. (*Kenya county climate risk profile series, 2015*)

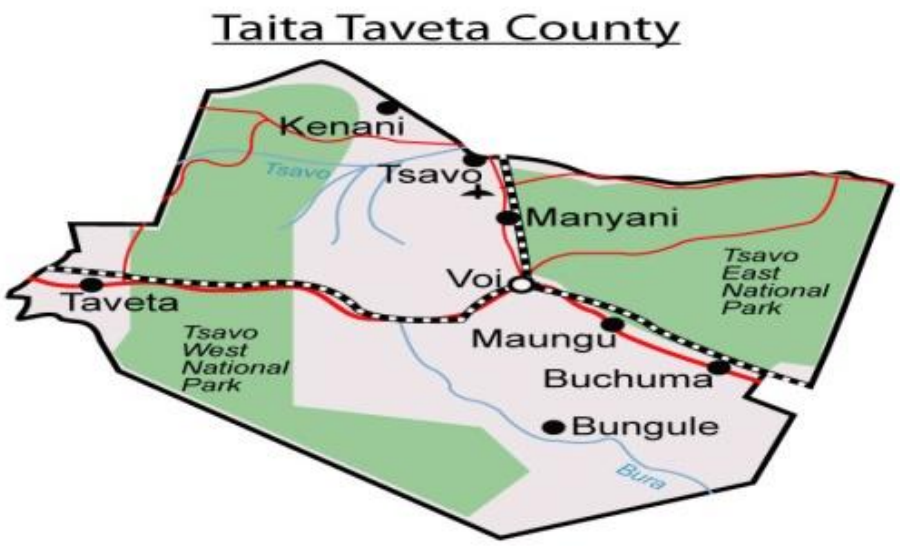


Figure 3. 1; Taita taveta county Map (source- learn.e-limu.org)

3.2.2 Kilifi County

It is one of the counties fronting the Indian Ocean and is known for its best beaches that attract both local and international tourists. It hosts a population of around 1,109,735 people as per the census done in the year 2009. It has a geographical size of 12,245 km2 according to Kenya's independent electoral and boundaries commission. It is quite large and has seven sub-counties counties including Magarini, Malindi, Ganze, Rabai, Kaloleni, Kilifi South, and Kilifi North.

The county is dominated by the largest community referred to as the Mijikenda but other communities including Swahili, Bajuni, Indians, Arabs and European settlers are found here.

Crops grown in this region include coconut, cassava, cashew nuts, maize, sweet potatoes and legumes. The county has a warm climate with temperatures ranging from 21^{o}C during the cold month and up to 32^{0}c during the hottest months. It receives an annual rainfall of 900mm to 1000mm, with long rains experienced in April to June and short rain experienced in October to December. (*Kenya county fact sheet, 2011*)

This county has four agro-ecological zones as described by (Jaetzold *et al*., 2010)

I. The Coconut-Cassava zone is also referred to as the Coastal Lowland zone (CL3). It has the highest potential for crop production with precipitation of 1,300 mm per annum and a mean annual temperature of 24^{o} C. The altitude ranges from 1-450m above sea level.

II. The Cashew nut-Cassava zone is also referred to as the Coastal Lowland zone (CL4). This zone has an average precipitation of 900 mm and an annual mean temperature of 24^{o} C. The zone has similar crop types like the medium potential zone (CL3) but with less production. In between the above two zones, there is the Coconut Cashew nut-Cassava zone that has the potential for the crops grown in both the Coconut-Cassava zone and the Cashew nut-Cassava zone.

III. The Lowland Livestock-Millet zone also referred to as the Coastal Lowland zone (CL5) this zone is of lower agricultural potential with precipitation of 700-900mm and temperatures of 27.0-25.2^{o} C. Areas of this zone are suitable for dry land farming especially drought-tolerant crops and livestock ranching.

IV. The Lowland Ranching zone is also referred to as the Coastal Lowland zone (CL6). This zone varies in altitude of 90-300m with a mean annual temperature of 27^{o} C and annual precipitation of 350-700mm. The major activities include ranching and wildlife.

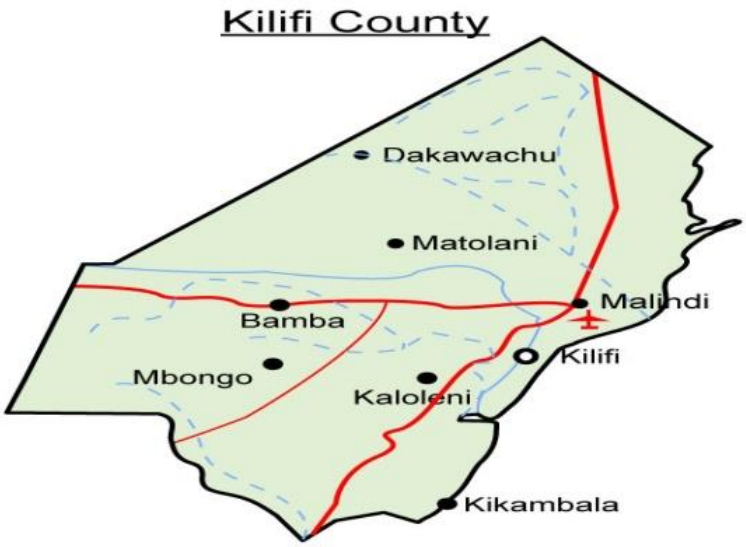


Figure 3. 2; Kilifi county Map (source- learn.e-limu.org)

3.3 Materials and methods

3.3.1 Focused group discussion (FGD)

A focused group discussion was conducted along the coast in Kilifi and Taita Taveta counties between 21st and 25th May 2018. Information obtained from farmers includes agronomic information, preference on cassava over other crops, varieties grown, history on cassava farming, their interaction with cassava diseases as indicated in appendix II.

It involved 166 farmers; 87 from Kilifi and 79 from Taita taveta all from the existing farmers' groups in the study regions. Out of these farmers, 95 were women and 71 were male all of them, adult. Children were not allowed to participate in the discussion. Predetermined questions on general cassava farming and cassava diseases were prepared and asked randomly to the present farmers during the FGD. Farmers were allowed to respond as an individual and collectively as a

group. Farmers were also grouped into two groups based on gender and asked questions differently. (Escalada & Heong 2014).

Printed colored photos showing disease symptoms were used to make FGD effective, easy to understand and more interactive. All the responses to each question were written on a flip chart and noted in books by the moderator of the FGD for better analysis basing on the objective of the study (Khan & Manderson, 1992).

3.3.2 Conducting of baseline Survey for CBB existence in Kilifi and Taita Taveta County

A survey was conducted in two counties of Taita Taveta and Kilifi. This was done in July 2018 and was achieved through the administering of a semi-structured questionnaire to the randomly selected 250 farmers who grow cassava. Purposive random sampling was done whereby farmers were selected randomly at an interval of 2km from each farmer for questionnaire administering. A sample size of 250 farmers determined basing on the previous studies done in the region, was used during the survey. For a uniform representation of farmers in the two counties, each county had a represented population of 125 sampled farmers. All the questionnaires were perused through, data was cleaned and entered for analysis using the social science statistical package (SPSS). A distribution map was developed using ARCH GIS software and the incidence was grouped into four classes of 1-25%, 26-50%, 51-75% and 75-100% (Odongo *et al.,* 2019). The geographical coordinates used to develop the map were taken using a GPS tool at the point of sample collection in the field.

3.3.3 Assessment of cassava bacterial blight and collection of plant samples

All the visited cassava farms/plots were inspected for cassava bacterial blight and infected plant samples were collected from plants showing cassava bacterial Blight symptoms. Sampling was

done on crops of all ages. Plant samples (leaves and stems) were picked from the cassava showing symptoms of the following symptoms: blighting, water-soaked lesions, leaf wilt, and defoliation. A total of seventy plant samples were collected by handpicking from the plants. They were then stored in brown envelopes and put in a cool box and transported to the pathology laboratory at the College of Agriculture and Veterinary Science, the University of Nairobi for isolation.

Disease assessment was done in each cassava farm/plot that recorded the presence of the disease to ascertain prevalence and distribution. Incidence was obtained by counting all the symptomatic plants in each visited farm. Then this was divided against the total number of plants in the field obtained by multiplying the number of plants on the width and length of the farm. (Bensal *et al.*, 1994)

$$\text{Disease incidence (DI \%)} = \frac{Number\ of\ diseasedplants}{Total\ number\ of\ plants}\chi 100$$

Severity assessment was done on the whole plant by examining the plant from the lower leaves to the upper leaves. From each farm 20 plants were selected diagonally for assessment. Severity was scored using the following scale. 1 - No symptom, 2 - Angular leaf spots, 3 – Angular leaf spots, blight, wilt, defoliation, and sometimes exudates on stems, petioles or leaves, 4 –Blight on leaves, leaf wilt, defoliation, exudates and tip dieback, and 5 - Blight on leaves, leaf wilt, defoliation, exudates, abortive lateral shoot formation, and stunting, complete dieback(Wydra *et al.*, 1998)

Severity was calculated as shown in the formula below. (Song *et al*., 2004)

$$SI\% = \frac{(\Sigma\text{Scale} \times \text{No of plants infected}}{\text{Highest scale} \times \text{Total number of plants}} \times 100$$

Cassava bacterial blight prevalence was obtained by calculating the total number of farms that had the diseaes over the total number of farmers which were visited during the study in each county. This was later calculated into percentage for each county to obtain prevalence for the study regions.

To establish farmers' knowledge and perceptions on CBB in the two counties, information on name(s) of the cultivar(s) grown in the fields, source of planting material, age of the crop, recognition of the CBB and its management approaches used, source of cassava information and the duration for which they have grown the crop were asked during the survey.

3.3.5 Isolation of the pathogen from collected plant samples

Isolation was done according to (Ogunjobi & Fagade, 2008). Infected plant samples stored at -4 ^{0}c were cut into small pieces of 2mm^{2} and washed in sterile distilled water. The pieces were then surface sterilized using 3% sodium hypochlorite for 3 minutes and triple rinsed in sterile distilled water using the universal bottles. Yeast peptone glucose (YPG) broth was prepared containing yeast 1%, peptone 1% and glucose1%. The cut-infected parts were placed in universal bottles containing 10ml broth and placed on the rotary shaker at a speed of 100rpm for 48 hours at room temperature to allow the bacteria to ooze into the broth. The broth was then serial diluted to 10^{-6} and then from each dilution, a drop of 100µl was spread on plates containing freshly prepared selective media containing Yeast1% peptone1%, glucose1% and agar1.5% (YPGA). The plates were then placed in an incubator for 28^{o}c for 48 hours.

The bacterial colonies obtained were identified and used for pathogenicity tests on clean symptomless plants multiplied through tissue culture technique from tissues culture laboratory in the department of plant science and crop protection. After four weeks the symptoms were recorded and photos were captured both for the infected plants and the control which were not infected. Re-isolation was done using YPGA to fulfill Koch's postulates.

3.3.6 Raising of tissue culture plants

Tissue culture plants were propagated in the tissue culture lab. After five weeks of germination, the seedlings were transplanted to pots of 1-liter capacity and hardened for 4 weeks. They were then selected based on morphological appearance and fast growth rate for pathogenicity testing.

3.3.7 Pathogenicity test

Clean disease-free plants were selected from the already propagated tissue culture seedlings, they were then put in a greenhouse and watered well. The bacteria that had been isolated from the plant samples collected during the field survey was used to inoculate on the leaves using a sterile inoculation needle. Both The pathovars were inoculated on with the control being inoculated with sterile distilled water. For each experiment 8 plants were selected and used. After 4 days observations were made for symptom appearance on the plants at the point of inoculations.

3.4 Results

3.4.1 Focused Group Discussion (FGD) output

From the discussions made in two counties, farmers acknowledged having seen several cassava diseases in their farms namely Cassava mosaic virus, Cassava brown streak disease, Cassava brown leaf spot and cassava bacterial blight after being showed symptoms of the diseases using photographs of the said diseases during FGD. Up to 80% of the farmers involved in the focused group discussion accepted to have seen the disease characteristic symptoms of cassava bacterial blight either in their farms or neighbor's farms. The majority of the farmers, 55% did not know it was cassava bacterial blight disease (Fig.3.2). About 20% had never observed such symptoms of the diseases in their farms while 25% of them had some knowledge of cassava diseases in general but didn't know any management strategies to control these diseases (Fig.3.2).

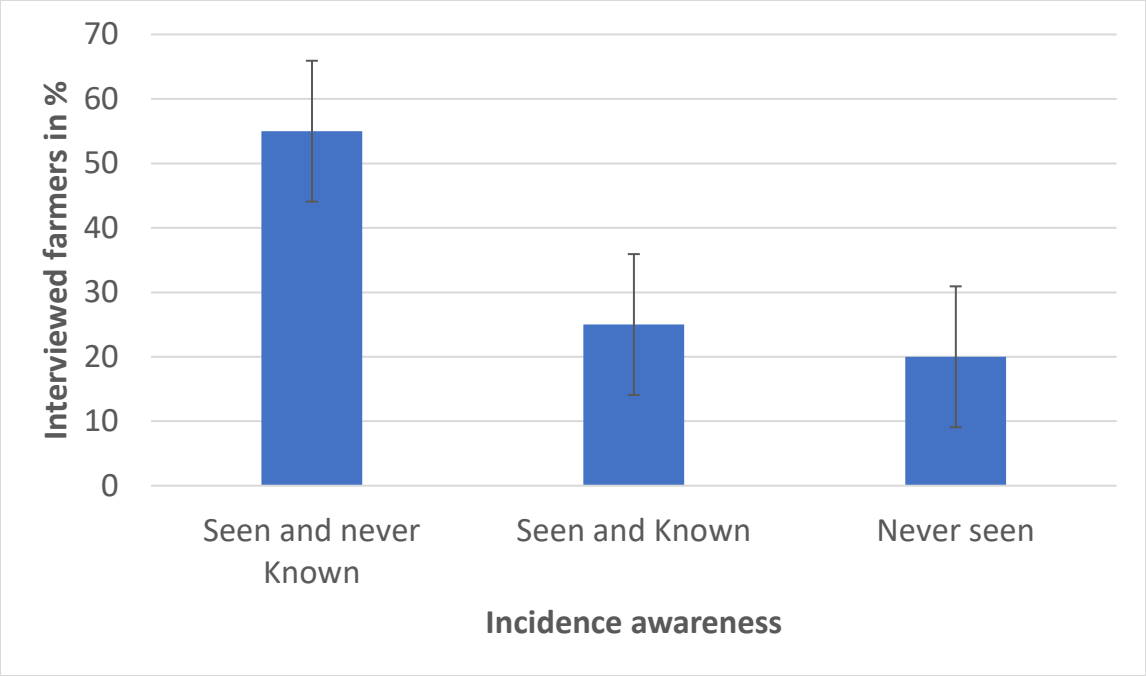


Figure 3. 3; Graph showing incidence awareness of CBB among farmers in 2018.

All the farmers could not tell how the Cassava bacterial blight is transmitted. Cassava bacterial blight is expressed in a range of symptoms and therefore some farmers did confuse it with other diseases or other symptoms caused by environmental stress. No farmer was practicing scouting for diseaes in their farms this is because the cassava crop has never been given that close attention when compared to managing diseases in other crops such as horticultural crops or maize.

Figure 3. 4; Focused group discussion in Progress in Bahari in Kilifi North on 26th May 2018.

3.4.2 Baseline survey

During the survey, a representative population of 250 cassava growing farmers were interviewed. Just about 50 % were interviewed in each county. Among the respondents, 61.6% in both counties had occasionally seen symptoms similar to CBB in their farms. Among the interviewed farmers, 43.6% reported to have experienced cassava bacterial blight in their farms every season they plant cassava. Few farmers 17% had never seen CBB symptoms in their farms. About 40% of farmers were not able to tell whether they have ever seen CBB in their farms.

About 40% of farmer respondents reported that *Kibandameno* was the variety highly susceptible to CBB followed by *Tajirika* which was named by 13.2% of the respondents. All the named varieties that the farmers grew that is *Shibe*, *Girikacha* and *Kaleso* were susceptible to CBB as per the farmer's responses. No resistance variety was reported by the farmers in both regions. Most of the farmers in both counties (82.5 %) don't manage cassava bacterial while a few of them practice rouging 8%, biological control 7% and chemical control 2% as a way of managing cassava bacterial blight

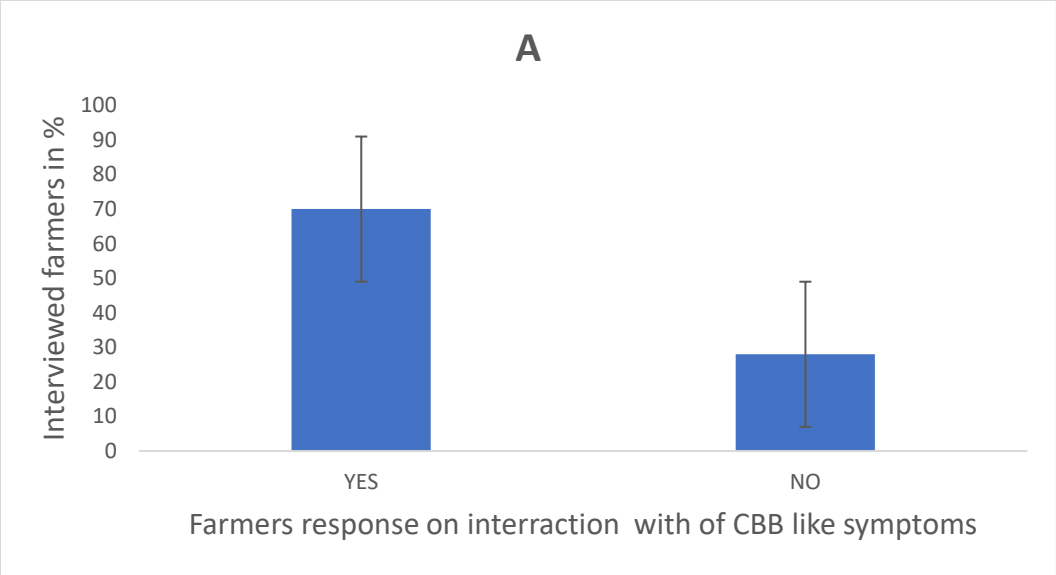


Figure 3. 5; Showing % of farmer's responses on if they have interacted with CBB or not.

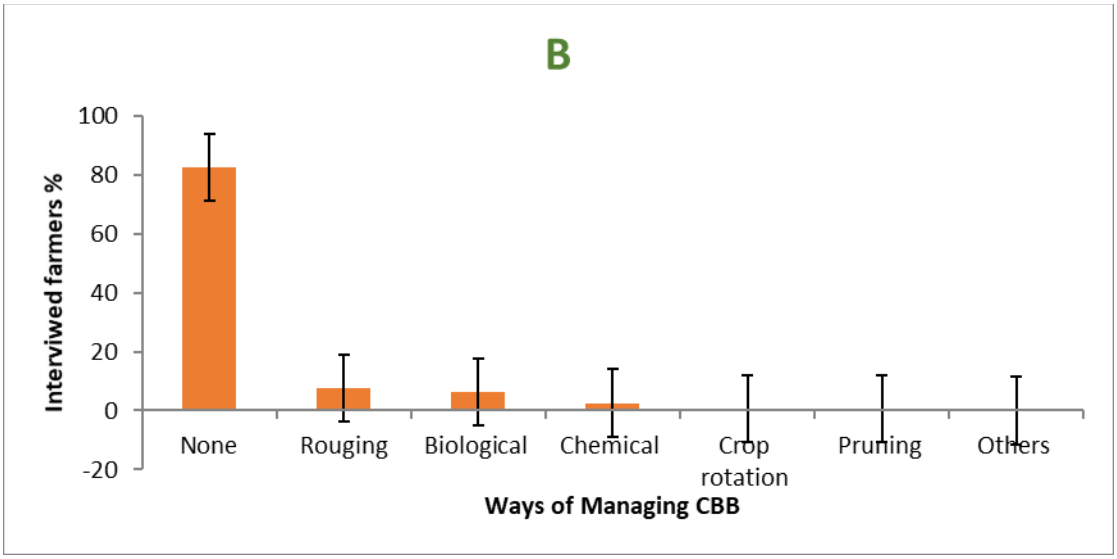


Figure 3. 6; Showing how farmers responded to different ways of managing CBB.

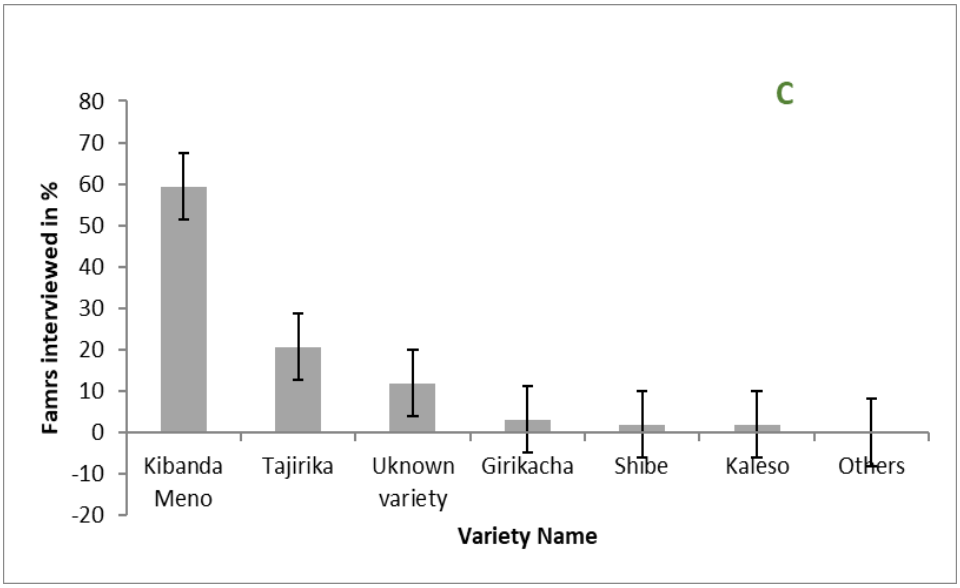


Figure 3. 7; Showing in % how farmers responded to the susceptibility of different varieties of cassava to CBB.

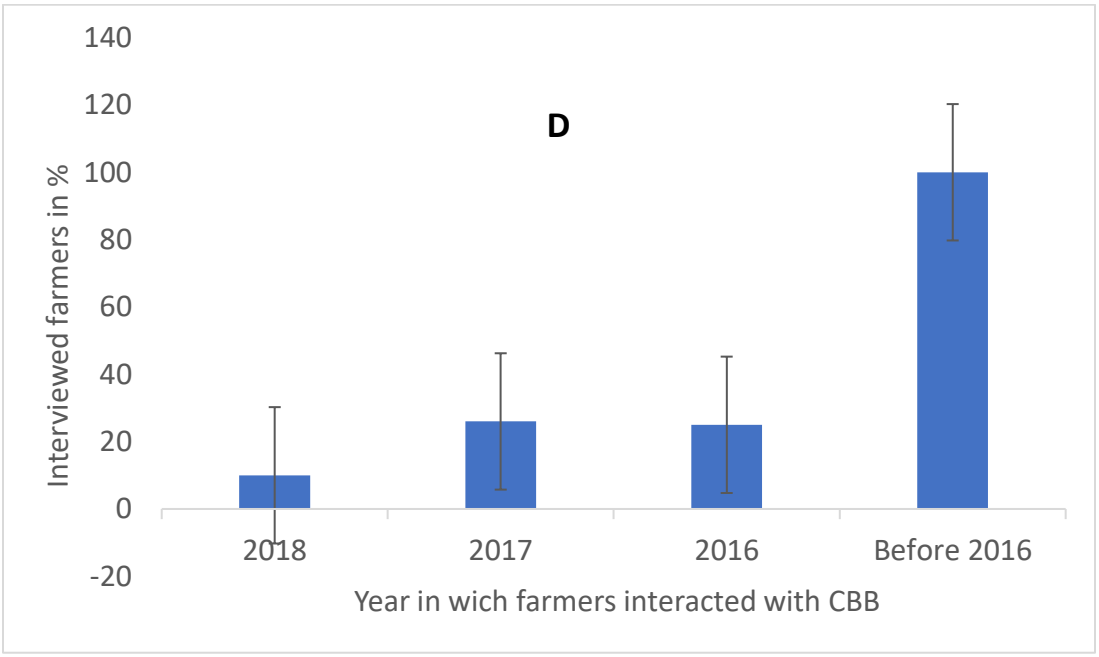


Figure 3. 8; Showing in percentage farmers who interacted with CBB in different years.

3.4.3 Prevalence, incidence and severity of cassava bacteria blight

There was a widespread of the disease in both counties with the prevalence of 28% being recorded for both counties. On incidence there was a significant difference in the incidence of the diseaes

in the two counties with Kilifi County having a higher incidence of 22% and Taita Taveta having 13% disease incidence. Kilifi County had a higher disease severity of 8% and that of Taita Taveta was 5% hence showing a significant difference in the diseaes severity of the two regions. (Fig 3.4.) During the survey, a range of symptoms was observed as captured in Fig.3.5, with the highest score of symptoms remaining at 3 on the disease scale used. Angular leafspot, blighting, water-soaked lesions were visible but

Defoliation, bacterial exudates, abortive lateral shoot formation, stunting, and die-back was not recorded as per the scale used.

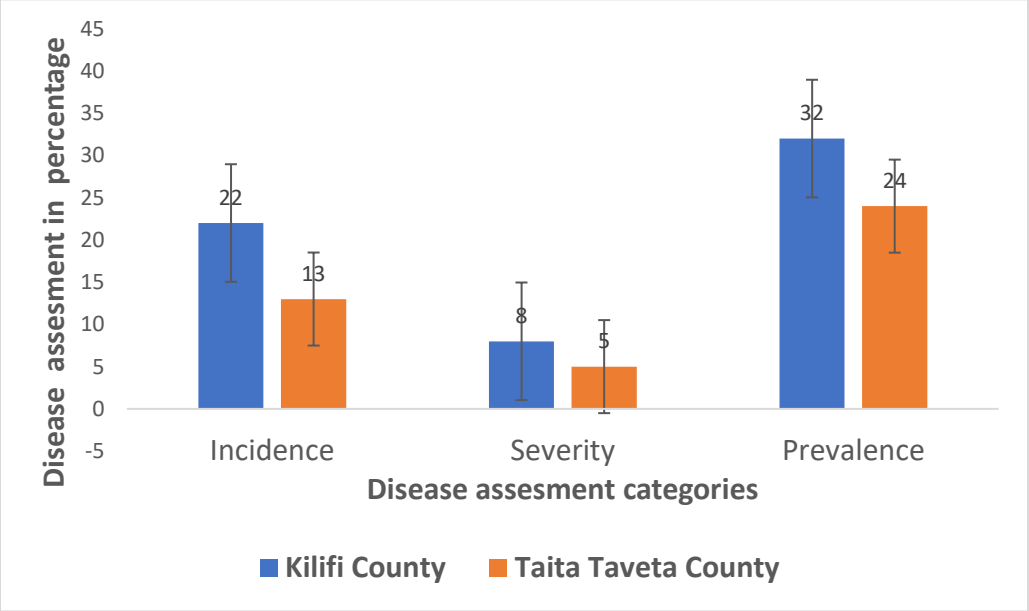


Figure 3. 9; Graph showing the percentage of incidence severity and prevalence in the two counties of study.

Blighting and angular leaf spot from the margin

Angular leaf spot clearly seen on the leaf

Disease free plant with no symptoms.

Figure 3. 10; Symptoms of infected plants and clean non-infected plants.

3.4.4 Distribution of CBB in coastal Kenya

There was a wide distribution of cassava bacterial blight in the two study regions of Kilifi and Taita taveta counties. Both pathovars were found in the two counties. The incidence for CBB was recorded in all counties in variation from 1 % to 100%. Out of the 40 samples found to be positive of CBB, 21 samples were well distributed in Kilifi County and 19 samples were well distributed in Taita Taveta counties. Samples from *Xanthomonas axonopodis* pv. *cassavae* were more distributed in Kilifi which had 7 samples while Taita taveta had fewer samples of 3 confirmed for *cassavae* pathovar. Some farms did not record any incidence of the CBB diseaes as seen in the Distribution map below.

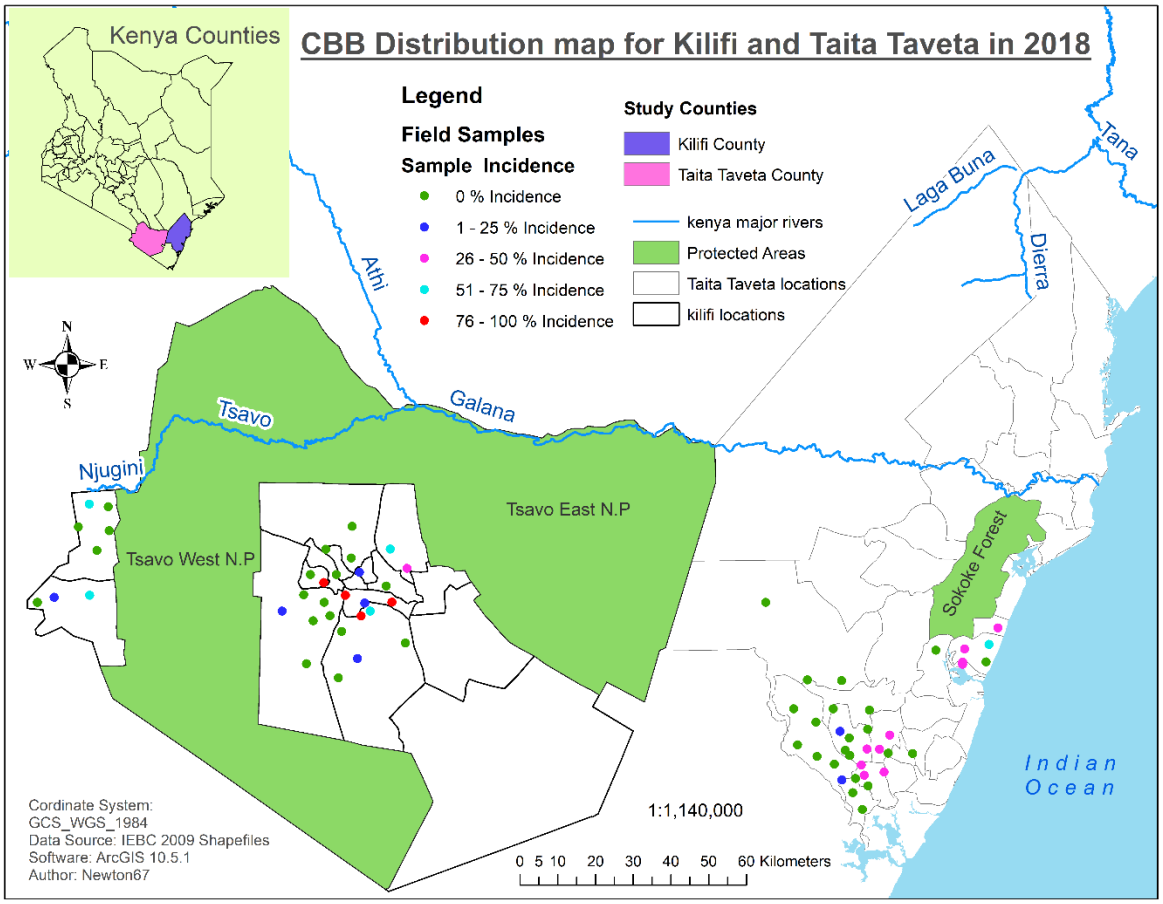


Figure 3. 11; Map on distribution and incidence of CBB in farmer's fields in the study region

3.4.5 Pathogenicity test

The isolated bacteria colonies ranged between whitish, creamish and yellow with convex shape, shiny and mucoid (Wydra *et al*., 1990). Inoculation of the bacterial on the plants after 2 weeks showed blighting around the point of inoculation with the control showing no blighting at the point of inoculation as seen in figure 3.12 below. This proved that the bacteria were pathogenic and it is the causal agent of Cassava bacterial blight.

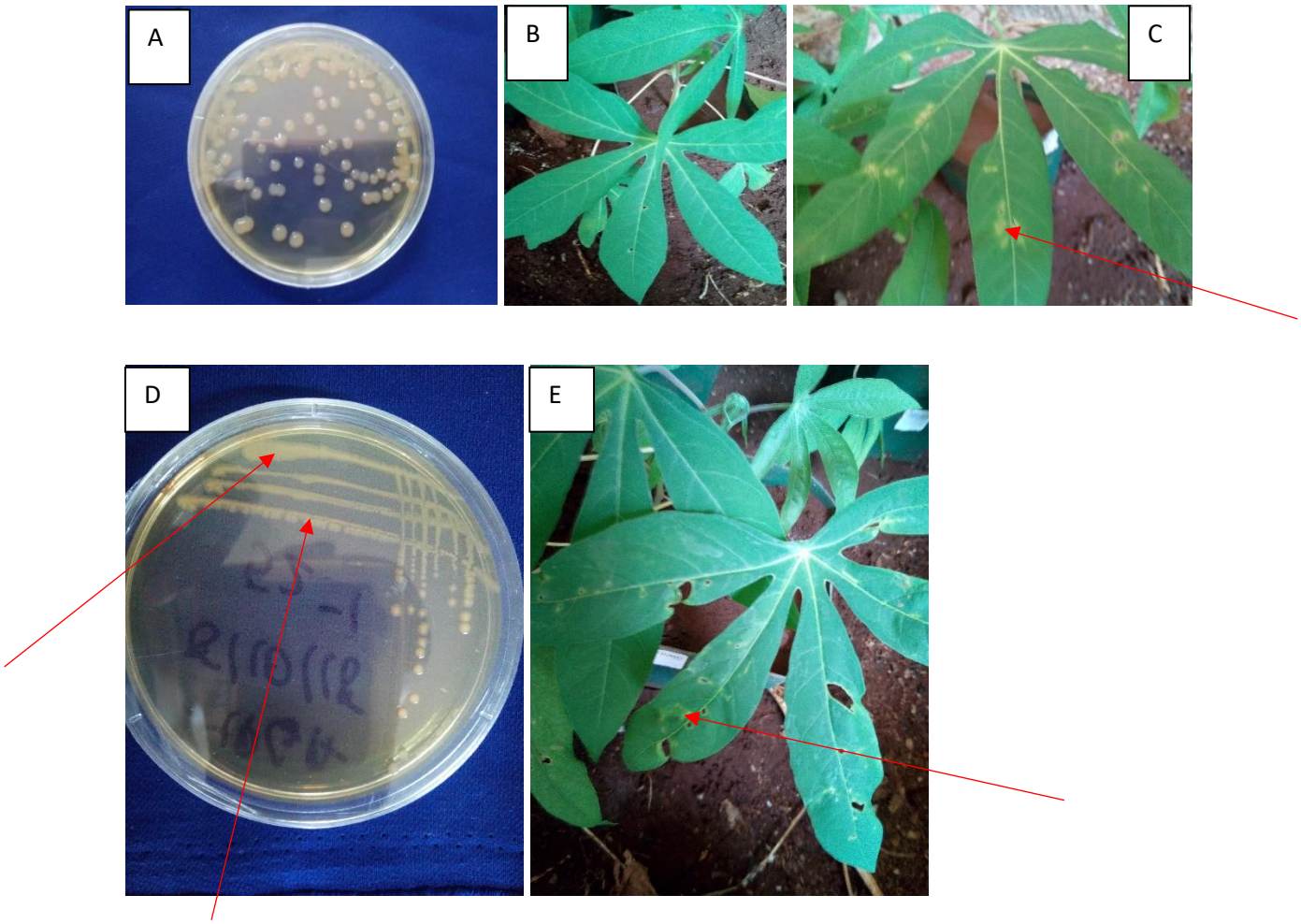


Figure 3. 12 **A**; Showing bacterial colonies of Xanthomonas *axonopodis pv cassavae* used in Koch's Postulate. **B**; Control plant used in Koch's postulate showing no symptoms, **C**: Tissues culture Plant treated with *Xam* showing symptoms as seen at the arrow point **E**: Bacterial colonies of *Xanthomonas axonopodis pv cassavae* on a plate used in Koch's postulate as indicated by the arrows. **E**: Plant treated with *Xac* colonies showing symptoms as pointed by the arrow.

3.5 Discussion

From the focused group discussion, farmers confirmed to have seen cassava bacterial blight alongside other cassava diseaes in their farms. This was a clear confirmation that cassava bacterial blight is present at the Kenyan coast with the majority (80%) of the farmers engaged in focused group discussion confirming this. Farmers still have a challenge in diseaes diagnosis, the majority of them (55%) engaged in FGD have interacted with characteristics symptoms of the diseaes but

have never known neither associated these symptoms to any cassava diseaes. A few of the farmers (17%) have never interacted with cassava diseaes and have never seen symptoms of the diseaes in their farms. During the questionnaire interview it came out clearly cassava diseaes awareness is still low among a majority of the farmers. This is attributed to the lack of extension services and the low priority of cassava over other crops grown for commercial purposes.

Kilifi County had a higher incidence of cassava bacterial blight as compared to Taita Taveta County. There was a higher severity Kilifi country as compared to Taita Taveta County. This could be explained by the fact that Kilifi farmers grow cassava extensively compared to Taita taveta. Therefore this implies that there is more awareness needed to be done in Kilifi on cassava diseases since the uptake of cassava in Kilifi is high compared to other coastal regions.

The severity of cassava bacterial blight is relatively low since no scoring was done above scale 3 on a scale of 1 -5 used during disease assessment in the field. This indicates that no high losses have been recorded in the two regions due to cassava bacterial blight compared to other regions in Africa (Onyeka *et al.,* 2004; Banito *et al.*,2007; Bamkefa *et al* .,2011; Kone *et al.*,2017)

In the questionnaire interview, 61.6% of the farmers confirmed to have interacted with cassava bacterial blight characteristics symptoms. This confirms that cassava bacterial blight exists in the coastal region among the cassava farmers' fields. This concurs with the studies done on the distribution of CBB across Kenya. (Odongo *et al* ., 2019). A good number, (43.6%) of the interviewed farmers confirmed to have experienced CBB in their farms every season they grow cassava. This is attributed to the recycling of cassava planting materials, a common practice amongst farmers in the region. There is a need for increasing awareness in crop rotation and access to new planting materials in every season to stop the seasonal existence of CBB in farms.

Some farmers were not able to tell if they have experienced cassava bacterial blight in their farms, and this was attributed to the fact that they don't give much attention to cassava as compared to other crops. This could be due to the low economic value perception given to cassava among most farmers in Kenya. This shows there is a high ignorance on information for cassava diseaes, therefore, calling for more extension services and increased awareness so as farmers can grow diseaes free crops and be able to increase yield.

All the varieties grown in the region are susceptible to cassava bacterial blight, the local varieties are highly susceptible with Kibandameno ranked the most susceptible by farmers as compared to other varieties which are improved like Tajirika, Shibe Girikacha and kaleso. Although Kibandameno is the most grown by farmers as compared to other varieties this also could be the reason why it was highly ranked susceptible by many of the farmers. This confirms a similar study done previously on the resistance of various varieties to cassava bacterial blight. (Mbaringong *et al* ., 2017). This shows that cassava farming in the region is at risk and therefore resistant varieties are needed as a measure to manage cassava bacterial blight.

The majority of farmers (82.5%) don't practice disease management in cassava farming which could have contributed to the continued spread of the diseaes in most of the cassava fields in the coastal region. Although few farmers engage in management practices like rouging (7.5%), chemical (6.25%) and crop rotation (0.63%). This is just done mostly for cassava intercropped with other crops hence cassava benefits from management practices targeted or intended for the neighboring crop. One of the reasons for these findings is inadequate extension services to guide farmers in the region. This implies that the spread of the disease is likely not to be contained hence one of the ways to counter this is to do capacity building for farmers.

Farmers had interacted with CBB in their farms as early as 2016. This was seen by more farmers 62.3% reporting to have seen cassava bacterial blight for the first time before 2016 as compared to 6.17 % of the farmers who have seen CBB for the first time in 2018. The diseaes has been in existence for a while and this agrees with other studies done by other scientists (Odongo *et al*., 2019; Mbaringong *et al.,* 2017; Mukunya *et al*., 1980) on the existence of CBB in Kenya. This information implies that CBB is on increase as far as distribution and existence are concerned hence needs attention by relevant phytosanitary institutions on management.

The distribution map shows that cassava bacterial blight is widely distributed along the coastal region. This confirms work done before on cassava bacterial blight distribution in Kenya (Chege *et al* ., 2017(Odongo *et al.,* 2019);). In both, regions 0% incidence was reported in some farms hence confirming that there are farms in the regions which are free from cassava diseaes. Kilifi was more leading with 22% incidence which was attributed to many varieties embraced as compared to Taveta. Prevalence of 28% was recorded for both study regions indicating that the diseaes is quite prevalent in the region. This gives room for mapping diseaes free farms and using them as seed multiplication farms. This information also keeps Kilifi county on a black spot as a highly CBB prevalent place within the coastal region.

3.6 Conclusion

The study concludes that cassava bacterial blight is widely distributed in Kilifi and Taita Taveta counties. This was seen through a range of symptoms observed during the field visits. The disease is more prevalent in Kilifi as compared to Taita taveta. There is a variation of both incidence and severity in all the counties. Both incidence and severity were high in Kilifi County as compared to Taita taveta. Some farms in both regions were found free from the disease, therefore, offering an opportunity for phytosanitary measures intervention. Farmers in Kilifi have many cassava varieties

as compared to farmers in Taita Taveta. All varieties grown in the two regions were found to be susceptible to cassava bacterial blight which raises the need for breeding resistant varieties. Both the pathovars *Xac* and *Xam* were present and prevalent in the two study regions. The study concluded that there is a big gap in farmers' knowledge and awareness of cassava bacterial blight something that was seen by a majority of farmers not being able to carry out any management activities on the diseases, this calls for intervention through extension services.

CHAPTER FOUR: CHARACTERIZATION OF THE TWO BACTERIAL PATHOVARS CAUSING CASSAVA BACTERIAL BLIGHT.

4.1 Abstract

Differentiating the two bacterial pathovers responsible for Cassava bacterial blight remains a big challenge. This is due to a lot of similarities and their origin from the same family. Therefore characterization will create more understanding and give room for studying the two pathogens. These two: *Xanthomonas axonopodis pv. cassavae* (Xac) and *Xanthomonas axonopodis pv. manihotis* (Xam), both infect cassava foliage causing blight in cassava plantations and creating a lot of similarity on symptomatology hence creating the need for intervention to differentiate the pathovars. A study was conducted to establish the difference between the two pathovers. This involved chemical, physiological and phenotypic characterization of the colonies obtained from leaf samples collected from farmer's fields in the coastal area of Kilifi and Taita taveta.

Forty cassava plant leaf samples were collected from farmer's fields and taken through cultural isolation to obtain bacterial colonies from each sample. During isolation yellow colonies are characteristic to *Xanthomonas axonopodis pv. cassavae* (Xac) and white colonies which are characteristic to *Xanthomonas axonopodis pv. manihotis* (Xam) were obtained from the samples proving the existence of both pathovars from the region where the samples were collected. *Xanthomonas axonopodis pv. cassavae* (Xac) was isolated from 10 samples and *Xanthomonas axonopodis pv. manihotis* (Xam) *was* isolated from 30 samples out of the 70 samples obtained from the field during the survey. From the isolations done, no leaf sample showed both pathovars from the same plant.

From the biochemical tests conducted both pathovars were gram-negative, motile, caused potato rot, hydrolyzed starch, hydrolyzed gelatin, tested negative for florescent pigment production,

catalase-positive, didn't grow in pH below 4.5. None of the pathovars was able to grow at temperatures above 40^{o}c when the ability of the colonies to grow at different temperatures was tested. There was a difference in utilization of cellobiose and maltose which offered an opportunity to differentiate the two pathovars. Pathovar *manihotis* did not utilize cellobiose while pv. *cassavae* utilized cellobiose. Pathovar *manihotis* utilized maltose while pv. *cassavae* did not utilize maltose. For all the sugars tested that is starch, sucrose, asparagine and lactose both pathovars were able to utilize the sugars. Therefore making it possible to differentiate the two pathovars based on the carbohydrate test. The use of biochemical and cultural characterization is not exhaustive in differentiating the two pathovars. Hence need for deploying other techniques like molecular techniques.

4.2 Introduction

Cassava bacterial blight was first reported in Brazil in 1912 but it has spread to other regions of the world: Africa, Asia and South America (Lozano 1976). The causal pathogen has been renamed several times from *Bacillus manihotis* to *Xanthomonas axonopodis pv manihotis*. Two pathogens namely *Xanthomonas axonopodis pv manihotis* and *Xanthomonas axonopodis pv cassavae* are pathogenic to cassava and are responsible for cassava bacterial blight (*Maraite et al.*, 1987). Both the pathovars have been found to induce angular leaf spots on cassava leaves on infected leaves.

Previously the pathogens were differentiated through colony colors but currently, several attempts have been applied in distinguishing the two pathogens using biochemical and pathogenic characteristics, serology, membrane profile analysis and Rebotyping(Verdier *et al.*, 1994). *Xanthomonas campestris pv cassavae* is phenotypically more heterogonous than *Xanthomonas axonopodis pv manihotis* (Mooter *et al*., 1987).

Xanthomonas axonopodis pv cassavae (Xac) is yellow-pigmented, it hydrolyses starch, forms acid from maltose but not from ribose. The pathogen also grows on maltose. *Xac* grows on Saccharic acid, does not grow on DL-glyceric acid, does not hydrolyze tween 60, and form yellow pigment on nutrient media. *Xam* does not grow on saccharic acid, grows on DL- glyceric acid hydrolyses tween 60 and forms white pigmentation (Van den *et al*., 1987)...

The objective of this study was to characterize the bacterial isolates from cassava using cultural methods and to observe if the different isolates observed in culture were the two pathovers when chemical, physiological and phenotypic characterization characterizations are conducted.

4.3 Materials and methods

4.3.1Biochemical characterization

4.3.1.1 Gram staining

This test was conducted to confirm if bacterial isolates were gram-positive or gram-negative. The technique was conducted as described by Ongujobi and others (2010). Bacteria previously obtained from plant samples and stored in nutrient broth was inoculated on Petri dishes and after 24 hours it was picked from the petri dish plate using a sterile wire loop, Then spread on the slide to form a thin film and allowed to dry for 2 minutes. The slide having the bacteria was held up and passed across a Bunsen burner flame five times with care not to overheat the slide. The entire area of the bacterial film was covered with gram crystal violet and left at room temperature for 1 minute. The slide was then rinsed for 5 seconds under slow-running water from a wash bottle. The bacteria were then covered with gram iodine and left at room temperature for 1 minute. The slide was then rinsed for 5 seconds under slow-running water from a wash bottle. Gram stain was added dropwise until the blue-violet color was cleared on the sample. The slide was then rinsed under slow-running water from a wash bottle. The bacteria film was then covered with gram safranin and left at room

temperature for 1 minute. The slide was then rinsed for 5 seconds under running water from a wash bottle to remove excess dye. Blotting was done using blotting paper to remove excess moisture then viewing was done under 60x magnification

4.3.1.2 Motility tests

This was conducted to ascertain if the bacterial isolates were motile. The test was conducted as described by Ongujobi and others (2010). Using a wire loop both bacterial isolates of the two pathovars were picked from 24hour culture and dipped in a drop of sterile distilled water on a hanging drop slide. Using a microscope at 100× magnification observation was done to view the motility of the bacteria.

4.3.1.3 Gelatin hydrolysis test

The medium was prepared using Peptone 5 gm, Beef extract 3 gm and Gelatin 120gm and the pH was adjusted to 6.8. All were mixed in 1 liter of sterile distilled water and autoclaved at 121^{o}c and 15 psi for 20 minutes. The medium was then dispensed in Petri dishes 10ml on each and allowed to cool and solidify. U sing wire loop the bacterial isolates of each pathovar were streaked on four plates each and the control was streaked with sterile distilled water. After 24 hours the inoculated plates were observed along the line of inoculation for any clearing zone (Dela Cruz *et al.*, 2012)

4.3.1.4 Starch hydrolysis test

The test was conducted according to Ongujobi and others (2010). The medium was prepared by mixing 2gm starch, 5gm peptone, 3gm beef extract, 20 gm agar all the ingredients were mixed with 1000ml distilled water. All the contents were autoclaved at 121^{o}c and 15 psi for 15 minutes. The media was then poured on Petri dishes and allowed to cool. Bacterial colonies of 24 hours were streaked on the Petri dishes 4 Petri dishes for each pathovar with the control Petri dishes

streaked with sterile distilled water and incubated at 28°c for 48 hours. After 48hours of bacterial growth, the plates were then flooded with Lugol's iodine solution to test for hydrolysis of the starch.

4.3.1.5 Catalase test

This test was conducted to determine the presence of catalase enzyme in the target pathogen. The test was done as described by Reiner in 2010. Hydrogen peroxide was prepared to 3% concentration by taking 3 gm of sodium hydroxide and dissolving in 100ml of sterile distilled water. Then 24-hour culture was put on two slides for each pathovar and four drops of sodium hydroxide were dropped on each slide to check for the formation of an effervescence.

4.3.1.6. Fluorescent pigment production tests

King's B media was prepared by mixing 20gm protease peptone,2.5 gm K_2HPO_4.7HO,1.5gm $MgSo_4$ $7H_20$, 20gm Agar, 10 ml Glycerol and 1000ml distilled water. (Kings *et al.,* 1954). The mixture was autoclaved at 121^0c and 15 psi for 20 minutes and cooled to 45 ^{0} c then poured on the plates to solidify. The plates were then inoculated with the bacteria *Xam* and *Xac* four plates for each pathovar and incubated at 28^0 c for 48hours then examined under an ultraviolet lamp to check for diffusible fluorescent yellowish green pigment. Four Uninoculated plates were used as the control plates.

4.3.1.7 Potato rot test

Irish potato-free from any infection was sliced into small pieces of 1 by 4cm pieces then washed in 3% sodium hypochlorite and triple rinsed in sterile distilled water. The pieces were then placed on sterile Petri dishes and using a sterile toothpick the culture was picked and pierced in the potato tissues for both the *PV. cassavae* and *pv. manihotis* in 2 different tissues for each pathovar with

the other 2 control slices inoculated with sterile distilled water. They were then sealed and incubated at room temperature for 48 hours. They were then checked after 48 hours at the point of inoculation for any rotting or change of color emerging from the point of inoculation for all the plates used. The potato was used as part of the LOPAT tests.

4.3.1.8 Utilization of carbohydrates

The ability of the bacteria to utilize different carbohydrates was tested for the following carbohydrates: cellobiose, Lactose, Maltose, sucrose, mannitol, arginine, asparagine and starch.

The broth YPG (made from yeast peptone and agar) 500ml was prepared and mixed with bromothymolblue indicator. The pH was adjusted to 7 and then the mixture was autoclaved cooled and dispensed 9 ml in universal bottles. Sugars were prepared at the rate of 20% for each concentration filtered and dispensed 1 ml for each sugar in every universal bottle. 1 ml of bacterial broth was added to each universal bottle containing the sugars except in the control bottles. The Durham tubes were inserted into all the bottles. The anaerobic treatments were sealed with wax at the top to limit oxygen availability (Ongujobi *et al.,* 2007). The experiment was incubated at 28^0c for 3 days and monitoring was done for color change and formation of bubbles in the Durham tubes.

4.3.2. Cultural characterization

The bacterial isolates which had earlier been obtained from collected plant samples were preserved in broth form and stored at 4^0c in the fridge. The broth was later cultured and the bacterial colonies obtained were incubated for 18 hours. After 18 hours the colonies in each of the 40 plates were characterized by observing the growth rate, the shape of the colonies and color of the colonies, appearance, structure and form (Chege *et al.*, 2019). Characterization was done for all the bacterial

colonies obtained from the plant samples. A detailed study was done on each phenotypic category with different scales as outlined below.

- Growth: 1 –less growth, 2- moderate, 3-high growth, 4 –very high growth, 5 no growth.
- Color: *white, pale yellow, yellow, brown, and green, cream.*
- Size: *small, medium, large.*
- Surface description used: *smooth, wavy, rough, granular, and papillate.*
- Form description used: *circular shaped, fussy form, Rhizoid, irregular, filamentous*.
- Elevation: *convex, concave, raised, domed, flat.*
- Structure description used: *opaque, translucent*, *transparent*.
- Texture: *dry moist, mucoid, viscous, battery, coily*.
- Edges description: *Curled, entire, crenated.*

4.3.3 Physiological characterization

4.3.3.1 pH Test

This was conducted by testing different levels of pH at which the pathogen can grow (3, 4.5, 6, 7.2, 7.5, and 8,9,10.5. One liter of YPGA (Yeast, Peptone, glucose and Agar) media was prepared by mixing all the reagents. It was then heated in an oven to evenly mix the components. The media was then divided into 7 portions in a conical flask of 250ml each to represent the seven levels of pH test used. Using pH meter Hcl solution and NaOH solution were used to establish the following pH levels: 3, 4.5, 6, 7.2, 8.9, 10.5, and 7.2 as control. The media in each conical flask with different levels of pH was sealed and autoclaved. After autoclaving, it was cooled and 10ml poured into every Petri dish with each pH level having four plates and allowed to solidify. The plates were then inoculated with both *Xac* and *Xam,* 2 plates for each pathovar on every level of pH, and incubated at 28^0C for 48 hours (Chase *et al.,* 1992; Suresh *et al,* .2013)

4.3.3.2 Growth temperature tests.

The pathogen's ability to grow in different temperatures was tested at four levels that are 23^0c, 28^0c. 35^0c, 40^0c. Each pathovar was inoculated on two Petri dishes containing 10ml of YPGA media, therefore, making four Petri dishes for each temperature level. A temperature level of 28^0c was used as a control. Inoculation and incubation of each temperature level were done on different days because only one incubator was used and which could not accommodate the four levels of temperature at once. (Suresh *et al.,* 2013)

4.3.3.4 Salinity test

The pathogen was tested at different levels of NaCl salt. Bacterium isolates were grown in 0.5% NaCl, 1%NaCl and 2%NaCl, 3% NaCl and 4% NaCl. Using sterile distilled water various concentrations of NaCl were made in different universal tubes with each universal bottle containing 10ml of the salt concentration. For every level of salt concentration, four universal bottles were used with two bottles holding each of the bacteria pathovars. Distilled water with no sodium chloride salt was used as the control experiment. The bacteria broth (1ml) for each pathovar *Xac* and *Xam* was introduced in each universal tube containing 10ml NaCl of each concentration level. Incubation was done for 3 days to observe for any indication of bacterial growth in the universal bottles. (Chase *et al.,* 1992)

4.4 Results

4.4.1 Biochemical characteristics of bacterial blight pathogen isolates.

When the bacterium isolates were observed under the microscope they appeared pink for both pathovars *cassavae* and *manihotis* as seen in figure 4.1 below. This indicated that they were both

retaining the color of the purple stain of crystal violet hence proving to be gram-negative bacteria for both pathovars *casaavae* and *manihotis.*

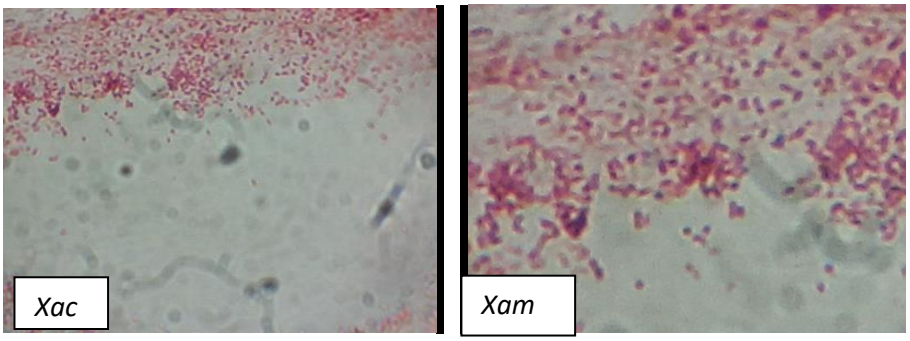


Figure4. 1; Microscopic view of gram-negative bacteria *Xac* and *Xam* at × 100 magnification.

The two pathovers were motile when seed under the microscope, he tested for gelatin they all produced gelatinase which hydrolyzed gelatin around the line of bacterial growth for both the pathovers. There was an appearance of brown color along with the bacteria growth for both *Xac* and *Xam* when lugos iodine was poured on the plates having bacterial growth showing that they can hydrolyze starch.

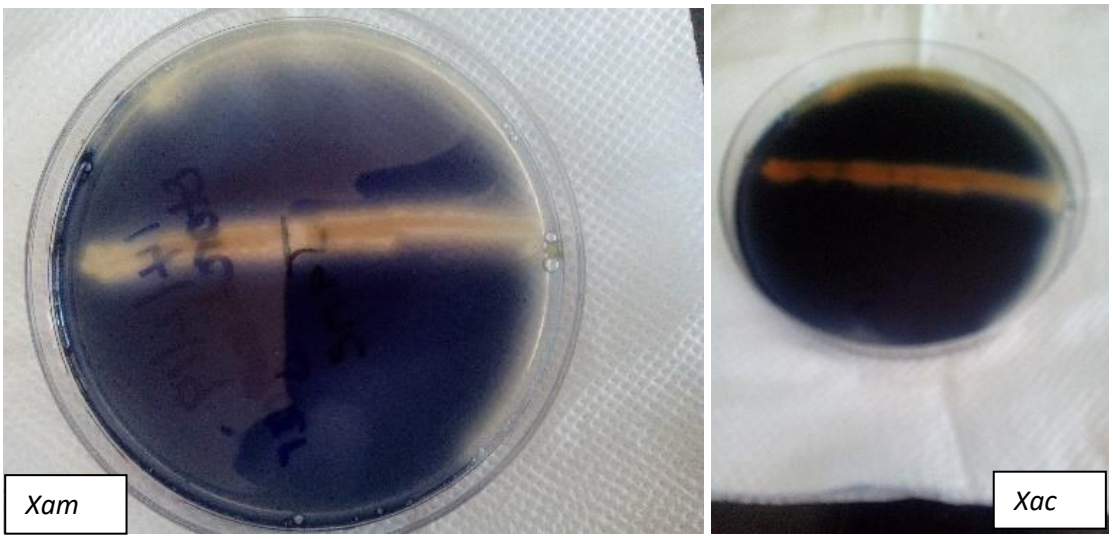


Figure4. 2; Starch hydrolysis of *Xac* and *Xam*

There was no fluorescence observed under the ultraviolet light when the bacteria were grown on Kings B media for 24 hours and placed in the UV light machine. The two pathovars proved negative for the fluorescent pigment production test. There was also the formation of effervescence immediately after sodium hydroxide solution was poured on the bacterial cultures proving positive for the catalase test.

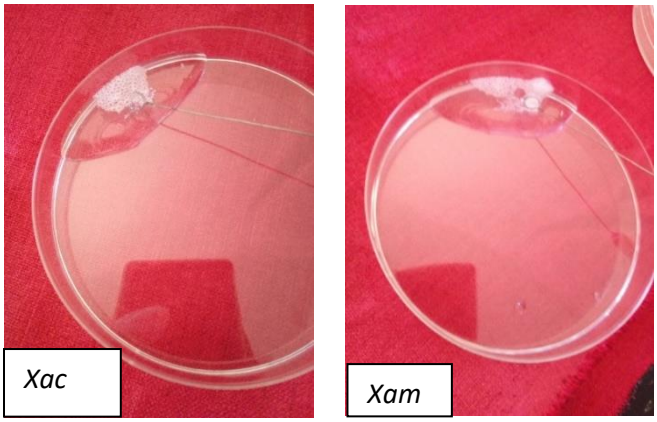


Figure 4. 3; Showing positive catalase test for *Xac* and *Xam*

Table 4. 1;Summary of the results of biochemical tests conducted.

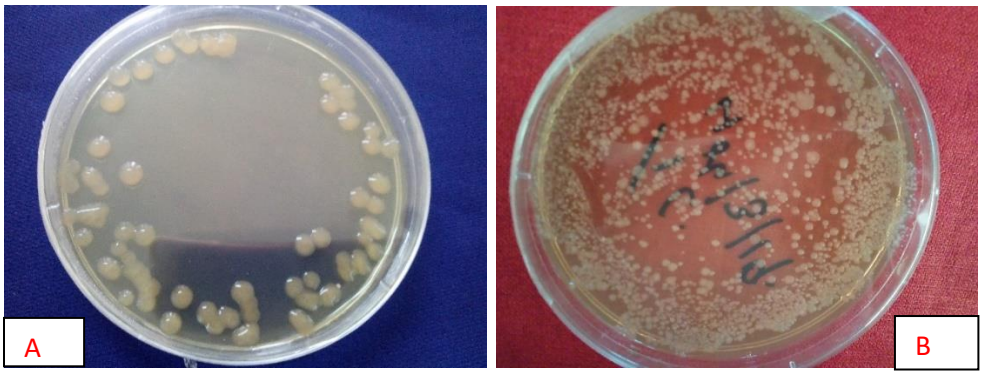
Test conducted	X.pv.*manihotis*	X.pv. *cassavae*
Gram staining	__	__
Motility test	motile	motile
Gelatin hydrolysis test	+	+
Starch hydrolysis test	+	+
Catalase test	+	+
Fluorescent pigment production test	__	__
Potato rot test	+	+

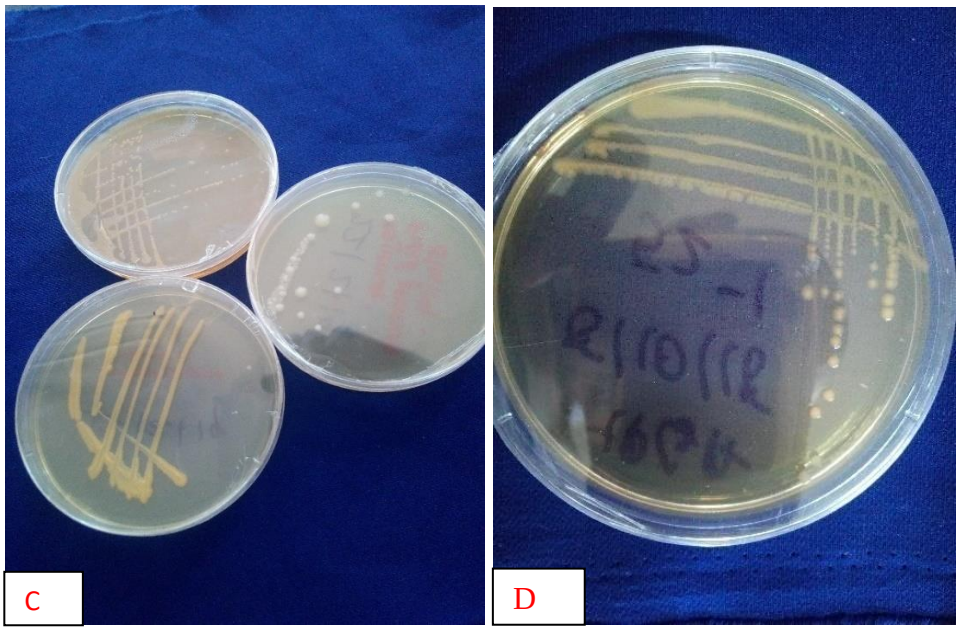
4.4.2 Phenotypic characterization

Table 4. 2; Phenotypic characterization of Xam and Xac isolates grown on YPGA media.

Sample no.	Form	Color	Elevation	Size	Surface	Margin	Structure	Growth degree	Texture
8	Circular	White	Convex	Small	Smooth	Entire	Opaque	Less	Mucoid
12	Circular	Cream	Flat	Small	Smooth	Entire	Opaque	Less	Moist
31	Irregular	White	Flat	Large	Smooth	Entire	Opaque	Less	Moist
7	Circular	Yellow	Convex	Large	Smooth	Entire	Opaque	High	Mucoid
8	Circular	White	Convex	Large	Smooth	Entire	Translucent	High	Moist
42	Circular	Yellow	Convex	Large	Smooth	Entire	Opaque	High	Moist
40	Circular	White	Convex	Small	Smooth	Entire	Opaque	Less	Moist
69	Circular	Cream	Convex	Small	Smooth	Entire	Opaque	Less	Moist
67	Circular	cream	convex	large	smooth	Entire	Opaque	High	mucoid
59	Circular	cream	convex	small	smooth	Entire	Translucent	Less	Mucoid
13	Circular	Cream	convex	Small	smooth	Entire	Translucent	Less	Mucoid
44	Circular	Cream	convex	Small	Smooth	Entire	Translucent	Less	Mucoid
70	Circular	cream	convex	Small	smooth	Entire	Translucent	Less	Mucoid
14	Circular	cream	convex	small	Smooth	Entire	Translucent	Less	Mucoid
1	Circular	cream	convex	small	smooth	Entire	Translucent	Less	Mucoid
12	Irregular	White	Flat	Large	Rough	Curled	Translucent	High	Moist
6	Circular	cream	convex	Large	smooth	Entire	Translucent	High	Moist
63	Irregular	white	Flat	Large	Rough	curled	Opaque	High	Dry
66	Circular	Yellow	convex	large	smooth	entire	opaque	High	moist
51	Circular	yellow	convex	large	smooth	Entire	Opaque	High	Moist
13	Circular	yellow	Convex	large	Smooth	Entire	Opaque	High	Moist
43	Irregular	White	flat	Large	Rough	Curled	Opaque	High	Dry
60	Circular	Cream	Convex	Small	smooth	Entire	Opaque	less	Mucoid
63	Irregular	White	Flat	Large	Rough	Curled	Opaque	High	smooth
67	Circular	Cream	Convex	Small	Smooth	Entire	Translucent	Less	Smooth
50	Circular	white	Flat	large	Rough	Entire	Opaque	High	smooth
60	Circular	Cream	convex	small	smooth	entire	Translucent	Less	smooth
25	Circular	cream	convex	small	smooth	entire	Translucent	Less	Smooth

The yellow-colored isolates were *cassavae* while the creamish to whitish colonies were *manihotis* as seen earlier in the studies.





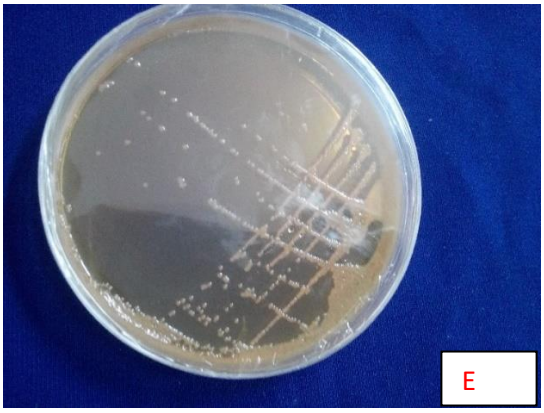


Plate 4.4; A& B, showing cream colonies of Xam. *C* Showing comparison of *Xac* colonies yellow in color and *Xam* colonies which are cream and White. D: showing cassavae while E: showing manihotis.

4.4.3 Physiological characterization

Bacterial colonies for both pathovars were seen growing on plates with a pH ranging from 6 to

10.5. There were no bacterial colonies for both pathovars seen in plates of pH 3 and 4.5.

Table 4. 3; Bacterial growth at different pH levels

Bacteria Pathovers	pH 3	pH 4.5	pH 6	pH 7.2	pH 7.5	pH 8	pH 9	pH 10.5
pv cassavae	-	-	+	+	+	+	+	+
pv manihotis	-	-	+	+	+	+	+	+

There was no bacterial growth at 40C as compared to 23^0c, 28^0c and 35^0c where growth was seen for both the pathovars.

There was bacterial growth for all the bacterial pathovars in 0.5 % 1 %, 2%, 3% but there was no growth in 4% and 5% concentration of sodium chloride. This was indicated by a cloudy appearance in the bottles with bacterial growth and a clear appearance in the bottles without bacterial growth as seen in the figure below. A cloud appearance was seen getting stronger from 4% concentration as you move towards the lowest concentration of 0.5%.

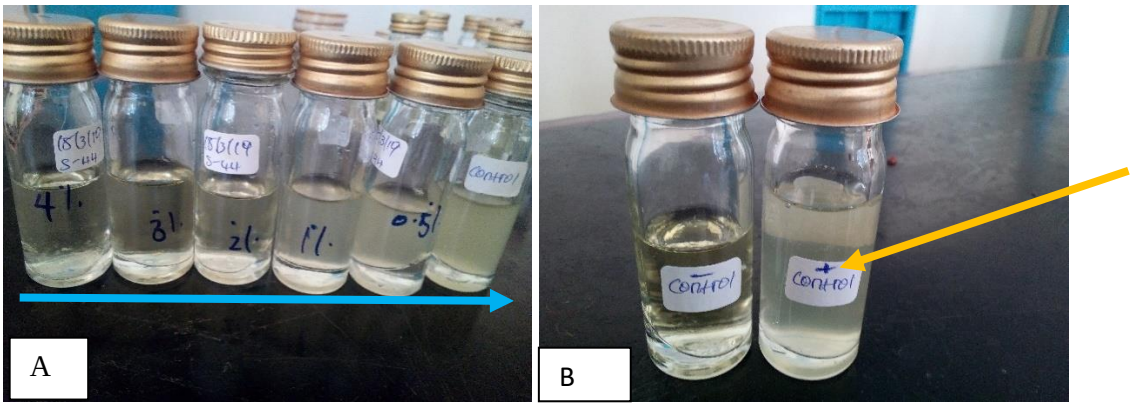


Plate 4. 5; **A,** shows bacterial growth manifested by cloudiness at different levels of salt concentration while **B,** shows the positive and negative control.

After 48 hours all the *Xanthomonas* species caused rot on potatoes beginning from the point of inoculation. There was no rot on the control potato pieces as seen in the figure below.

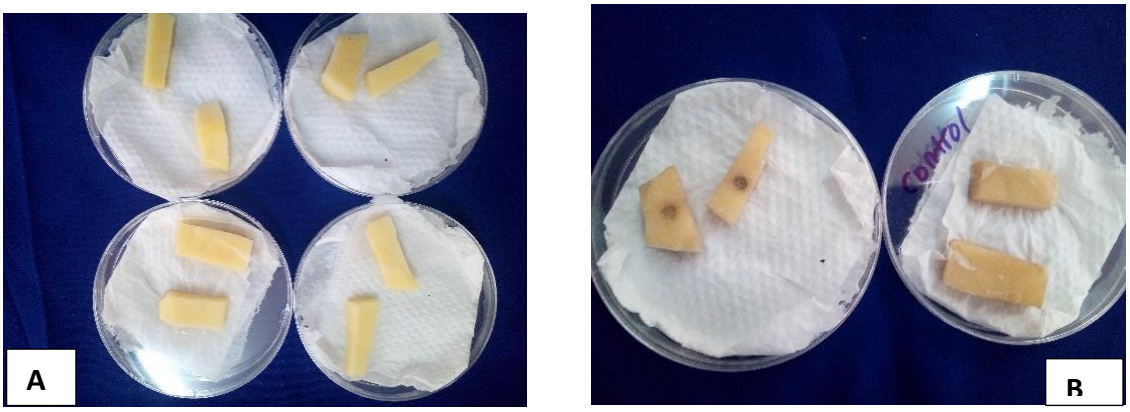


Figure 4. 6; Potatoes before inoculation A and after inoculation B with pv *cassavae* and pv *manihotis* .

Table 4. 4 Utilization of carbohydrates for both the pathovars.

Pathovar (pv.)	**Cellobiose**	**Starch**	**maltose**	**sucrose**	**Asparagine**	**Lactose**
pv. manihotis	-	+	+	+	-	-
pv. cassavae	-	+	-	+	-	-

There was gas production for the two bacteria pathovars in all the sugars used except on cellobiose where Xam was negative. This was indicated by the formation of a bubble in all the Durham tubes except for the control bottles.

Plate 4.7; Experiment on utilization of different sugars by the bacteria

4.5 Discussion

A biochemical test conducted, showed two pathovers were gram-negative, as indicated by a pinkish color appearance in the observation of the bacteria under the microscope. This confirms studies done by Ogunjobi in 2008 on physiological studies of *Xanthomonas axonopodis p.v manihotis* and Chege in 2017 on Phenotypic and genotypic diversity of bacteria causing cassava bacteria blight in Kenya. The two pathovers were motile, this was seen by a continuous movement of the bacteria under the microscope hence confirming work done by *Ongujobi et al.* (2008). The study confirms the bacteria isolated belongs to *Xnthomanadaceae* family depending on the output of the tests conducted.

The two bacteria pathovars hydrolyzed gelatin, hydrolyzed starch and tested negative for fluorescent pigment production test. They were also positive for the catalase test as indicated by the formation of effervescence when sodium hydroxide solution was poured on both bacteria pathovars. These tests were not able to differentiate the two pathovars, resulted in similarities hence could not be used to differentiate Xam and *Xac*. This confirms work done by (Ongujobi *et al.*, 2008 and Odongo *et al.*, 2019).

The 40 isolates obtained from the field during the survey had a variation in the phenotypic features of their colony. The colony color ranged from yellow, white and cream. The 10 isolates produced yellow colonies similar to Xac characteristics while 18 isolates produced white colonies and 12 isolates producing cream colonies similar to Xam characteristic. Most of the colonies had a convex elevation, entire margin, translucent structure, smooth surface, circular form and mucoid texture. There was variation in size and growth rate with many colonies having large colonies while few isolates had small colonies. Most colonies showed a high growth rate and others slow in growth rate. The manifested characteristics were not specific to any pathovar apart from the Yellow pigmentation which was specific to the 10 isolates of Xac and white to creamish pigmentation which was specific to 30 isolates of Xam. This confirms work done by Chege *et al*., 2017 on the phenotypic diversity of bacterial causing CBB in Kenya. These results give room for more intervention on different strains of the pathogen and how they could be infecting the cassava plant.

The two pathovars grow at similar pH and none was able to grow at pH 3 and pH4.5.There was continuous growth for all the bacteria from a range of pH 6 to pH 10.5. The two pathovars can grow well in both neutral and alkaline conditions. Acidic conditions are not suitable for the pathogen hence subjecting the CBB bacterial to acidic conditions of Ph below 4.5 can manage the disease completely. The PH range can be used to manage the bacteria and also can be adopted for treating infected planting materials.

Both the bacteria can grow at any salt concentration range from 0.5 to 3 % but beyond 4% concentration, there was no growth for both the bacterial pathovars. All the pathovar were able to cause potato rot when the colonies were inoculated on clean potato tissues. This confirms the ability of the *Xanthomonas* species to cause potato rot. This could also indicate that potatoes can

be an alternate host for the bacteria and if the pathogen finds its way on potato crop it can cause infection to bring losses to the crop.

Pathovar *manihotis* did not utilize cellobiose while pathovar *cassavae* utilized cellobiose. This differentiates the two pathogens basing the utilization of cellobiose. Pathovar *manihotis* utilized maltose while pv. *cassavae* did not utilize maltose, this brings another difference in characterization of the two. For all the sugars tested that is starch and sucrose both pathovars were able to utilize the sugars. Asparagine and lactose tested negative for both pathovers. Other studies done by (Ongujobi *et al*., 2008) show that pathovar *cassavae* is not able to produce acid faster from maltose. These basic tests on sugar prove that scientists can separate the two pathovars when working with them.

4.6 Conclusion

The two pathovars show a lot of similarities in the tests conducted. This included: gram stain, motility test, gelatin test, starch hydrolysis test, fluorescence test, temperature tests, pH test, and salinity test. Also, similarities were seen in the utilization of starch sucrose asparagine and lactose sugars. Although there was a difference witnessed in the utilization of some sugars recorded on maltose and cellobiose. Pathovar *manihotis* did not utilize cellobiose while pathovar *cassavae* utilized cellobiose which gave an opportunity in differentiating the two pathovars. Generally, the two pathovars can grow well in alkaline conditions, cannot grow well in high temperatures above 4^0c and cannot survive in any salt concentration above 4%. This, therefore, offer a basic characterization of the two pathovars responsible for cassava bacterial blight.

CHAPTER FIVE: RECOVERY OF CASSAVA BACTERIAL BLIGHT (CBB) INFECTED PLANTING MATERIALS THROUGH USE OF ANTIBIOTICS IN TISSUE CULTURE PROPAGATION MEDIA

5.1 Abstract

Cassava bacterial blight is mainly disseminated through infected planting materials. This is when farmers share infected planting materials from one cassava farm to another within or outside the country. The biggest challenge in the seed system is insufficient supply of disease-free planting materials, this has led to increased incidence of cassava bacterial blight in cassava growing regions in Kenya. Tissue culture techniques can be instrumental in the rapid production of cassava planting materials and also useful in producing diseaes free planting materials.

A study was conducted to propagate tissue culture cassava diseaes free planting materials through tissue culture technique in combination with antibiotics. Three antibiotics were used that is, Tetracycline, doxycycline and Streptomycin at different levels of concentration of 5mg/l, 10mg/l, 15mg/l and 20mg/l. The different concentrations for each antibiotic were incorporated into the tissue culture media. The experiment was monitored for 7 weeks while recording the vital data.

After sprouting the seedlings were isolated and then randomly selected for hardening in the greenhouse. They were then transplanted in pots and monitored for any CBB symptoms and their rate of establishment. Both tissue culture-raised cassava seedlings in the greenhouse were monitored for CBB infections and no symptoms were seen. All the antibiotics used in tissue culture were effective but tetracycline had a lower infection rate of 40% followed by Streptomycine and doxycycline and control which had 45%, 50%, and 70% respectively. However, Doxycycline and tetracycline at 15mg/l and 20mg/l were most effective with plants treated with this antibiotic not showing any growth of the bacteria in the media. All the antibiotics affected plant height and as

the concentration was increasing the rate of plant growth reduced. The study shows that antibiotics have the potential for the early management of cassava bacteria blight in tissues culture when raising cassava plantlets hence offer an option for disinfecting CBB infected plants and raising healthy cassava planting materials for farmers.

5.2 Introduction

Cassava bacterial disease caused by *Xanthomonas axonopodis PV cassavae* and *Xanthomonas axonopodis pv manihotis* is the major bacterial disease of cassava in Kenya. The major transmission of the disease is through the use of infected planting materials which in most cases harbor latent infection (Lozano *et al.,* 1986). Due to this farmers have continued to spread the pathogen as they reuse and share the cassava planting materials from one farmer to another farmer.

The use of stem cuttings and tissue culture seedlings is a common practice in preparing planting materials for cassava in Kenya and relatively use of tissues culture cassava seedlings is being integrated. One of the ways to minimize the spread of cassava bacterial blight causal agents is to ensure the planting materials are disease-free and those infected can be cleaned in tissues culture media and propagated as cassava cuttings.

This is either by using proven clean planting materials or suppressing the bacteria when using planting materials sourced from infected mother plants. Suppression can be done by killing the bacteria or inhibiting their growth using antibiotics.

This research was aimed at cleaning CBB infected cassava plant materials by propagating them on media treated with antibiotics using tissues culture technique. Various studies have shown the effectiveness of antibiotics used *invitro* against many plant pathogens (Ketznelson and Sutton,

1951). Antibiotics application has been practiced widely especially in sprays to control fruit tree bacterial diseases but is not cost-effective when applied under field conditions

5.3 Materials and methods

5.3.1 Sensitivity of bacteria to antibiotics in culture

Antimicrobial susceptibility test was conducted using 3 antibiotics namely streptomycin, doxycycline, tetracycline using the disc diffusion method. Mueller Hinton media was prepared by adding 38 grams of Mueller Hinton powder in 1000ml of distilled water, the mixture was dissolved evenly by heating to boiling. After cooling the pH was adjusted to 7.3 and later autoclaved at 15 psi, 121^{o}C for 15 minutes. It was then cooled to 45^{o}c, mixed well and poured onto clean Petri plates.

Young bacterial colonies of *Xanthomonas axonopodis pv. manihotis* (Xam) and *Xanthomonas axonopodis pv. cassavae* (Xac) aged 24 hours were used. Using a sterile swab the bacteria was picked from the Petri plates and smeared to the plates containing Mueller Hinton media. This was done on 8 plates, 4 plates for *Xanthomonas axonopodis pv. cassavae* (Xac) *and* four plates for *Xanthomonas axonopodis pv. manihotis* (Xam). Discs for each antibiotic were introduced by picking with sterile forceps and placing gently on the plates smeared with the bacteria. All the discs were arranged separately at an equal distance of 3 cm as seen in Plate 5.1 below to give room for the formation of the zone of inhibition. The discs used had the following manufacturer concentrations: Doxycycline 30mcg per disc, Streptomycine 10 mcg per disc and Tetracycline 30mcg per disc. The plates were then sealed and incubated at 28^{o}C for 24 hours

Observations were made after 24 hours and the results were captured for each plate by measuring the zone of inhibition for each antibiotic disc using a 30cm ruler and the results recorded in cm.

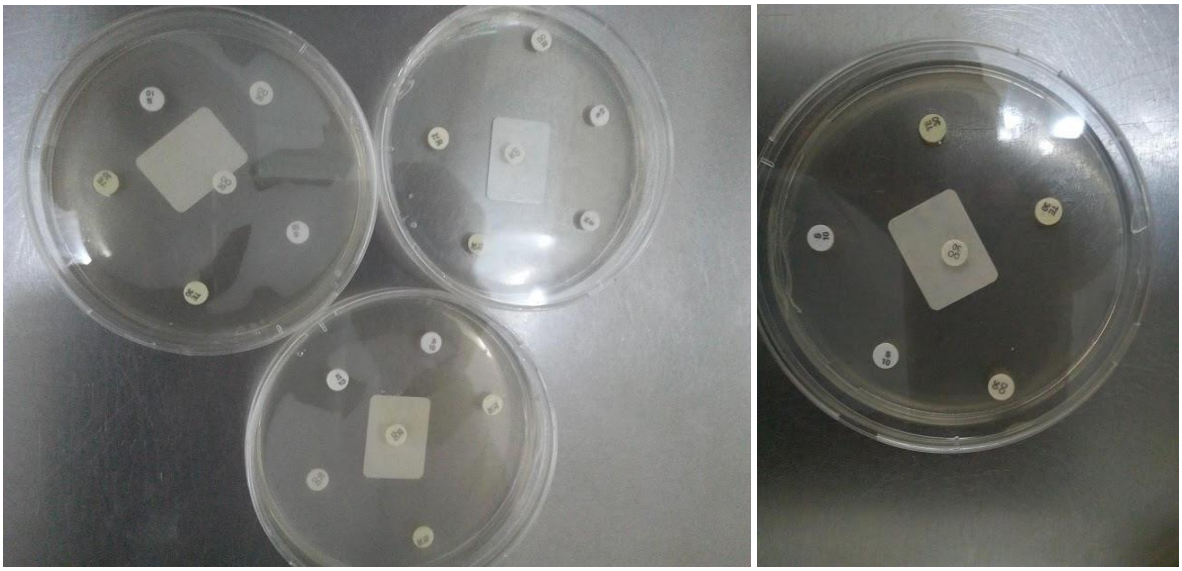


Figure 5. 1; Antibiotic discs arranged on the media inoculated with bacteria isolates

5.3.2 Antibiotic optimization and Tissue culture media preparation

The selected three antibiotics tetracycline, Streptomycine and doxycycline, were optimized at four levels of concentration 5mg/l, 10mg/l, 15mg/l, 20mg/l and 0mg/l as a control treatment. Using the formula C1 V1= C2 V2 the volumes were down calculated to have 100ml for each antibiotic concentration to be used in the experiment. Using a weighing scale each of the antibiotic powder was measured to the required volume, then mixed with sterile double distilled water to make the antibiotic solution. A randomized complete block design in a factorial arrangement with 10 replications, 3 blocks and 5 treatments was used to set the experiment. Each treatment had 10 bottles initiated with explants. The explants were harvested from diseased mother plants which had earlies tested for the presence of CBB.

Murashige and Skoog media (Murashige and Skoog, 1962) was prepared and mixed with different antibiotics to form 15 different treatments of the Experiment. Tissue culture media components included Sucrose 30g/L, agar 8.5g/l, Murashige 4.406g/l, pH 5.8. All the components of the media were weighed and put in a 1-liter media bottle and filled up to a total volume of 1000ml. Then the

mixture was heated on a lampstand to boiling point to enhance even mixing. After boiling the pH was adjusted to 5.8 and the media was dispensed into 15 conical flasks of 250 ml to represent each treatment. All the contents were autoclaved up to 121^{o}c at 15 psi for 15 minutes.

After autoclaving, the media was allowed to cool to 45^{o}c and the temperature was maintained using a water bath. In each conical flask, the treatments were established by adding three antibiotics: Doxycycline, Tetracycline and Streptomycine at different concentration levels of 5mg/l, 10mg/l, 15mg/l, 20mg/l and the labeling was done for every level of antibiotic on the conical flask. The concentrations were chosen basing on previous studies which showed high phytotoxicity experienced in tetracycline concentration above 20mg/l therefore concentration below this was highly preferred (Caervalho *et al* .,2017). After establishing each treatment in every conical flask, the media was dispensed into 10 universal bottles for each treatment containing 10 ml and allowed to cool under sterile conditions.

5.3.4 Explant initiation procedure

Plant materials for the experiment were obtained from cassava bacterial infected plants, grown under greenhouse conditions and confirmed to be infected through observing field symptoms and isolation. Explant harvesting was done by cutting the plant at the tip with at least four to five internodes using a sterile scalpel. The harvested plants were put in a glass vessel containing distilled water and transferred to the tissue culture lab to begin the initiation process.

In the laboratory, the explants were washed in twin 20 for 20 minutes to remove field dust and other contaminants, to soften the plant tissues and to open plant pores. Rinsing was done with distilled water until foaming stopped. Ethanol 70% was added for 3 minutes and poured off, then 0.5% sodium hypochlorite was added for 20 minutes with frequent observation not to bleach the

explants. The explants were then put in a glass beaker and then triple rinsed in sterile distilled water in the laminar hood.

Using sterile forceps and scalpels the explants were put on sterile Petri dishes, the bleached ends were cut and removed using a scalpel. The ex-plant was then reduced to double internode and initiated in universal bottles containing media of each treatment in an upright manner with the potential node points facing up. The experiment was set at room temperatures in a tissue culture laboratory at a photoperiod of 16hours light and 8 hours darkness for seven weeks. The following data was taken during the experiment every week. Number of plants showing infection, the height of the plant in centimeters, number of roots on each plant, plants showing roots.

Figure 5. 2 ; Tissues culture experiment layout showing bottles with initiates inside

5.3.5 Hardening of tissue culture seedlings in the greenhouse

Before hardening, plants from different treatments aged 10 weeks were picked randomly. They were then assessed for bacterial infection through cultural isolation to confirm presence of the bacteria. The confirmed clean plants were then moved to the hardening process to observe the rate of establishment for each treatment. The Prefield experiment was conducted for 4 weeks and data

on growth rate involving plant height, the number of leaves and stand count were scored for the seedlings under the experiment.

5.4 Results

5.4.1 Sensitivity of bacteria to antibiotics

After 24 hours of incubation under 28^{o}c, there was bacterial growth in all the plates but no growth around the antibiotic discs showing a zone of inhibition for all the antibiotics. *Xanthomonas axonopodis pv manihotis* (Xam) showed an equal zone of inhibition for all the tested antibiotics while *Xanthomonas axonopodis pv cassavae* (Xac) showed an equal zone of inhibition for doxycycline and tetracycline but a smaller zone of inhibition for Streptomycine.

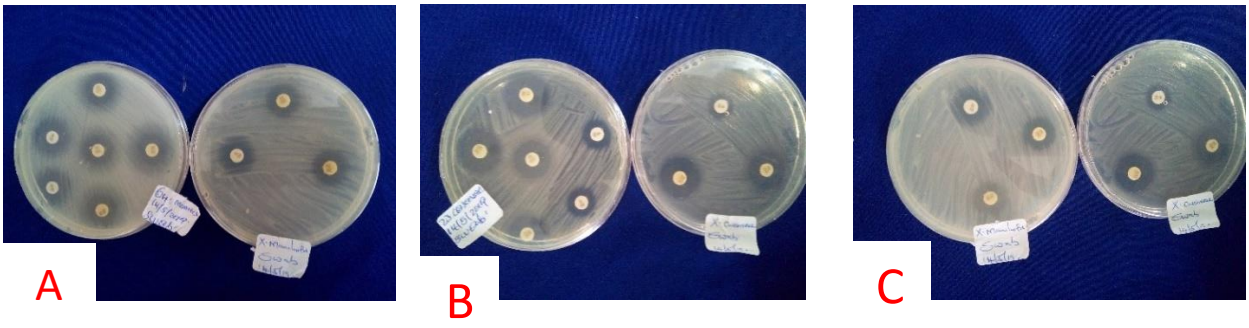


Figure 5. 3 ; Zone of inhibition: A; for *manihotis* (Xam), B; for both *cassavae* (Xac) and *manihotis* (Xam) and C; for *cassavae* (Xac) taken after 28 hours of incubation...

From the measures of the zone of inhibition in cm, tetracycline had the highest zone of inhibition of 1.97cm on cassavae while Streptomycine offered the smallest radius for the zone of inhibition of 1.28cm. There was an equal length in the zone of inhibition for tetracycline and Streptomycine of 1.63 as seen in Figure 5.3 around the discs and also demonstrated in graphs of figure 5.4.

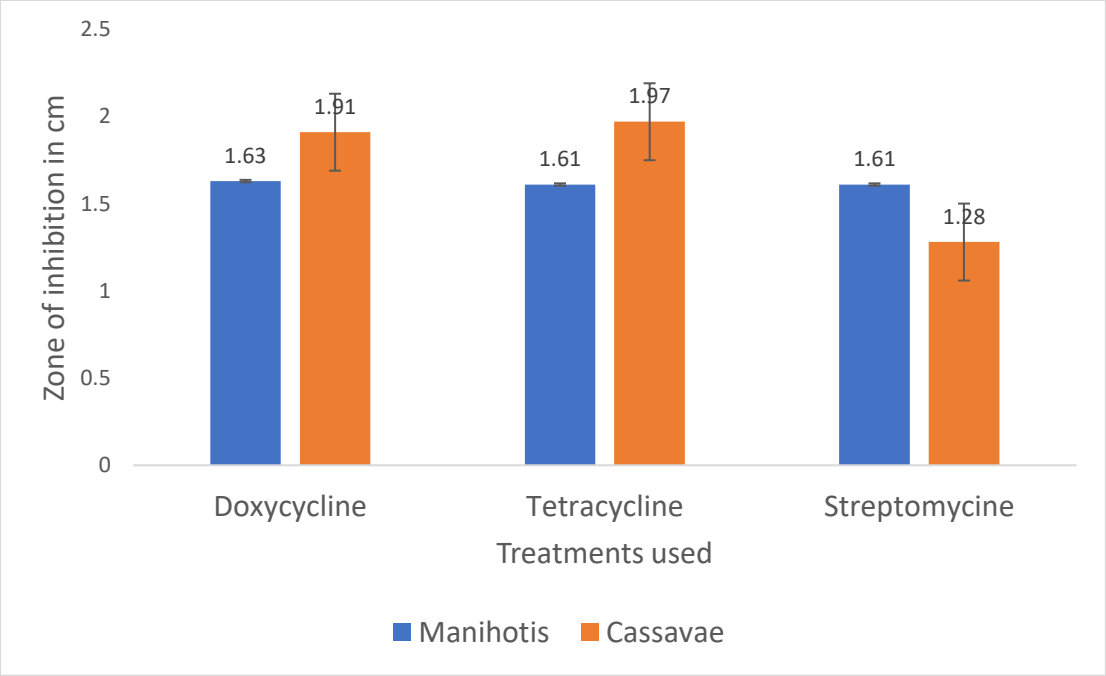


Figure 5. 4; Graph on the variation of the diameter in cm for the zone of inhibition.

5.4.2 Effect of antibiotics in suppressing Bacterial infection in initiated tissue culture cassava plants

Tetracycline had a great potential of inhibiting bacteria growth therefore reducing the infection rate to 40% as seen in Figure 5.5 below. There was a higher infection rate of up to 70% in the control. Tetracycline and Streptomycine had almost similar ability in eradicating bacteria from already infected ex-plants when incorporated in tissue culture media. Those two Tetracycline and Streptomycine showed no significant difference in the rate at which they reduced bacterial infection in explants.

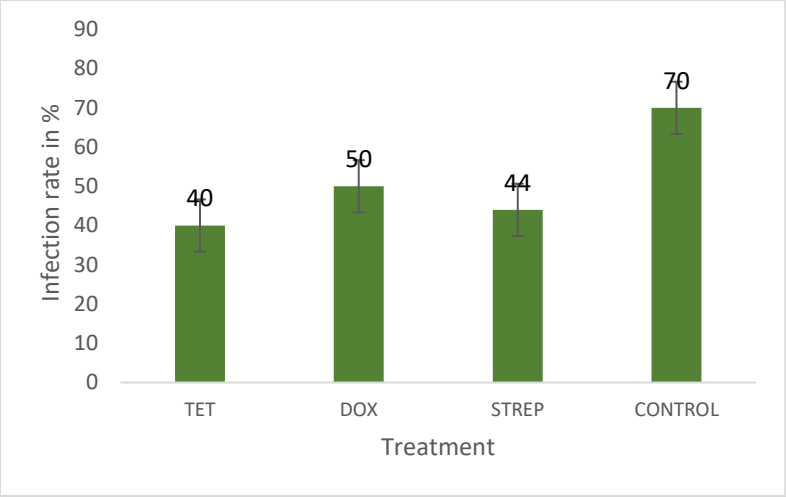


Figure 5. 5; Effect of antibiotics on the rate of cassava bacterial infection.

5.4.3 Effect of antibiotics on bacterial infection in initiated tissue culture cassava plants.

There was zero infection for the two antibiotics tetracycline and doxycycline at the concentration of 15mg/l and 20mg/l. There was an infection in all the antibiotic treatments at the lowest concentration of 5mg/l and 10mg/l. There was no bacterial growth at the concentration of 20mg/l for doxycycline and tetracycline and concentration of 15mg/l of doxycycline. The graph also shows no growth at 15m/l for tetracycline too. As seen in Figure 5.6.

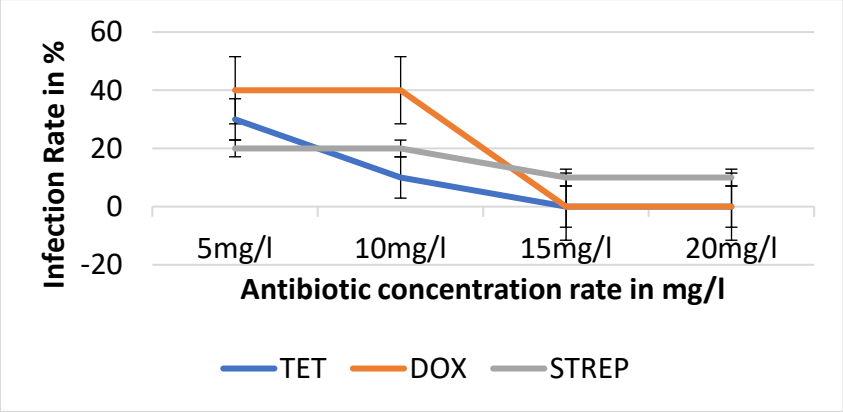


Figure 5. 6 ; How different antibiotic concentrations are affecting bacterial infection rate on TC seedlings.

There is more bacterial growth in the control but no growth at concentration 20mg/l of doxycycline, concentration 20mg/l of tetracycline, and concentration 15mg/l of doxycycline as seen in Figure 5.7.

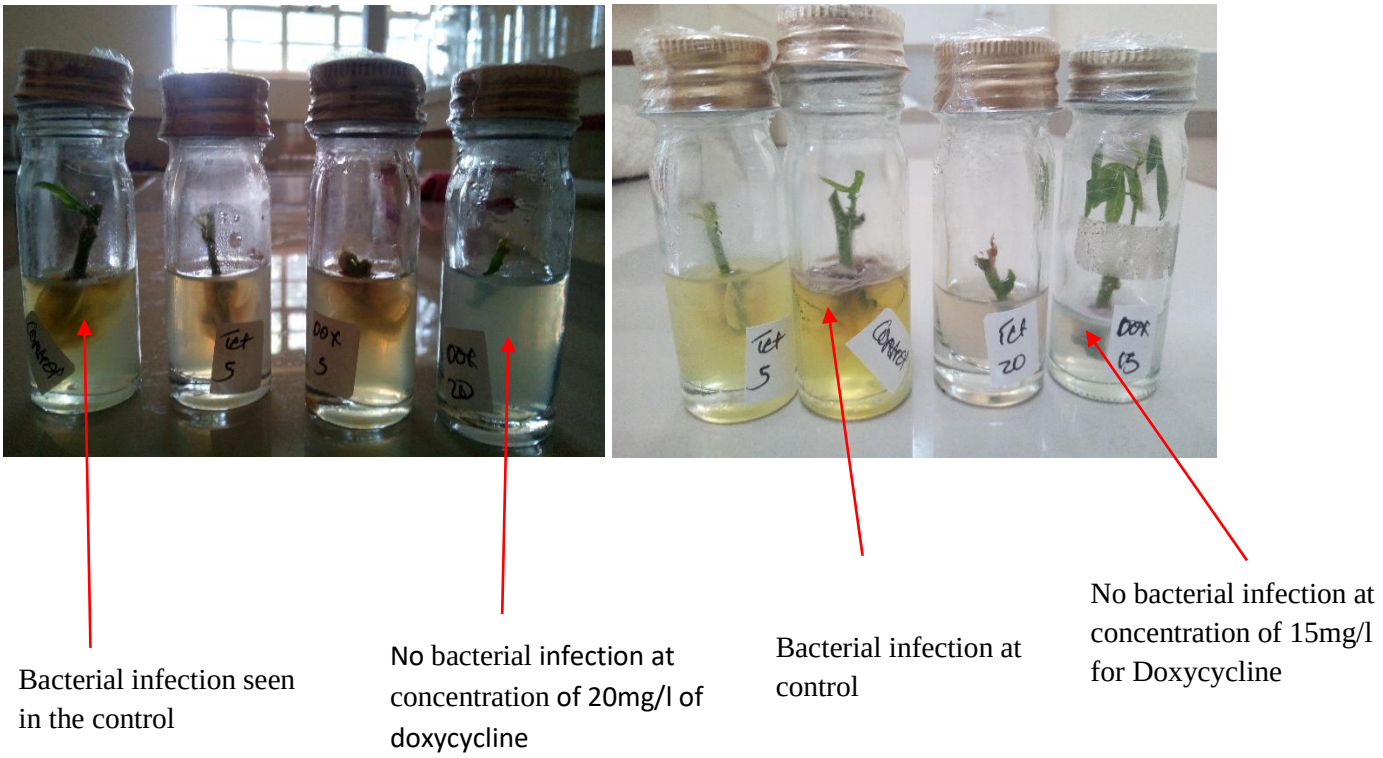


Figure 5. 7; Different antibiotics concentrations from 0mg/l to 20mg/l inhibiting bacterial growth.

5.4.4 Antibiotic concentration effect on plant height in vitro.

All the antibiotics used did not hinder the sprouting of the plant tissues. But they affected plant height at different levels. At 5mg/l concentration, there was a higher plant height with Tetracycline treated plant leading with an average height of 2cm. The highest concentration 20mg/l resulted in the lowest average plant height with doxycycline-treated plants having the lowest plant height of 1cm. An increase in concentration for all antibiotics used caused a reduction in average plant height for all the plants as showed in Figure 5.8.

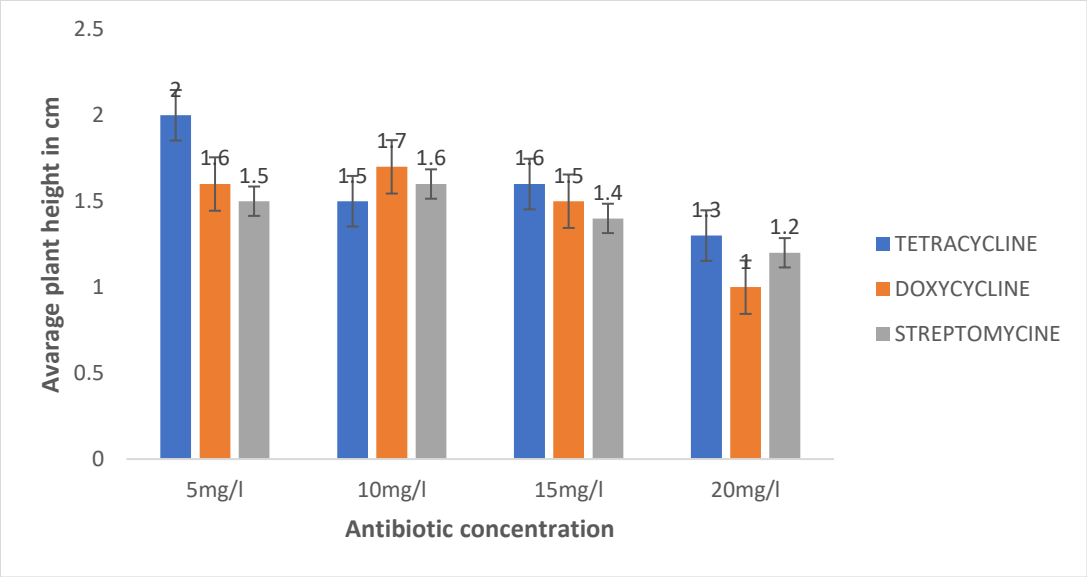


Figure 5. 8; Effect of each antibiotic concentration on plant height in the seven weeks of the experiment.

5.4.5 Effect of the three antibiotics on growth rate

At all the concentrations of 5mg/l to 20mg/l, there was a positive increase every week in terms of growth rate. The lowest concentration of 5mg/l had the fastest growth rate as compared to the highest concentration of 20mg/l in which the plants had a slow growth rate. At week 7 concentration of 5mg/l had the highest average plant height of 2.9 while 20mg/l concentration reported the lowest average height of 2.1 cm as seen in figure 5.9 below.

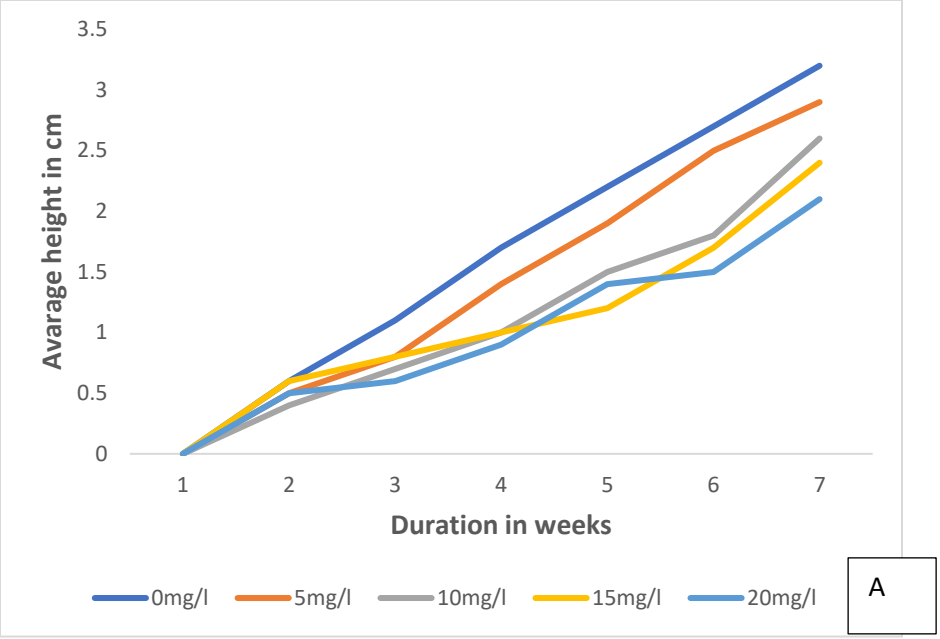


Figure 5. 9; How different concentrations level of Tetracycline affects the height of Tissue culture seedlings within seven weeks.

On doxycycline, there was a continuous increase in growth for all the concentrations. For all the concentrations there was no stable continuous growth as compared to the control. The lowest concentration of 5mg/l had the highest average plant height of 2.5 cm while the highest concentration of 20mg/l had the lowest average plant height of 1.9 cm as seen in figure 5.10 below.

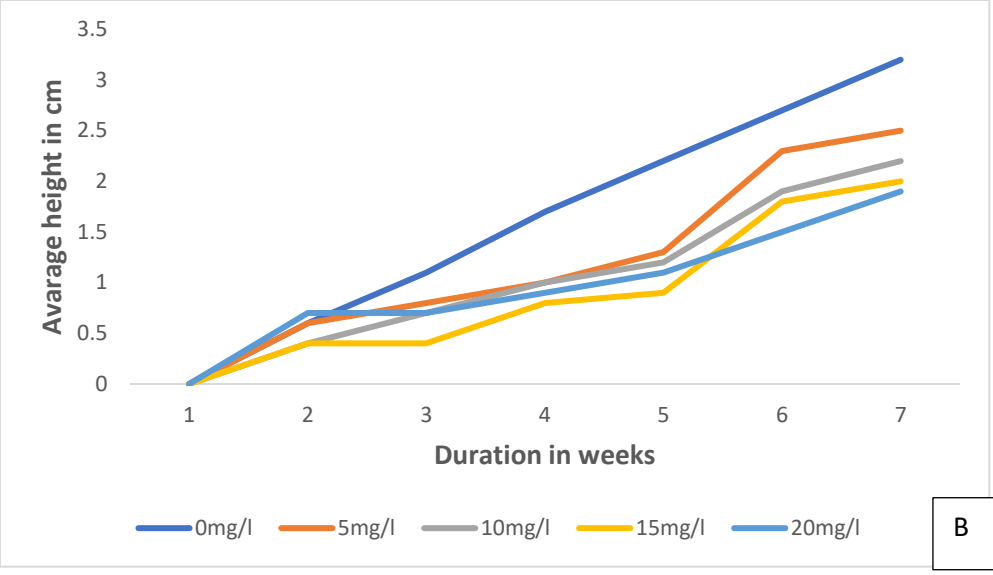


Figure 5. 10; How different concentration levels of Doxycycline affect the growth of tissue culture seedlings in 7 weeks.

Streptomycine showed unstable but continuous growth for all the concentrations from week one towards week 7. There was a reduction in height at week seen as you compare the four different concentrations. Concentration at 5mg/l had the highest average plant height of 3cm as compared to concentration at 20mg/l which had the lowest average plant height of 2cm as seen in Figure 5.11 below.

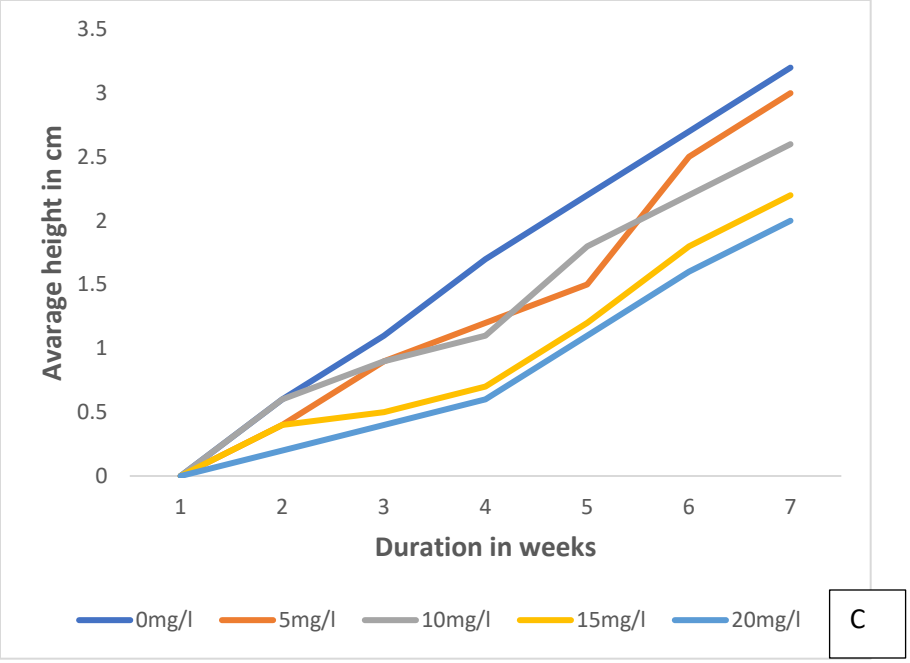


Figure 5. 11; How different concentration levels of Streptomycine affect the growth of Tissue culture seedlings within 7 weeks.

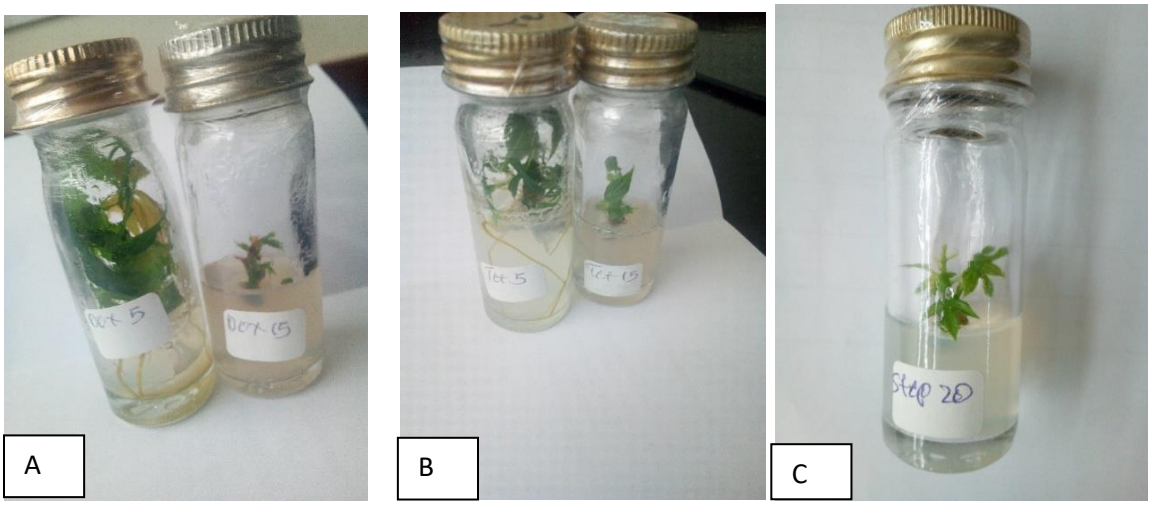


Figure 5. 12; **A**: Plants in Doxycycline at 5mg/l is taller than at 15mg/l. **B**: Plants in Tetracycline at 5mg/l taller than at 15mg/l, **C**; retarded growth in Streptomycine at concentration 20mg/l

There was a faster growth rate in the control as compared to plants treated with antibiotics. Plants treated with doxycycline had the lowest growth rate as compared to those treated with tetracycline and Streptomycine. A gradual continuous growth was seen for all the treatments indicating that no

antibiotic caused stagnation in the growth of the treated plants. This shows that the use of antibiotics on plant tissues influences the growth rate.

5.4.6 Effect of antibiotics on Tissue culture seedling establishment at hardening stage.

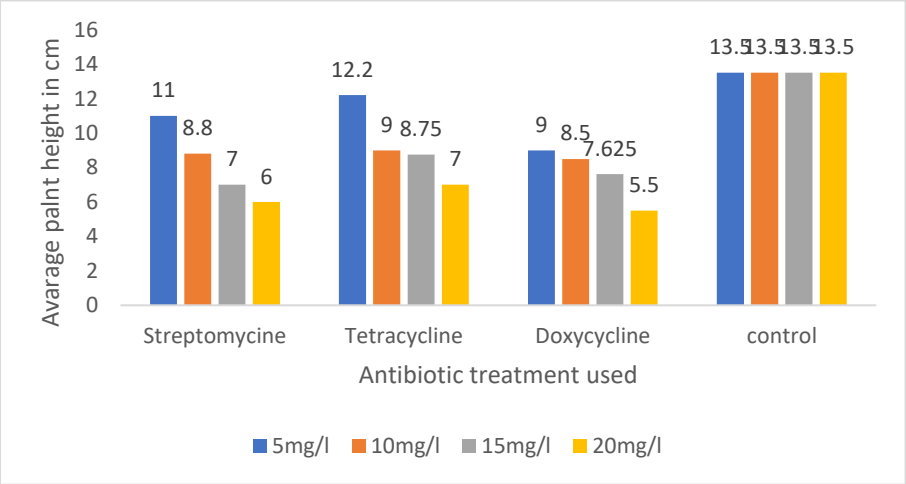


Figure 5. 13; Total average height of treated Tissues culture seedlings in the greenhouse after 4 weeks.

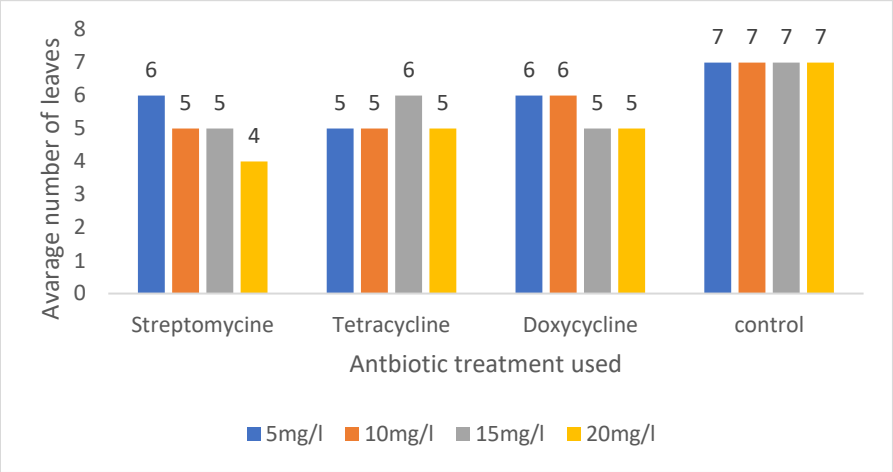


Figure 5. 14; Total average number of leaves for the treated Tissue culture seedlings after 4 weeks.

Figure 5. 15; Hardening of Tissue culture treated plants in green house

5.5 Discussion

Sensitivity tests conducted showed the formation of a zone of inhibition which confirmed that the antibiotics used in the study had the potential to inhibit or eradicate the growth of the two bacterial pathovars. Xanthomonas *axonopodis pv. manihotis* offered a uniform susceptibility to the three used antibiotics by having an equal zone of inhibition for all the antibiotics used on all the plates. *Xanthomonas axonopodis pv*. *cassavae* offered a varied susceptibility to the three antibiotics used with a high susceptibility being to tetracycline followed by doxycycline and streptomycin, respectively. This finding proves that the three antibiotics used had the potential to manage cassava bacterial blight. The zone of inhibition created by tetracycline on *cassavae* indicates that the antibiotic has a high potential for eradicating the pathogen hence can be used in managing the disease effectively. How the three antibiotics responded towards *manihotis* proves that they can all be adapted to manage pathovar *manihotis*.

Tetracycline proved to be most effective in suppressing and even eradicating the bacterial infection on tissue culture media followed by Streptomycin and Doxycycline respectively. There were few

plants on average found to show bacterial infection as compared to other treatments. This showed the potential of antibiotics in eradicating the bacteria from infected cassava plant tissues. Therefore antibiotics can be used to completely clean planting materials from these bacterial diseaes.

Antibiotic concentration is critical in inhibiting bacterial growth, the highest levels used in this study 15mg/l and 20mg/l were more effective as compared to low concentrations of 10mg/l and 5 mg/l. Each antibiotic used was more effective at the highest concentration of 20mg/l. The strength of every antibiotic was seen to increase with the increase in concentration as seen in Figure 5.6. All the antibiotics have the potential of suppressing CBB but with different potentials. Tetracycline and Doxycycline can completely eradicate CBB in the media if used at the concentration of 15mg/l and 20mg/l. While the lowest concentration used of 5mg/l did not eradicate the pathogen,bacterial growth was seen on most of the surface and even within the TC media when observations were made against light through the transparent media. This means the adoption of these antibiotics can be utilized well at a concentration of either 15mg/l or 20mg/l.

Plant height was recorded lowest in the 20mg/l concentration with 1.9cm, 2cm and 2.1cm respectively for Doxycycline, Streptomycine and Tetracycline. Tetracycline had the best results on plant height by showing 3.2cm at the lowest concentration and 2.1cm at the highest concentration. Doxycycline had 1.9 cm plant height at highest level concentration and 2.5 cm at lowest concentration. Streptomycine had 2cm at highest concertation level and 3cm plant height at lowest concertation level. Considering that the strength of the antibiotics in eradicating the bacteria infection increases with an increased concentration, then 15mg/l is well-preferred in offering the best growth rate and also eradicating the bacteria at the same time.

The three antibiotics individually affected growth rates differently. They all showed continuous increased growth every week. At week 7, Streptomycine had recorded 3 cm in seedling height, Doxycycline recorded 2.5 cm while Tetracycline recorded 2.9 cm. Therefore on weekly growth, the lowest concentration of 5mg/l had the best results with Streptomycine leading at a height of 3cm on week 7.

At the hardening stage, Tetracycline offered the best height variations which included 8.75cm as compared to doxycycline, Streptomycine which had 6.25cm and 7 cm average plant height respectively. On leaf count, tetracycline had an average of 6 leaves per plant as compared to doxycycline and Streptomycine which had 5 leaves, and 5 leaves respectively. This showed that plants treated with antibiotics can establish well in the greenhouse. The study established that Tetracycline had good results when it came to hardening as compared to the other two antibiotics used. The study allowed for incorporation of antibiotics into cassava tissue culture media as a strategy to lower transmission by producing healthy planting material in case of no resistant cultivars.

5.6 Conclusions

Antibiotics have the potential to be used in managing cassava bacterial blight when incorporated in tissue culture media. The most preferred concentration was 15mg/l. This concentration gave the best growth rate and was the most effective in eradicating the bacteria from the plant tissues. Tetracycline was the best-preferred antibiotic in the incorporation of antibiotics in tissue culture to manage cassava bacterial blight from infected cassava planting materials. The other two antibiotics Doxycycline and Streptomycine were not as effective as compared to Tetracycline in both the growth rate of the seedlings and eradication of the bacteria from the plant tissues. Therefore the study concludes that Tetracycline is the most preferred at a concentration of 15mg/l

to be incorporated tissue culture media when propagating CBB free cassava planting materials. The application of antibiotics in tissue culture to propagate diseaes free cassava planting material is a viable practice and should be adopted by other researchers and practitioners in the cassava seed system.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

With the distribution of Cassava Bacterial blight in 24 counties in Africa, Kenya is not an exception. The diseaes are now in all cassava growing regions in Kenya. The study showed a wide distribution of the diseaes across the Kenya coast. This calls for an integrated approach in management ranging from the use of resistant varieties, understanding the genetic diversity of the various strains, use of disease-free material, use of adequate cultural practices and careful selection of planting materials from sites confirmed to be disease-free (Lozano and Sequeira, 1974).

From the survey studies conducted in the Kilifi and Taita Taveta counties, many farmers were sharing planting materials and this was a common practice in the regions that had contributed to the spread of cassava bacterial blight. Farmers were less informed on cassava diseases and the study showed that farmers did not give attention to cassava diseases and most of them did not even practice management of cassava diseases. This contributed to the spread of the disease from one farm to another unknowingly. Farmers in the study region did not have access to any varieties that proved to be resistant to cassava bacterial blight. All the varieties found in the study region during the survey were susceptible to the disease. This is offering a great threat to cassava farming in the region. Therefore the spread of the disease through sharing or selling of infected cassava cuttings should be stopped to avoid great losses which are likely to occur in the region.

The study showed the distribution of the disease in both counties with a variation in incidence for each county. This confirms studies done in 2016 on the distribution of the disease in Kenya which showed Cassava bacterial blight to be present in Kilifi and Taita Taveta counties (Odongo *et al*.,

2019). This study showed that both Xanthomonas *axonopodis pv cassavae* and *Xanthomonas axonopodis pv manihotis* are well distributed in study counties with pv. *manihotis* being the dominant pathogen in cassava farms. This concurs with studies done by (Chege *et al.*, .2017) on morphological and genetic variability of the pathogen in Kenya, where yellow isolates were obtained from the samples collected in different regions of cassava farming in Kenya.

The two pathovars *cassavae* and *manihotis* can be differentiated using colony morphological appearance whereby pv. *cassavae* has yellow pigmentation and pv. *manihotis* has a white to creamish pigmentation (Mariete *et al*., 1984). Biochemical characterization of the two pathovars showed a lot of similarity between the two pathogens. This was not reliable in differentiating them since most of the tests conducted produced similar results for the two pathovars. The two pathovars only differed on the utilization of maltose and cellobiose. *X. axonopodis* pv. *manihotis* utilized maltose while *X. axonopodis* pv *cassavae* did not utilize maltose. *X. axonopodis* pv *cassavae* utilized cellobiose. Molecular fingerprinting remains the sure way in differentiating the two pathovars (Ongujobi *et al*., 2005).

Antibiotics have been widely used since the 1950s in managing plant diseases in many countries including the United States of America (Stockwell *et al*., 2012). Streptomycine, Oxytetracycline, and penicillin are some of the antibiotics applied to control plant diseases including fire blight in apples, crown gall, necrosis of giant cactus and many more diseases. The greatest fear remains on-resistance of antibiotics and their effect on human health when applied to plants. Currently, most of the existing antibiotics are for human use and very few antibiotics have been developed for use on plants. Successful results have been obtained in using antibiotics to control contamination in plant tissue culture experiments. In this study, all the used antibiotics namely: Doxycycline,

Streptomycin and tetracycline were effective in controlling the CBB causing pathogen in tissue culture plants albeit at different concentrations.

6.2 Conclusion

The study concludes that cassava bacterial blight is well distributed in Taita Taveta and Kilifi Counties. Farmers in this region were less informed on cassava diseases, a factor that was seen to greatly contribute to the spread of the disease. The two pathovars responsible for CBB were well distributed in the two counties. The most dominant pathovar was *X. axonopodis* pv. *manihotis* while *X. axonopodis* pv *cassavae* was less prevalent in the two counties. The incidences in the two counties were noted as being at an average of 20%. This creates need for need for better disease management practices to lower increase in incidence which has the possibility for going to more than 80% as reported in West African countries like Ghana, Nigeria, and Guinea (Lozano *et al.*, 1987; Nkongolo *et al*., 2014;).

The two pathovars, X. *axonopodis* pv. *cassavae* and *X. axonopodis pv manihotis*, causing CBB are similar in many features. Cultural and physiological/biochemical characterization were not exhaustively reliable in differentiating the two pathovars although they are used for basic differentiation. That is cultural in which colors were used to differentiate the pathovars signified by yellow and white pathovars. Sugar utilization offered difference in the characteristics of the pathovars where by X. *axonopodis* pv. *manihotis* utilized maltose while *X. axonopodis pv cassavae* did not utilize maltose.

The use of antibiotics in tissue culture media eradicated the pathogens from planting materials infected with CBB through the Tissues culture technique. This gave room for the production and distribution of disease-free planting materials to farmers. Doxycycline and Tetracycline were

effective at the concentration of 15mg/l and 20mg/l in suppressing CBB infection. These concentrations eradicated the pathogens and still allowed the plant to establish well in the hardening process. There was no significance difference in the antibiotics used to eradicate bacterial infection from the plantlets. This proved the ability to recover CBB infected planting materials in tissue culture using antibiotics as viable scientific approach. Tetracycline at the concentration of 15mg/l was the best preferred in recovery of CBB infected plant materials through tissues culture process.

6.3 Recommendations

I. Cassava bacterial blight surveillance in Kilifi and Taita-Taveta counties should be continued through educating the farmers on diseaes identification.

II. There is a need to use this data and establish disease-free farms within the two counties to be used for seed production purposes.

III. Farmers should be trained on the importance of sourcing clean planting materials every season to avoid reoccurring of CBB in their farms.

IV. Future studies are needed on characterizing the two pathovars from isolates collected across the country to establish strain variation.

V. Further studies need to be done on more sugars to establish more differences from the bacteria pathovars in utilizing the sugars.

VI. Antibiotics should be deployed to disinfect cassava plantings in tissue culture and the seedlings supplied to cassava nurseries to raise cuttings for plantings or selling.

VII. The established cassava tissue culture laboratories should ensure mass production of disease-free cassava seedlings for farmers to start new plantations. Plantlets can be supplied to initiate disease-free mother cassava plants in the counties for farmer uptake.

VIII. There is a need for developing a clean seed production program that will be disseminated planting materials to farmers.

IX. It is recommended that proper phytosanitary measures should be followed from the tissue culture level to mother plants in the fields or greenhouses to ensure a disease-free supply of planting cassava materials at a community level.

X. In the long run, there is a need for a rapid diagnostic protocol that can be adopted in monitoring the movement of cassava planting materials from one source to another.

XI. More research on different antibiotics can be done to establish the most economic antibiotic to be routinely used in the tissue culture as an initial option for managing CBB pathogens

XII. The use of sensitivity tests can be exploited further in differentiating closely related bacteria pathovars.

XIII. There is a need for studies on the role of vectors in spreading the cassava bacterial in two counties where cassava is a major crop.

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APPENDICES

Appendix I. Questionnaire used during the baseline survey

<table>
<tr>
<td>1</td>
<td>S

County .....

Sub-county .....

Location .....

Village .....</td>
<td>Ward.

<u>GPS Coordinates</u>

Longitude (E).

Latitude (S).

Altitude.</td>
</tr>
<tr>
<td>2</td>
<td>Name of Farmer

…….

Sex: Female [0]Male [1]</td>
<td>Age of farmer: Years

<table>
<tr><td>Youth</td><td>< 35</td><td>[1]</td></tr>
<tr><td>Middle aged</td><td>36 - 50</td><td>[2]</td></tr>
<tr><td>Upper middle aged</td><td>51 - 60</td><td>[3]</td></tr>
<tr><td>Retiree</td><td>> 60</td><td>[4]</td></tr>
</table>
</td>
</tr>
<tr>
<td>3</td>
<td>Head of household (sex)

Female [0]Male [1]

Household size (Number of members) .....</td>
<td>Own Farm size acres

<table>
<tr><td>< 2</td><td>[1]</td></tr>
<tr><td>2 - 5</td><td>[2]</td></tr>
<tr><td>6 – 15</td><td>[3]</td></tr>
<tr><td>> 15</td><td>[4]</td></tr>
</table>
</td>
</tr>
</table>

<table>
<tr><td></td><td></td><td>Rented farm size (if any)acres</td></tr>
<tr><td>4</td><td>
<table>
<tr><td rowspan="6">Respondent main occupation</td><td>Formal Employment</td><td>[1]</td></tr>
<tr><td>Casual Employment Time</td><td>[2]</td></tr>
<tr><td>Business Person</td><td>[3]</td></tr>
<tr><td>Full Farmer</td><td>[4]</td></tr>
<tr><td>Other</td><td>[5]</td></tr>
<tr><td colspan="2">(Specify)……</td></tr>
</table>
</td><td>Do you participate in other off-farm activities?
Participate [1] otherwise [0]
Specify.</td></tr>
<tr><td>5</td><td>Academic Qualification</td><td>Years of schooling ……..
<table>
<tr><td rowspan="4">Level of education attained</td><td>None</td><td>[1]</td></tr>
<tr><td>Primary</td><td>[2]</td></tr>
<tr><td>Secondary</td><td>[3]</td></tr>
<tr><td>Tertiary</td><td>[4]</td></tr>
</table>
</td></tr>
</table>

SECTION 2

Land preparation

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

TYPE /TIME	START(month)	END (month)
Long rains		
Short rains		

2. In which months do you prepare land?
    a) Long rains

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
1	2	3	4	5	6	7	8	9	10	11	12

    b) Short rains

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
1	2	3	4	5	6	7	8	9	10	11	12

3. How do you prepare your land for crop production?

    Tractor [1]

    Ox-plough [2]

Hand hoe [3]

Minimum tillage [4]

Other [5]

(Specify)…….

4. a) Do you practice any soil conservation management?

Yes [1] No [2]

b) If yes, which soil conservation measures do you practice?

Cover crops [1]

Terraces [2]

Gabions [3]

Mulching [4]

Contour planting [5]

Minimum tillage [6]

Other [7]

Specify……

5. What is the total current area under crops in acres? ..... Acres

< 0.5 [1]

>0.5 to 1 [2]

>1-2 [3]

> 2 [4]

6. Do you grow cassava on your farm? Yes [1] No [2]
7. How many years of experience do you have in cassava farming? ..... Years
8. In which months do you plant cassava?

   Long rains

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
1	2	3	4	5	6	7	8	9	10	11	12

   Short rains

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
1	2	3	4	5	6	7	8	9	10	11	12

9. a) What is the total current area under cassava? .....Acres

   < 0.5 [1]

   >0.5 to 1 [2]

   >1-2 [3]

   > 2 [4]

10. a) What quantity do you harvest from the farm in one season? .....Kg

    b) What is the quantity harvested per plant? .....Kg

    a) Why do you grow cassava? (You may choose more than one)

Food [1]

Income [2]

Soil conservation [3]

Others [4]

Specify……

11. a) Which varieties have you grown for the last two years?

Varieties grown		Rank best 2
Tajirika	[1]	
Shibe	[2]	
Kibandameno	[3]	
Nzalauka	[4]	
Karibuni	[5]	
Karembo	[6]	
Girikacha	[7]	
Others	[8]	
(Specify)……		

12. a) Do you intercrop cassava?

Yes [1] No [2]

b) If yes, which crops do you usually intercrop with cassava? (List)

Crop	
Maize	[1]
Cowpea	[2]
Green gram	[3]
Pigeon pea	[4]
Beans	[5]
Okra	[6]
Other (Specify)…….	[7]

c) Why do you intercrop? (List reasons)

Reasons	
Small land size	[1]
Reduce the cost of production	[2]
Food security	[3]
Soil conservation	[4]
Other (Specify)……	[5]

13. a) Do you use fertilizer or manure on cassava?

Yes [1] No [2]

b) If yes, which one and when do you apply the fertilizer and/or manure?

Which one?	
Fertilizer	[1]
Manure	[2]
Both	[3]

14. A). Do you ever receive any information on cassava production?

Yes [1] No [2]

b) If yes, what is/are the source(s) of the information, what kind of technical information do you receive? And how often?

information	**Source of information**	**Frequency**
	Extension staff [1] Media - Radio/T.V/Newspaper [2] Agro input dealer [3] From other farmers [4] Research [5]	Weekly [1] Monthly [2] Quarterly [3] Semiannually [4] Annually [5]

Sources of Planting materials		
New varieties		
Crop husbandry		
Pest and disease management		
Marketing		
Utilization/processing		
Others		

15. a) Are you a member of any cassava related organization? (group or co-operative)

Yes [1] No [2]

b) If yes, which one? .....

Yes [1] No [2]

c) A) Do other members of the family work on the family farm? Yes [1] No [2]

b) If Yes, How many members of your family are involved in the farm activities?

16. What challenges do you face in cassava farming? (you may tick more than one)

Challenge	
Drought	[1]
Floods	[2]
Inadequate planting materials	[3]

Pest and diseases	[4]
Low market prices	[5]
No standardized measure of produce when selling	[6]
Perishability	[7]
Others Specify.....	[8]

SECTION 3 (CASSAVA BACTERIAL BLIGHT SECTION)

1. a) Do you know cassava bacterial blight?

   Yes [1] No [2]

   b) If yes, when was the first time you saw cassava bacterial blight in your farm?

This year	[1]
Last year	[2]
2 years ago	[3]
More than 2 years ago	[4]

2. Do you usually experience CBB every season you plant cassava on your farm?

   Yes [1] No [2]

3. On which cassava varieties have you seen the cassava bacterial blight?

Tajirika	[1]
Shibe	[2]
Kibandameno	[3]
Nzalauka	[4]
Karibuni	[5]
Karembo	[6]
Girikacha	[7]
Others	[8]
Specify ……	

4. When do most cassava plants show the symptoms of CBB infection?

Young crops <3 months	[1]
Mid aged crops 3-6 months	[2]
Older crops > 6 months	[3]

5. How do you manage cassava bacterial blight? (May tick more than one)

None	[1]
Roughing	[2]
Biological	[3]
Chemical	[4]
Crop rotation	[5]

Others	[6]
Specify ……	

Appendix II. Focus group discussion questions

General questions

1. Which crop do you plant in this region?
2. What is the order of preference?
3. Why do you prefer the crop above? (preference 1 above)
4. Do you get any information about crop production? From which source?
5. What is your general view of cassava?

Planting

1. Which varieties are grown in this region?
2. What is your preference for the above varieties?
3. And why do you prefer the above varieties?
4. Where do you source your planting material? And why?
5. What is the cost of cassava planting material and what is the price per unit.
6. How do you prepare your land for planting?
7. When do you prepare your land?
8. When do you plant?
9. How much land does cassava occupy?

Maintenance

1. Which farm inputs do use for cassava.
2. When do you apply the above inputs?
3. Do you weed your cassava crop?
4. When do you weed after planting? And how many times do you weed?
5. How do you weed ox plow, hoe, herbicides
6. Do you experience pest and disease on cassava
7. Which pest and diseases do you find on cassava
8. How do you manage pests and diseases?

Harvesting

1. When do you harvest?
2. How do you harvest the cassava roots?
3. How do you transport the cassava roots from the farm to the homestead?
4. Yields
5. Losses they incur

Storage

1. Do you ever store the cassava roots after harvesting?
2. How do you store the cassava roots after harvesting? And for how long do you store the cassava roots?

Marketing

1. Do you sell your cassava roots?
2. Where do you sell? (Find out if there are aggregation centers)
3. And who do you sell to?
4. How do you transport the cassava roots to the market? And how far is the market? (indicate village, towns,)
5. In what form do you sell the cassava? And at what price?
6. When do you fetch high prices of cassava?

Utilization

Advantages of cassava and disadvantages of cassava as human food

What are the foods and feeds replaced by cassava?

How do you utilize cassava leaves?

Intercrops with cassava and cowpea

1. In which forms do you utilize the cassava?(boiling, roasting, mixing with legumes)
2. How do you make your cassava flour?

What are the utilization forms of cassava flour?

SEPARATE GROUPING IN TERMS OF GENDER DISCUSSION

1. What are the challenges you encounter during cassava production?
2. On the challenges, what are some of the coping strategies and possible solutions that can be done?
3. Where do you source your labor? And what are the wages of the labor?
4. What are the resources available within the community? Who controls the resources?
5. Gender daily calendar
6. Sources of livelihood in order of priority

Appendix III. Table showing data set used for scoring disease incidence (CBB) in the study area of Kilifi and Taita Taveta counties.

DATE----------

COUNTY----------

DATA COLLECTED BY ----------

FARM NUMBER	LOCATION	FARMER NAME	FARM SIZE IN M2	PLANT POPULATION	AFFECTED PLANTS	VARIETY	AGE OF THE CROP IN MONTHS	

Appendix IV. Datasheet on tissue culture initiates in media treated with antibiotics.

Treatment	Parameters taken weekly for 7 weeks							
	Infected plants	Plant height	Number of Leaves	Dead plants due to scorching	Plant with roots	Number of roots	Confirmed after isolation	Comments
CONTROL								
TET 5								
TET 10								
TET 15								
TET 20								
CONTROL								
DOX5								
DOX10								
DOX15								
DOX 20								
CONTROL								
STREP 5								
STREP 10								
STREP 15								

Appendix V. Table of Numerical scores of morphological colonies of the isolated bacteria

PHYNOTYPIC CHARACTERISTICS	DISTINCTIVE	SCORE
Form	Circular	1
	Fusiform	3
	Rhizoid	2
	Irregular	3
	Filamentous	4
Color	White	1
	Pale yellow	2
	Grey	3
	Brown	4
	Green	5
	Cream	6
Elevation	Convex	1
	Concave	2
	Raised	3
	Umbonate	4
	Domed	5
	Flat	6
Size	Medium	1
	Small	2
	Large	3
Surface	Smooth	1
	Wavy	2
	Granular	3
	Papilate	4
	Rough	5
Edges	Entire	1
	Undulate	2
	Crenated	3
	Fimbriate	4
	Curled	5
Structure	Opaque	1
	Translucent	2
	Transparent	3
Degree of growth	Less growth	1
	Moderate growth	2
	High growth	3
	Very High growth	4
	No growth	5

Appendix VI: Datasheet on hardening tissues culture treated plants.

Parameters	Week 1				comments
	Streptomycine	Tetracycline	doxycycline	control	
Plant height					
No of leaves					
symptoms					

Parameters	Week 2				comments
	Streptomycine	Tetracycline	doxycycline	control	
Plant height					
No of leaves					
symptoms					

Parameters	Week 3				comments
	Streptomycine	Tetracycline	doxycycline	control	
Plant height					
No of leaves					
symptoms					

Parameters	Week 4				comments
	Streptomycine	Tetracycline	doxycycline	control	
Plant height					
No of leaves					
symptoms					