PREVALENCE AND CHARACTERIZATION OF *Xanthomonas campestris* pv. *musacearum* AND EVALUATION OF BANANA GENOTYPES FOR RESISTANCE TO BANANA WILT IN THARAKA NITHI COUNTY, KENYA

MOGAKA MELKZEDEK ONYAMBU

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CHUKA UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

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

Signature: ..... Date: 06/04/2021

Mogaka Melkzedek Onyambu
NM23/28942/16

Recommendation

This thesis has been examined, passed and submitted with our approval as University supervisors.

Signature: ..... Date: 06/04/2021

Dr. Moses M. Muraya, PhD
Chuka University

Signature: ..... Date: 6/04/2021

Dr. Benson O. Onyango, PhD
Jaramogi Oginga Odinga University of Science and Technology

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DEDICATION

I dedicate this thesis to my wife Annritah for providing unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis.

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ABSTRACT

Banana (*Musa spp*.) is one of the most grown food crops in the world, and is rich in carbohydrates, vitamins and minerals. A major constraints to banana production in Tharaka Nithi County is banana *Xanthomonas* wilt (BXW) caused by *Xanthomonas campestris* p.v *musacearum*. The pathogen attacks photosynthetic leaves and young fruits reducing palatability and marketability. Currently, there is limited information on severity and prevalence of BXW in Tharaka Nithi County. Different strategies are used in the region to curb the wilt, including use of chemicals, as well as cutting and uprooting infected plants, although effective management of BXW is yet to be achieved. In other parts of the world, an integration of resistant cultivars with other control methods has been the most effective method for BXW management. However, data on banana varieties that are resistant to BXW in Nithi region is still limited. The objective of this study was to determine the prevalence of BXW in Nithi region, isolate and characterize the pathogen using morphological and biochemical tests and to assess local banana genotypes for resistance to BXW. The prevalence of BXW was assessed on-farm by symptoms inspection and a survey design using structured questionnaires to banana farmers in five villages (Kiang'ondu, Marima, Mitheru, Gibumbu and Giampampo). Laboratory isolation and characterization of BXW pathogen at Chuka University was arranged in Completely Randomized Design based on site of sample origin, with three replicates. Susceptibility tests were conducted under controlled green-house conditions arranged in a Randomized Complete Block Design (RCBD) with five banana varieties and one pathogen, replicated five times. Prevalence data from the questionnaire was analysed on SAS version 9.4 version using chi square t-test of association, while data on genotype susceptibility data was analysed using a general linear model on SAS version 9.4 and significant means separated using Least Significance Difference at $\alpha = 0.05$. Study revealed that percentage prevalence of BXW in the studied villages was not significantly different ($p > 0.05$), with the highest value of 21.14% at Giampampo while Mitheru had the least value of 11.24%. Majority of the farmers (92%) were unaware of existence of BXW in the region. Morphological and biochemical tests (Catalase test, Starch hydrolysis test, Gelatine liquefaction test, Ammonia production test) revealed the banana wilt pathogen is *Xanthomonas campestris pv. musaceaerum*. Susceptibility of five banana varieties to *Xanthomonas* wilt pathogen isolate was statistically significant ($p < 0.05$), with Gasukari variety having the mean of 70.37% followed by Kiganda (60.74%) while Muraru had the lowest susceptibility mean of 45.19%. In terms of resistance, Muraru variety may be a good source of breeding material due to its relative resistance to BXW and is recommended for farmers in the region. This study broadens the understanding BXW prevalence in Nithi region and provides opportunities for selection of resistant banana varieties for better yields and improved food and nutritional security in the region.

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ABBREVIATIONS AND ACRONYMS

AMT	Ammonia production test
BXPS	Banana *Xanthomonas* Wilt Pathogen
BXW	Banana *Xanthomonas* wilt
CCA	Cellobiose Cephalexin Agar
CFU	Colony forming units
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FAME	Fatty acid methyl ester
FlT	Fluorescent test
FTA	Flinders Technology Associates
GCA	Growth Count Agar
GLT	Gelatine liquification test
H_2S	Hydrogen Sulphide
IPT	Indole production test
LB	Lysogeny Broth
MRD	Maximum Diluent Agar
MRT	Methyl red test
O.D	Optical Density
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PGT	Protein digestion test
T3Es	Type III effector proteins
VpT	Voges-Proskauer Test
Xcm	*Xanthomonas campestris* pv. *musacearum*
YDC	Yeast Dextrose Carbonate
YEPG	Yeast Extract – Peptone - Glycerol
YGPA	Glucose Yeast Peptone Agar
YTS	Yeast Tryptone Sucrose

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Banana (*Musa spp*.) is one of the most grown food crops in the world, taking the fourth position after maize, rice and wheat (FAOSTAT, 2018). India is the largest producer of bananas in the world with 27,575,000 tons per annum (p.a) followed by China with 12,075,238 tons per annum and Philippines with 8,645,749 tons p.a (Tripathi *et al*., 2010; Dale *et al*., 2017). In Kenya, bananas are produced by small and large-scale farmers, mainly for the local market and household consumption. Banana crop is grown in various regions in Kenya. Regionally, Meru County takes the first position in banana production (19%), Kirinyaga County takes the second position (14%). Embu takes the third position (12%). Other banana producing counties include: Tharaka Nithi (6%), Bungoma (5%), Kakamega (5%), Kisii (6%), Taita Taveta (9%) (Agwara, 2017).

Banana is an important dietary source of carbohydrates and vitamins (Tripathi *et al*., 2010), and other nutrients such as fibers, potassium, proteins and lipids (Joan *et al.*, 2012). The banana crop is a source of income for many rural households. Despite its nutritional and economic importance, the production of bananas is threatened by a variety of biotic and abiotic factors. In particular, pests and diseases are major biotic constraints to high banana yield and overall quality of the fruit. The major diseases that constrain banana production in the world are *Fusarium* and *Xanthomonas* wilts (Dale *et al*., 2017).

Banana *Xanthomonas* wilt (BXW) is a bacterial disease caused by *Xanthomonas campestris* pv. *musacearum* (Kwach, 2012). Symptoms of fruits include internal discolouration and premature ripening. The inflorescence may show gradual wilt and yellowing accompanied by shrivelling of male buds and bracts (Dale *et al*., 2017). The disease may cause up to 100% yield loss if effective control measures are not taken (Tushemereirwe *et al*., 2003). This is because the disease mainly affects the leaves hindering photosynthesis leading to eventual death of the plants (Ochola *et al*., 2015), decreased yields and thus lowering farmers' income.

Xanthomonas wilt disease is a major constrain in banana production globally (Ocimati *et al*., 2013a). The disease has been reported in African countries such as Uganda, Tanzania as well as Kenya (Blomme *et al.,* 2014; Nakato *et al.,* 2018). In East Africa, economic losses as a result of BXW ranging from $2 to $8 billion have been reported (Nkuba *et al*., 2009; Tripathi *et al*., 2015). In Kenya, BXW is widely spread and has been reported in Teso, Bungoma and Busia, Bumula, Yala, Bondo, Siaya, Mumias, Butere, Kisumu and Mt Elgon areas (Tripathi *et al*., 2007a; Mbaka *et al*., 2009; Onyango *et al*., 2012; Kwach *et al*., 2012), where it has caused up to 100% yield losses without an effective control strategy (Geberewold and Yildiz, 2019). However, information on the occurrence and distribution of the disease in many regions of Kenya, including Tharaka Nithi County is limited. Knowledge of BXW occurrence in the County can be useful in development of its management strategies (Tripathi and Tripathi, 2009).

Between 2011 and 2014, Kenya produced 1.4 million tons of bananas on an average of 50,281 hectares. Tharaka Nithi County accounts for 6% of total banana production in Kenya (Agwara. 2017). The major banana varieties grown include 'Muraru', 'Kiganda', 'Israel', 'Kampala' and 'Gasukari' (Kwach, 2014). However, the production is below potential, which may be attributed to infectious diseases such as BXW. Up to 54% of losses occur when *Xanthomonas* wilt is not managed (Kayobyo *et al.,* 2005) and may exceed up to 100% (Geberewold and Yildiz, 2019). Little work has been done to evaluate the susceptibility or resistance of these varieties to BXW. As such, the impact of BXW in bananas production is not fully evaluated (Geberewold and Yildiz, 2019). Farmers have no clear criterion on selection of appropriate varieties with respect to threat by BXW.

The symptoms of BXW show marked similarity with other fungal and bacterial wilts of banana such as *Fusarium* wilt caused by *Fusarium oxysporum* (ProMusa, 2014). The pathogen may be present in the plant without easily manifesting visible symptoms (Mahuku, 2004). Studies have shown that there is inconsistent application of recommended management which includes; rouging of infected banana plants, orchard replanting, sterilization of farm implements, cultural control measures and removal of male banana flowers (Shimwela *et al*., 2016). In addition to management strategies,

there is limited resistant source in banana germplasm (Tripathi *et al*., 2017). Exploration of plant cultivars which are resistant to disease is a principle aspect in increasing banana production by curbing diseases as well as reducing cost of disease control (Geberewold and Yildiz, 2019). Selection of resistant banana varieties requires a rapid and reliable screening method (Smith *et al.,* 2008). I*n-vitro* and *in-vivo* screening techniques which involve the inoculation of plantlets with the pathogen have been used to assess varietal response to BXW infection (Tripathi *et al*., 2007b).

BXWs have tremendous effects on banana production since the disease targets photosynthetic leaves of the plant and reduces quality of banana fruits, hence threatening survival of the crop. Livelihoods of the rural farmers are directly affected through reduced income and lack of the much-needed dietary carbohydrates and vitamins usually provided by bananas. Reduced supply of bananas as a result of wilt disease has negative effects both domestically and industrially, since banana serves as a raw material in various food industries like in beer brewing, wines and jam industries, crisps production, livestock fodder production and a source of giving food (Kayobyo *et al.,* 2005; Iralabati *et al.,* 2014). Eventually, there is a negative impact on the economy of the County, due to loss in revenue usually obtained when bananas are exported to other counties for sale. This study was conducted to phenotypically characterise *Xanthomonas* bacteria isolated from BXW infected plants in selected areas in Tharaka Nithi County. Further, tests were conducted to evaluate the resistance of five banana genotypes to BXW infection.

1.2 Statement of the Problem

Banana *Xanthomonas* wilt (BXW) is a devastating disease of banana. If not controlled, BXW may cause losses of between 54-100%. Infestation of banana plantation decreases banana yield affecting farmers' source of income. A preliminary survey of banana plantations in Tharaka Nithi County revealed the presence BXW in most farms. However, the information on prevalence and severity of BXW in the County is scanty. Further, local farmers were not only aware of *Xanthomonas* symptoms but could also not tell the extent of the damage due to banana *Xanthomonas* wilt. There are various management strategies that farmers use to control this disease, however, there is scanty information on the best management practices in Nithi area within Tharaka Nithi

County. There are a number of banana varieties grown in Tharaka Nithi County which include 'Muraru', 'Kiganda', 'Israel', 'Kampala' and 'Gasukari'. Nonetheless, there is little information on resistance level of banana varieties grown by farmers. Lastly, information on strains of *Xanthomonas campestris* that attack banana in Nithi part of Tharaka Nithi County is scanty. Shortage of this information hinders disease management strategies in the area.

1.3 Objectives

1.3.1 General Objective

To assess the prevalence of banana wilt disease, characterize the causative pathogen and screen banana genotypes for resistance to *Xanthomonas campestris pv. musacearum* in selected areas in Tharaka Nithi County, Kenya

1.3.2 Specific Objectives

i. To assess the prevalence of BXW infection in selected villages in Tharaka-Nithi County, Kenya.
ii. To determine morphological and biochemical characteristics of *Xanthomonas campestris* pv. *musacearum* isolates obtained from selected areas in Tharaka-Nithi County, Kenya.
iii. To evaluate five banana genotypes grown in selected areas in Tharaka-Nithi County, Kenya for resistance to *Xanthomonas* wilt pathogen.

1.4 Hypotheses

$H0_1$: There is no significant difference in prevalence of banana *Xanthomonas* wilt in selected villages in selected areas in Tharaka-Nithi County, Kenya.

$H0_2$: There is no significant difference in morphology and biochemical characteristics of isolates of Banana *Xanthomonas* wilt pathogen from selected areas in Tharaka-Nithi County, Kenya

$H0_3$: There is no significance difference in resistance to *Xanthomonas* wilt among five banana cultivars grown in selected areas in Tharaka-Nithi County, Kenya.

1.5 Justification of the Study

Banana *Xanthomonas* wilt (BXW) is a devastating disease with the potential of causing banana yield loss of up to 100% leading to loss of income for farmers (Tushemereirwe *et al*., 2003; Ochola *et al*., 2015). The impact of BXW in bananas production is not fully evaluated in most banana production areas including Tharaka Nithi County (Geberewold and Yildiz, 2019). A critical step in disease management begins with diagnosis that creates understanding of the causative agent (Ssekiwoko *et al.*, 2006). Diagnosis of the diseases may involve inspection for disease symptoms during farm survey (Blomme *et al.*, 2017). Survey for plant diseases such as Banana *Xanthomonas* wilt and identification of associated pathogen is significant in generating information on the extent of disease intensity to facilitate management strategies (Riley *et al*,. 2002). Further, proper identifiction of the disease causing pathogen is necessary in choosing the best managent strategy (Abdulkhair and Alghuthaymi, 2016). Despite the existence of different banana varieties including 'Muraru', 'Kiganda', 'Israel', 'Kampala' and 'Gasukari' (Kwach, 2014), information on the level of resistance of these banana varieties remains scanty. Knowledge of caltivar susceptibility to the disease is necessary as they may provide breeding material for disease resistance (Donga and Ronald, 2019). Additionally, identification of resistant banana varieties in this study will help in selection of propagating materials for improved productivity. Smallholder farmers will benefit by accessing the information on BXW banana resistant varieties thus may help improve yield and income. The results of the study will add on the knowledge of *Xanthomanas* wilt of banana in the region as will serve as source of reference to scholars and researchers.

CHAPTER TWO
LITERATURE REVIEW

2.1. Overview of Banana Production

Banana plant is native to South East Asia (Fuller *et al.,* 2015). It is a widely cultivated perennial monocot belonging to the genus *Musa* and family *Musaceae* (ProMusa, 2014). Banana cultivation occurs in over 130 countries both in the subtropical regions and tropical regions (Reay, 2019). Globally, banana cultivation ranks fourth after rice, wheat and maize (Agwara, 2017). The total annual banana production in the world is approximately 130 million tons (Food and Agriculture Organization (FAO), 2017), with India (11 million tons) being the largest producer. In Sub-saharan Africa, the largest production and consumption of banana is in East Africa. Up to 20 million tons of banana are produced in Central and East Africa annually (FAOSTAT, 2019).

Banana is a major source of food in developing countries. About seventy five percent of carbohydrates in the diet is provided by bananas (Tripathi *et al*., 2005; Karamura *et al*., 2008). Banana takes the fourth position as a source of food besides rice, maize and wheat crops (Kwach *et al*., 2000). Sub-Saharan Africa produces 35% of the world banana plantains (Tripathi *et al*., 2008). In East and Central Africa, banana production is manifested in the great lake regions covering Tanzania, Congo, Burundi, Rwanda, Uganda and Kenya. The total production per year is 15 million tons (Food and Agriculture Organization (FAO), 2017). The East and Central Africa region has the highest per capita consumption of banana estimated at 200-250 kg annually (Tushemereirwe *et al*., 2002; Jones *et al*., 2007).

In Kenya, up to 74,000 hectares of land is used for banana production with yield of over 510,000 tons annually (Ministry of Agriculture and Livestock (MoALF), 2016). According to Horticultural Validated Report (2017) banana is among the leading important fruits that has dominated the market with a share of 35.6% ahead of pineapples with market share of (20%), mangoes at (17%), avocados at (6%), paw paws at (6%), passion fruits at (3.6%), oranges at (3%), water melons at (3%), and tangerines with market share of (2%)**.** Majorly, **b**anana farming in Kenya is for income generation and has got attention just like coffee, tea among other regarded conventional crops (Mbuthia *et al.,* 2018). Several factors influence banana cultivar and distribution in

Kenya. These considerations include planting materials supply, area suitability, efficiency, and market demand (Caroline *et al*, 2021). However, the crop is still faced with a number of production constraints (Kayobyo *et al.,* 2005). Key a mong them being BXW which can cause absolute yield loss (Kubiriba *et al*, 2014).

2.2 Banana Taxonomy and Germplasm

Banana taxonomically belongs to the family of *Musaceae* and genus *Musa.* It is grouped in the order *Zingiberales* (Kress and Specht, 2006). Over 60 wild banana species that are diploid (2*n*) with varied number of chromosome found in this genus (Shepherd, 1999). The banana in *Musa* genus is further grouped into sections that include *Australimusa, Callimusa, Rhodochlamy, Incertae sedis and Eumusa* (Karamura, 1998). However, species grouped in the two sections of *Callimusa* and *Rhodochlamy* are majorly ornamentals since they are non-fruit forming. The species grouped in the section of *Australimusa* are edible seedless bananas. *Musa* in the section *Eumusa* is a true banana which has about 15 species and is the mostly cultivated banana species (Karamura, 1998). The cultivated banana originated from wild *Musa acuminata Colla* (genome A) and *Musa balbisiana* Colla (genome B) which are wild species (Simmonds and Shepherd, 1955). Though triploids banana are the mostly grown and widespread with high economic value, some diploid banana are still common and grown today.

Tetraploids bananas with genome AAA are artificially bred by crossing seeded banana diploids (AA) which is edible severally with a number of *Musa acuminata* subspecies. Other hybridizations involving *Musa balbisiana* have resulted in sterile banana hybrid progeny such as dessert bananas (genomes AB), plantains (AAB) and cooking bananas [ABB] (Kagy and Carreel, 2004). Edible bananas are grouped into several groups, sub groups and clones. According to Cheesman *et al.* (1933), three subgroups of *Musa* AAA are Cavendish, Green-Red and Gros Michel. Cavendish subgroup is further divided into the clones (Giant Cavendish, Dwarf Cavendish and Grand Naine (Stover and Simmonds, 1987; Daniels *et al.,* 2002). The *Musa* AAB has several subgroups and include; Popoulu, Plantains, Mysore, Pome, Pisang Raja and Silk (Lebot *et al.,* 1994).

2.2.1 Industrial Uses of Banana

Banana is an important food crop fed on by nearly 400 million people particularly in Africa (Mbuthia *et al.,* 2018). It is a major staple food in many developing countries in Africa, Asia and Latin America. Up to 1 kg of banana is consumed in households daily in the East African. Banana is used whole either cooked or ripen, milled into flour or may be fermented for production of beer wine and vinegar, also it is blended into banana juice (Pillay and Tripathi, 2007).

In East African countries such as Rwanda, Burundi and Uganda, about 65% of commercial beer is produced from banana. Banana psuedostem, flowers, and the leaf buds are utilized as vegetables. Banana sap is a significant raw material for dye and ink production (Nelson *et al*., 2006). Banana foliar may be a source of fibre, wrapping food for steaming while, strips are useful for weaving or roofing. Banana fibre is used to make cords, baskets and mats, which are a reliable source of income in banana-growing areas. (Olango *et al*., 2014). Banana parts such as pseudostem, leaves, fruit peel offs are used as animal feed. Banana plays a significant role in management of soil erosion particularly in steep landscape thus contributing in soil formation and fertility (Gold *et al.,* 1999). Banana has also been used for treatment abdominal complications such as deworming and ulcers (Karamura *et al*., 1998).

2.2.2 Nutritive Values of Banana

Banana provides important nutrients that are required for human and animal health (Ashokkumar, 2018). Key components of banana fruits include; 0.5% fats, 1% protein, 2.5% fibre, 74% water, and 23% carbohydrates (Kahariri, 2016). Ripe banana is rich in; vitamin E, carotene, niacin, thiamine, pyridoxine, riboflavin, pantothenate, folic acid, vitamin C and biotin (Kahariri, 2016). Banana also provides minerals; potassium, magnesium, phosphorous, copper, sodium, iron, calcium, zinc, manganese iodine and chloride (Kahariri, 2016; Englberger *et al*., 2010) reported that eight five banana cultivars have riboflavin of about 2.72 mg/100 g. Carotenoids concentration in banana has been reported to vary. Amorim *et al.* (2009) reported average concentration of carotenoid of 4.7 µg g^{-1} and 1.1-19.2 µg g^{-1} respectively in 42 banana accessions. In 21 banana accessions, Englberger *et al*., 2003) reported average carotenoid concentration

of 11.1 µg g^{-1}. Banana offers a greater percentage of nutritive values as compared to other food crops as shown in Table 1.

Table 1: Comparison of Banana Nutritional Content to Other Food Sources

Content %	Banana	Mango	Apple	Pineapple
Fats	0.33	0.38	0.17	0.12
Protein	1.09	0.82	0.26	0.54
Calories	89	60	52	50
Vitamin C	8.7	36.4	46	47.8
Carbohydrates	5.38	7.34	10.39	9.85

Source: Joan *et al.,* (2012)

2.2.3 Banana Propagation

Generally, edible banana have no seeds and are propagated using plantlets, split corms or suckers which can be facilitated through tissue culture (Swennen, 1990). Rapid multiplication when done through *in-vitro* propagation may enable raising suckers that are free from diseases (Kulkarni *et al.*, 2007). Banana plantlets may also be produced from the meristem by tissue culture (Swennen, 1990). Traditionally, banana plant materials are obtained from suckers.

2.2.4 Conditions Favoring Banana Cultivation

Bananas are grown in wide a range of altitudes of up to 1800 m above sea level and rainfall ranging from 1000 mm to 2500 mm p.a. that is well distributed throughout the year with shorter dry seasons. The amount of bananas produced in Kenya's various counties varies. Cultivation of banana is done between 30° N and 30° S on the equator that experiences monthly temperature mean of 27 ºC. Whereas plantains are known to perform well in lowlands, East African highland bananas commonly known as *Matooke* grow best at the altitudes of between 1000 and 1800 m above the sea level (Mbuthia *et al.,* 2018). A deep and well drained loam soil with high soil humus content is suitable for growing banana but can grow on varied soils types (Zake *et al.,* 2000). Larger quantities of minerals such as Nitrogen, Phosphorus and Potash are required to improve banana yield (Yako *et al.,* 2011).

2.2.5 Banana Morphology and Reproduction

Banana is a perennial plant that has an underground corm stem. The banana corm forms lateral bud that grows into aerial shoots which develop into eyes and later forming

suckers (Olango *et al*., 2014). Suckers grow continuously forming a pseudostem of 2 - 8 m high depending on growth conditions and banana the variety. The banana pseudostem is formed by large overlapping leaves rolled tightly round one another to form a cylindrical structure. The roots which are from the length 50 to 100 cm in length, emanate from basal corn (Blomme and Ortiz, 2000). The banana corm also has apical meristem tissue that gives rise to the long leaves as well as flowers. Each banana plant may produce an average of 35 to 50 leaves. Within eight to eighteen months, the terminal bud transforms into the female and male spikes of un-branched inflorescence and moves through the banana pseudostem to the banana tip (Irei, 2015).

The inflorescence consists of two flower rows that are on top of each other and appressed together (Robinson *et al*., 2010). The flower row is covered by a bract which is large and subtending. The bracts together with axillary flowers are spirally arranged round the centre (axis). The bracts overlap closely to each other and form conical inflorescence that is tighten at the tip (Irei, 2015). The female flowers are enclosed by the lower bracts on axis, at the middle of the bract, flowers develop into a hand of the fruit. The male flowers are aligned at the bract tip of banana inflorescence (Endress, 2010). In a few cases, hermaphrodite flowers have been reported in *M. acuminata* sp. *M. acuminata* sp. *errans and M. schizocarpa* (Sharrock *et al*., 2001).

The banana bunch with about 4 – 12 clusters (hands) that is made of at least 10 fingers develop from the female inflorescences. Male and female flowers of wild banana flowers may produce a mass of pollen and nectar unlike in domesticated (cultivated) bananas which may not produce pollen. Pollens of banana which are small and sticky are coated (lined) with protein and waxes that steadily fix them in place. According to Dumpe and Ortiz (1996), the number of pollen is important for the germination of pollen grain. Female flower of banana have ovaries that develop without prior fertilization (parthenocarpy) forming edible pulp

2.3 Constrains in Banana Farming

2.3.1 Fusarium Wilt Disease

Fusarium wilt disease is also known as the panama disease. Its causative agent is *Fusarium oxysporum f. sp. Cubense Foc*, fungi which is soil-borne. The fungus is

capable of infecting banana via the roots and end up blocking the plant's xylem vessels therefore causing wilting of the infected banana and eventually affecting banana production (Viljoen, 2002). Fusarium race known as Tropical Race 4 has been reported to cause untold losses to Cavendish' (genome AAA) in South Africa (Hwang and Ko, 2004). In East and Central Africa, *Fusarium oxysporum* 'tropical race 1 and 2' are the most destructive (Tushemereirwe and Bagabe, 1998). *Fusarium oxysporum f. sp. Cubense Foc* fungal wilt pathogen is disseminated through planting of infected seeding in areas that grow banana (Ploetz and Pegg, 2000). Contaminated equipment, irrigation water and soils are also key diserminater of the pathogen. The fungus *Fusarium oxysporum f. sp. Cubense* forms chlamydospores that enables the pathogen to survive in the organic matter and soil for longer duration. Use of chemical to manage *Fusarium oxysporum f. sp. Cubense* has proved difficult. However, success in managing *Fusarium oxysporum f. sp. Cubense* has been partially met by the introduction of banana that are resistant to the disease (Hwang and Ko, 2004; Daly and Walduck, 2006).

2.3.2 Black Leaf Streak

Black leaf streak caused by *Mycosphaerella fijiensis* is a fungal disease that affects banana leaves causing loses of over 50% banana (Ferreira *et al.*, 2004). In Africa, Black leaf streak disease was first reported in Gabon in 1978 and in Tanzania in 1987 (Carlier *et al.,* 2000). According to Carlier *et al. (*2000), Black leaf streak disease replaced yellow sigatoka leaf spot that was caused by fungus *Mycosphaerella musicola* in the tropical Africa. This disease was a bit milder (Carlier *et al.,* 2000). This severe disease affects varieties of bananas and plantains (Tushemereirwe and Bagabe, 1998). Wind and water are the principal agents of dispersing Black leaf streak ascospores and conidia (Carlier *et al.,* 2000).

2.3.3 Viruses affecting bananas

Banana is also affected by several viral diseases leading to reduced banana yield. Globally, *Banana bunchy top virus* (BBTV), *Banana bract mosaic virus* (BBrMV), *Banana streak virus* (BSV), *Cucumber mosaic virus* (CMV), *Banana virus X* (BVX) and *Banana mild mosaic virus* (BanMMV) are viral diseases that has been reported to infect banana (Swennen and Vuylsteke, 2001; Adams *et al*., 2004; Geering *et al*., 2005).

Banana bunchy top virus (BBTV) the causative agent of Banana bunchy top disease (BBTD) is an economically destructive pathogen that causes complete banana yield loss globally (Dale, 1987; Dale and Harding, 1998). Eleven African Sub Saharan countries have been affected by the Banana bunchy top disease (Lockhart and Jones, 2000; Thomas and Iskra- Caruana, 2000). South African nations have been heavily affected by Banana bunchy top disease. In Malawi, the disease accounts for 40% banana loss. The first case of Banana bunchy top disease in Africa occurred in 1901 but have since spread across the Africa banana producing regions. In the year 2011. In West Africa, Nigeria was first to report the occurrence of Banana bunchy top disease. Infested planting materials together with insect such as aphids are key player in Banana bunchy top disease dissemination (Lockhart and Jones, 2000; Thomas and Iskra-Caruana, 2000).

2.3.4 Bacterial diseases affecting bananas

Bacterial diseases have been reported as contributors to lower banana production. Among the bacteria pathogens that affect banana yield are *Xanthomonas campestris* pv. *musacearum* that causes *Xanthomonas* wilt and *Ralstonia solanacearum* that causes banana wilt by affecting banana vascular system (Swennen and Vuylsteke, 2001). In Africa, *Xanthomonas* wilt is rampant and spread in banana production system.

2.3.5 Causes of Banana Wilts

Besides Fusarium wilt, other causes of banana wilt include:

(a) Moko disease is caused by *Ralstonia solanacearum* major symptoms are chlorosis and collapse of the younger leaves. With Moko, the vascular discolouration is concentrated near the centre of the pseudostem and not periphery which is common with *Fusarium* wilt (Buddenhagen, 2009). The disease is also characterised by fruit rot and fruit stalk discoloration and bacterial ooze may exude as droplets from the cut tissue of vascular tissues (Ploetz, 2006). Bacterial ooze distinguishes Moko disease from Fusarium wilt disease *Xanthomonas* wilt disease is caused by *Xanthomonas campestris p.v musacearum.* The symptoms can be expressed in various parts of the plant including the leaves, fruits and the stem of the plant. According to Jones *et al*. (2007) and Tripathi *et al*. (2009), the earliest symptoms expressed by a plant invaded by the pathogen include progressive yellowing and complete wilting of entire plant, uneven yellowing,

premature ripening and rotting of the bunch which leads to the death of banana plants (Plate 1). Infected plants show leaf wilting and as well as death of plant. Fruit infection is characterized by brown internal colorations (Tripathi *et al*., 2013). Also, internal cross sections of pseudo-stems show yellow bacterial ooze and fruit cross-sections show rusty brown stains (Tushemereirwe *et al*., 2004; ProMusa, 2014).

2.3.6 Diagnosis of Banana *Xanthomonas* Wilt

BXW disease results in yellowing and wilting of leaves and yellowing of immature and mature fruits. However, symptoms are cultivar-specific and also determined by the route and stage of infection (Ndungo *et al*., 2005). Foliar symptoms include yellowing and wilting, these symptoms often resemble those presented by *Fusarium* wilt but excretion of yellowish bacterial ooze from cut tissue is characteristics for bacterial wilt (Tushemereirwe *et al*., 2004). Depending on the route of infection, leaves of some plants may show yellowing and wilt symptoms (Plate 2) but the bunch may appear green and normal outwardly though internally, the fruits often exhibit reddish brown discoloration and they are inedible.

Plate 1: Symptomatic banana wilt

There are various methods that can be used in the diagnosis of the disease-causing bacteria depending on the aim and resources available, some of the methods are simple and less costly while others are relatively costly. For instance, method like polymerase chain reaction requires PCR special facilities that might be costly.

Xanthomonas wilt disease may be diagnosed in the field by scouting and observing plant symptoms. The diagnosis process involves field observation where visible

symptoms expressed on infected bananas are noted e.g. yellowing of leaves, wilting of male bud bracts followed by drying of the rachis, premature and uneven ripening of fruits brown internal colorations. When a cross section of an infected pseudostem is carried out, pockets of pale-yellow bacterial ooze are observed (Triphathi, 2013; and ProMusa, 2014).

Several biochemical tests may be applied for the diagnosis of bacteria wilt pathogen. They include: Aesculine hydrolysis, Potassium hydroxide test, Indole test, Urease production, Catalase production, H_2S production and starch hydrolysis. The most commonly used biochemical tests to diagnose the bacteria includes: Kovac's reaction gram reaction and pathogenicity tests (Kwach *et al*., 2014)

Molecular techniques are available for diagnosis of *Xanthomonas* wilt pathogen. Studies have shown that several methods can be used in diagnosis including the scientific technology like using polymerase chain reaction (PCR). The Enzyme-linked Immunosorbernt Assay (ELISA) for testing specificity and sensitivity are some of the methods (Ssekiwoko *et al*., 2006; Kwach *et al*., 2014).

2.3.7 *Xanthomonas campestris'* Taxonomy, Genetic Diversity and Host Range

Xanthomonas campestris belongs to genus *Xanthomonas* and subclass *Proteobacteria*. It is a rod shaped, Gram negative bacteria (Smith *et al*., 2008), measuring between 0.7–0.9 µm and 1.8–2.0 µm. A single polar flagellum is used by the bacterium for movement (Bradbury, 1986).In nutrient medium, the ideal growth temperature for Xanthomonas campestris is 25–28 °C.

At 3 days after incubation, *Xanthomonas campestris* forms distinct yellow, circular, convex and mucoid colonies in growth culture (Ssekiwoko *et al*., 2006). The presence of chemical brominated aryl polyenes (xanthomonadins) causes the yellow colour, according to Holt *et al.* (1994) and Wasukira *et al.* (2012).

There is no poly-β-hydroxybutyric acid in *Xanthomonas campestris*, and it fails oxidase, gelatinase, starch hydrolysis, nitrate reduction and tyrosinase tests.

Xanthomonas campestris has no poly-β-hydroxybutyric acid and is negative for oxidase, tyrosinase, nitrate reduction, starch hydrolysis and gelatinase tests.

Xanthomonas campestris pv. *musacearum* belongs to *Xanthomonas campestris* based on biochemical characterization such as urease production, catalase test, hydrogen sulphide test, hydrolysis of aesculin, and utilization of sorbitol, dulcitol and salicin (Parkinson *et al*., 2009). Fatty acid methyl ester (FAME) study of *Xanthomonas campestris* pv.*musaceaerum,* which included sequencing of the *gyrB* gene, revealed that the pathogen is not as closely related to *Xanthomonas vasicola* pv. *vasculorum* and *Xanthomonas vasicola* pv. *holcicola* (Wasukira *et al*., 2014)

Various molecular finger printing methods and markers have been used to study *Xanthomonas spp* populations. Odipio *et al*. (2009) used spontaneous amplification of polymorphic DNAs (RAPDs), Aritua *et al*. (2008) used Rep PCR, Wasukira *et al*. (2012) used single nucleotide polymorphisms (SNPs), and Lewis et al. (2010) used enterobacterial repetitive intergenic consensus PCR (ERIC PCR).

The strains of *Xanthomonas campestris* are genetically monomorphic, according to molecular fingerprinting. The SNPs revealed two big sublineages, which may indicate that there was more than one introductory session. Aritua *et al*. (2008) used Rep PCR to show that *Xanthomonas campestris* pv. *musaceae* strains in Rwanda, Uganda, Rwanda, DRC, and Ethiopia have similar profiles and therefore are homogeneous and clonal. Wasukira *et al*. (2012) divided *Xanthomonas campestris* pv. *musaceae* into two sublineages based on 86 SNP positions, with sublineage I containing isolates from Ethiopia, DRC, and Rwanda, and sublineage II containing isolates from Kenya, Burundi, Uganda, and Tanzania

Xanthomonas bacteria species infect wide range of plants such as, banana, sweet potato, tomato, sugarcane, capsicum, maize, beans and sorghum (Destefano *et al*., 2003; Mkandawire *et al*., 2004; De Cleene, 2008; Todorović *et al*., 2008). Study by Bhat *et al.* (2010) showed that *Xanthomonas* campestris may attack Crucifers some of which are grown together with banana. It attacks *Brassica oleracia var. capitata L.* (Cabbage), *B. oleracia var. botrytis* (Cauliflower), *B. oleracia var.caulorapa* (Knol khol), *B. oleracia var. acephala (*kale), *B. rapa* (turnip), *B. campestris* (mustard), *Raphanus*

sativus (radish), domesticated cruciferous crops, wild crucifer i.e. shepherd's purse (*Capsella bursa-pastoris*) and *Rorippa indica (*wild crucifer weed).

Studies under artificial inoculation have proven that *Musa (M.) zebrina*, *M. ornata*, *Canna (C.) indica*, *C. orchoides* as well as maize may serve as an alternative hosts of *Xanthomonas campestris* pv. *musaceae* (Ssekiwoko *et al*., 2006). Further, it is thought that *Xanthomonas vasicola* which includes pathovars *holcicola* and *musacearum* that attack banana originated from sugarcane and sorghum (Aritua *et al*., 2008). In contrary to this thought, According to Smith *et al*. (2008) *Xanthomonas campestris* pv. *musacearum* evolved to attack banana plant. Karamura *et al*. (2015) through experiments reported that banana may asymptomatically host *Xanthomonas vasicola* that includes pathovars *holcicola* and *musacearum* and other *Xanthomonas* species. Karamura *et al*. (2015) further observed that maize and sugarcane when grown in same farm with bananas may serve as alternative hosts to *Xanthomonas campestris* pv. *musacearum*.

2.3.8 Propagation of Xanthomonas campestris

Many plant bacterial pathogens propagate and survive on floral parts, leaves and stems as epiphytic populations that play a significance role in disease epidemiology (Agrios, 2005). The major sources of inoculum are; plant residue, contaminated soils and water, infected mats and traded products including fruits leaves and planting materials. The spread of BXW is mostly carried out by contaminated insects that come into contact with infected banana plants. Among the most common insects that act as vectors for the bacteria are stingless bees *Pletbenia denoiti* family *Aridea,* fruit flies (family *Drosophilidae*) and grass flies (Family *Choloropidae*) and honey bees (*Apis mellifera*) (Gold *et al*., 2006; Bioversity, 2009; Tripathi *et al*., 2009). The nectar sucking birds and bats have also been suspected for transmission of the bacterium (Smith *et al*., 2008) and use of contaminated farm tools (Mwangi and Muthoni, 2008). Also, it was noted that the bacteria is viable on metallic tools up to 3 days or more. The spread has also been attributed to the movement of infected banana planting materials such as banana suckers for planting or replanting (Mwangi *et al*., 2007).

Fresh banana leaves that contain the bacteria when used as cushion for packing harvested banana bunches to the market can spread the pathogen from home to market place. This occurs when the leaf debris are disposed in banana plantations thus forming sources of transmission (Tripathi *et al*., 2013). Bacteria can penetrate the plant through injured roots that have been infested by nematodes and weevils (Mwangi *et al*., 2007; Tripathi *et al*., 2009).

The bacterium is able to survive for 35 days in an unsterilized environment (Mwebaze *et al*., 2006; Ssekiwoko *et al*., 2006). Once debris have been buried in the soil, the bacteria can survive for only 21 days. In cold environments like a refrigerator, it can survive for more than four months (Tripathi *et al*., 2010).

2.3.9 Infection and Colonization of Banana by *Xanthomonas* Wilt

The bacterium has limited ability to gain access into a plant unless there is injury on the plant (Tripathi *et al*., 2013). The bacteria is greatly challenged to penetrate through an intact cuticle thus, entry is either through wounds or natural openings such as stomata and hydathodes hence protective cultural practices are encouraged to prevent infections (Kidist, 2003). After penetration, the bacterium moves to the tissues and multiply in the intercellular spaces and cell death follows due to pectolytic enzymes produced by the bacteria and as well as toxins (Agrios, 2005). The spread of the disease depends on the rate of multiplication of the pathogen, motility, ability to produce pectolytic enzymes, the host factors and as well environmental factors like temperature have great effect (Kidist, 2003).

2.3.10 *Xanthomonas* Pathogenicity and Virulence Factors

Pathogenicity factors enables *Xanthomonas campestries* pv. *musaceae* to adopt and infect banana plant (Genin and Denny, 2012; Lonjon *et al*., 2017). The Type III secretion system (T3SS) secretes and transports Type III effector proteins (T3Es), which have been related to *Xanthomonas* virulence (Wasukira *et al*., 2012; Jacques *et al*., 2016). The T3SS is the molecular syringes that facilitate the injection of bacterial proteins referred to as type III effectors (T3Es), into the plant cytosol (Büttner, 2012; Lonjon *et al*., 2017). The T3Es plays significant role in host defense suppression of

plant pathogenic bacteria. T3Es therefore promote the proliferation and invasion of pathogen in the plant (Studholme *et al*., 2010; Jacques *et al*., 2016).

In *Xanthomonas* species, T3Es an essential pathogenicity and virulence factor, induces effector triggered immunity (ETI) (Jacques *et al*., 2016). AvrGfl, AvrBs2, XopF, XopL, XopK, XopN, XopQ, XopP, and XopR are some of the T3E analogues encoded by *Xanthomonas campestries* pv. *musaceae* (Ryan *et al*., 2011; Studholme *et al*., 2010). Pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) are thought to activate T3Es proteins, which inhibit innate immune responses (Huffaker *et al*., 2006; Boller and Felix, 2009; Sinha *et al*., 2013).

Other than the role of suppressing the invading pathogen, T3Es are able to recognize receptors coded by plant resistance genes (*R-genes*) for a virulence function (Studholme *et al*., 2010). Nakato *et al*. (2018) investigated the role of *R* genes particularly pathogenesis related (*PR*) genes and the NPR1 in the development of *Xanathomonas* wilt in banana. According to Ssekiwoko *et al*. (2015) study, banana *Xanthomonas* wilt pathogen may deactivate plant's pathogen detection system making plant susceptible to pathogen attack hence disease development (Ssekiwoko *et al*., 2015).

2.3.11 Factors favouring *Xanthomonas campestris* bacteria

Symptoms of *X. campestris* infection progress faster during the wet season than the dry season. Also, the times taken by different banana cultivar to show symptoms differ with environmental conditions like temperature (Tripathi *et al*., 2009). Plants are able to show symptoms within three to four weeks of infection under favourable conditions (Tripathi *et al*., 2013). Also, the altitude of a given area and land topography that affect the local environmental conditions like temperature and rainfall are key contributing factors in banana growth and as well as vector population (Mwangi *et al*., 2007). The male bud infection is mostly transmitted through airborne vectors. There is an indication that the disease has not been experienced at altitudes over 1700 m above the sea level, this show that climatic factors affect greatly the spread of BXW (Addis *et al*., 2004; Ndungo *et al*., 2005; Kumakech *et al*., 2013).

2.3.12 Occurrence, Distribution of Banana Wilt and its Economic Significance

The disease was first described on Ensete (*Ensete ventricosum* pv. *musaceae*) in 1968 (Kidist, 2003) later it was reported in the Ethiopian highlands (Ssekiwoko *et al*., 2010). According to Tripathi (2013) the spread outside Ethiopia remained unreported for about four decades until when an epidemic was reported outside Ethiopia in Ugandan districts of Mukono and Kayunga (Tushemereirwe *et al*., 2003).

In Kenya, reports indicate that the disease was encountered at the first time in the year 2006 in Bungoma, Busia and Teso and in Siaya Counties (Onyango *et al*., 2012). A year later, it was noted in Bondo, Mumias, Butere and Elgon (Kubiriba *et al*., 2014). The disease is believed to have advanced to more other parts in Emulaya, Gem, Kakamega, Ugenya, Kisumu and Vihiga (Kwach *et al*., 2012; Tripathi *et al*., 2009). The disease is believed to be epidemic in areas of western Kenya (Onyango *et al*., 2012). However, there is less information on the occurrence of the disease in eastern parts of Kenya. Most of research work has been carried out in the western parts of Kenya with minimal research on eastern parts of the country especially in Tharaka Nithi County (Kwach *et al*., 2014).

According to Tushemereirwe *et al.* (2003) wilting disease of banana was reported for the first time in East Africa in Uganda in the year 2001. The disease has since been reported in a number of East and Central Africa such as in Eastern Democratic Republic of Congo (Ndungo *et al.*, 2004), in Rwanda, in Tanzania (Carter *et al*., 2010), in Kenya and in Burundi (Carter *et al.*, 2010). In Ethiopia, banana wilt disease first attacked *Ensete* banana and developed rapidly and spread covering Eastern, North-western and Central districts of the Country. In Democratic Republic of Congo, banana wilt disease first occurred in the year 2001. However, the identity of the disease pathogen was confirmed in 2004 (Ndungo *et al*., 2006).

Xanthomonas wilt of banana was for the first time identified in Kagera region of Muleba District in early 2006. The disease has since spread and has been observed in other regions of Tanzania that include Bukoba, Karagwe, Biharamulo and in Mara region. However, the disease has not been reported in other regions in Tanzania (Zerfu, 2019). These regions which are perceived free of banana wilt pathogen include; Meru-

Kilimanjaro Kigoma region, the southern highlands that border Malawi and as well as Mozambique. Islands of Zanzibar and Pemba have also been free of wilt disease of banana.

In Kenya, Teso, Busia and Bungoma formerly of Western province were the first regions that reported cases of banana *Xanthomonas wilt* disease in 2006 (Zerfu, 2019). The disease was afterword reported in Siaya and Kakamega and districts, formerly of Western province and Nyanza province respectively in 2007. Coastal region, Kisii, Central Kenya and most of Rift Valley so far has not reported cases of *Xanthomonas* wilt diseases (Zerfu, 2019).

Infestation of banana farms by the *Xanthomonas* wilt affects food security of many households who practice banana farming for lively hood (Yadav *et al.*, 2006). In Tanzania and in Kagera particular, banana contributes up to 50% of the diet for many households. In Rwanda, 32 % of the households grow banana that contributes about 50% of the diet. The *Xanthomonas* wilt outbreak in banana farms in the villages of Kagera in 2009 and 2011 affected households earning at 34 and 44% respectively (Zerfu, 2019). Yadav *et al.* (2006) reported that 70% of the farmers who were interviewed acknowledged that the outbreak significantly changed household's dietary patterns.

Karamura *et al.* (2008) reported that the yield loss of banana due to *Xanthomonas* wilt in Uganda was estimated to range from 30-52 % between 2001 and 2004. Banana *Xanthomonas* wilt has spread widely in most districts of Uganda (Tushemereirwe and Opolot, 2005). It has spread from Central Uganda, where banana production is less intensive and mainly subsistence oriented to the high-production areas in Western Uganda. In Central Uganda *Xanthomonas* wilt of banana infestation ranges at 18 - 27% (Tushemereirwe and Opolot, 2005). If *Xanthomonas* wilt of banana is uncontrolled infection is estimated to spread at the rate of 8% annualy (Zerfu, 2019). Should the spread of the disease remain constant at 8% p.a., total bananas production loss should be expected to be approximately 56% for the period of ten years. This loss is estimated at 4.5 million tons and eventually 2.1 million tons per annum (Zerfu, 2019). If the *Xanthomonas* wilt spread becomes extended in the whole of Uganda it will translate to

2 billion dollars economic loss over one decade. Increased disease infestation rate will lead to higher prices that will eventually affect diet in many households (Tushemereirwe *et al.,* 2006; Abele and Pillay, 2007). Bananas in Rwanda, Uganda and Burundi based on food security studies constitute daily per capita caloric intake of over 30% - 60% in some regions. In the Great Lakes region, bananas are an income generator to farmers (Okech *et al.,* 2004).

2.4 Management of Banana Diseases and Challenges

Identification of banana germplasm that is resistant to diseases for use in development of resistant varieties through breeding is one of the challenges facing management of banana diseases (Geberewold and Yildiz, 2019). Varied responses of the banana cultivars to different diseases pathogen have been reported. A germ-plasm screening study indicates that some of banana varieties may escape disease occurrence due to the morphology of their inflorescence (Geberewold and Yildiz, 2019). Another setback in managing banana diseases is farmers' reluctance to destroy their banana plants especially the best performing ones. In Uganda even in times of infection, farmers have resisted cutting down "kayinja" and "Bluggoe" banana cultivars. Since the banana mat may still be producing though infected persuading farmers to uproot infected ones has not been easy (Geberewold and Yildiz, 2019).

In places where uprooting of infected mat is practical scarcity of formal seed-system that may be used for reestablishment of uprooted fields has been pointed to be a hindrance (McCampbell *et al.,* 2018). Scarcity of diseases free planting material such as those of tissue culture or suckers, forces farmers to rely on readily available seedlings (McCampbell *et al.,* 2018). The spread of banana diseases has been worsened by the cultural practice where farmers freely obtain suckers from neighbors (McCampbell *et al.,* 2018). Farmers have also been observed to obtain suckers from within their farms which may already be infected (Tripathi *et al.,* 2009; Tinzaara *et al*., 2013).

In banana growing regions, trans-boundary transmission of pathogen may not be easy to prevent due to sharing of planting materials across the border (McCampbell *et al.,* 2018). Further, control and management of banana diseases such as *Xanthomonas* wilt is hindered by ineffective surveillance methods (Tinzaara *et al.*, 2016). There is limited

effective predictive and early-warning system that may be used to alert concerned institutions on possible disease occurrence and spread from hot spot (Bouwmeester *et al.*, 2016).

Plant diseases are managed using various methods that include uses of innovative cultural practices, integrated cultural practices inscribed with sensitive specific diagnostic technologies and use of conventional breeding (Blomme *et al.,* 2017). A critical step in disease control begins with diagnosis to understand the causative agent (Ssekiwoko *et al.*, 2006). In banana, diagnosis of the diseases may be done through inspection of the farm regularly for the appearance of symptoms (Blomme *et al.,* 2017). However, the appearance of the symptoms may differ from one banana cultivar to the next. Variation of symptoms may be attributed to prevailing environmental conditions, banana cultivar planted, banana growth stage and pathogen or disease transmission mode (Ssekiwoko *et al.,* 2006). Symptoms of *Xanthomonas* wilt have been reported within 2 weeks to 1 month following infection (Ocimati *et al*., 2013). However, the manifestation of the symptoms may depend on inoculum point of entry, pathogen strain as well as plant age (Addis *et al.*, 2010).

2.4.1 Application of Cultural Methods to Control Bacteria Wilt of Banana

Control methods of plant disease entails human activities focused on controlling disease by cultural manipulation of plants (Blomme *et al.*, 2017). In areas where the pathogens have not been introduced, efforts are made to avoid introducing them through exclusion strategies. These methods include planting of clean seed material, ensuring sanitation and legislations such as quarantine (Geberewold and Yildiz, 2019).

Regions that are endemic to plant diseases require a wide area integrated approach to control diseases. Some of the control activities that may be integrated include a limited access to area infected by pathogen and laborious use of sterilized farm equipment and implements (Nakato *et al.,* 2013). Additional practices that may be integrated are removal (uprooting) of diseased plants, regular spraying (Disinfection), elimination of alternative host plants and de-budding (Removal of male flowers) Blomme *et al.,* 2017. Failure or improper sterilization of farm implement has been reported to contribute for

the rising cases of *Xanthomonas* wilt infection in regions such as east Africa (Shimwela *et al*., 2016).

Removal of male inflorescence which is the primary infection sites in banana may lower or cut off infection (Blomme *et al.,* 2017). The male inflorescence attracts insect vectors that visit the plant for nectars and end up introducing pathogens (Blomme *et al.,* 2017). To ensure effective control of bacterial wilts of *banana,* de-budding using forked stick (Wooden) should be done immediately the last hand is formed (Shimwela *et al*., 2016). The choice of forked stick is strategically to prevent cross infections that may arise from use of farm implements like contaminated knives. Effective use of de-budding to control has been reported and also demonstrated that early male de-budding solely was effectively in reducing banana Bugtok infections in Philippines (Molina, 2006).

De-budding has been effectively practiced in commercial banana plantations (Blomme *et al.,* 2017). However, in East and Central Africa, where *Xanthomonas* wilt is rampant, its application by farmers has not been consistence (Mwangi and Nakato, 2007). Where de-budding is practiced, it has been applied sporadically and late thus leading to reported failure to prevent insect vector interaction with male buds (Kagezi *et al.,* 2006; Muhangi *et al.,* 2006).

Insect vectors of banana diseases may be prevented from introducing pathogens to the plant through bagging of inflorescence (Ocimati *et al*., 2015). Bagging is done shortly after the emergence of inflorescence using Nylon mesh bag, muslin cloth or polyethylene bag (Blomme *et al.,* 2017). Once the fruits are set and male flowers are no longer there, the bags are removed. Latin America is an example of regions where the bagging is practiced in commercial plantations and cuts off all insect vectors. Bagging is particularly effective where other sanitation such as removal old dead leaves is observed (Blomme *et al.,* 2017).

Farm equipment should routinely be cleaned and sterilized since they are major disease pathogen transmitters. Equipment used in farms that are infested with plant pathogens such as in *Ralstonia Solanacearum* or with *Xanthomonas campestris* may be sterilized using 5.5% sodium hypochlorite (Ocimati *et al.,* 2015). Use of ammonia related

disinfectants are not only effective in bacteria infected farm tools, but they are also non corrosive, has more stability when compared to sodium hypochlorite also, it is bio-degradable (Blomme *et al.*, 2017). Suitability of ammonia based disinfectants is supported by study done by Nakato *et al.* (2013).

A study carried out to compare various methods for management of *D. paradisiaca* in plantain reported that regular decontamination of farm equipment with 5.5% NaOCl is efficient in reducing plantain disease intensity to 80% (Fernández *et al*., 2013). Disinfection by flaming is widely practiced in east and central Africa countries to control *Xanthomonas campestris* in banana (Blomme *et al.,* 2017). Roughing is a widely practiced disease control method even in modern farming practice. Roughing has not gotten wide application in plantain and banana farming due to its laborious nature (*Ocimati et al., 2015*). In banana farming uprooting of banana mat may be tedious and also severely compromises effectiveness of roughing technique. According to Mwangi (2007) complete removal of two banana mats may take an individual a full day.

The removal of the banana debris is a major hindrance for the control of *Xanthomonas* wilt in East and Central Africa region. Though burying of infected banana debris may be cumbersome, burning of uprooted debris requires much time and fuel (Blomme *et al*., 2014). Nonetheless, Countries such as Indonesia, farmers have successfully managed to control banana blood disease by uprooting and burning infected banana tissues (Setyobudi and Hermanto, 1999).

According to Blomme *et al.* (2014) and Ocimati *et al.* (2013) *Xanthomonas campestris* pv. *musacea* does not colonize all the lateral root of bananas. Further, infection of bananas with *Xanthomonas campestris* pv. *musacea* may not necessarily cause symptoms (Geberewold and Yildiz, 2019). Thus, the disease may spread in the field without being noticed especially where banana mats are not replaced. For effective control, single diseased stem removal from the mat should be done using clean equipment and should be supplemented with chemical application and de-budding (Blomme *et al.*, 2014).

Farmers should actively monitor occurrence of weeds or other alternative hosts that may harbor and promote survival of banana diseases such as bacterial wilt (Rutikanga *et al.*, 2016). Banana should be rotated from one farm location to the next in a period of two to three years. However, aspect of rotation may be determined by the level of farm contamination pathogen survival and area climatic conditions (Denny, 2006; Turyagyenda *et al.*, 2008). Leaving land fallow for up to 18 months may reduce the incidences of *Ralstonia to* less than 1% in 12 months following replanting. However the duration of fallowing may be determined by intensity of infection and may range from between 6 and 12 months especially in cases of *Xanthomonas* wilt infestation (Sivirihauma *et al.*, 2013; Rutikanga *et al.*, 2016).

Alternative hosts are the reasons why pathogen may persist in an area after development of infection (Geberewold and Yildiz, 2019). Alternative hosts are a reservoir for inoculum that facilitate infection and hinders the implementation and success of control strategies like crop rotation and fallowing (Aritua *et al.*, 2008). Removal and ultimate destruction of alternative pathogen hosts is recommended for effective disease management (Romo *et al*., 2012). According to Ssekiwoko *et al.* (2006) evaluation of 20 plant species for their capability to host *Xanthomonas campestris* pv. *musaceae* showed that the pathogen only infect monocots that belong to Musaceae and Cannaceae families.

Soil nutrition status may affect the development of plant pathogens in an area (Ochola *et al.*, 2014). The soil should be amended by addition of inorganic fertilizers or supplemented with humus derivative of organic matter such as bagasse, compost and rice husk powder (van Elsas *et al.*, 2005). Soil amendment modifies soil microbial community for instance addition of oyster shell powder in soil suppresses the activity of the *Ralstonia solanacearum* population (Arenas *et al.*, 2004; Saddler, 2005). According to Arenas *et al.* (2004) when *Tagetes patula* is incorporated in the soil, the population of *R. solanacearum* in plantains reduces by 85%. Application of nutrients such as potassium, calcium and nitrogen have been seen to reduce the incidence of *Xanthomonas* wilt of banana under laboratory set up (Atim *et al*., 2013).

2.4.2 Application of Synthetic Chemical to Control Bacterial Wilt of Banana

Methyl bromide has been used for many years to control development of Bugtok disease in banana (Blomme *et al.*, 2017). The methyl bromide is applied through a dug hole at the mat of infected banana (Geberewold and Yildiz, 2019). However due to environmental and health concerns, methyl bromide have since been banned from use (Blomme *et al.*, 2017). Following the ban on the use of methyl bromide, Dazomet a soil sterilizer has been used as it reduces development of Bugtok disease (Cronshaw, 1998). Drenching soil with formalin has also been observed to minimize infection of Cavendish banana with *Ralstonia* in the Philippines (Pava *et al.*, 2003).

The success of table salt has been documented for the control of control of Bugtok banana disease (Pava *et al.*, 2003). Table salt is applied through a bored hole into the supporting banana pseudostem as has been done on Saba' and 'Cardaba' banana cultivars. Table salt should be applied about ten days prior to and five days after banana flowering (Blomme *et al.*, 2017). Five hundred grams should be poured in a dug hole and water added poured into a hole bored into the supporting pseudostem (Pava *et al.*, 2003). According to Blomme *et al.* (2008), Pestcide may provide an alternative control option to bacterial wilt of banana better than rouging. Success of herbicide use as sprays against Moko banana disease has been reported in Central America (Lehmann-Danzinger, 1987).

2.4.3 Application of Biological Methods to Control Bacteria Wilt of Banana

Use of antagonistic microorganism termed as Biological control agents have been reported in management banana *Xanthomonas* wilt (Blomme *et al.*, 2017). However, successful application of antagonistic bacteria has not been widely explored (Geberewold and Yildiz, 2019). Countries where application of bacteria antagonist is used to control *Xanthomonas* wilt include Ethiopia (Abayneh, 2010). Four bacterial isolates to antagonise development of *Xanthomonas* wilt. Bacteria antagonism reports indicates 56 to 75% reduction of disease incidence (Abayneh, 2010). In Uganda, laboratory study by Were (2016) showed potential of *Burkholderia* spp., *Herbaspirillum* spp banana isolates in suppressing *Xanthomonas campestris musaceae*.

2.4.4 Application of Resistance Banana Cultivar in Management of Banana Diseases

Use of resistant or tolerant cultivars is considered sustainable, both economically and environmentally in disease management (Boshou, 2005; Geberewold and Yildiz, 2019). Due to polygenic nature of host plants to pathogens such as *Xanthomonas campestris*, it has become challenging to develop cultivars with higher resistance (Blomme *et al.,* 2017). This is because identification and transfer of all quantitative trait loci to the desirable plant cultivars has become difficult (Blomme *et al.,* 2017). The difficulty in identification or transfer is due to their close linkage to undesirable traits (Boshou, 2005; Geberewold and Yildiz, 2019). Crops that are tolerant to Ralstonia wilt have been developed through traditional (conventional breeding. The newly developed crops with tolerance to wilt include eggplant (Gopalakrishnan *et al.,* 2005), potato (Lemaga *et al.,* 2005), tobacco (Prior *et al.,* 1994) and tomato cultivars (Wang *et al.,* 1998). However, there has been little success in developing banana cultivars with tolerance or resistance to wilt disease (Ssekiwoko *et al.,* 2006).

Screening of banana cultivars in East Africa against *Xanthomonas* wilt showed all as susceptible to the disease (Ssekiwoko *et al*., 2006). Study by Stover (1972) observed that commercial banana varieties and plantain studied were not resistant to the Moko disease. Banana cultivars Java, Mundo,' 'Turangkog,' 'Paa Dalaga,' Inabaniko and Biguihan,' that comprise of ABB/BBB genome were susceptible to Bugtok (Blomme *et al.,* 2017). The variety 'Madu-ranga,' 'Katsila,' 'Gubao,' 'Pelipita,' with genome ABB as well as Giant Kalapua' that has ABBB genome were also susceptible (Blomme *et al.,* 2017). Commercial varieties of banana that include' 'Petit Naine,' Grande Naine, 'Valery and 'Gros Michel,' containing AAA genome are also susceptible to bacteria wilt diseases (Blomme *et al.,* 2017). Varieties comprising plantains with genome AAB such as *M. ornata, M. acuminata, M. balbisana, M. salaccensis* (AAB) have shown tolerance or resistance to wilt pathogens (Baharuddin, 1994).

Sahlan and Nurhadi (1994) and Setyobudi and Hermanto, (1999) surveyed banana field in Indonesia for the blood disease of banana, and observed that cooking *balbisiana* are infected. Banana varieties evaluated by Sahlan and Nurhadi (1994) were Jimbluk, Pisang Kepok, 'Kapas,' 'Nangka,' and 'Kepok Besar' among others. *Xanthomonas* wilt is common with ABB type bananas such as 'Pisang Awak' that has dehiscent bracts

because attracts many insect vectors (Tripathi, 2009). 'Escape' cultivars that possess physical barriers on their inflorescence are able to avoid pathogen entry as they lack the male inflorescence (Denny, 2006; Buddenhagen, 2009. Other 'escape' varieties may have un-attractive flowers or abscission wounds that dry or heal prior to the fall of bracts fall, avoiding penetration by bacteria pathogens (Addis *et al.,* 2004; Mwangi and Nakato, 2007).

2.5 Screening of Banana Genotypes Tolerant to *Xanthomonas* Wilt

2.5.1 Banana Genotypes Grown in Kenya

In Kenya, the major banana varieties grown include Muraru, Kiganda and Gasukari. These banana genotypes have proved to be well adapted to various agro-ecological zones in the country. Improved cultivars include: Apple, Gross Michel, Kampala, Dwarf Cavendish, Giant Cavendish, Williams, Grand Nain, Vallery, Poyo and Lacatana while medium variety include Valery, Paz and Williams (Mbaka *et al*., 2008). In Tharaka Nithi County, the most commonly grown varieties include Muraru, Gasukari, Kampala, Israel and Kiganda.

2.5.2 Tolerance of Banana Genotypes to *Xanthomonas* Wilt

Host resistance is the most cost effective and simplest method of controlling any disease caused by a pathogen. Previous research has shown that there is no entirely natural immunity to BXW in cultivated Musa species (Tripathi *et al*., 2008). Some of the cultivars that lack the male bud pose a challenge for the pathogen to infect under natural conditions (Ssekiwoko *et al*., 2009; Lewis *et al*., 2010). Thus, prospects of developing cultivars with resistance to BXW through concentration breeding are limited because so far, no source of cultivated banana has been found to exhibit full resistance against *Xanthomonas campestris* (Tripathi *et al*., 2008 and Ocimati *et al*., 2013). However, *Musa balbisiana*, a wild variety of banana has been identified to be the most resistant to the pathogen in Uganda (Ssekiwoko *et al*., 2006). In Ethiopia and East Africa, some studies carried out have shown that even though no natural banana cultivars and genome groups possess complete genetic resistance to BXW, but the varieties differ in the degree of susceptibility (Tripathi *et al*., 2008). Therefore, further exploration on resistant banana genotypes in Kenya will be a base of further study for developing resistant cultivars through conventional breeding.

CHAPTER THREE
MATERIALS AND METHODS

3.1 The Area of Study

The study on banana *Xanthomonas* wilt was carried out in Tharaka Nithi County in Kenya within Nithi region which comprise of Maara and Chuka Igambangombe Sub Counties. Tharaka Nithi County borders Embu, Nyeri, Kitui and Meru Counties (Fig. 1). Tharaka Nithi County is divided into 4 sub counties that is: Maara, Tharaka North, Tharaka South and Chuka Igamban'gombe. The County is divided into lower region (semi-arid area) and upper region. Lower region is semi-arid and covers Tharaka area. The lower region receives low amount of rainfall ranging between 100 mm to 600 mm pa (Funk *et al*, 2010). The upper region of Tharaka Nithi County comprises of areas bordering Mount Kenya forest such as Chuka. The upper region receives adequate rainfall that ranges between 150 mm to 1100 mm (Funk *et al*, 2010). The upper zone is suitable for agriculture and it includes areas like Chuka and Maara (Nithi). Chuka Igambang'ombe has the highest altitude in the county of 5200 m while the lowest is Maara with an altitude of 600 m. In Tharaka Nithi County, long rains falling pattern is experienced in the months of April to June while short rains in October to December. The two upper zone sub counties receive adequate rainfall hence provide adequate environment for banana growth unlike the other two sub counties of the lower zone. The geographical location of all the farms surveyed were recorded using Garmin etrex 30x GPS machine (Appendix I). The coordinates obtained for the farms were used to generate the map for the study area (Figure 1)

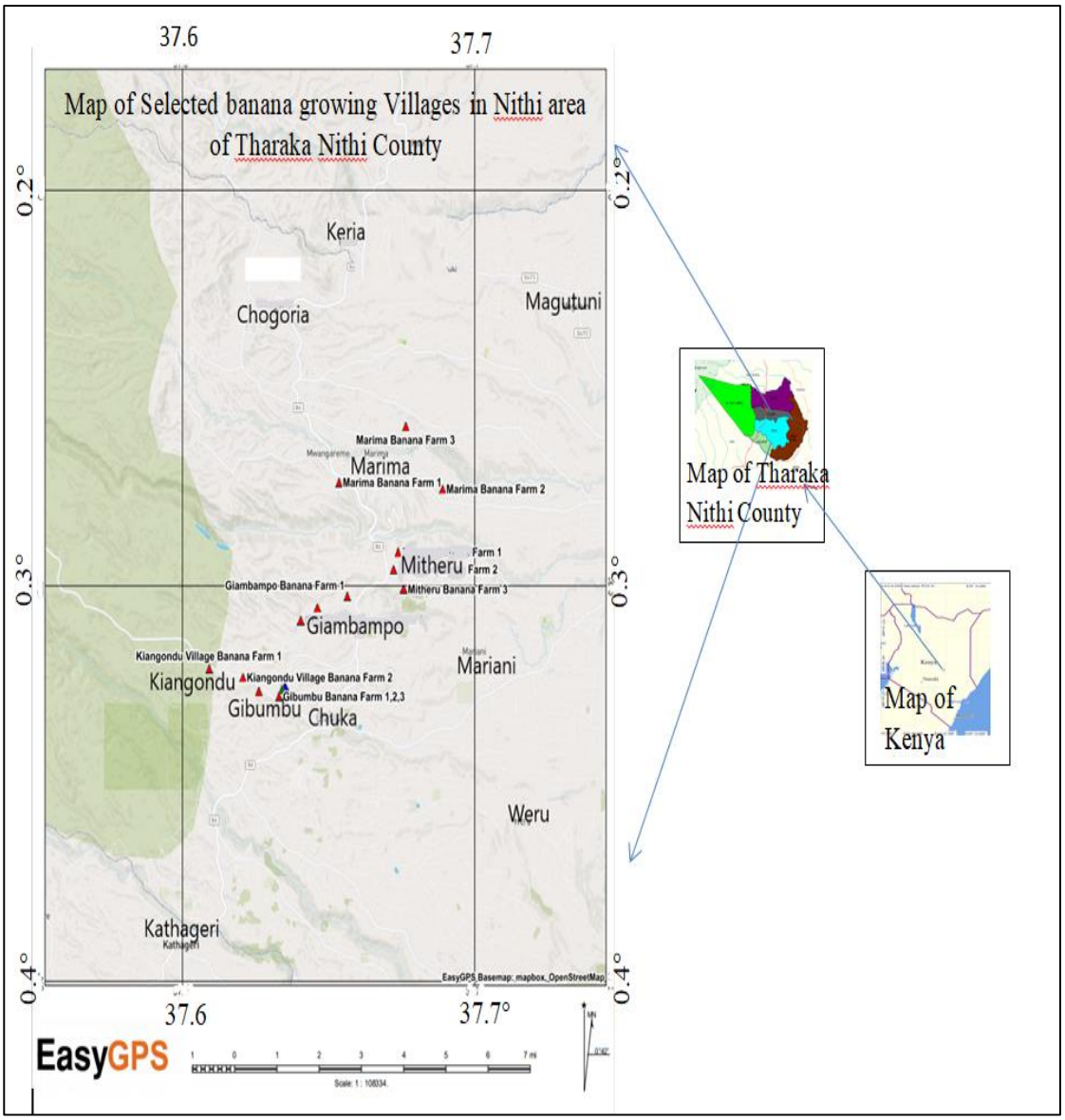


Figure 1: Map of Tharaka Nithi County showing selected villages surveyed for banana Xanthomonas wilt.

3.2 Research Design

3.2.1 Survey Method

Study on *Xanthomonas* wilt of banana in the farms was done using Descriptive Survey Design. The design is appropriate because it identified the banana varieties grown, infested plants, disease severity, susceptibility, disease management, and occurrence of banana diseases in the farmers' fields at the time of the survey. Farms surveyed were purposively sampled. In individual farms, transect were established where banana were assessed for infestation of *Xanthomonas* wilt.

3.2.2 Experimental Design

Susceptibility of banana cultivars to *Xanthomonas* wilt was done in the greenhouse using a 5 x 3 factorial experiment laid out in Randomized Complete Block Design (RCBD) and replicated five times. Factor A was varieties of banana at five levels (Israel, Kiganda, Kampala, Muraru and Gasukari). Factor B was pathogen concentration at four levels (3 concentration of isolate BXPS 12 at 10^{-1}, 10^{-2} cfu and 10^{-3} cfu and control, i.e., distilled water.

3.3 Target Population, Sampling Procedure and Sample Size

3.3.1 Target Population

The target population in this study was 100 banana farmers who have banana farms of 0.5 acre in size in five villages in Maara and Chuka Igambang'ombe Sub-Counties, Tharaka Nithi County. The five villages included Marima, Mitheru, Giampampo, Gibumbu and Kiangondu. The five villages were selected purposefully for the study since they are main banana producing areas in Maara and Chuka Igambang'ombe Sub-Counties and majority of the banana farms measures about 0.5 acres.

3.3.2 Sample size

The sample size for the banana farmers to participant in this study was obtained using Cochran (1977) formula as follows,

$$n^0 = \frac{z^2 pq}{(e)^2}$$

where n^0 = sample size, Z^2 = Z score (95%), Error level of (0.057), p = estimated proportion presenting population (5%) and q = 1-p.

$$n^0 = \frac{1.96^2(0.057)(1-0.057)}{(0.05)^2} = 82.59$$

The actual sample size for the banana farmers to be included in the survey as respondent using the questionnaire was further calculated using the Cochran (1977) smaller sample modified formula as follows,

$$n = \frac{n^o}{1 + \frac{n^o - 1}{N}}$$

$$n = \frac{83}{1 + \frac{83 - 1}{100}} = 45.7$$

Thus the sample size of 46 banana farmers was used in this study.

3.3.3 Sampling Procedures

The banana farmers to be interviewed by filling a questionnaire were obtained using a cluster random sampling. Therefore, the 46 banana farmers were distributed as follows, 9, 10, 9, 9 and 9 farmers in Marima, Mitheru, Kingondu, Giampampo and Kibumbu villages, respectively.

Out of 46 banana farmers' interviewed, 15 of their farms were randomly picked for actual farm assessment of BXW. Three farms were randomly picked per village. In every sampled farm, a transect was laid across the banana plantation and six (points) locations were established at approximate equidistance of 5 m. Individual banana plants in a mat in each of the six points in the farm were then inspected for the symptoms of banana *Xanthomonas* wilt. The geographical location of all the farms surveyed were recorded using Garmin etrex 30x GPS machine (Appendix I).

3.4 Research Instruments

The questionnaire was administered to banana farmers to collect information on: Banana varieties grown, infected plants, disease severity, susceptibility, disease management and occurrence of banana diseases in their farm. Farmers' knowledge of *Xanthomonas* wilt was also assessed by the questionnaire (Appendix II).

3.4.1 Pilot Study

A pilot study was conducted in farms at Mitheru village in Nithi region that boarders Chuka Igambang'ombe Sub County in Tharaka Nithi County. The aim of the pilot study was to identify potential problem areas during the actual study, deficiency in the research protocol prior to implementation. The pilot study enabled familiarization of research procedure and facilitated the assessment of the efficiency of the prepared questionnaire.

3.4.2 Validity Test

The research instruments used were evaluated though a judgmental approach through thorough literature reviews and follow-ups with the evaluation by experts in the same field of research. Also the research work was presented to various panels for scrutiny for effective validation.

3.4.3 Reliability Test

A pre survey was conducted to test the efficiency of the questionnaire used in the study. The results of the pretest were used to adjust the questionnaires to ensure accuracy, clarity and consistency of information collected.

3.5 Data Collection

3.5.1 Evaluation of Prevalence of Banana *Xanthomonas* Wilt

Symptoms of BXW were identified and disease was scored on scale of 1 - 7 (Muhinyuza *et al.,* 2007): where 1 = no symptom, 2 = yellowing leaves, 3 = wilted leaves, 4= dry male buds with no wilting symptoms, 5 = wilted banana leaves on banana mat and dry male bud, 6 = heavily wilted leaves, drying or dried male bud and premature fruit ripening and 7 = yellow leaves necrosis. A banana mat includes the parent plant and its suckers (stool). The Global Positioning System (GPS) was used to mark the altitude, latitude and longitude of locations where sampling was done (Appendix I). Percent disease prevalence was calculated using the formula below as used by Muhinyuza *et al*., 2007

$$\text{Disease prevalence} = \frac{\text{Number of individual ratings}}{\text{Total number of banana assessed}} x100$$

3.5.2 Isolation and Characterisation of *Xanthomonas campestris*

3.5.2.1 Sample Collection for Pathogen Isolation and Characterization

Five symptomatic banana leaf samples were randomly collected from every village surveyed (Marima, Mitheru, Giampampo, Gibumbu and Kiangondu). The banana leaf samples were cut using sterile forceps and packed in ziplock bags. In total, 25 diseased banana samples were collected. Samples collected were yellow, wilted and excreting yellowish bacterial ooze (Tushemereirwe *et al*., 2004; Tripathi *et al*., 2013). Samples

were labelled with details such as place of collection, sample number and type of tissue collected. The bags were packed in a cool box and transported to Chuka University botany laboratory for isolation and characterisation of the pathogen. In the laboratory, samples were stored in the fridge at 4°C prior to pathogen isolation.

3.5.2.2 Media Preparation

The culture media was constituted by adding peptone water (10 g), sodium chloride (10 g) and yeast extract (10 g) respectively into 1000 mL distilled sterile water. Once the media dissolved completely, 15 g of agar was added to facilitate gelling. The pH of reconstituted medium was adjusted to the pH of 7.4 by adding a drop of 1 M sodium hydroxide solution. The media was then sterilized in an autoclaved (Model: IX8.35L) for 121 °C at 15 psi for 15 minutes. At the end of sterilization, the media was cooled to 50°C in the water bath and dispensed in petri plates and allowed to gel in the biosafety cabinet.

3.5.2.3 Pathogen Isolation and Purification

Isolation of *Xanthomonas campestris* pathogen was done from 24 symptomatic banana leaves collected during farm survey. The symptomatic leaves were passed through 5% sodium hypochlorite for surface-sterilization for two minutes and rinsed in sterile water. The portion of the infected leaves were aseptically cut using sterile scalpel and placed on pre sterilized pestle. Normal saline (1 ml) was added to the pestle and the leave sections crashed. Sap (1 ml) from crushed leave tissue was serially diluted to 10^4 CFU in a Maximum Diluent Agar (MRD) so as to ensure dispersal of the pathogen to allow growth of single colonies. To achieve 10^{-4} CFU concentrations, initial inoculum (crushed leaves) 1 ml was transferred to 9 ml of MRD and mixed to make 10^{-1}. Dilution 10^{-2} was prepared from 10^{-1}, dilution 10^{-3} was prepared from dilution 10^{-2}. The inoculum prepared was inoculated in nutrient broth medium and incubated overnight at 37°C. After 24 hours of incubation, the single colonies which grew were sub cultured in a fresh LB agar medium. The sub cultured plates were incubated at 37°C for 17 hours at the temperature of 37°C. The pure colonies were stored at 4°C for two days prior to biochemical analysis.

3.5.2.4 Morphological and Biochemical Characterization of Bacteria Pathogen Isolates

Gram staining, Oxidase, Starch hydrolysis, Gelatine liquefaction, Is Proskauer, Methyl red, Fluorescent test, Hydrogen sulphide, Potassium hydroxide and Catalase tests were done to characterize the isolated pathogen. Based on growth characteristics, the isolates were placed into two groups. The first categories were those which were yellow in colour while the other group comprised of isolates which were cream in colour.

Differential staining was used for identification of the isolates. A smear preparation and staining was adopted for Gram staining of the pure isolates according to Ogolla and Neema (2019). A thin smear on a clean slide was prepared from the pure colonies by placing a drop of normal saline on the slide and adding a loop of bacteria colony on it. The smear was air dried and passed over the flame for 30 seconds. Upon fixing, the smear was stained with crystal violet stain as the primary stain and rinsed. Gram iodine a mordant was poured on the smear and left to stand for 1 minute then rinsed. The smear was then flooded with 80% ethanol for 1 minute to remove the mordant and primary stain from gram negative bacteria then rinsed. Decolourisation was followed by flooding the smear with safranin to counter stain the decolourised gram-negative cells. The slide with the stained smear was finally blot dried and mounted on the microscope for viewing using oil immersion and x100 lens. Cells that stained purple in colour were be considered as gram positive while those that stained pinkish to reddish were considered gram negative bacteria. Positive bacteria samples were recorded as (+ve) while the negative colonies were recorded as (–ve)

Oxidase test was done by placing Whatman No. 42 filter paper on a petri plate and adding four drops of dimethyl p-phenylenediamine hydrochloride an oxidase test reagent which differentiates bacteria based on their ability to utilize the dye. On filter paper moistened by dimethyl p- phenylenediamine hydrochloride aliquots of pure bacterial colony (yellowish in colour) was smeared and reaction observed in three replicates. Development of purple colour on the isolates were observed within 10 seconds, 60 seconds and more than 60 seconds. Isolates that formed purple colour were considered oxidase positive. Those which did not develop purple colour were considered oxidase negative (Rafi *et al*., 2013; Ogolla and Neema, 2019).

Starch hydrolysis test was performed using starch agar. Starch agar media was prepared from 10 g of potato starch and 8.8 g nutrient agar dissolved in 1000 ml distilled water (Deshmukh, 1997). The media was sterilized and from it 20 ml was dispensed in individual sterile petri dishes for use in starch hydrolysis test. Media on plates was left for about 10 minutes to solidify after which spot inoculation of the pure isolates was done using flame sterile inoculation wire. The plates were incubated (Memmert; Model: INB 200) at 28°C for 5 days and at the end of incubation period, iodine was poured on the surface of the inoculated plates to determine bacteria's starch utilization activity (Gamangatti and Pati, 2013).

Gelatine liquefaction test was conducted using Nutrient Agar (NA) amended with gelatine. Twenty-eight grams of NA media was weighed using an electronic balance (TP-B500) and transferred into a 500 ml round bottomed flask. About 100 ml of distilled water was added and then thoroughly shaken to obtain a homogenous mixture. The mixture was sterilized at 121^{o}C and 15 psi for 15 minutes in an autoclave (IX8.35L). After the media had cooled, 5 g of gelatin was added and mixed using a stirrer. Cooled media was dispensed in clean sterilized petri dishes. The medium was left to solidify for 20 minutes then spot inoculation was done using sterile inoculation wire. Inoculated plates were then inoculated for 48 hours at the temperature of 28°C. At the end of incubation freshly prepared acid mercuric chloride solution (10 ml) was poured on the surface of the media with grown colonies. Colonies that formed hallo zone around a round them were considered positive (Naik *et al.,* 2018)

Media for indole test was prepared by dissolving 10 g of tryptophan and 5 g of sodium chloride in one litre of sterile distilled water. The medium was then dispensed in test tubes autoclaved at 121°C at 15 psi and for 15 minutes. Whatman filter paper was cut into strips and soaked in warmed saturated oxalic acid solution. The strips were cooled then covered with the oxalic acid crystals then dried at room temperature. The banana wilt pathogen isolates were then inoculated in tryptophan broth tube into which two oxalic acid test strips were inserted. The test tubes were then incubated at room temperature for 14 days. Observation for formation of pink colour was monitored after every two days during the 14 days incubation period (Gamangatti and Pati, 2013).

Isolates were subjected to the Voges-Proskauer Test indicator. The indicator was made of glucose phosphate broth comprising of 0.5% peptone, 0.5% glucose and 0.5% K_2HPO_4 was used. Pure pathogen isolates were re-cultured in IMVIC broth and incubated for five days at 27°C. At the end of incubation period, the culture (1 ml) was dispensed in a sterile test tube which had 0.6 ml naphthol and 0.2 ml of 40 % potassium hydroxide solutions then shaken vigorously. The tube's content were allowed to stand for ten minutes and observed for the formation of colour that was considered positive test. Absence of red colour formation after 10 minutes was regarded a negative test (Ogolla and Neema, 2019).

The isolates were subjected to the methyl red test using methyle red (MR) indicator. The indicator (MR) was prepared by dissolving 0.1 g methyl red in 300 ml (95%) ethanol. The MR indicator was added in the bacteria culture grown in IMVIC broth media. The change of indicator colour from yellow to red was considered to be positive reaction. Failure of indicator to turn red was considered as negative reaction.

Fluorescent test was conducted on the banana wilt pathogen isolates. Fluorescent test was constituted using King B medium made of 20 g Protease peptone medium, 1.5 g K_2HPO_4, 10 ml Glycerol, 15 g agar and $MgSO_4$ 1.5 g. The media was prepared, autoclaved and dispensed in petri dish. Pure isolates of the pathogen were streaked on King' B medium and incubated for two days at 28 °C. The plates after incubation period were observed for fluorescence.

Pathogen isolates were subjected to the Hydrogen sulphide (H_2S) Production test. Hydrogen sulphide was done using peptone broth upon preparation and sterilized. The 48-hour old pure colony of the pathogen culture of 48 hours was inoculated on a slant of peptone broth. Whatman No. 42 filter paper discs that have been impregnated with 10% neutral lead acetate solution were placed hanging on the inoculated test tubes and the tube sealed with sterile cotton. The tubes were then incubated for 72 hours at 28°C. Lead acetate impregnated strips were assessed for signs of blackening as an indicator for positive H_2S production test (Naik *et al.*, 2018; Ogolla and Neema, 2019).

Xanthomonas wilt pathogen isolates were tested for production of gas and bubble. Banana wilt pathogen pure isolates (loop-full) of 48 hours growth were smeared on a clean sterile glass slide. The bacterial loop on the slid was flooded with two drops of hydrogen peroxide (H_2O_2) and left to stand for 30 seconds. The reaction was observed for formation of gas bubbles which was considered positive reaction (Emitaro *et al.,* 2017).

Potassium hydroxide (KOH) solubility test was performed was carried out on the pathogen isolates. A loop of Pathogen isolates was aseptically picked using sterile applicator stick and placed on a clean glass slide on KOH (3%) solution. The KOH and loop of pathogen colony were stirred and mixed for ten second in a circular motion. The mixture was raised by the applicator stick slightly above the slide and formation of viscid strand is common with Gram negative bacteria observed (Gamangatti and Pati, 2013).

Nutrient broth was used to do ammonia production test on the banana wilt pathogen isolates. Sterile test tubes were filled with 8 ml of nutrient broth and inoculated with a loop of pure bacteria pathogen isolate cultures and tightly closed with sterile cotton wool. Inoculated tubes with one un-inoculated test tube used as test control were incubated for 48 hours at 28°C. At the end of incubation period, red litmus paper strip was sling inside the test tube and test tube re-sealed with cotton that held the litmus paper in place. The litmus paper strip was observed for colour change (Gamangatti and Pati, 2013; Ogolla and Neema, 2019).

Protein digestion test was done on the isolates using both liquid test and solid agar plate test. Liquid test was done by preparing the medium from powdered skim milk has been supplemented with 0.004 per cent bromocresol purple. The media was then sterilized through steaming at 60°C for 30 min for three successive days. Milk solution in the tubes were inoculated with a loop of pure colonies of banana wilt pathogen isolates and incubated at 20°C. Observation of the media for the clearing reaction which indicate digestion of casein was made. In solid agar plates test, powdered skim milk was added to autoclave sterilized yeast extract nutrient agar (YNA) and dispensed in sterile petri dishes. The banana wilt pathogen isolates were spot inoculated on by on the media. The

media was observed for the occurrence of clear zones around the colonies on day 3rd, 5th and 7th (Gamangatti and Pati, 2013).

3.5.3 Evaluation of Banana Genotypes Susceptibility to *Xanthomonas* Wilt of Banana in the Greenhouse

3.5.3.1 Inoculum and Media Preparation

The bacteria culture media and glassware were sterilized using an autoclave (Model: IX8.35L) and then dried in hot air oven. Also, the soil media comprising of soil and farm yard manure at a ratio of 1:2 used in the bucket experiments was sterilized to ensure it is free of disease-causing organisms. *Xanthomonads campestris* pv. *musacearum* isolate BXPS 12 isolated from banana samples as described in section 3.5.2.4 was used for the study since the isolates showed similar reaction to biochemical tests. Pure colony of the isolate (Isolate BXPS 12) was sub cultured in 25 ml of Yeast Tryptone Sucrose medium which was made of, 1% tryptone, 1% yeast extract and 1% sucrose. The broth was incubated at 28°C for 48 h and shaken at 150 revolutions per minute in an orbit shaker. At the end of incubation, the culture was centrifuged at 4500 rpm for 10 minutes. The resulting pellets were finally re-suspended in double distilled sterile water. Using a UV- spectrophotometer (Shimadzu UV-1800), the bacteria suspension was adjusted using optical density of 600 nm to the concentration of 10^{-1}, 10^{-2}, and 10^{-3} cfu ml^{-1} with peptone water. The concentration made was kept in the fridge at 4°C prior to use for inoculating the banana varieties in the green house.

3.5.3.2 Inoculation of Banana Suckers

Plastic buckets measuring 35 cm height and diameter of 27 cm were used for planting banana suckers in the greenhouse at Chuka University. The buckets were filled with 3 kilogram of soil media which was pre-sterilized in an oven at 120^{o}C for 4 hours and cooled for one day. Soil media comprised of soil and farm yard manure at a ratio of 1:2 (World Agroforestry Centre, 2012)

Banana suckers (Kampala, Kiganda, Israel, Gasukari and Muraru) of height 40 cm were used to evaluate five banana varieties for their susceptibility to *Xanthomonas* sp. under artificial infection. A total of 75 banana plants (five banana cultivars X 3 concentrations of isolate (BXPS 12) x 5 replication) were used in the study. The distance between each banana pot to the next was 1 m. Each plant was injected with 20 ml of *Xanthomonas*

bacteria inoculum at the base of the pseudo stem as described by Tripathi *et al.* (2008). The control experiment, banana plants for each of the five varieties (Israel, Kiganda, Kampala, Muraru and Gasukari) were injected with distilled water. The inoculated plants were observed for two months for development of *Xanthomonas* wilt symptoms. Banana under test were watered with distilled water (1000 ml) at 1600 hr every day. Banana plants which showed wilting symptoms were selected and the pathogen re-isolated from them on a Nutrient Agar as described in section 3.5.2.4 to confirm if the characteristics of isolates were similar to the initial bacteria isolates on banana. Disease severity for every banana variety inoculated was calculated using percentages in order to determine the variety that was more susceptible to the disease using (Tripathi *et al.,* 2009).

Data on the banana bacteria wilt incidence was obtained through observing wilting symptoms and dead banana plants. Banana wilt disease severity was rated on a scale of 1-5 (Table 2). Scale of 1= 0% indicated no wilting symptoms, 2 = 1 - 25% indicating mild banana leaf wilt symptoms, 3 = 26 - 50% indicated mild bacteria wilt symptoms on banana suckers which were either stunted and burnt, 4 = 51 - 75% indicated mild bacteria wilt symptoms on suckers with severe wilting of suckers, 5 = 76 - 100% indicating that all banana suckers were stunted and dying (Tripathi *et al.,* 2009). Disease severity was calculated using the formula below as used by Amin *et al.*, 2014

$$\text{Disease severity} = \frac{\text{Number of individual ratings}}{\text{Total number of banana assessed}} x \frac{100}{\text{Maximum score (5)}}$$

Table 2: Scale used to score disease in the greenhouse

Disease rating	% of leaf surface infected	Intensity of symptoms
1	0	Free from disease
2	1-25	Slight symptoms
3	26-50	Moderate symptoms
4	51-75	Moderately severe symptoms
5	>75	Severe symptoms

Source: Tripathi *et al*. (2009)

3.6 Data Analysis

Data obtained on prevalence of *Xanthomonas* wilt was subjected to one way analysis of variance in SAS version 9.4 and significance means separated using least significance difference at 5% probability level. The data were log transformed (log_{10}) to meet the requirement for analysis of variance. Data collected using questionnaire on diseases aspects were analysed using Chi-square test of independent at 5% significant level. The Chi-square test was used to test the relationship between banana varieties grown and occurrence of banana diseases.

Percentage severity data collected to determine banana susceptibility in the greenhouse susceptibility test was analysed using general linear model and significance means separated using least significant difference (LSD) at 5% significance level in SAS version 9.4.

3.6.1 Statistical Models

The statistical model for analysis of prevalence of banana *Xanthomonas* wilt for different villages was as shown below,

$$Y_{ij} = \mu + \tau i + \epsilon ij$$

where Y_{ij} is the response from the i^{th} treatment and j^{th} experimental unit.

μ is the population mean

τi is the i^{th} effect of disease prevalence

e_{ij} is the error from i^{th} treatment and j^{th} experimental units.

The statistical model for analysis of prevalence of banana cultivars to determine their susceptibility to *Xanthomonas* wilt under greenhouse study.

$$Y_{ij} = \mu + ai + b_i + (ab)_{ij} + e_{ij}$$

where Y_{ij} is the response from the i^{th} treatment and j^{th} experimental unit.

μ is the population mean

a_i is the effect of pathogen concentration.

b_j is the effect of banana variety.

ab_{ij} is the effect of factor interaction of factor a and b treatment.

e_{ij} is the error from i^{th} treatment and j^{th} experimental units.

3.7 Ethical Issues Considerations

Clearance was sought from Chuka University Ethics Review Committee, Tharaka Nithi county agriculture department and a research permit from NACOSTI (Appendix III, IV and V). Farmers consent was sought prior to farm visits for survey and sample collection. During administering of questionnaire, farmers were assured that the information collected were of no ill intention and was to be used strictly for this study purpose. The experiment was carried aseptically in a greenhouse to ensure that there was no pathogen escape. Further, all the wastes from the study including banana used were drenched with 6% Lysol and buried (Chattopadhyay, 2017). The procedures were followed as outlined to avoid biasness. During data collection, write up and reporting, all the source of the information used in the document was acknowledged by citation to avoid plagiarism.

CHAPTER FOUR

RESULTS

4.1 Prevalence of Banana *Xanthomonas* Wilt in Nithi in Tharaka Nithi County

There was no significant difference ($p > 0.05$) in BXW prevalence between the five villages (Appendix VI). Prevalence at Giampampo was 21.14% followed by Kiang'ondu village 17.54%. Mitheru village had the least prevalence of 11.24% (Table 4). The overall mean for the prevalence was 15.09% and only 3 villages (Giampampo, Marima and Kiang'ondu) recorded prevalence above the overall mean (Table 3).

Table 3: Prevalence of *Xanthomonas* banana wilt in selected villages in Nithi

Village	BXW Prevalence (%)
Giampampo	21.14
Kiang'ondu	17.54
Marima	15.49
Gibumbu	12.14
Mitheru	11.24
Mean	15.095
LSD ($p \leq 0.05$)	1.743
CV (%)	30.856

The banana infected by *Xanthomonas* produced yellow pigmented exudates on cutting the pseudostem, the leaves were yellowish in colour while the banana bunch had poorly developed banana fruits (Plate 2)

Banana *Xanthomonas* wilted plant

Xanthomonas effect on banana bunch

Xanthomonas Yellow exudates on psuedostem

Plate 2: Observed symptoms of banana *Xanthomonas* wilt

4.1.1 Varietal Effect on Disease Occurrence

There was significant relationship (X^2 (8, N = 46) = 19.93, Cramer's V = 0.4654, p = 0.0034) between banana variety grown and occurrence of banana diseases in Nithi.

Twenty five percent (25%) of the respondents who grow mainly Kiganda and Israel banana variety reported disease occurrence in their farm. Twenty percent (20%) of the respondents who grow mainly Kampala banana variety reported disease occurrence in their farm. Seventy-five percent (75%) of farmers who could not tell the variety of banana they grow reported occurrence of diseases in their farm. Lastly, seventy six percent (76%) of farmers who grows mixed varieties reported occurrence of banana diseases in their farms (Figure 2).

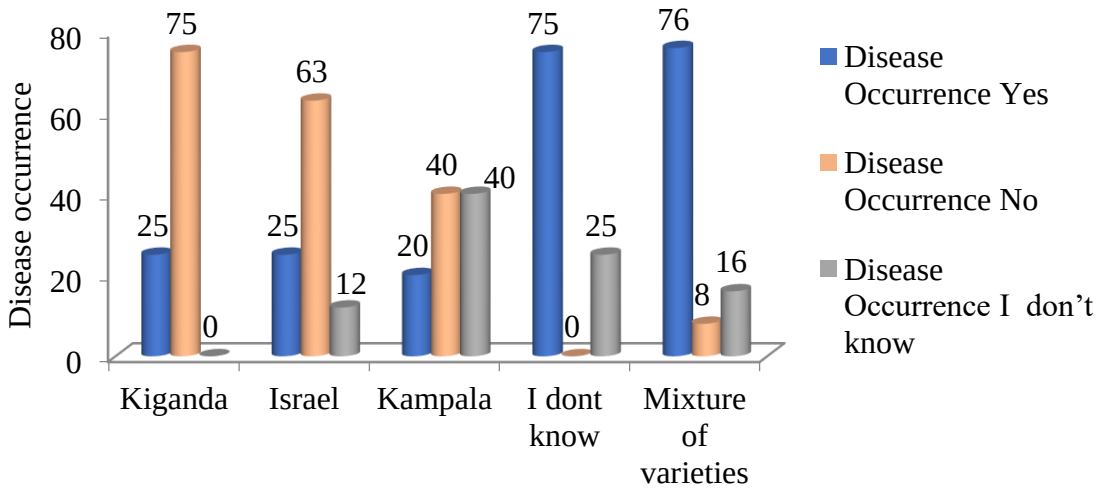


Figure 2. Relationship between banana variety grow and diseases occurrence

4.1.2 Banana Variety Grown and Susceptibility to Diseases in Nithi

The results indicated that there was significant relationship (X^2 (8, N = 46) = 31.165, p = 0.0053) between banana variety grown and susceptibility to diseases in Nithi. A hundred percent (100%) of farmers who grow purely Kiganda, Eighty eight percent (88%) of Israel growing respondents, eighty percent (80%) of farmers who purely grow Kampala, Seventy five percent of respondents who lacks knowledge on variety that they grow and sixteen percent (16%) of farmers who grow mixed varieties reported that they did not know variety susceptible to banana diseases. (Figure 3)

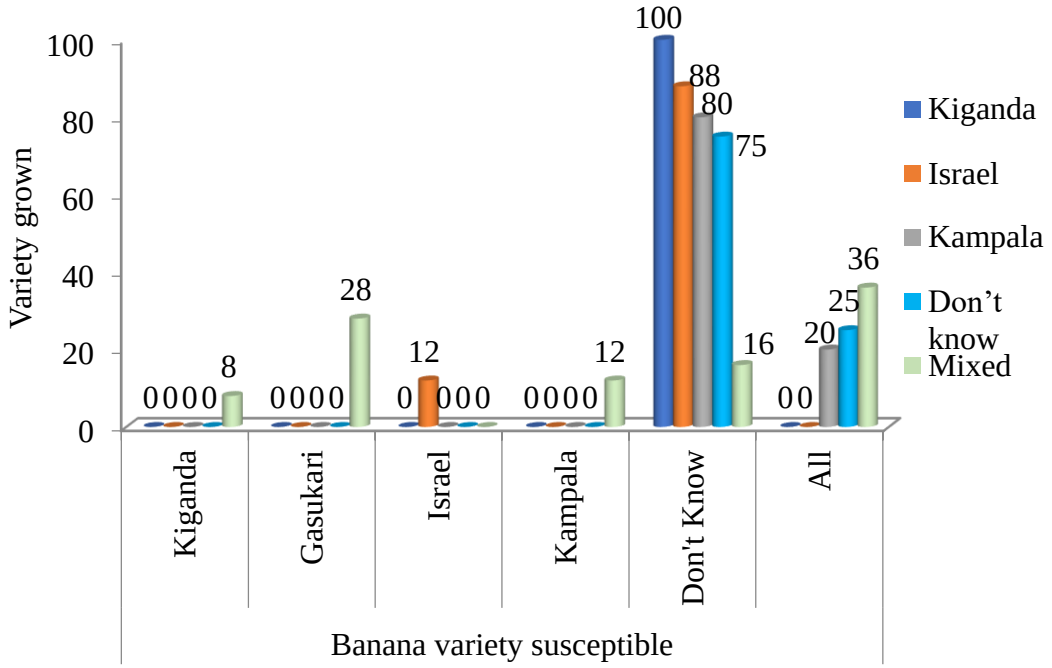


Figure 3: Graph of banana variety grown by farmers and susceptibility

4.1.3 Prevalence of Banana Diseases with Years of Banana Farming

The results indicated that relationship between years of growing banana in the same farm and occurrence of banana diseases in Nithi was significant ($X^2(6, N = 46) = 8.761$, $p = <0.0001$. Thirty three percent (33%) of farmers growing banana for 1 - 3 years, reported occurrence of disease. Farmers that have grown banana for between 4 - 7 years, fifty percent (50%) reported occurrence of banana diseases in their farm. On the other hand, twenty three percent (23%) of farmers that have grown bananas for over ten years did not report occurrence of banana diseases in their farms while eight percent (8%) of farmers who have grown bananas for over ten years do not know whether banana diseases occurred in their farm (Figure 4).

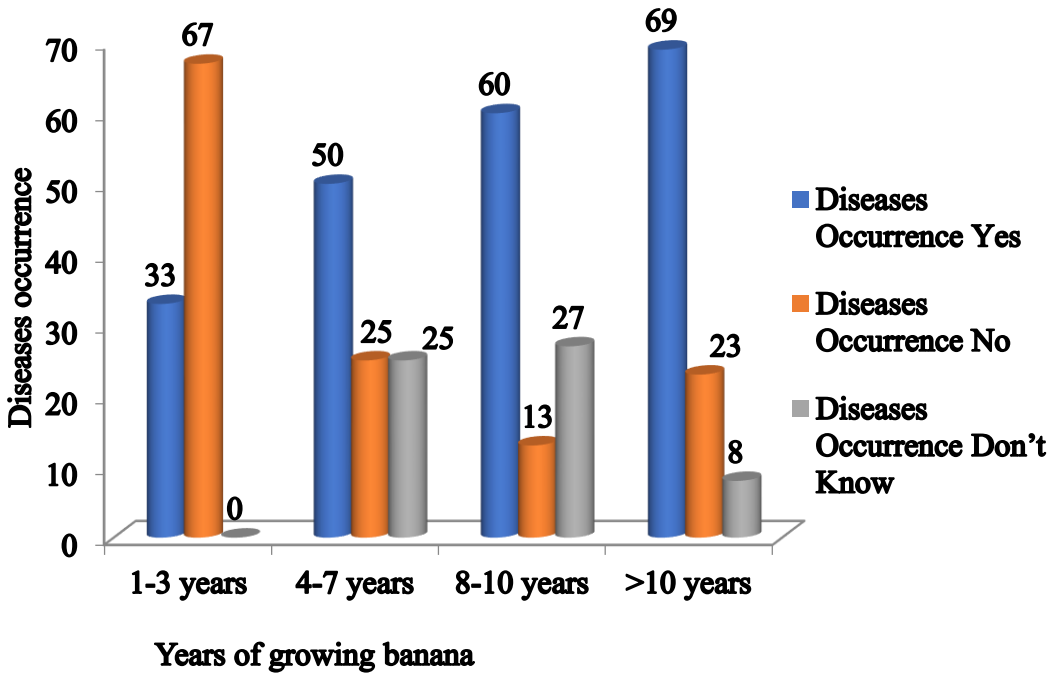


Figure 4: Association between years of growing bananas in the same farm and disease occurrence

4.1.4 Disease Occurrence across different Seasons of the Year in Nithi

The results indicated that the relationship between occurrence of banana diseases and season of the year in Nithi was significant (X^2 (8, N = 46) = 32.4591, p = <0.0001). Sixty five (65%) of farmers who reported occurrence of banana diseases in their farms noted that diseases were most common during the dry season. Sixty three (63%) of farmers reported no knowledge of diseases occurrence and season that diseases are common (Figure 5).

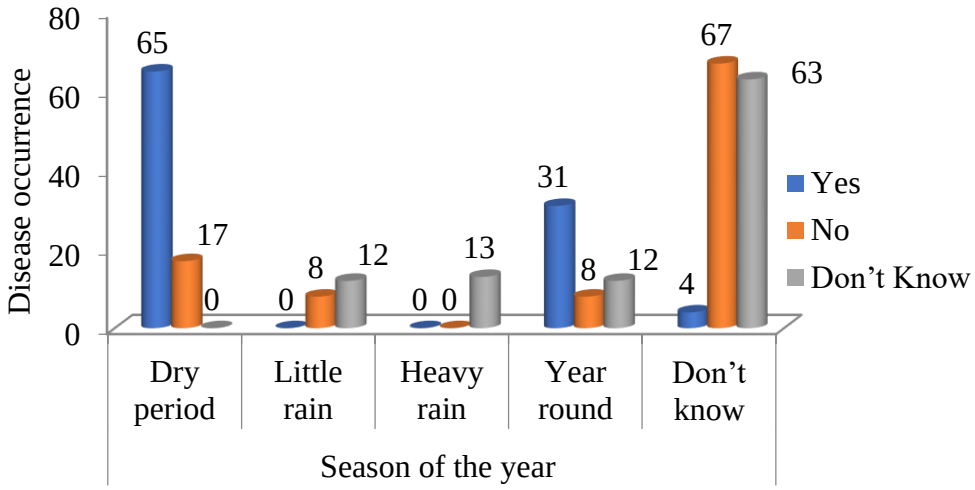


Figure 5: Association between season of the year and occurrence banana diseases

4.1.5 Disease Management Methods Applied for Different Banana Diseases

The results indicated that relationship between occurrence of banana diseases and management option in Nithi was significant (X^2 (4, N = 46) = 6.9758, p = 0.0025). Out of the total number of farmers who reported disease occurrence in their farms, nineteen percent (19%) uproots diseased tuber to control the diseases, fifteen percent (15%) apply chemicals to control the diseases while sixty six (66%) do not do anything to control diseases (Figure 6).

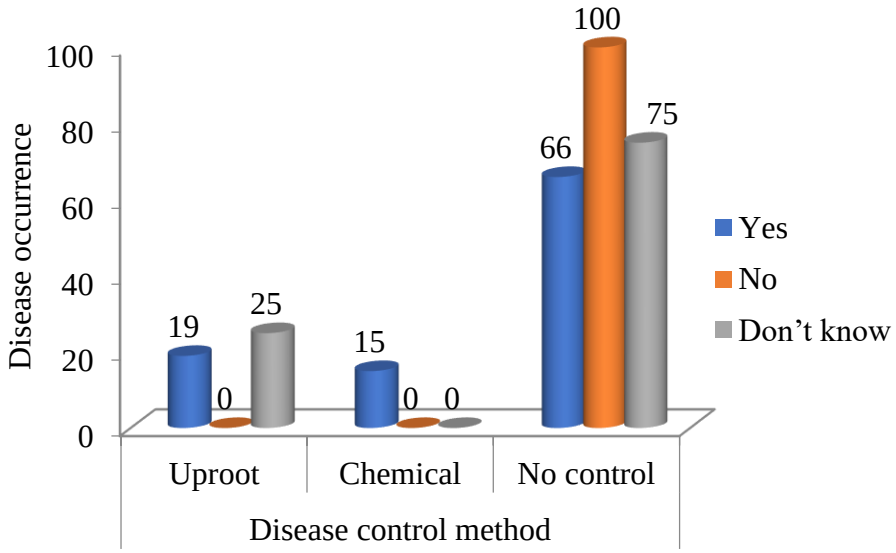


Figure 6. Disease management methods applied for different banana diseases in Nithi

4.1.6 Farmers Knowledge of Banana *Xanthomonas* Wilt in Nithi, Tharaka Nithi County

The results indicated that relationship between occurrence of banana diseases and knowledge of *Xanthomonas* wilt of banana in Nithi was significant (X^2 (2, N = 46) = 6.212, p = 0.0448). Out of the total number of farmers who reported occurrence of diseases in their farms, ninety two percent (92%) lack knowledge of *Xanthomonas* wilt of banana while eight percent (8%) knowledge of *Xanthomonas* wilt of banana. Hundred percent (100%) of farmers who did not know whether diseases occur in their farm also reported no knowledge of *Xanthomonas* wilt of banana (Figure 7).

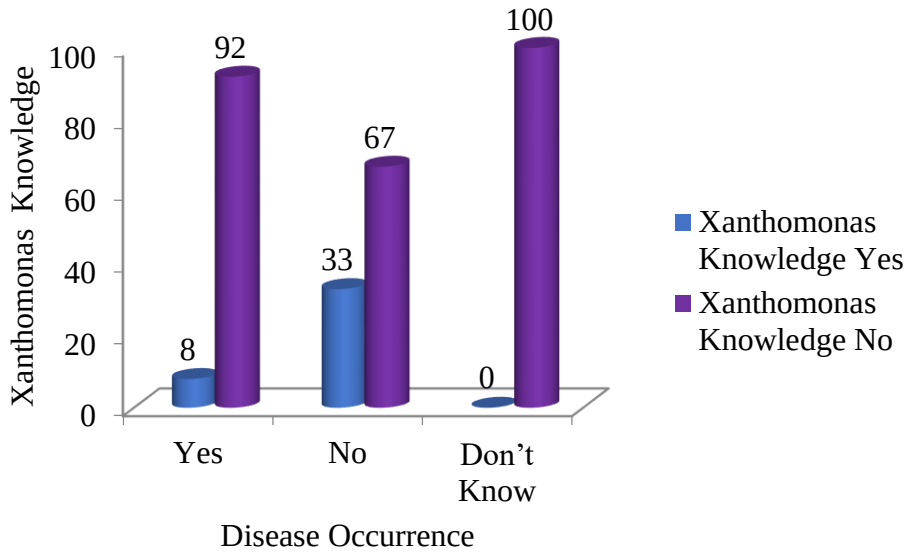


Figure 7. Knowledge of banana *Xanthomonas* wilt and disease occurrence in banana farms in Nithi, Tharaka Nithi County.

4.2. Morphological and Biochemical Characterisation of *Xanthomonas campestris*

4.2.1 Biochemical Characteristics of the *Xanthomonas* Isolate

A total of 19 *Xanthomonas* isolates were isolated from 24 symptomatic *Xanthomonas* infected banana leaf samples (Table 4). The isolates were slightly varied in colour for instance, isolate BXPS 12 was deep yellow while isolate BXPS 8 was pale yellowish colour (Plate 3). All the nineteen *Xanthomonas* isolates were oxidase negative as well as gram negative rods (Table 4). However, isolates BXPS8 and BXPS9 from Giampampo tested negative for KOH production. Results of Starch hydrolysis test on the isolates were both positive and negative. Isolates BXPS1, 5, 6, 10, 14, 16 and 17 tested negative for starch hydrolysis while the rest of the isolates tested positive. All the isolates *Xanthomonas* wilt pathogen isolates produced gas bubbles when a drop of hydrogen peroxide was added on to the colony on the slide and mixed with sterile toothpick. Thus, the isolates were considered catalase positive.

Table 4: Biochemical tests on banana *Xanthomonas* wilt pathogen isolates from banana in Nithi, Tharaka Nithi County

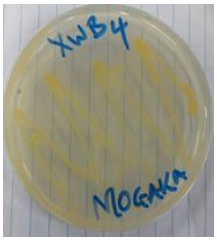
Isolate Accession Number	Place of Sample Collection	FlT	Cclr	CT	Gs	OT	KOH	ShT	GLT	AMT	IPT	VpT	MRT
BXPS1	Mitheru	-ve	Light yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS2	Mitheru	-ve	Medium yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS3	Mitheru	-ve	Deep yellow	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
BXPS4	Giampampo	-ve	Medium yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS5	Giampampo	-ve	Medium yellow	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve
BXPS6	Giampampo	-ve	Medium yellow	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
BXPS7	Giampampo	-ve	Deep yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
BXPS8	Giampampo	-ve	Pale Yellow	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS9	Giampampo	-ve	Pale yellow	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS10	Gibumbu	-ve	Pale Yellow	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
BXPS11	Gibumbu	-ve	Medium yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS12	Gibumbu	-ve	Deep yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS13	Kiangondu	-ve	Medium yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
BXPS14	Kiangondu	-ve	Pale Yellow	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
BXPS15	Kiangondu	-ve	Medium yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS16	Marima	-ve	Deep yellow	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
BXPS17	Marima	-ve	Medium yellow	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
BXPS18	Marima	-ve	Medium yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
BXPS19	Marima	-ve	Medium yellow	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve

Key: Cclr = Colony colour, CT = Catalase Test, Gs = Gram Stain, OT = Oxidase Test, KOH = Potassium hydroxide, ShT = Starch hydrolysis test, GLT = Gelatine liquefaction test, IPT = Indole production test, MRT= Methyl red test, AMT = Ammonia production test, PGT = Protein digestion test, VpT = Voges-Proskauer Test, FlT = Fluorescent test

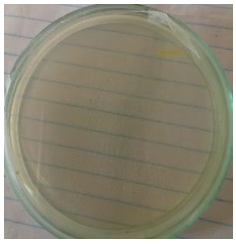
BXPS 1

BXPS 2

BXPS 3



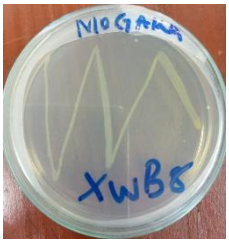
BXPS 4



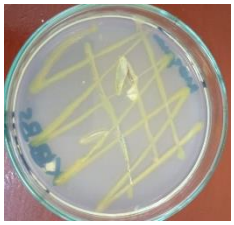
BXPS 5

BXPS 6

BXPS 7



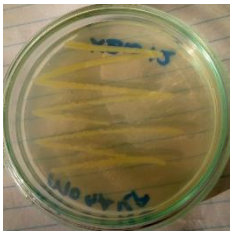
BXPS 8



BXPS 9

BXPS 10

BXPS 11



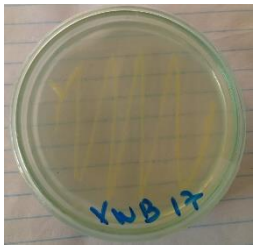
BXPS 12

BXPS 13

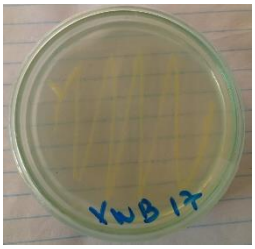
BXPS 14

BXPS 15

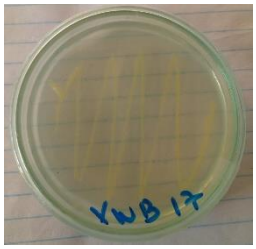
BXPS 16



BXPS 17



BXPS 18



BXPS 19

Key: BXPS = Banana *Xanthomonas* Wilt Pathogen; Sample 1-19

Plate 3: Cultures of *Xanthomonas* wilt of banana isolates

4.3 Susceptibility of Banana Variety to *Xanthomonas* Banana Wilt Pathogen under Greenhouse Experiment.

The five banana varieties were significantly ($p < 0.05$) susceptible to *Xanthomonas* wilt pathogen isolate (Appendix VII). Gasukari variety had the highest susceptibility mean of 70.37% followed by Kiganda with susceptibility mean of 60.74% (Table 5). Muraru had the lowest susceptibility mean of 45.19% (Table 5; Plate 4).

Table 5: Varietal Susceptibility to BXW

Banana Variety	Susceptibility Mean (%)
Gasukari	70.37 [a]
Kiganda	60.74 [b]
Kampala	54.81 [c]
Israel	50.37 [d]
Muraru	45.19 [e]
LSD (p<.05)	3.798
Mean	56.3
CV (%)	6.988

[a]Means covered by the same letters in the column are not significantly different at 5% probability level.


Kampala variety


Kampala variety


Israel variety


Israel variety


Gasukari variety


Gasukari variety


Muraru variety


Muraru variety


Kiganda variety


Kiganda variety

Plate 4: Images of banana variety reaction to *Xanthomonas* pathogen inoculation

4.3.1 Effect of pathogen index of *Xanthomonas campestris* on Susceptibility of Banana Varieties

Pathogen index significantly ($p<0.05$) affected banana susceptibility to *Xanthomonas* wilt pathogen isolate (Appendix VII). Concentration of 10^{-1} had the highest (76.0 %) while concentration of 10^{-3} caused lowest banana susceptibility.

Table 6: Effect of pathogen index on banana susceptibility to *Xanthomonas* Wilt disease

Pathogen index	Susceptibility Mean (%)
10^{-1}	76.0 [a]
10^{-2}	58.67 [b]
10^{-3}	34.22 [c]
LSD (p<.05)	2.943
Mean	56.3
CV (%)	6.988

[a]Means covered by the same letters in the column are not significantly different at 5% probability level.

CHAPTER FIVE
DISCUSSION

5.1 Prevalence of *Xanthomonas* Wilt in Nithi

Xanthomonas wilt of banana was observed in most of the farms surveyed. Prevalence of *Xanthomonas* was consistent from village to village with Giampampo having higher severity mean of 21.14% and Mitheru village recorded the lowest severity. According to Jaworski and Hilszczański (2013) and Mwangi *et al.* (2006), areas which are below 1700 m above the sea level have many insects which may contribute in rapid spreading of the disease across farms. Closeness of banana farms in the area of study may also be a contributing factor for increased incidence of *Xanthomonas* wilt (Uwamahoro *et al.*, 2019). Since the survey was conducted during the wet season the observed prevalence values may be attributed to precipitation. Higher precipitation have been pointed out as the prevailing factor in occurrence of *Xanthomonas campestris* pv. *musacearum* pathogen (Biruma *et al.*, 2007). For instance, farms have been observed to experience higher *Xanthomonas* wilt prevalence during rainy season unlike during drier seasons (Biruma *et al*., 2007). Precipitation encourages *Xanthomonas* pathogen survival, spore production, spore germination, multiplication and dispersion (Kang *et al*., 2010; Aung *et al.*, 2018).

5.1.1 Effect of Banana Variety on Occurrence of Banana *Xanthomonas* Wilt

Chi square test on the relationship between banana variety grown and occurrence of banana diseases in Nithi was significant X^2 (8, N = 46) = 19.93, p = 0.0034 (α = 0.05). Majority of farmers were found to grow mixed banana varieties and reported the highest number (76%) of occurrence of banana diseases. The results of this study are similar to those of (Uwamahoro *et al.*, 2019). According to Tooker and Frank (2012), genetically diverse banana cultivars attract various categories on insects' pollinators that hasten disease spread.

5.1.2 Banana Varieties Grown and their Susceptibility to Diseases in Nithi

Chi square test results indicated that relationship between banana variety grown and susceptibility to diseases in Nithi was significant X^2 (20, N = 46) = 31.165, p <0.0001 (α = 0.05). However, response on banana perceived to be susceptible was varied among the interviewed farmers. The result of this study is supported by Tripathi *et al.* (2009)

and Ocimati *et al.* (2013) that banana cultivated are susceptible to diseases. Most susceptible ones lack persistent bracts that minimize pathogen infection naturally (Lewis *et al.,* 2010). According to Tripathi (2009) and Mwangi and Nakato (2007), flowers of some banana varieties are less attractive to diseases vectors or may not be easily be penetrated by the bacteria which minimizes infection.

5.1.3 Prevalence of Banana Diseases with year of growing banana Plants

Chi square test indicated that relationship between years of growing banana in the same farm and occurrence of banana diseases in Nithi was significant X^2 (6, N = 46) = 8.761, p = <0.0001 (α = 0.05). According to Ocimati *et al.* (2019), some suckers in a mat may be free of pathogen despite the parent sucker showing *Xanthomonas* wilt symptoms. Likewise, in cases where parent banana plants may not succumb to *Xanthomonas* wilt some of its suckers might be attacked by the diseases.

5.1.4 Disease Occurrence across Different Seasons of the Year in Nithi, Tharaka Nithi County

Chi square test of independence indicated that relationship between occurrence of banana diseases and season of the year in Nithi was significant X^2 (8, N = 46) = 32.4591, p = < 0.0001 (α = 0.05). Sixty five percent (65%) of farmers reported that banana diseases mostly occur during the dry season. Sixty three (63%) of farmers reported no knowledge of disease occurrence and season that diseases are common. Observation from majority of the respondents that diseases occur mostly in dry season correlates with those of (Tushemereirwe *et al*., 2004). However, results differed to those of Ewané *et al*., 2013). Conditions that are favorable promote pathogen reproduction, development and pathogen persistence (Ochola *et al*., 2015). Development of bacteria wilt associated with the bacteria that affects the plant xylem may be significantly increased by water deficit (McElrone *et al.,* 2001). Increased temperature due to drought may lead to the breakdown resistance genes that are sensitive to heat in plants (Gijzen *et al.,* 1996; Bonnett *et al.,* 2002) and it can be difficult to discriminate between effects on host resistance genes and effects on pathogen virulence.

5.1.5 Disease Management Methods Applied for Different Banana Diseases

The chi square test showed that relationship between occurrence of banana diseases and management option was significant X^2 (4, N = 46) = 6.9758, p = 0.0025 (α = 0.05). However, sixty six percent (66%) of the respondents who reported the occurrence of diseases in their farm do not apply any control mechanism. These results are supported by the report of Rutikanga *et al*. (2013) but differed to those of Hashim (2013) where majority of farmers reported cutting down banana plant once infected. Farmers have the tendency to ignore diseases occurrence favoring persistence of the pathogen in the farm (Rutikanga *et al*., 2013). Infected pseudostem remains a potential source of inoculum and a key factor in spread of banana diseases (Shimwela *et al.,* 2016; Ocimati *et al.,* 2019). According to Bagamba *et al.* (2006), due to fear of loosing income from banana, farmers may not be willing to uproot the infected bananas even when infected. Disease management is important in regulating the pressure of infection on farms. Prevention of diseases in the farm requires concerted effort to prevent pathogen entry and spread. Thus, sterilization of equipment, timely removal of infected plants is necessary (Blomme *et al*., 2017; Biruma *et al*., 2007). Regular use of farm equipment that is not sterilized is likely to increase the frequency of disease occurrence particularly during the wet season (Blomme *et al.*, 2014).

5.1.6 Farmers Knowledge of Banana *Xanthomonas* Wilt in Selected Areas in Tharaka Nithi County

The relationship between occurrence of banana diseases and knowledge of *Xanthomonas* wilt of banana in Nithi was significant (X^2 (2, N = 46) = 0 6.212, p = 0.0448). Out of the total number of farmers who reported disease occurrence, Eight percent (8%) had knowledge of *Xanthomonas* wilt of banana while (92%) reported lack of knowledge of *Xanthomonas* wilt of banana. Thirty-three percent (33%) of farmers who reported no occurrence of diseases in their farms, had knowledge of *Xanthomonas* wilt and the rest had no knowledge. These results on knowledge of *Xanthomonas* wilt of banana differ with those of Uwamahoro *et al.* (2019) in which majority of the respondents were aware of *Xanthomonas* wilt of banana.

5.2 Characterization of *Xanthomonas* Wilt Pathogen of Banana in Selected Areas in Tharaka Nithi County

Results of Starch hydrolysis test on the isolates were both positive and negative. Isolates BXPS3, 5, 6, 10, 14, 16 and 17 tested negative for starch hydrolysis while the rest of the isolates tested positive. The finding of this study on hydrolysis of starch by the *Xanthomonas* isolates differs with the findings of Guvera and Marsella (1999) who failed to make such variations. The result on hydrolysis test is supported by the report by Swings *et al.* (1990) that *Xanthomonas* isolates are capable of hydrolysing starch. The bacterium hydrolyzes starch only in the areas surrounding their colonies in the media while the remaining areas have non-hydrolyzed starch. On addition of iodine solution, hydrolyzed parts of the media fails to forms dark blue color unlike non hydrolyzed parts (Yazdanparasat, 1993).

All the isolates *Xanthomonas* wilt pathogen isolates produced gas bubbles when a drop of hydrogen peroxide was added on to the colony on the slide and mixed with sterile toothpick. Thus, the isolates were considered catalase positive. Catalase test is useful for differentiating bacteria that are morphologically similar. A gram-negative bacterium produces bubbles when mixed with hydrogen peroxide due to activity of catalase enzyme breaking down hydrogen peroxide into water and oxygen. Catalase enzyme is vital for detoxification of hydrogen peroxide toxicity in bacteria (Abdo-Hasan *et al.,* 2008).

Methyl red is a test that differentiates high acidic from low to non-acidic producing bacteria (Zaoti *et al.,* 2018). Five days after incubation three drops of methyl red reagent was added to the culture and all the isolates tested negative. Negative methyl red reaction has been reported for other isolates of *Xanthomonas* (Ogolla and Neema, 2019). Failure by the *Xanthomonas* isolates to metabolize the glucose and production of acetoin is the reason for negative methyl red test. In positive methyl red test like those of sample, PXBS5, PXBS7 and PXBS13, acetoin is always oxidized to diacetyl that forms red colour with naphtol at an alkaline pH.

All the *Xanthomonas* isolates tested negative for VP test. Negative test suggests that they did not produce acetymethylcarbinol. Voges – Proskaure test when positive

indicate acetylmethylcarbinol production in the process of fermentation of glucose (Arshad *et al.*, 2015). Indole test for all the isolates was negative indicated by failure of the isolates to form red ring. The negative indole test observed in this study corroborates with those of Arshad *et al.* (2015). Positive indole reaction results from bacteria isolate production of endoenzyme tryptophanase. Endoenzyme tryptophanase hydrolyses amino acid tryptophan and produces ammonia, indole and pyruvic acid (Ponpom *et al.*, 2005). Indole combines, in the presence of a tryptophan rich medium, with pDimethylaminobenzaldehyde at an acid pH in alcohol resulting in reddish ring (Ali, 2013).

5.3 Susceptibility of Banana Genotypes to *Xanthomonas* Wilt Pathogen

Susceptibility of five banana varieties to *Xanthomonas* wilt pathogen isolate was statistically significant ($p<0.05$). All the banana varieties screened in this study were susceptible to *Xanthomonas* wilt. However, Gasukari was most susceptible when compared to the rest of the varieties. Variation in susceptibility of different banana varieties may be attributed to variation in rate of immune response and response magnitude that depend on expression of resistant genes (Tripathi *et al.*, 2019). Timely activation of proteolysis process in plant helps trigger plant defense response once invaded by pathogen (Xia, 2004). Delay in production of proteins such as Osmotin-like protein, a PR5, Germin-like protein may aggravate plant susceptibility to pathogens (Breen and Bellgard, 2010; Chowdhury *et al.*, 2017). The finding of this study is supported by the studies of Ssekiwoko *et al.* (2006) and those of Tripathi and Tripathi (2009) who observed susceptibility of various banana varieties to *Xanthomonas campestris* pv. *musacearum* pathogen. From the result of this study, it is plausible to report that the five banana varieties have no resistant characteristic towards *Xanthomonas* wilt of banana.

Pathogen concentration had significant effect on the susceptibility of banana cultivars ($p<0.05$). Similar results on the effect of pathogen concentration on plant disease severity has been reported by a number of researchers for different pathogens (Raftoyannis and Dick, 2002; Papadaki and Ladomenou, 2018). According to Agrios (2005) higher pathogen density increases the frequencies of colonization of plant tissue by the pathogen hence high plant susceptibility.

CHAPTER SIX

SUMMARY OF KEY FINDINGS, CONCLUSION AND RECOMMENDATIONS

6.1 Summary of the Key Findings

Forty six banana famers were surveyed in the month of October 2019 for the prevalence of *Xanthomonas* wilt of Banana in Nithi region of Tharaka Nithi County in Kenya. Questionnaire was used to gather farmer's knowledge of *Xanthomonas* wilt of banana, management practices, and type of Banana they grow and the source of planting material among others (Appendix II). The villages where survey was conducted were Marima village, Mitheru village, Giampampo village, Gibumbu village and Kiangondu village. Three banana farms of 0.5 acre in size were selected for banana sample collection for laboratory evaluation. In every farm, transect was laid across the banana plantation and six (points) locations were established at approximate equidistance of 5 m.

Prevalence of Xanthomonas wilt of banana in selected villages Nithi were not significantly different. However, higher diseases prevalence was observed at Giampampo. Only three (3) villages recorded severity mean above the overall mean. The Chi square test for independence revealed a significant relationship between banana variety grown, years of banana growing, and the occurrence of banana diseases. Chi square test of independence indicated that there was significant relationship between occurrence of banana diseases and season of the year in Nithi was significant being the majority reported that banana diseases mostly occur during the dry season. There was significant relationship between occurrence of banana diseases and management option in Nithi was significant.

Chi square test of independence indicated that there was significant relationship between occurrence of banana diseases and knowledge of *Xanthomonas* wilt was significant. Out of the total respondents who reported occurrence of diseases in their farm, 8% reported knowledge of *Xanthomonas* wilt of banana while the remaining 92% reported lack of knowledge of *Xanthomonas* wilt of banana. Farmers need to be made aware of banana diseases including BXW.

A total of nineteen *Xanthomonas* isolates were isolated from 24 symptomatic *Xanthomonas* infected banana leaves samples. Five of the isolates were light yellow, ten were medium yellow while four were deep yellow. Biochemical testes Catalase Test, Gram Stain, Oxidase Test, Potassium hydroxide, Starch hydrolysis test, Gelatine liquefaction test, Indole production test, Methyl red test, Ammonia production test, Protein digestion test, Voges-Proskauer Test, Fluorescent test was carried out on the isolates.

The nineteen *Xanthomonas* isolates were oxidase negative as well as gram negative rods (Table 4). The isolates were gram negative bacterium since the primary purple colour of crystal violate was washed out by acid alcohol and counter stained with crystal violet appearing reddish. *Xanthomonas* isolates tested positive to gelatine hydrolysis. Gelatine medium was observed to flow at the tilting of the test tube. In King's B medium inoculated with *Xanthomonas* isolates there was no fluorescence under the UV- light, indicating that the isolates were negative to fluorescence test. Seventeen isolates out of 19 tested positive for KOH by forming sticky slime which when raised using toothpick formed thread like elongation. Isolate BXPS8 and BXPS9 from Giampampo tested negative for KOH production. Thirteen isolates tested positive for Starch hydrolysis test while isolate BXPS1, 5, 6, 10, 14, 16 and 17 tested negative. All the isolates *Xanthomonas* wilt pathogen isolates produced gas bubbles when a drop of added on to the colony on the slide and mixed with sterile toothpick. Thus, the isolates were considered catalase positive.

Susceptibility of five banana varieties to Xanthomonas wilt pathogen isolate was statistically significant. Gasukari variety had the highest susceptibility mean of 70.37%. Kampala, Israel and Muraru varieties had lower means when compared to the susceptibility overall mean score of 56.3.

6.2 Conclusion

This study concludes that the majority of farmers are not aware of existence of *Xanthomonas* wilt of banana. Furthermore, farmers who were interviewed expressed lack of adequate knowledge on methods of managing occurrence and spread of diseases in their banana farms. The respondents appeared not being able to identify common

diseases of banana. Based on these observations, there is need to educate farmers on best banana farming practices, disease identification and management. Biochemical tests, Catalase Test, Gram Stain, Oxidase Test, Potassium hydroxide, Starch hydrolysis test, Gelatine liquefaction test, Indole production test, Methyl red test, Ammonia production test, Protein digestion test, Voges-Proskauer Test, Fluorescent test carried out on the isolates confirms that the causative agent banana wilt is *Xanthomonas campestris*. All the banana varieties screened are susceptible to *Xanthomonas* wilt of banana though at different frequencies and Gasukari variety is more susceptible.

6.3 Recommendations

This study recommends that:

i. Farmers should be educated on the symptoms and management of *Xanthomonas* wilt.
ii. The respondents seemed to be unable to recognize various banana diseases. Based on these findings, farmers should be educated on the best banana farming methods, as well as disease detection and management.
iii. Since Muraru variety showed a high level of resistance to BXW, it could be a good source of breeding material for farmers in the region.

6.4 Suggestions for Further Study

i. Study on the prevalence of *Xanthomonas* wilt of banana should in future cover the whole of Tharaka Nithi County to determine the extent of spread since banana is grown throughout the county.
ii. Characterization of the pathogen should be done using molecular tool to determine the genetic variations on the pathogen.
iii. Screening for banana resistance that include molecular tool should include all the varieties grown in Tharaka Nithi and neighboring Counties. Such extended screening may provide resistance source for banana breeding

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APPENDICES

Appendix I: Coordinates of Sample Collection Area

Village	Longitudes	Latitudes	Height above sea level (M)
Marima	S00°27.391'	E037°65.361'	4669.11
Marima	S00°27.556'	E037°68.903'	4710.9
Marima	S00°25.991'	E037°67.651'	4348.83
Mitheru	S00°29.155'	E037°67.392'	4244.92
Mitheru	S00°29.588'	E037°67.245'	4408.69
Mitheru	S00°30.093'	E037°67.587'	4372.46
Kiang'ondu	S00°32.104'	E037°60.921'	5119.32
Kiang'ondu	S00°32.321'	E037°62.073'	5013.89
Kiang'ondu	S00°32.674'	E037°62.629'	4897.43
Gibumbu	S00°32.545'	E037°63.525'	4797.35
Gibumbu	S00°32.635'	E037°63.420'	4816.18
Gibumbu	S00°32.545'	E037°63.525'	4797.35
Giampampo	S00°30.275'	E037°65.662'	4550.33
Giampampo	S00°30.566'	E037°64.641'	4645.16
Giampampo	S00°30.874'	E037°64.055'	4686.8

Appendix II: The Questionnaire for the Evaluation of Banana Distribution

(a) General Information

Form No:____ Gender [ ] Male Occupation_____ Level of education_____

[ ] Female

District_____ Division_____Location_____ Sub location_____

Village_____

Type of farming being practised; Small scale [ ]

Large scale [ ]

Crops grown by the farmer besides bananas

1 Maize 2 Beans, 3 Sugarcane, 4Cofee, 5Tea,

(b) Survey Questions

1. How long have you been growing bananas?

   1 (1-3 years), 2 (4-6 years), 3 (7-10 years), 4 (More than 10 years),

2. Which variety do you grow? 1 Muraru,
   2 Kiganda,
   3 Gasukari,
   4 Israel,
   5 Kampala,
   6 Mixed,
   7 Don't know,

3. Where do you obtain your planting materials from?

   1 Market,
   2 Research institutions,
   3 Other farmers,
   4 Others…….

4. (a) Have you ever experienced any banana disease in your farm? [ ] Yes
   [ ] No
   [ ] Don't know

   (b) If yes, what are the symptoms?

5. (a) what is the total number of banana plants in your farm? 1 (1-10), 2 (10-30),

3 Others

6. (a) Are you able to differentiate the diseases present in the farm? [ ] Yes
[ ] No

(b) If yes. What are the symptoms?

7. Do you have any knowledge on banana *Xanthomonas* wilt disease? [ ] Yes
[ ] No

8. Have you ever experienced banana *Xanthomonas* wilt disease in your farm?

[ ] Yes

[ ] No

[ ] Don't know (not aware)

9. (a) Which is the most prone variety to diseases?

1 Muraru

2 Kiganda

3 Gasukari

4 Israel

5 Kampala

6 Don't know…….

(b) Which period (season) of the year do your banana plants experience diseases.

[ ] Little rain, [ ] Dry period, [ ] Heavy rain, [ ] Year round, [ ] Don't know

10. How do you manage banana diseases in your farm? 1. Uprooting the plant

2 Chemicals

3 Removal of infected sections

4 No control……

5 Others (specify)……

10. (a) Have you ever been visited by any research person checking diseases? [ ] Yes
[ ] No

(b)If yes, how many times? 1. Once. 2. Twice. 3. More than twice

Appendix III: Chuka University Institutional Ethics Review Committee Clearance

CHUKA UNIVERSITY

Knowledge is Wealth *(Sapientia divitia est)* Akili ni Mali

CHUKA UNIVERSITY INSTITUTIONAL ETHICS REVIEW COMMITTEE

Telephones: 020-2310512/18
Direct Line: 0772894438
Email: ierc@chuka.ac.ke,
P. O. Box 109-60400, Chuka
Website: www.chuka.ac.ke

REF: CUIERC/ NACOSTI/041
TO: MELKZEDEK MOGAKA ONYAMBU

23rd September, 2019

Dear Sir/madam

RE: CHARACTERIZATION AND EVALUATION OF BANANA GENOTYPES RESISTANT TO XANTHOMONAS WILT DISEASE (XANTHOMONAS COMPESTRIS PV. MUSACEARUM) IN THARAKA NITHI COUNTY

This is to inform you that ***Chuka University IERC*** has reviewed and approved your above research proposal. Your application approval number is ***NACOSTI/NBC/AC-0812.*** The approval period is **23rd September, 2019 – *31st August, 2020.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***Chuka University IERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***Chuka University IERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***Chuka University IERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***Chuka University IERC.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

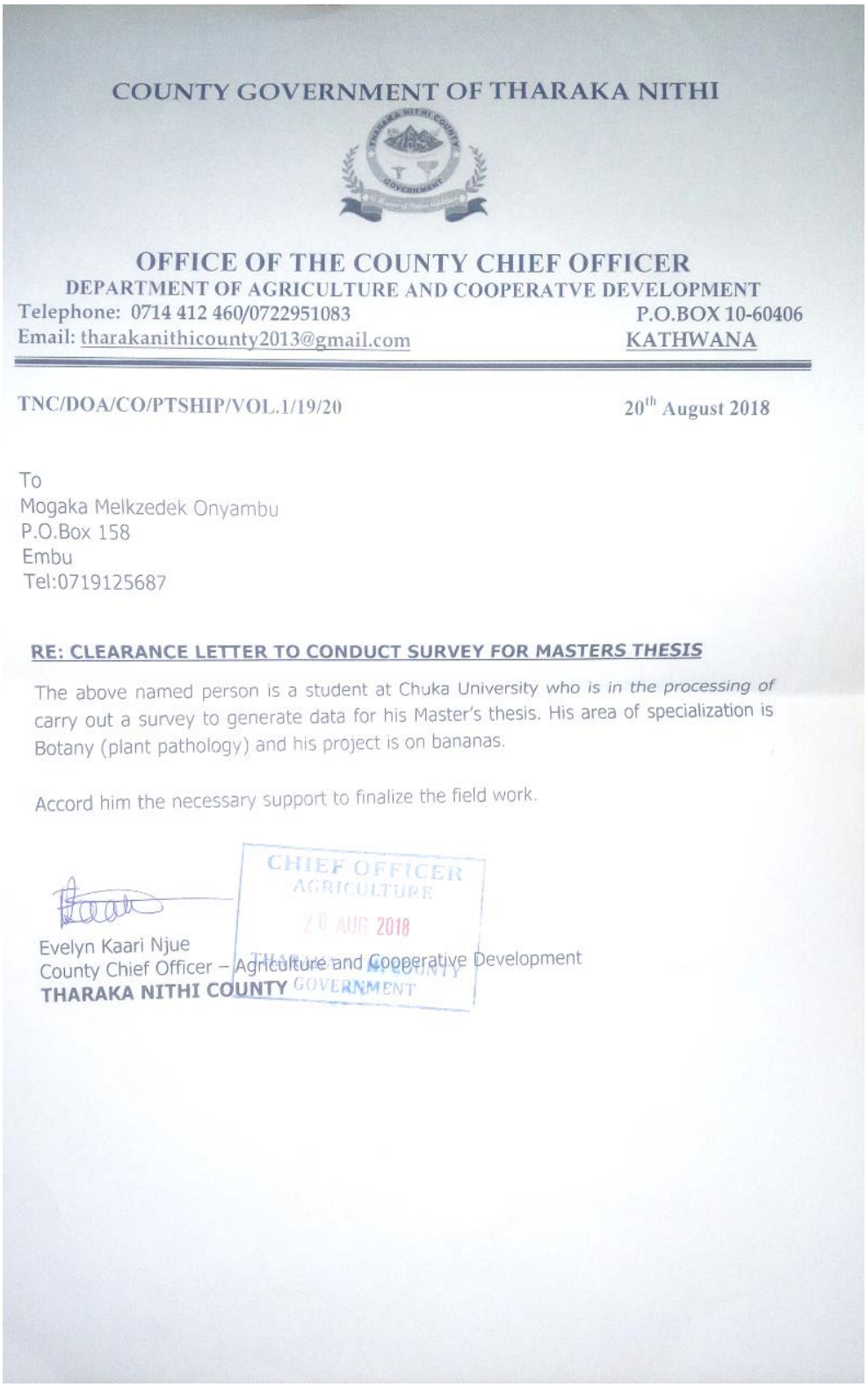
Yours sincerely

PROF. ADIEL MAGANA
CHAIRMAN CHUKA UNIVERSITY IERC

Chuka University is ISO 9001:2015 Certified...

Inspiring Environmental Sustainability for Better Life

Appendix IV: Tharaka Nithi County Agriculture Department Clearance



COUNTY GOVERNMENT OF THARAKA NITHI

OFFICE OF THE COUNTY CHIEF OFFICER
DEPARTMENT OF AGRICULTURE AND COOPERATVE DEVELOPMENT
Telephone: 0714 412 460/0722951083 P.O.BOX 10-60406
Email: tharakanithicounty2013@gmail.com KATHWANA

TNC/DOA/CO/PTSHIP/VOL.1/19/20 20th August 2018

To
Mogaka Melkzedek Onyambu
P.O.Box 158
Embu
Tel:0719125687

RE: CLEARANCE LETTER TO CONDUCT SURVEY FOR *MASTERS THESIS*

The above named person is a student at Chuka University who is in the processing of carry out a survey to generate data for his Master's thesis. His area of specialization is Botany (plant pathology) and his project is on bananas.

Accord him the necessary support to finalize the field work.

CHIEF OFFICER
AGRICULTURE
20 AUG 2018
THARAKA NITHI COUNTY GOVERNMENT

Evelyn Kaari Njue
County Chief Officer – Agriculture and Cooperative Development
THARAKA NITHI COUNTY

Appendix V: National Commission for Science Technology and Innovation Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **529669**

Date of Issue: **07/April/2020**

RESEARCH LICENSE

This is to Certify that Mr.. MOGAKA ONYAMBU of Chuka University, has been licensed to conduct research in Tharaka-Nithi on the topic: CHARACTERISATION AND EVALUATION OF BANANA GENOTYPES RESISTANT TO XANTHOMONAS WILT DISEASE (Xanthomonas campestris pv. musacearum) IN THARAKA NITHI COUNTY for the period ending : 07/April/2021.

License No: **NACOSTI/P/20/4686**

529669

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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Appendix VI: Analysis of Variance for Prevalence of *Xanthomonas* wilt of Banana in Selected Villages in Nithi

Source	df	Sum of Squares	Mean Square	F Value	p-value
Model	9	6.199.	0.689	0.98	0.461
Error	80	56.115	0.701		
Corrected Total	89	62.3124			
Source	df	Type III SS	Mean Square	F Value	p-value
Replication	5	1.322	0.2644	0.38	0.863
Villages	4	4.877	1.2191	1.74	0.149

Appendix VII: Analysis Variance for Severity of Banana Varieties to *Xanthomonas* wilt of Banana Pathogen Isolates under Greenhouse Experiment

Source	df	Type III SS	Mean Square	F Value	p-value
Rep	2	11.03162	5.51581	0.36	0.7033
Conc	2	13213.98945	6606.99473	426.88	<.0001
Variety	4	3398.45578	849.61394	54.89	<.0001
Conc*Variety	8	657.51169	82.18896	5.31	0.0004

Appendix VIII: Raw Data on Banana *Xanthomonas* Wilt Severity

Observation	Replication	Village	No of sucker	No. of infected suckers	Severity (%)
1	1	Marima	6	0	0.0
2	2	Marima	8	2	25.0
3	3	Marima	9	2	22.2
4	4	Marima	5	1	20.0
5	5	Marima	6	0	0.0
6	6	Marima	5	1	20.0
7	1	Marima	11	4	36.4
8	2	Marima	7	0	0.0
9	3	Marima	9	1	11.1
10	4	Marima	6	1	16.7
11	5	Marima	8	2	25.0
12	6	Marima	4	1	25.0
13	1	Marima	10	2	20.0
14	2	Marima	6	0	0.0
15	3	Marima	8	1	12.5
16	4	Marima	10	1	10.0
17	5	Marima	7	1	14.3
18	6	Marima	5	0	0.0
19	1	Mitheru	9	0	0.0
20	2	Mitheru	5	1	20.0
21	3	Mitheru	8	1	12.5
22	4	Mitheru	4	0	0.0
23	5	Mitheru	5	1	20.0
24	6	Mitheru	8	0	0.0
25	1	Mitheru	5	0	0.0
26	2	Mitheru	4	1	25.0
27	3	Mitheru	7	3	42.9
28	4	Mitheru	12	1	8.3
29	5	Mitheru	8	0	0.0
30	6	Mitheru	6	0	0.0
31	1	Mitheru	6	0	0.0
32	2	Mitheru	12	4	33.3
33	3	Mitheru	9	2	22.2
34	4	Mitheru	11	0	0.0
35	5	Mitheru	7	0	0.0
36	6	Mitheru	7	1	14.3
37	1	Kiangondu	7	0	0.0
38	2	Kiangondu	14	4	28.6
39	3	Kiangondu	6	0	0.0
40	4	Kiangondu	10	2	20.0
41	5	Kiangondu	9	1	11.1
42	6	Kiangondu	11	1	9.1
43	1	Kiangondu	4	1	25.0
44	2	Kiangondu	10	2	20.0
45	3	Kiangondu	5	0	0.0
46	4	Kiangondu	7	2	28.6

Observation	Replication	Village	No of sucker	No. of infected suckers	Severity (%)
47	5	Kiangondu	6	1	16.7
48	6	Kiangondu	9	3	33.3
49	1	Kiangondu	3	0	0.0
50	2	Kiangondu	7	2	28.6
51	3	Kiangondu	9	1	11.1
52	4	Kiangondu	6	1	16.7
53	5	Kiangondu	5	1	20.0
54	6	Kiangondu	7	2	28.6
55	1	Gibumbu	13	3	23.1
56	2	Gibumbu	7	0	0.0
57	3	Gibumbu	8	1	12.5
58	4	Gibumbu	7	0	0.0
59	5	Gibumbu	9	0	0.0
60	6	Gibumbu	10	3	30.0
61	1	Gibumbu	3	0	0.0
62	2	Gibumbu	10	4	40.0
63	3	Gibumbu	5	0	0.0
64	4	Gibumbu	9	2	22.2
65	5	Gibumbu	9	1	11.1
66	6	Gibumbu	11	2	18.2
67	1	Gibumbu	9	0	0.0
68	2	Gibumbu	13	2	15.4
69	3	Gibumbu	6	0	0.0
70	4	Gibumbu	7	1	14.3
71	5	Gibumbu	9	2	22.2
72	6	Gibumbu	5	0	0.0
73	1	Giampampo	8	1	25.0
74	2	Giampampo	5	0	0.0
75	3	Giampampo	9	4	44.4
76	4	Giampampo	14	2	14.3
77	5	Giampampo	10	3	30.0
78	6	Giampampo	9	1	11.1
79	1	Giampampo	3	1	33.3
80	2	Giampampo	6	0	0.0
81	3	Giampampo	5	1	20.0
82	4	Giampampo	7	2	28.6
83	5	Giampampo	9	3	33.3
84	6	Giampampo	10	4	40.0
85	1	Giampampo	4	1	25.0
86	2	Giampampo	8	3	37.5
87	3	Giampampo	7	4	57.1
88	4	Giampampo	5	0	0.0
89	5	Giampampo	9	1	11.1
90	6	Giampampo	8	0	0.0

Appendix IX: Raw data of Susceptibility of Banana Varieties to *Xanthomonas* wilt of Banana Pathogen Isolates

Observation	Replication	Concentration	Variety	Severity
1	1	10^1	Gasukari	40.00
2	2	1	Gasukari	46.67
3	3	1	Gasukari	40.00
4	1	1	Muraru	26.67
5	2	1	Muraru	26.67
6	3	1	Muraru	33.33
7	1	1	Kiganda	33.33
8	2	1	Kiganda	33.33
9	3	1	Kiganda	33.33
10	1	1	Kampala	33.33
11	2	1	Kampala	33.33
12	3	1	Kampala	33.33
13	1	1	Israel	33.33
14	2	1	Israel	33.33
15	3	1	Israel	33.33
16	1	2	Gasukari	73.33
17	2	2	Gasukari	66.67
18	3	2	Gasukari	80.00
19	1	2	Muraru	46.67
20	2	2	Muraru	46.67
21	3	2	Muraru	40.00
22	1	2	Kiganda	66.67
23	3	2	Kiganda	66.67
24	3	2	Kiganda	73.33
25	1	2	Kampala	53.33
26	2	2	Kampala	60.00
27	3	2	Kampala	53.33
28	1	2	Israel	60.00
29	2	2	Israel	46.67
30	3	2	Israel	46.67
31	1	3	Gasukari	100.00
32	2	3	Gasukari	93.33
33	3	3	Gasukari	93.33
34	1	3	Muraru	66.67
35	2	3	Muraru	60.00
36	3	3	Muraru	60.00
37	1	3	Kiganda	80.00
38	2	3	Kiganda	80.00
39	3	3	Kiganda	80.00
40	1	3	Kampala	73.33
41	2	3	Kampala	73.33
42	3	3	Kampala	80.00
43	1	3	Israel	66.67
44	2	3	Israel	66.67
45	3	3	Israel	66.67

Where, concentration 1 = 10^1, concentration 2 = 10^2 and concentration 3 = 10^3

Appendix X: Letter of Introduction

MOGAKA MELKZEDEK ONYAMBU
P O BOX 158,
EMBU.
TEL: 0719 125687
Date……

TO…….

Dear Sir/Madam,

RE: PERMISSION TO CARRY OUT FARM STUDY

This is to let you know that I Mogaka Melkzedek Onyambu is a student at Chuka University currently undertaking a master's degree in Botany (Plant Pathology). My registration number is NM23/28942/16. I kindly wish to carry out a farm study in your banana farm.

The research questionnaire and farm observation on your bananas is of no ill intention but it's geared to assess Banana *Xanthomonas* wilt Disease in Tharaka Nithi County, the information obtained will be highly appreciated and will be used for educational purposes in the University.

Your assistance will be warmly regarded. Thank you.

Yours Faithfully,
Mogaka Melkzedek Onyambu